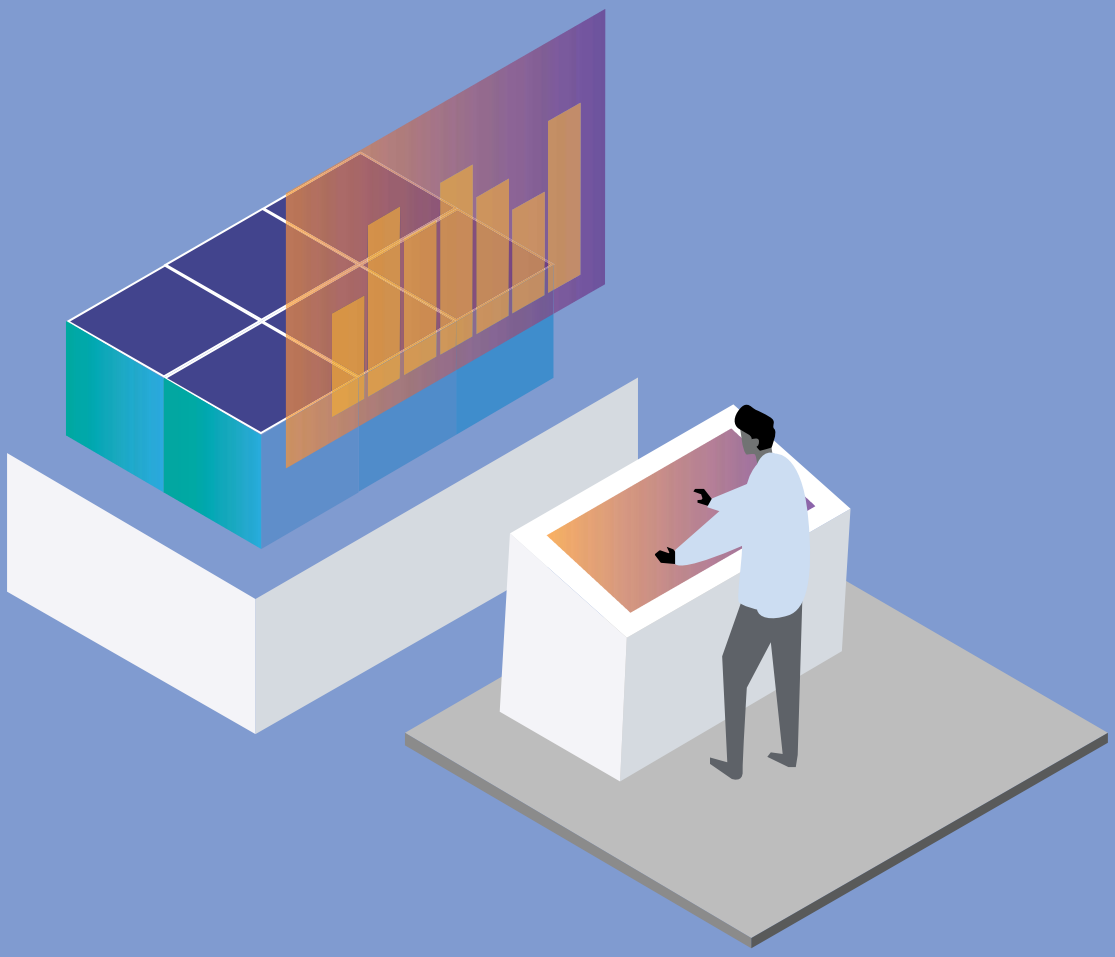

DESIGN FOR AN AUTOMATED SYSTEM FOR RESEARCH ON GENETIC DISEASES

System Concept development for the automation of iPSC culture

Erasmus MC

Master thesis - Natasa Rikalo
Integrated Product Design
August 2020, Delft

TU Delft

Graduate student and author
Rikalo, N.
Master Integrated Product Design
Faculty of Industrial Design Engineering
TU Delft

Chair
Prof. dr. ir. Goossens, R.H.M.
Section Applied Ergonomics and Design
Department of Industrial Design
Faculty of Industrial Design Engineering
TU Delft

Mentor
Prof.dr.ir. A. Bozzon
Human-Centered Artificial Intelligence
Department of Design Engineering
Faculty of Industrial Design Engineering
TU Delft

External Mentor
Mehrnaz Ghazvini
Department of Developmental Biology,
Erasmus MC

Delft University of Technology - August 2020

DESIGN FOR AN AUTOMATED SYSTEM FOR RESEARCH ON GENETIC DISEASES

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Acknowledgements

This project concludes the path of my official education: six overall years of studying the amorphous discipline of design and adapting it to new topics to create something tangible, hopefully innovative, and serving a good cause.

I am grateful to all the people that inspired me in these two last years: it has been a time of constant redefinition and exploration that opened my view a little wider on even more possibilities to come.

I thank my supervisors, Richard and Alessandro, for showing support also in the moments of its redefinition and doubt. I am thankful to Joost and Mehrnaz from Erasmus MC for the patience and comprehension with which they let me sneak in a world I would have never thought to come in contact with. Thank you to all the ones that have participated in the ideation sessions and contributed to my research.

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To my friends here, there and back home, thank you for checking in during this project, making me laugh, sharing a story, and making me part of your lives.

The biggest thank you goes to my sister, for the attention she gives me, the love she never hides and having always trust in me.

Executive summary

Human induced pluripotent stem cells (hiPSCs) offer the possibility to model human disease and study their behavior. They help scientists track the early disease-causing events in cells and are therefore used in discoveries about premature aging, congenital heart disease, cancer, and all the other disorders that are connected to fetal development. Because of their characteristics, these cells can be employed to create any cell of the body and since they are derived by patient cells, potentially they can be manipulated to fix disease-causing defects and create healthy cells for transplants.

However, the production of these promising cells is usually done by the tedious and time-consuming work of technicians in a lab who, basing decisions on their experience and knowledge on cell behavior, bring further the production of good quality cell lines for partnering researchers.

An increase in interest in population studies means that there is more request for the production of cell lines, but in order to keep up with the need, the IPS Facility in Rotterdam would need to transition to automation.

At the moment there are a limited number of systems for the automated production of IPSC cell lines. However, these robots are not able to readapt to changes of the process, or of the spaces where they are installed and do not follow exactly the process needs of the IPS Facility in Rotterdam, who initiated with this project the set up of an own automated system. The system design objective has been reached by combining several fronts of investigation.

On one side, the process of production has been initially investigated in order to identify functional needs. The results of this analysis have been merged with observations of the daily activities and work organization of the laboratory, which contributed to the understanding of the human factors necessary within biological production. An extensive comparative analysis of other automated systems has been conducted in order to identify the system structure that comes closest to the needs of the IPS Core Facility production needs. Further analysis and realignment have been conducted on the final components necessary for the satisfaction of the functional needs of the cell production process. On the other side, ideation sessions, and considerations on modularity, management of throughput and composition lead to the development of system architecture concepts. The combination of these two research sides and synthesis of the strengths of four initial concepts lead to one final proposition for the architecture layout of the automated system for IPSC production: the RXF. This is a system composed of three modules, each of which is used during one section of the overall IPSC production process, giving possibilities of repurposing the remaining modules for other needs.

A final evaluation phase of the project made it possible to highlight risks and most relevant cost factors, to be used in further stages for further development and decision making.

RELEVANT INSIGHTS:

1. .

REQUIREMENT DECISION:

1. .

Figure 2: Comunication on relevant insights and Requirement decisions

Preface

Reading guide

These two initial pages provide the reader with an overview of the visual language used in this document to support the understanding of its content.

The initial introduction paragraph explains its following Chapter. Here the reader can find informations on the nature of the content and the reasoning used for grouping such topics together.

On the same page, a dialog box as the one showed in Figure 1 recalls the Table of Contents relative to the current Chapter.

At the end of each paragraph, dialog boxes as in Figure 2 summarize the relevant insights, sky blue when on a general level, midnight blue when connected to requirements.

At the end of each Chapter a synthesis is provided.

Figure 1: Chapter TOC

Project Brief

The problem
The process of production of human induced pluripotent stem cells (hIPSCs) is time consuming. It requires constant monitoring and it is dependent on the precision of researchers working physically on the cells.

Therefore, there is need for automation of production of IPSC lines in order to keep up with the demand and facilitate population studies in the dish.

There have been attempts in automation of the process which led to the development of three robots for the automation of hIPSCs cells.

These robots do not allow for modularity of the system and flexibility in the methods they are used for in case of future rearrangement of activities in the facility that uses them.
Further on, each facility has its own needs in terms of functions necessary for the process and management of the workflow, therefore the systems alread existend could be not suitable for the IPS Core Facility at the Erasmus MC.

Initial Brief
The first design brief, deriving from the initial envisioning of the project, can be summarized in the following problem statement:

"Design the concept of a set-up in which the generation, expansion and storage of iPSCs is automated by connecting existing platforms (incubator, microscope, pipetting robot etc) physically and set up the development of a Machine Learning software and a software interface for the IPSCs machine. As a proof of concept design the embodiment of one section of the IPSCs machine."

Brief readjustment
After the initial research phase, the nature of the process analyzed revealed that the embodiment section of the original brief had to be re-defined. Concept development required more exploration due to the fact that the client found himself at an early stage of the transition towards automation.
Five development directions have been identified (Appendix I). These design possibilities were graded based on:

- Desiderability for Erasmus MC
- Feasibility in the graduation project time frame
- Influence on overall decision making for automation
- Learning opportunities
- Area of competence for Industrial Design engineering

The direction selected to go further is also the stepping stone for further development of the project in the future. It would allow learning on the side of system design, although being more limited for what concerns learning

of coding of systems and it is based on the envisioning of the concept for the automated system rather then the development of its details. For this reason, the final brief didn't focus on the development of a machine learning software as initially stated but the problem statement developped in the following way:

"Define the layout design concept for a set-up in which the generation, expansion and storage of iPSCs is automated by planning the connection of commercially available platforms and define with more detail the necessities of the IPSCs machine's interfaces."

Table of contents

Chapter 1

General Introduction

This chapter provides the reader with the essential elements for the project understanding: the context, the IPS Core facility, and the primary stakeholders, the involved parties in the project.

Later, the assigment is framed within the context of the process of automation on a temporal roadmap. This will provide a macro understanding of the project boundaries.

Research objectives will be then be identified for each primary stakeholders and the main research question is stated.

Lastly, the research and design methodology adopted to accomplish the objectives are explained. This will also provide the reader of a clear overview on the coming chapter.

The chapter concludes by giving definitions useful for the comprehention of the rest of the report.

In this chapter:

1.1 Context - Involved parties

1.1.1 Erasmus MC

The customer of the project is the department of Developmental Biology of the Erasmus Medical Center (Erasmus MC).

The department executes basic research and participates in the teaching and training programs of the Erasmus Rotterdam University aiming to discover the epigenetic mechanisms regulating cell fate decisions in the cycle of life. Their aim is to expand the comprehention of deregulations that may occur in X-linked diseases, cancer, infertility, and aging.

With this purpose, in 2010, the Erasmus MC IPS Core facility was founded. The lab assists fundamental and translational researchers, within and outside the Erasmus MC, and provides them with high quality induced pluripotent stem (iPS) cell lines and embryonic stem (ES) cell lines.The high-quality iPS cell lines are generated by experienced researchers, applying high-end technology to standard operating procedures. The services needed to generate iPS cell lines are skin biopsies, cultured fibroblasts, erythroblasts, and bone marrow.

1.1.2 TU Delft

In the last 10 years, TU Delft has been involved in the research of healthcare-related projects, which has led to the establishment of the Delft Health Initiative in order to facilitate scientists to find each other and collaborate. In the faculty of Industrial design Engineering, the specialization Medesign gathers designers working on projects that facilitate the medical field both in research and clinical practice. With this research field in mind, TU Delft has intensified the close collaboration with Erasmus University Rotterdam and Erasmus MC in disciplines of medicine, technology, and social sciences.

How can TU Delft help Erasmus MC

The technologies used by the IPS Core facility enable it to produce high-quality biological material useful for research. As the request for research on population studies increases in the last years, there is increasingly more need for higher volumes of production, which requests a shift in production methodologies.

Technician's work, although allowing for constant control over the quality of production and benefitting from a great amount of experience necessary in decision-making tasks, has limitations. These are regarding the quantities of material that can be handled simultaneously by a human being, the amount of variability between the work of each person due to a different sensibility and perception, and the number of people that can work within a facility in safe conditions, both for the technicians and for the culture itself.

Moreover, human power and its availability are subject to the variability of life events and the socio-cultural limits of our society, in the good and in the bad: the number of hours a day a person can work is (rightfully) legally limited; hopefully, there are life events that intertwine within the working calendar (marriages, pregnancies,..), or there could be eventuality of disruptions that force the human power to stop being available (as we have seen this year during the months of lockdown for safety needs due to Covid-19).

All these reasons are the laying ground for the shift to automation, as in other industries before, also within the bioproduction industry.

As will be seen in later stages of this report, this is not complete news, other facilities and centers have already started the adoption of automated systems and technologies. The market has developed itself to the

point of offering cutting edge technology for each functionality necessary within a Bioprocessing Lab.

The urge, therefore, is of screening the market, understanding the offer, but also, most importantly, the necessities of the IPS Core Facility, its users and stakeholders in the present and in its foreseeable future with the aim of depicting the ideal concept design that merges both the strengths of automation with the advantages of human control.

1.2 The problem

Different systems and laboratories have different automation goals: what works or worked for one laboratory could be not successful for others.

Highlighting what are the main reasons for which investment into the process of automation will be made is the starting point for understaning what are the main drivers that lay the ground for the project.

The motivations, in fact, lead to different decisional directions and can be used as a compass when comparing the overwelming offer that can be found from product vendor websites or choosing between concepts.

Before entering the design reseach territory, therefore, one overarching question has to be answered: "What is *the main* motivation for investment into automation of the systems for cell line culture?"

The only people that can answer are the managers of the facility due to their knowledge on the projects to be implemented in the future, the current practice and the possibilities of development of the department.

1.2 .1 Identifying the nature of the need for automation for the Erasmus MC:

The brief of the project is more clearly identified if, even prior to gaining more knowledge about the functioning of the systems, the possible drivers towards automation are ranked in a qualitative way.

This can be done easily by first moving the elements the Facility wishes for on a horizontal axis, in accordance with a near or far future vision.
By placing the same interest points on a vertical axis, from low to high interest, it is possible to identify how significant each opportunitity of automation is for the facility and therefore also what to prioritize.

The schematic at the following page have been made in this way, uppon discussion and in collaboration with the iPS Core facility manager Joost Gribnau and Mehnaz Ghazvini, the original graph can be found at Appendix II.

Analysing the outcomes of Figure 1 allows to define better the problem.

Erasmus MC is aiming to improve the consistency of their results, reducing the variability of the current procedures conducted by different operators. Their further objective is to increase the throughput of the Facility, and benefit from the possibility of exact documentation allowed by automated systems.

The plan of the Facility doesn't necessarely target the reduction on costs that derive from saving on human workforce, or automatization of specific tasks, but it is important to keep an eye on the advantages given from miniaturization and redirection of labor on result analysis tasks as benefits for the future.

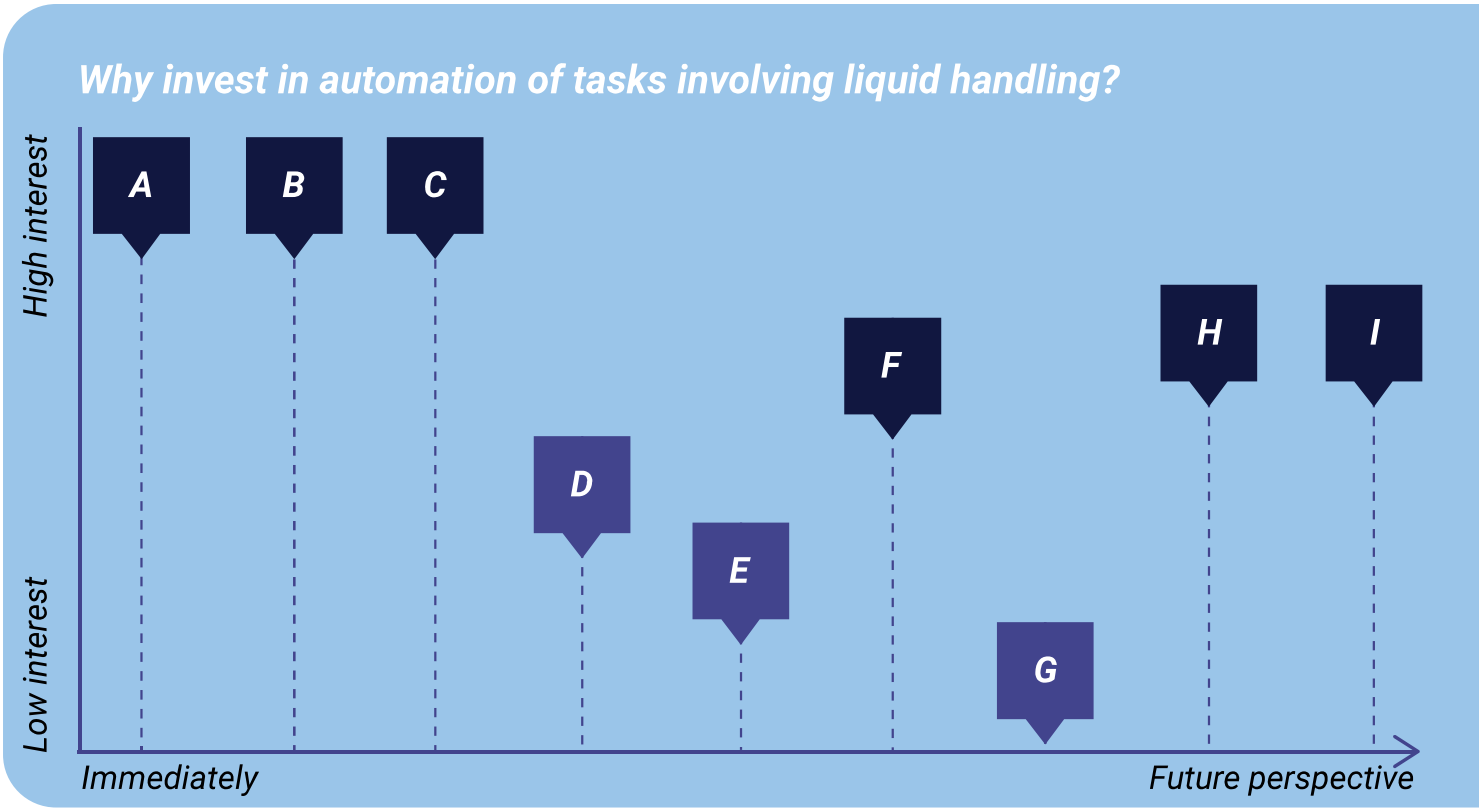

Figure 1: Rating of the reasons for which Erasmus MC aims to implement automation.

A - Reduction of variability - refers to the variability of results between different operators

B - Increase of throughput - intended as the increase thanks to parallelization

C - Improve assay reproducibility - specifically intended for the advantages derived from the implementation of technological ways or documentation of automated proceses.

D - Cost saving - Focus of the transition is on the reduction of the waste, labor tasks and screening of devices based highly on the product price.

E - Relieve the boredom of repetitive tasks - focus on the cooperation between human and machine by taking most advantage from the ability of machines to execute repetitive actions.

F - *Time saving* - The focus is in extending the working hours in which its possible to work on the cell lines with the system.

G - Relieve physically staff - focus on avoiding the potential for staff to be affected by repetitive strain injury.

H - Redirect labour - refers to the advantage given by being able to free up resources for other tasks.

I - Miniaturize assays - the adoption of machines makes it easier to handle smaller objects allowing therefore to use formats not achievable by hand

RELEVANT INSIGHTS:

1. The reasons for which the IPS Core facility wants to shift towards automation are connected to reduction of variability, increase of throughput and improvement of assay reproducibility.
2. Attention should be given also to parallelization of lines for time saving, redirection of labour and miniaturization of assays.

1.3 Roadmap to automation

1.3.1 Roadmap to automation

Transition towards automation of cellular production and reprogramming is a project of system design engineering, in which different necessities of the stakeholders, of the biological material itself and of the manufacturers have to be combined.

These typology of projects are usually carried out in teams of experts both on the manufacturing side and on the biological one.

In order to do so, companies such as Beckman coulter and Hamilton Robotics engage in a process that takes usually averagely 1,5 years to bring a research facility from the initial idea of transition from manual processes to automated ones.

The stages of this roadmap can be seen in Figure 3: the timings given are based on estimations of companies and therefore do not take into account varriable time that is spared due to their pregressed experience.

1.3.2 Focus of the research

The current project has the aim of using the possibilities given by a design research methodology in understanding the variable needs of a process-automating project and unifying them into a combination of devices that satisfies people, that are the technitians and operators working at the facility, businesses, by facilitating the efficiency of production, and technology, by identifying future integration of emerging technologies.

The focus of this project, therefore, is that of arriving to the definition of a desired concept for the masterplan for the automated system, to be implemented by the Erasmus MC.

The concept will be further used by the facility in order to contact selected manufacturing companies, check with them further details on requirements and possibilities and start the process of implementation.

The final outcome of the research deriving from the current report, therefore, should be considered as the starting point of further steps, documenting of the process to be automated and exploring workflow organisation possibilities and interactions.

RELEVANT INSIGHTS:

1. The transition from manual process to an automated takes one year to come to realization.
2. The first step for the process of transition towards automation is to analyse the manual processes that takes place in the facility
3. Combining the necessities of the manual process with informations relative to quantities of production will enable the definition of the initial layout plan.

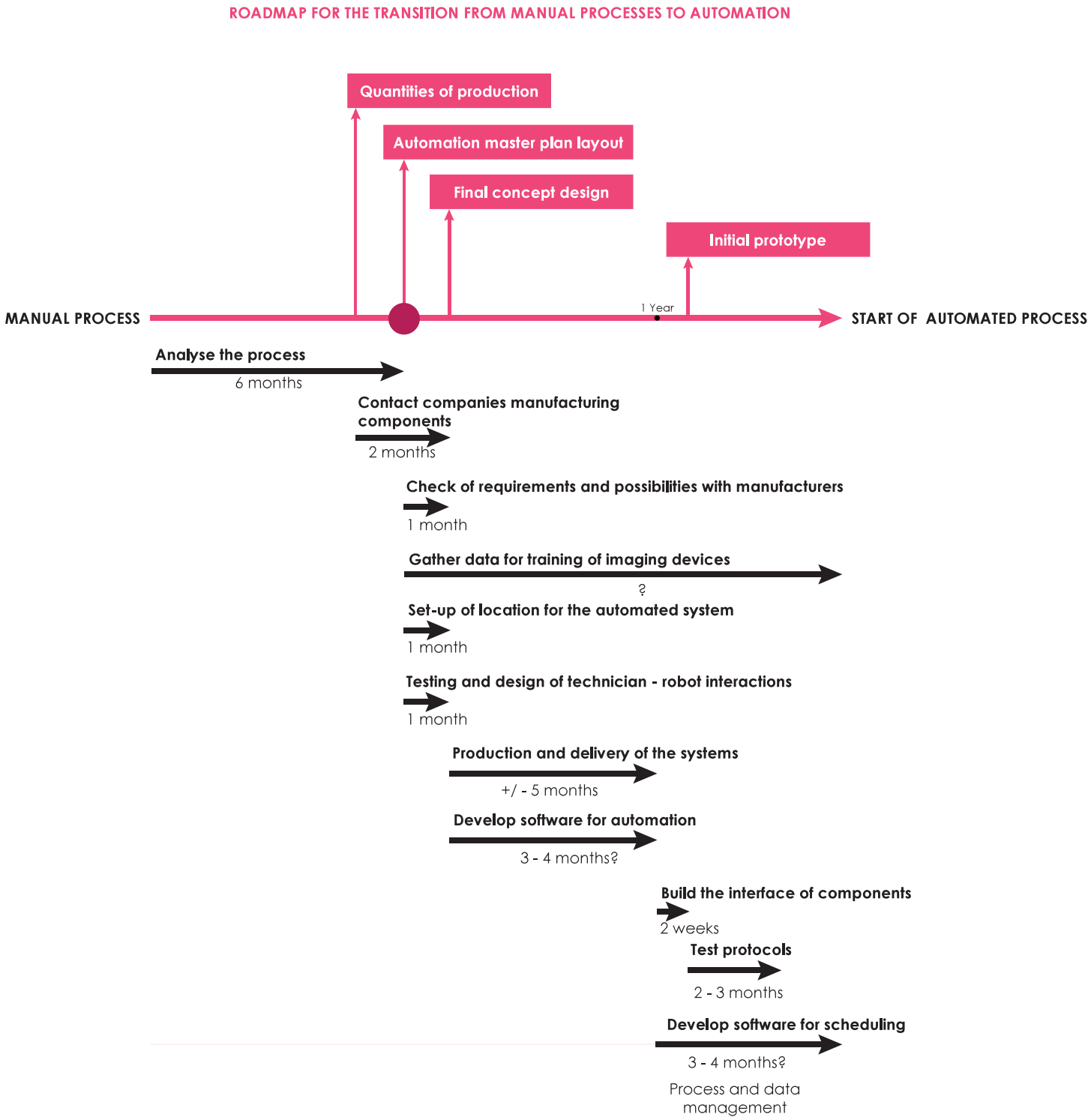

Figure 3: Automation roadmap
(*) Additional action to take by the facility, already developped if done in collaboration with service providing companies.

1.4 Research objectives

1.4.1 Erasmus MC project objectives:

1. Assess the iPS reprogramming process automation and the future steps to take in the direction of upscaling of the iPS Facility Lab throughput.
2. Define the design guidelines for the connection of exiting platforms for iPSC automation.
3. Determine the possible economic impact of the automation of IPSC generation, storing and reprogramming.

1.4.2 TU Delft Project objectives:

1. Investigate design features that can enhance the automation of health-research related projects
2. Define the method of research for key elements of design for automation within research field related projects.

1.4.3 Common research objectives:

Although the project has a design research approach nature, it is going to be highly focused on the needs and practices of the iPS Core facility.

The main Reseach Objective of the project, therefore, is:

MRO Define and test the configuration and interactions of an automated system at the IPSC Core facility.

This research objective can be subdivided in the following:

RO. 1 Identifying design aspects that most influence the transition of human-controlled processes towards automation.

RO. 2 Determine the roadmap towards automation of the process of iPSC culture to be adopted by the iPSC Lab Facility at Erasmus MC.

RO. 3 Evaluate the state of the art of technologies and different product architectures for automation of iPSC culture.

RO. 4 Identify the design opportunities for the automation of decision-making processes.

RO. 5 Identify risk factors which might obstruct automation of iPSC culture production in case of increase of throughput.

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RO. 3 Evaluate the state of the art of technologies and different product architectures for automation of iPSC culture.

RO. 4 Identify the design opportunities for the automation of decision-making processes.

RO. 5 Identify risk factors which might obstruct automation of iPSC culture production in case of increase of throughput.

1.5 Methodology

It is not possible to automate a process before understanding it. For this reason, observations and interviews are a necessary tool to gain initial knowledge. Focus groups, sessions in which relevant stakeholders (usually the users of the final product) are invited to discuss several topics concerning a specific product, are able to provide additional contextual information when used in the initial phases of a project.

The outcomes of observations, interviews, and focus groups can be schematized into the process trees and function analysis charts, enabling documentation of the gathered insights and structuring additional knowledge. A process tree is a schematic diagram usually focusing on the description of a follow-up of steps from the initial phase until the final stage and it allows to create criteria for further product development. A functional analysis allows developing the function structure of a new product concept and allows describing the intended functions of the product and relate them together.

Finally, existent and developing technologies can be analyzed in order to scan for possibilities of the fulfillment of the previously gathered functional needs.

After a first better understanding of the process that has to be automatized and of the typology of technologies present on the market, the direction of development of the project is easier to distinguish by the description of the requirements to be fulfilled.

Laying down a list of requirements enables an understanding of what elements to focus on during the development of the new product.

It is possible to ensure that the envisioned concept will prepare beyond the current stage into the future by reviewing future trends. The project developed is then related to the identified patterns of development of business and technologies in a schematic of horizons, in order to see what are the trends that could be interesting to relate to and embed in the project.

This phase both enlarges the spectrum of possibilities that are considered and at the same time centers the project in-between areas of influence and serves, therefore, also to scope the initial phases of research. In system design, the process of understanding embeds also the analysis of the relationships between elements, their interconnection needs, and the necessity of production of the process. This can be done with methods for layout planning, while ideation sessions are functional to the diversification of ideas.

After understanding what are the possibilities of design, the ideas have to be selected and merged. For this the morphological chart diagram can be used for further integration of principles and ideas, creating the first concepts.

Further evaluation of the concepts allows selecting the better scoring one against the rating criteria identified also in the initial interviewing phase. The outcome of this stage can help to identify future challenges for the realization of the automated system.

As can be seen, the here described process of the project has been developed within the renowned process of the Design Council's Double Diamond framework (Design Council UK, 2005).

Figure 4 describes this visually linked to the activities carried out and where to find them in the following chapters of the report.

In fact, the Double Diamond methodology two main activities take place:

Problem setting

In the initial section, the Discover Phase, the issue is explored with divergent thinking, discovering the characteristics of the problem.

In the second section, the Define Phase, once insights are collected, they are used for a better definition of the challenge.

Ideation

The second phase of the process is subdivided into two parts as well:

The first part, the Develop Phase, is focused on expanding the possibilities of the solutions offered to the defined problem.

The last part, the Deliver Phase, involves testing early different solutions, focusing on the one that satisfies criteria more clearly and improving it.

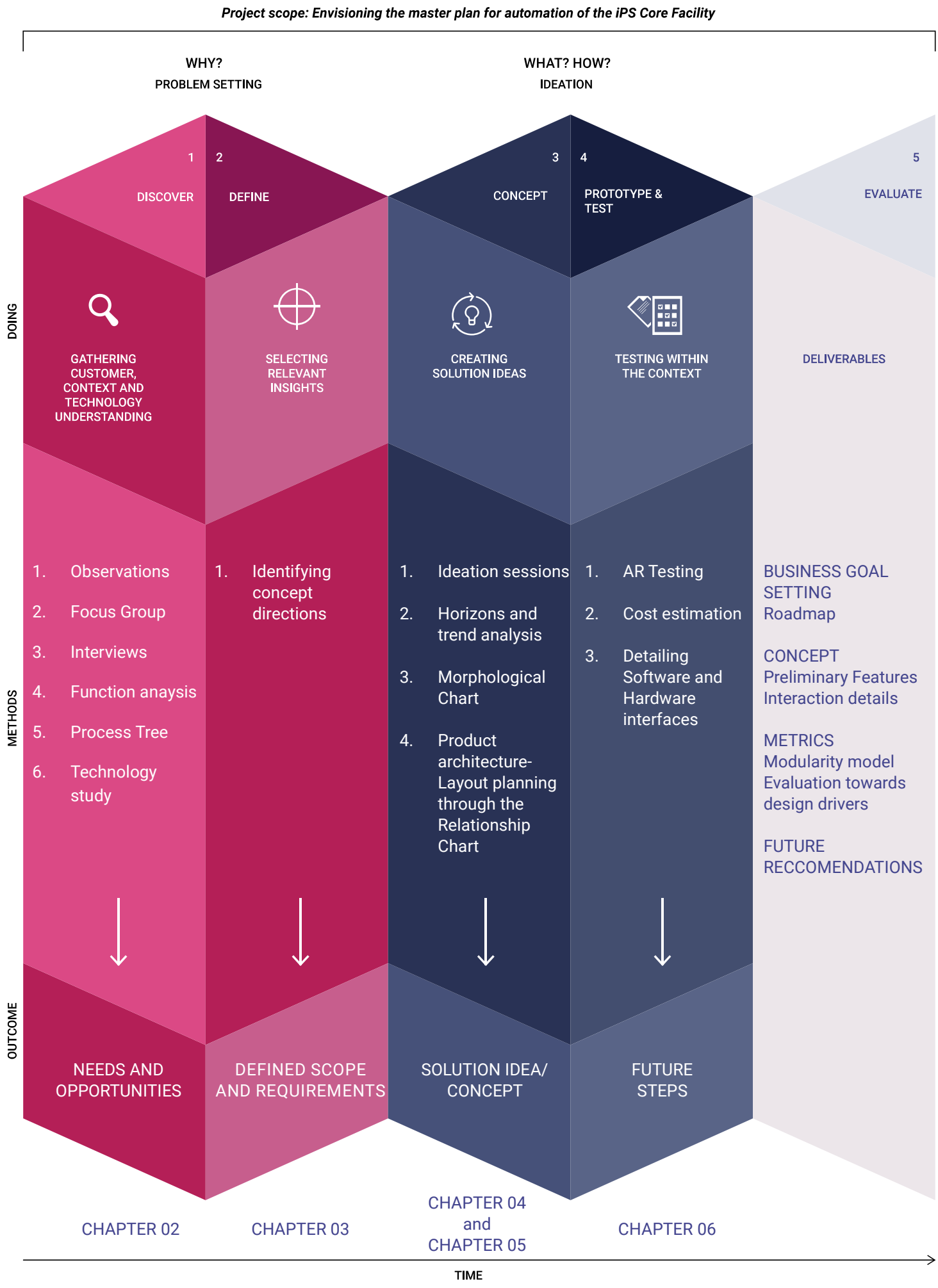


Figure 4: Methodology used correlated to the activities and their outcomes

1.6 Definitions

Stem cells
These cells are defined as one of the human body master cells, with the ability to grow into any one of the body's 200 cell types and create new cells of whatever tissue they belong to. ("Medical Definition of Stem cell", 2017)

Cell line
"a cell culture selected for uniformity from a cell population derived from a usually homogeneous tissue source (such as an organ)"
(Biological Abstracts, Merriam Webster online dictionary)

Automation:
Setting up a single task to run on it's own.

hIPSC Human-Induced pluripotent stem cells
Induced pluripotent stem cells (iPSCs) are adult cells that have been genetically reprogrammed to an embryonic stem cell–like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells (NIH Stem Cell Information Home Page, 2016).

Good Laboratory Practice (GLP)
"embodies a set of principles that provide a framework within which studies are planned, performed, monitored, recorded, reported and archived".

(2014, Verna S., Laboratory Manual for Biotechnology)

Chapter 2

Background and related work

In this chapter the background informations related to the three main aspects of the project are addressed: automation, cell culture and their convergence.

Initially the history of automation is going to be reviewed in order to give a picture of the framework of automation surrounding the project.

Later on, the process related to the cultivation of iPS lines is analyzed and explained.

Lastly, the two topics (automation and cell culturing) are related one to the other by describing the advantages of automation specifically for iPSC line production, the current market development. This background is going to be used later for the definition of the stystem requirements, in Chapter 3.

In this chapter

2.1 Automation

Manufacturing has adapted in history to new needs of people, their higher interconnection, the increasing demand, the globalized landscape and the request of performance having to meet always higher expectations.

Traditional supply lines could not meet this demand which forced manufacturers to change tactic: reducing their costs by making the process streamlined and adaptable and, on the other side, generating more value by introducing personalization.

The value-chain has been re-thought through the industrial revolutions and is finally embedded in Industry 4.0.

Integration of automation is at different stages in different industries. For the bio-medical cellular industry automation is still in the process of addoption.

By analyzing synthetically the developments of general manufacturing its possible to have an overview of the possibilities, benefits and challenges the industrial revolution of biological processes can have now.

2.1.1 History of manufacturing

The first industrial revolution from 1760 until 1830 responded to the need of increase in productivity and saw the introduction of the steam engine and the screw cutting lathe. This enabled production of standardization of interchangeable parts and mechanization of bulk material processing into continuous-production. Factories became full of specialized workers turning standardized -parts and processed-material into batch-production products.

The second industrial revolution (1870 until 1914) introduced developments in metallurgy, chemistry and physics that led to advances in technologies (combustion engine, the screw propeller, steam turbine and commercial dynamo)

The innovations done by Taylor and Ford introduced the first approach at scientific management for optimization of manufacturing and the assemply-line factory.

The digital revolution started in 1947 with the invention of the transistor and the first computers. Mass-production of integrated circuits was then invented as a result of the merging of this technologies with photographic-processing of silicon-crystal-slices.
Later developments gave life to the computer processors and the first home-computer, the digital image sensor and digital camera, the first interconnected network of computers and the World Wide Web.
Resulting exponential growth of digital storage and computing power accelerated the invention of software tools such as Computer-aided Engineering for modeling and optimization and pushed invention even more.
Programmable Logic Controllers (PLC's) were implemented to automate most production processes, and robotic manufacturing became a reality, performing repetitive complex tasks fast. This is the birth of automatization, but human labor for assembly or machine operation remained still necessary.

In the further industrial developments the transport sector grew and the modern mass-production value-chain became a reality.

With the growth of all factors (world population, manufacturing and the transport industry, material and energy demand) there has been increasing pressure on the existing supply lines and manufacturing chains.

Just-in-time Manufacturing, also called Lean Manufacturing, came from the rethinking of the production system for the reduction of waste and costs. In this stage the

RELEVANT INSIGHTS:

1. Automation means soley the substitution of manual work with robotized machinery
2. In order to benefit from the possibilities given by the advances of industrialization, such as flexibility and stream-line production, there is need to integrate elements coming from other manufacturing methods.

FIRST INDUSTRIAL REVOLUTION

Continuous-production

With this method the manufacturing process is able to produce final products starting from bulk materials and in a continuous flow. This allows to reduce takt times (average time between the start of production of one unit and the start of production of the next) and increase efficiency.

SECOND INDUSTRIAL REVOLUTION - TECHNICAL REVOLUTION

Mass production

Production is fully scheduled, uses specialized labor and standardization for the assembly-lines. Production of large quantities is enabled by setting strict and process dependent design requirements and the exact same product is produced each time. The consequence is that if the demand declines or there is need to produce new products, it can be incovenient to adapt the system and satisfy the desired changes.

THIRD INDUSTRIAL REVOLUTION - DIGITAL REVOLUTION

Robot Production or automation

In this method mechanisms are process-controlled in order to replace human workers within the assembly line. This allows an increase in efficiency , reduction of mistakes and reduction of costs. This can be the solution for simple repetitive tasks while human workforce is assigned to more creative problem-solving tasks.

Agile manufacturing

This method tries to make it possible to adapt production systems quickly and accomodate new products in competitive and volatile markets. The necessity of quick readaptation requests having strong supplier networks to correct supplies and correct quality issues early. The focus is still on understanding the times the consumers are willing to wait. On the same time high standards of quality should be mantained as well as the overall costs.

Lean manufacturing

Just-in-time (or Lean) manufacturing allows to streamline the supply chain. Between its characteristics it uses a build-to-order pulling strategy for each process step instead of the build-to-stock pushing strategy.
This approach makes the production costs lower, makes the system more efficient and able to readapt learning from mistakes. Sudden changes in the market affect strongly its abilities of production given the close codependance from supplies.

FORTH INDUSTRIAL REVOLUTION - DIGITAL EVOLUTION

Mass customization

This method has the ability of produce goods and services to meet individual customer's needs with near mass production efficiency (Tseng et al. 1996). It combines personalization of artisan production and low-cost output capacity of lean-manufacturing. This method requires: co-creation, in order to combine customer wishes into a personalized design, the setup of the supply-chain to deliver in time for the customer-order-cycle and minimizing upstream and downstream warehousing to reduce costs. For this purpose direct digital manufacturing (DDM) based on automated computer systems control such as computer-numerical-control (CNC) and additive-manufacturing (AM) are used.

supply-chain is streamlined by removing all non-value adding activities and waste, and by using a build-to-order pulling strategy for each process step, as opposed to a traditional build-to-stock pushing strategy, where every component and sub-assembly gets stored until needed

The current revolution is embedded in movements such as Industry 4.0 that is trying to push a paradigm shift in society for a more efficient, valuable, and sustainable supply chain and product life-cycle.

Resulting technologies are direct-digital-manufacturing, algorithm-aided-design, cyber-physical systems, the industrial-internet of things and 3D-printing allow to create configurable products in a mass production output capacity, which is the so called mass-customization.

Figure 5: Characteristics of each industrial revolution

2.1.2 Characteristics of industry 4.0

The analysis of the way manufacturing developed through time leads us to evaluate and understand the situation in which we find the manufacturing productions nowadays.

The already started Digital Revolution, is currently passing through a stage called Industry 4.0 which puts the focus on the implementation of information age technology into the existing methods and systems.

Through the last decades there has been an extensive digitalization of all manufacturing industries, a process defined as the fourth industrial revolution and which has completely changed the industrial production by making it more automated, intelligent and connected. The changes that have been registered are so fundamental that the whole industrial production is in need and in search of a rearrangement.

According to Hermann et al. (2016), Industry 4.0 scenarios are met by applying four design principles: interconnection, information transparency, Decentralized decision and technical assistance.

Interconnection

All the actors involved in production (people, modular machines, vendors) are connected through the Internet of Everything (IoE), this allows for communication between each other within the same factory of among different ones. In order for communication to be effective and productive it is organized through standards and results into a cyber-physical system. The interconnection of different factories is what makes possible nowadays the allocation of production streams and the optimization of manufacturing times or product transport distances.

Information transparency

The interconnectivity of all the actors involved leads to having to organize a large amount of data coming from their communications: factory sensor data, product drawing data, process simulation data, factory worker interruptions, management decisions. To ensure usability, trackability and usefulness of these informations, enable cooperation of the participants to the system, create the ideal process sequencing, identify possible failures, and underachieving phases or parts, their transparency and organization is crucial: all stakeholders, phases and products are embedded in the digital world as their Digital Twin. Digital twins are virtual representations defined as useful for the information organization and study.

Products can have a Digital Twin even after production, this allows the monitoring of their life-cycle, the clustering of lower-level information, and their evaluation. Troubleshooting, prevention and maintenance are much more direct and automated and reach near-zero downtime within a factory. The transparency of information makes Agile Manufacturing possible and allows a high percentage of one-off personalized products and mass customization based on collected data or inputs.

Decentralized decision

In the Industry 4.0, independence within the parts in standard phases is highlighted, by focusing the interactions among participants to the exceptional cases and in case of interferences, delegating tasks to further levels and decentralizing decisions.

Technical assistance

In order for this system to be sustainable, the industry should implement the technical assistance possibly needed by each participant to the Cyber-physical setting. The relation between technical assistance and the human participants is two sided since the human takes time to make decisions, act properly, while the machine uses

the experience and know-how of human participants to solve local and specific issues.

RELEVANT INSIGHTS:

1. Communication standards to allow interconnection between the modular machines
2. Interconnection between different factories and within a factory is possible for orchestration of bigger production throughputs or specialization within facilities.
3. Digital Twins allow better communication of data and one specific product tracking also after production in the whole product life-cycle
4. Decentralized decision making allows each element of the system to act independently.
5. Technical assistance and correlation between the human and the machine is necessary for maintainance of decision making reliability and solving local problems.

REQUIREMENT DECISION:

1. Interfaces between components of the system should allow interconnection through the use of standardised software and hardware interfaces.
2. Future increase of production should be backed by possibility of interconnection between facilities and integration of Digital Twins.

2.1.3 The control problem

People imagine modern Machine Learning aided automation as able to act completely independently from humans and not make mistakes. It has been designed to learn and rely on computational architecture modeled on the neurons and synapses of biological brains, and, to its advantage, it's data processing extracts statistical patterns from very large amounts of information.

In fact, thanks to its ability to run with vast datasets and use deep-learning, Machine Learning is used prominently in decision support systems.

However, the possibility of a person relying on the decision-making ability of a machine can vary depending on the training of the system, which could require even long periods of time such as decades.

Operators of complex machines can neglect this and fail to recognize their malfunctioning, an issue that has been identified by industrial psychologists.

The control problem is the "tendency of humans within a human-machine control loop to become complacent, over-reliant or unduly diffident when faced with outputs of a reliable autonomous system" (Zerilli, 2019) and it can affect both experts and novices.

The reasons why the control problem exists are between the subjects studied by human factors research related to human-machine systems.

Zerilli et al. (2019) pointed out four characteristics that are on the basis of creating difficulties for the monitoring function of people within the human-machine system:

1. Capacity problem: Humans have cognitive limits when compared to an autonomous system that is more sophisticated. This can be related to the speeds to which the calculations occur that sometimes can not be tracked by human monitors. In other examples, the system is so sophisticated that the risks of failure are so rare that they can not be foreseen by the designer and, therefore, operators can not be trained on how to react on them.
2. Attention problem: Humans are less likely to maintain attention towards a source that doesn't communicate anything for more than 30 minutes.
3. Attitudinal problem: This is linked to the automation bias in which the monitoring human supposes that the system can not be wrong with the improvement of it's learning. This difficulty doesn't disappear if the number of people involved is higher.
4. Currency problem: This difficulty is related to the translation of cognitive aspects into physical ones. It is more complicated to retrieve long term memory if it is not practiced or frequently used. Although an operator could have been able to detect issues in the functioning of a machine, he could be less attentive once the checking actions are done less frequently.

A human-machine system, in fact, should take advantage of the strengths of both of its components: humans have the power of intuition, while computers contribute by quickening, parallelizing, and making repetitive tasks precise.

One way of benefitting mutually from both elements is to dynamically allocate functions preserving attention resources in order to make automation enhance human skills rather than replace them completely.

After locating the control problem within control related issues, with this proposition in mind Zerilli et al. (2019) also identified the principles human-machine systems should reflect in their designs:

1. Division of labor: there should be a clear subdivision of responsibilities within systems that have automatizable subcomponents taking a role also on the side of decision-making factors.
2. Complementarity: Components that are better suited for humans, and therefore related to symbolic reasoning, concept, communication, empathy, and intuition, are not automated.
3. Dynamism: there should be a designed system of hand over and hand back that will prevent losing control skills. As an example, random checks or deliberate trial-errors could be designed in order to keep the operators careful of the decisions made by the system.
4. Co-evolution: the design of the system should allow for flexibility for user-requirements
5. Pragmatism: The tools that support decisions should be congruent with the existing practices.
6. Context sensitivity: The importance of the principles is related to the context in which the design is applied, therefore a different trade-off can be put according to the specific project needs.

RELEVANT INSIGHTS:

1. Specific human factors have to be taken into account when designing decision making interactions.
2. Human-machine system operators can fall into the "control problem and over-rely on the capabilities of the system
3. The automation bias doesn't depend on the number of people involved or on their expertise

REQUIREMENT DECISION:

1. The automated system should embed the 6 principles of division of labor, complementarity, dynamism, Co-evolution, pragmatism and context-sensitivity in the design.

2.2 Introduction to iPS cells and culture procedure

2.2.1 Background on the discovery of IPSC cultivation

In 2006 the Japanese researcher Shinya Yamanaka has discovered that mature cells, such as skin cells, can be converted into stem cells, undifferentiated or partially differentiated cells, using four key genes, the "Yamanaka factors" or currently called OSKM, for OCT3/4, SOX2, KLF4, and MYC. The remarkable breakthrough has lead Yamanaka to win the Nobel Prize for Physiology or Medicine in 2012.

The following decade and a half have seen an increase of the research in the field and significant advances in developmental biology, disease modeling, drug discovery, personalized and regenerative medicine. In 2014 the first clinical trial using a patient own iPSCs opened the door for direct therapeutic applications of the technology ("Reflecting on the Discovery of the Decade: Induced Pluripotent Stem Cells", 2016).

Since the initial discovery researchers have identified factors that can be added or substituted to enhance the transition to iPSCs or developed new technologies for direct cellular reprogramming by improving the reprogramming process. Other researchers have focused on the application of iPSCs for the study of disease and drug discovery, treatment of sickle cell anemia, Parkinson's disease, diabetes, spinal cord injuries, and heart failure. Personalized medicine has also taken contributions from iPSC research for the identification and testing of drugs on a patient's brain or heart cells derived from skin cells, which still carry the same genetic mutations but are more accessible.

2.2.2 What are iPSCs

Stem cells are *unspecialized* cells that have the *capability of renewing themselves through cell division.* They also have the possibility to become different cell types depending on the signals they receive from their environment.

Not all stem cells have the same specializing capacity:

Based on their ability to specialize to different cells the cells can be totipotent, if they can give rise to every cell type in the entire body, pluripotent, if they can develop in every cell type in the body except for tissues such as the placenta or multipotent, if they are even more restricted (Figure 6).

Stem cells can be extracted to be directly cultivated at the moments of development of an embryo or from bone marrow.

The ability of stem cells to self renew indefinitely while retaining their undifferentiated pluripotent state is a key feature of these cells. Cells from one petri dish can be used to seed many other petri dishes and if an appropriate signaling molecule is provided ES cells can be coaxed into becoming many different mature cell types: bone cells, pancreatic cells, muscle cells, nerve cells.

Scientists hope to replace cells in the body that have been lost or damaged by injury or disease (such as diabetes, Alzheimer's, Parkinson's, spinal cord injury…) but since ES cells generated from blastocysts, they have a different genetic background from the patient and therefore they may be rejected. Therefore it is desirable to transplant cells that are a match to the recipient.

Overcoming the hurdle of not being able to create cells from an adult animal to make them become another cell type is possible using somatic cell nuclear transfer (SCNT): the nucleus from a cell is moved and with it all the genetic material. The next step is to take a biopsy from the patient, the nucleus

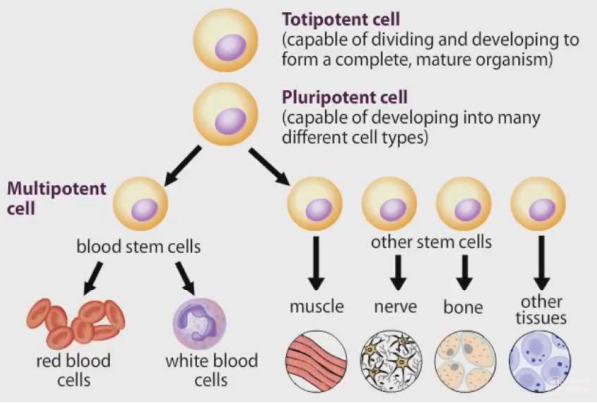


Figure 6 : Different level of differentiation achieved by cells

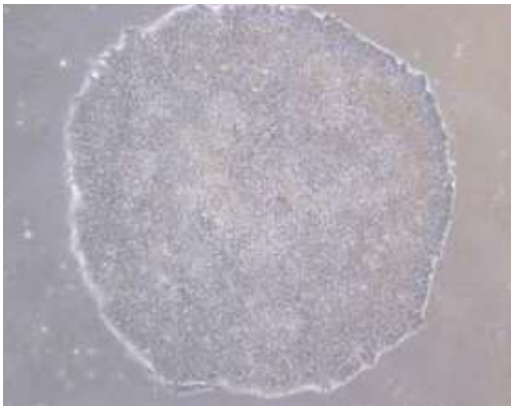


Figure 7 : Immage of a hIPSC colony, Source: HipSci

Figure 8: Picture of technician in a lab executing the cell culture procedure manually, Source: Erasmusmc.nl

of this other cell is transferred then in the denucleated egg. After activation, the egg, bearing the transplanted nucleus, divides and becomes a blastocyst, an inital embryonic cell. In this way, the developed embryo has the same genetic background as the donor of the genetic material of the patient and will, therefore, less likely be rejected when transported back to the patient.

A further development in the production of stem cells comes from the discoveries of Sheenya Jamanaka that showed the possibility of creating stem cells without first creating a blastocyst. By inserting 4 specific genes into adult cells the cells will revert to an embryonic pluripotent state. The technique uses viral vectors to insert genes into adult skin cells. The factors carrying the vector are selected and expanded in culture. Cells generated in this way are referred to as *induced pluripotent stem cells* (iPS cells).

They can self renew and specialize in different stem cell types.

2.2.3 The advantages of iPSC

Differently from embryonic stem cells, they do not pose ethical concerns since they are not derived from embryos but can be obtained simply by blood sampling.

They are suitable for auto-transplantation. Differentiated cells can be collected from a patient, induced to the pluripotent state, differentiated into a specific cell type and transplanted avoiding most of the complications of rejection.

The main problem for the use of iPSCs in therapy concerns safety

The time interval to induce the pluripotent state and the differentiation of cells to be

transplanted into the patient is quite long and this can lead to the occurrence of mutations that can be transferred to the patient. For this reason iPSCs are not yet used in clinical practice. To date, only one clinical trial has been opened and then suspended due to the presence of a mutation in the reprogrammed cells.

At the moment iPSCs are used to create a huge collection of stem cells called Biobanks they are derived from people from all over the world and can be potentially used for any type of transplantation.

Patient cells are reprogrammed and since they carry genetic alteration that causes the disease they are used to reproduce it in vitro in order to study the underlying molecular mechanisms.

2.2.4 Quality controls of hIPSC lines

By the definition of hIPSC derives also the need for controlling their quality.

In fact, as stated in the STEM cell definition a pluripotent stem cell is validated through self-renewal tests, differentiation (pluripotency), and genome stability checkpoints.

- Self-renewal: This property is tested through the RT-PCT technic, a process done outside of the process in the scope of this project but for which the steps are responsible for extracting RNA samples.
- Pluripotency: The ability to differentiate is checked through the process of staining, this method is also executed in later stages by technicians. Automatable steps that prepare for this stage consist of making the cells grow in contact with the three germ layers (endoderm, ectoderm, and mesoderm). These are primary cells that eventually would give rise to the tissues and organs. By putting the iPS cells in contact with the germ layers their ability to specialize can be tested.
- Genome stability checkpoints: They are done through DNA isolation, a process for which the DNA of the cells has to be extracted by first putting the cells in contact with lysis reagents. The process of DNA isolation is done in later stages by the technicians for the specific analysis of the patient's cells.

Summary:

- The discovery of Stem cells in 2006 lead to significant advancements and research in the treatment of degenerative deseases and personalized medicine.
  Stem cells are unspecialized cells that can self renew and that are pluripotent: they can specialize into different cells depending by external influences.

  The main problem in their clinical implementation is the possibility of rejection by the patient.
  To match genetic material Somatic cell nuclear transfer (SCNT) is used.
- Another technical discovery is that of induced pluripotent stem cells (iPS cells, optained by simple blood sampling and bre suitable for auto-transplatati

  Long time interval can induce mutations which leads also iPS cells to not be used in clinical practice but mainly to create collections called Biobanks, used to reproduce in vitro the diseases and provide research study material.
- Upon their creation cell properties are tested: self renewal through RT-PCT technic, pluripotency by putting the cells in contact with germ layers, genome stability through DNA isolation.

2.3 Performance of quality control

2.3.1 Quality of culture

IPS cells, unlike differentiated cells, grow in colonies and have a round shape, in the figure in the next page it is possible to see in the same well IPS cells and differentiated cells.

Three factors rule the quality of the colony: *The amount of differentiation, the size of the colonies, and the density in a well.*

There is always a certain amount of differentiation of the cells, but the number of differentiated cells should not exceed 10%. The quality of the culture is determined by the percentage of the differentiated cells present in the well. If the quantity of differentiated wells is too high, there is too much spontaneous differentiation.

Another information that derives the quality of the culture is the size of the colonies, the size of the aggregates of cells is never the same in the whole well, colonies that from the start of the culture are slightly bigger also grow faster, but 50 % of the colonies should be of the same size (in the images the correct size is represented by the cells pictured on day 6). The technicians train the eyes to go through the well and estimate which is the common size of the colonies in several points of the well and therefore of the well as a whole.

Third information that is interconnected to the previous two and that determines the quality of the culture is on how full the well is since if the well is too full there is possibility of it starting to differentiate. It could happen, for example, that the density and disposition of the aggregates are such that their proximity could mislead in seeing several colonies positioned close together as one bigger aggregate. Therefore the machine learning system should be able to recognize the variety of dispositions the cells can be placed into the well.

The information on size and density is also interconnected, for example, in the case in which the density of the cells is so high that the colonies have to be split in order to give them enough space to grow towards a bigger dimension.

2.3.2 Procedure of microscopic analysis

Three levels of magnifications are used: 20x 40x and 400x.

Because with the higher magnification it is not possible to analyze the full well in one step the technician takes a look at the well in several places in order to evaluate the size and density of the colonies. From the understanding of the size of the cells, the technicians decide if it is the time for the splitting of the cells; form the density of the cells the technician calculates the ratio of cells to take to the next plate.

The cell aggregate size can be adjusted by altering the number of times the cell aggregate mixture is pipetted up and down but avoiding the generation of single cells.

The colony confluency at the time of passaging can be changed by altering the split ratio at the time of plating while typical splitting ratios are 1 in 10 to 1 in 50 in the laboratory this can change by operators.

The methodology, in fact, is that of using the sensibility of the eye to estimate the cell confluency. Counting cell aggregates is an alternative way to determine and adjust the plating densities.

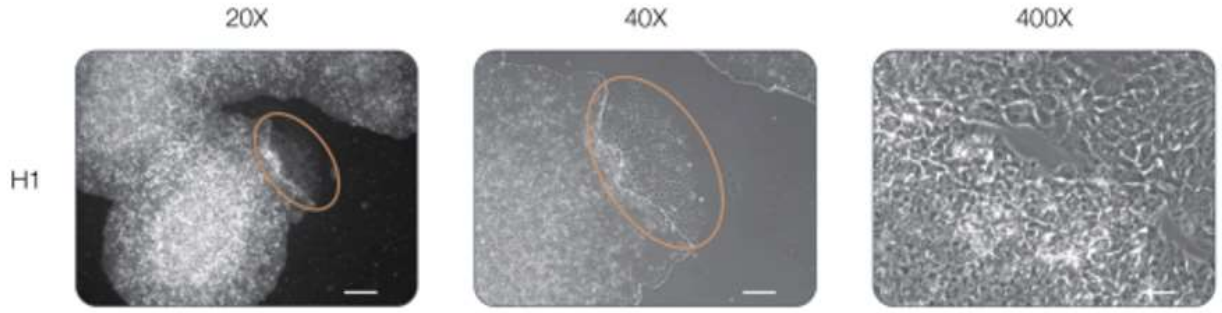


Figure 9:The orange circle highlights differentiated cells within the culture
Source tem Cell Technologies

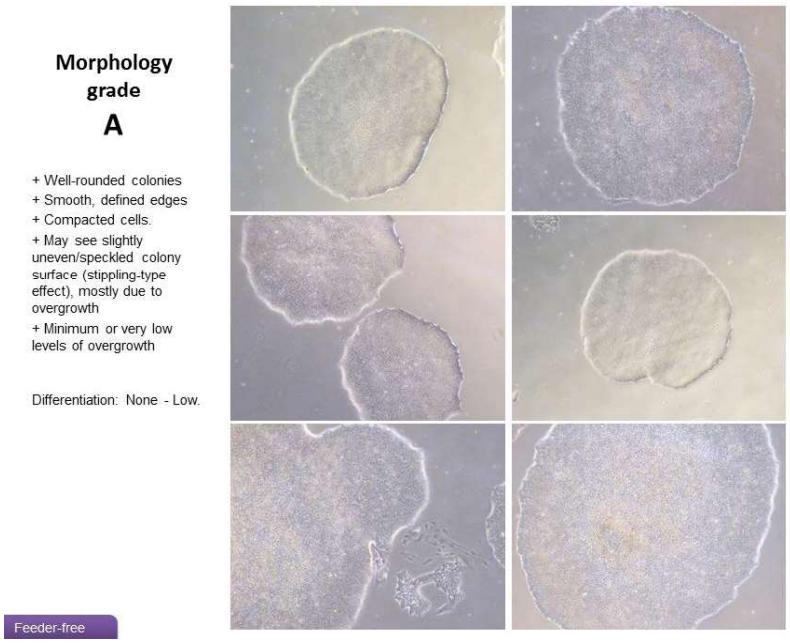


Figure 10: Morphology of the iPS colonies,
Source HipSci

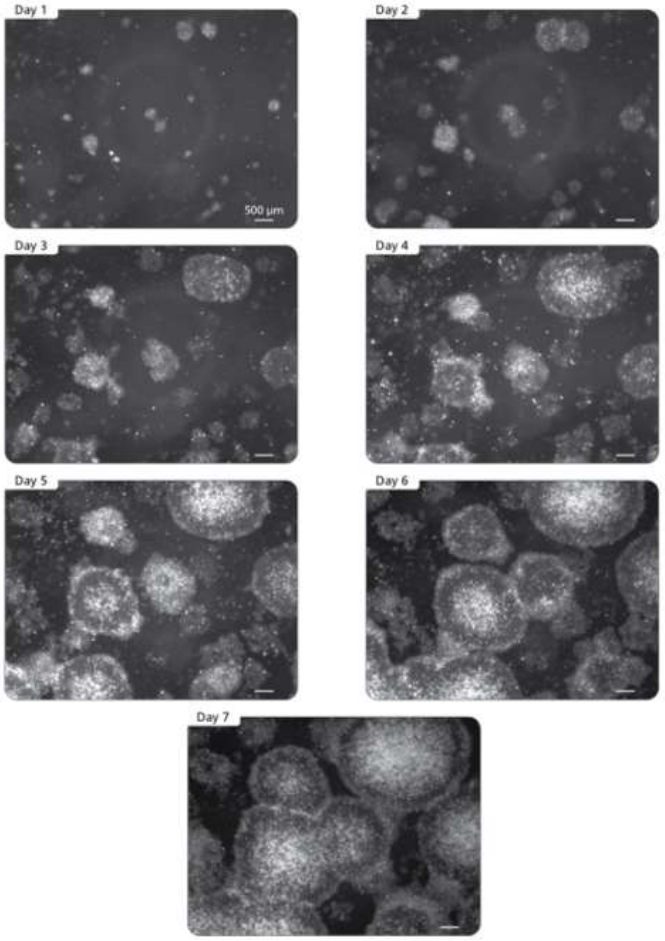


Figure 11: size and density of the cells
Source Stem Cell Technologies

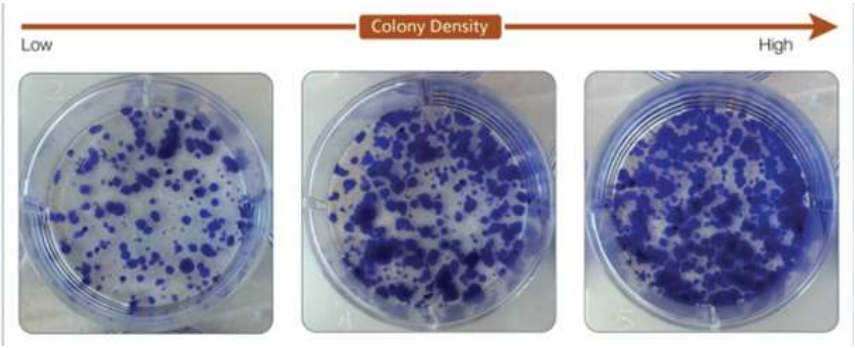


Figure 12: Confluency of cells, source Stem Cell Technologies
Source Stem Cell Technologies

Quality Control indicators: :

- Amount of differentiation: max 10%.
- Size of the colonies: 50% of the colonies having the same size.
- Density in a well: not too high to avoid uncontrolled differentiation.

Quality Control is performed with microscopic analysis, to each requirement met corresponds a subsequent passage in the cell cultivation. Decisions on such passages are based on the technician's experience.

REQUIREMENT DECISION:

1. The automated system should ensure quality control of the cell lines by checking the amount of differentiation, the density in a well and the size of the colonies.

2.4 Methodologies of reprogramming

2.4.1 General considerations

Various strategies have been developed to improve the technologies for culture since the first report of iPS cells generation from murine fibroblasts using retroviral transduction of a defined set of transcription factors. Therefore, the reprogramming of the cells can be executed in different ways.

All methodologies are following the same concept: a cocktail of stem cell reprogramming factors is ectotipically expressed and culture the cells untill they de-differentiate.

There are two main categories of reprogramming methods: chemical, involving no genetic modification of the donor cells, and transgene reprogramming, involving the integration of external genetic material. The most widely used methodology is the retroviral delivery of OCT4, SOX2, KLF4, and MYC (the OSKM set).

The choice of methodology depends on the donor cell type and reprogramming is achieved with different efficiencies and kinetics. The differences are attributed to variations in the endogenous levels of certain reprogramming factors, differentiation status, and other intrinsic epigenetic states.

In general terms, reprogramming requires the delivery of certain factors into a specific cell type and their adequate expression under defined culture conditions for a period of time, which varies depending on the cell type, species, and delivery method. Depending on the donor cell type, reprogramming is achieved with different efficiencies and kinetics. In the case of human foreskin fibroblasts, the process takes 20 to 25 days but not all cell typologies take the same amount of time.

The variability of methods is caused by the fact that there isn't a universal method that can handle all applications of iPSCs.

Most iPSC research falls into two categories (Kenkel, 2018): studies focused on better understanding the mechanisms of reprogramming, and studies with clinical endpoints. In the first scenario, robust and efficient generation of iPSCs is the main priority, with safety taking lower priority. Genomic integration is less of a concern, so viral vectors are better suited for these situations. The second scenario requires higher levels of safety and as little chance of genomic alteration as possible, with efficiency being the trade-off. Non-integrating approaches, such as episomes, RNA delivery, and protein delivery are better suited for these types of studies. What cells are reprogrammed will also affect the outcome, since not all cell types are easily attainable, or easy to reprogram.

2.4.2 Methodologies used at the iPS Lab Facility

The iPS Core facility at the Erasmus MC adopted three different methods for the reprogramming of the cells (Figure :

- RNA reprogramming
- Sendai virus reprogramming of fibroblasts
- Sendai virus reprogramming of Erythroid progenitors

The methodologies of reprogramming are then followed by the same process once the mechanical passaging phase is reached.

During reprogramming, cells are put in contact with a variety of reagents and growth mediums, the days and substances for the reactions are different but the process can be simplified to addition and subtraction of reagents to the wells and daily incubation in between the steps.

One of the variables of the reprogramming phase is given by the time necessary for the cells to develop, which can vary in relation

RELEVANT INSIGHT

- The priority of research laboratories is on the robust and efficient generation of iPSCs.
- Laboratories with clinical endpoints have efficiency as a trade off and prioritise safety and minimizing genomic alteration

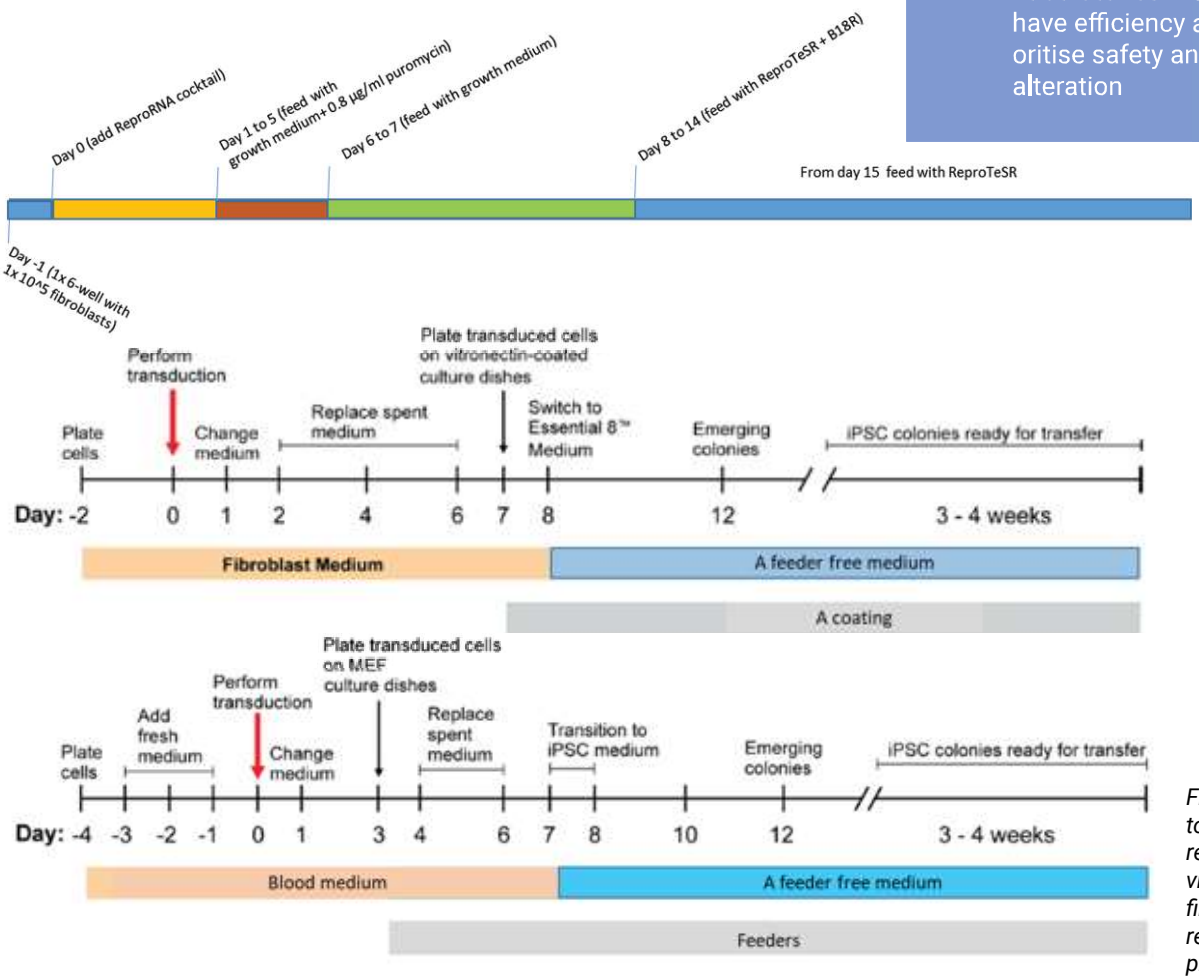


Figure 12: (From the top to the bottom) RNA reprogramming, Sendaii virus reprogramming for fibrblasts and Sendaii virus reprogramming of Erythroid progenitors.

to each typology of cell that is chosen at the start.

In all three methodologies, the reprogramming follows pre-established protocols with standard procedures and measurements. Their schematics can be seen in the pictures above.

In the case of RNA programming, the process takes from 20 to 28 days in average, depending on the development of the cells, with one additional day in the beginning for the preparation of one 1x6 well plate.

In the case of Sendai virus reprogramming, the phase of reprogramming lasts approximately 40 days, two days of preparation are needed upfront for the plating of the cells in case of fibroblasts reprogramming while four days of preplating, feeding, transduction and change of medium are needed upfront for the reprogramming with Erythroid progenitors.

After reprogramming, all three methodologies of cell culture share the same process: mechanical passaging, expansion through bulk passaging, and preparation for RNA and DNA isolation.

These steps have the final aim of checking the quality of the final iPSC cells and their properties.

As stated in the STEM cell definition, a pluripotent stem cell is validated through self-renewal tests, differentiation (pluripotency), and genome stability checkpoints.

Unlike the reprogramming phase which follows a prescribed protocol, the remaining stages of the procedure need to be customized for the specific needs of laboratory, which, in the case of this project, is reppresented by the iPSC facility of Erasmus MC.

2.5 The organization at Erasmus MC

2.5.1 The context

The context of the project, as said, is the IPS Core facility at the Erasmus MC.
The Context Diagram in Figure 13 illustrates stakeholders and institutions that are part of the context around the system under design (SUD). The following chapter describes the organization between the involved stakeholders.

Understanding the current organization, in fact, is going to give additional comprehention of the requirements of the SUD.

An interview with the IPS Facility head manager Mehrnaz Ghazvini and head of department Joost Gribnau was conducted regarding the current way of working and production of the facility and the final objective of the process of automation. The questions can be found in the Appendix.

2.5.2 What is the role of the Core Facility

Core Facilities and labs are centralized technology-based laboratories that maintain and support sophisticated equipment for use by their host institution's researchers or by external customers. Labs can be described as service centers for the local research community, providing access to sophisticated and expensive equipment so that individual principal investigators (PIs) do not need to buy hardware themselves, learn how to use it, train laboratory staff, manage repairs. Some laboratories provide training on technology; most also offer computational and statistical services (Gould, 2015).

2.5.3 Current and future throughput

The production of the facility is dependant on how many researchers choose them as service providers, and most importantly if the researchers get the funding they need for the production. Grants are of national and international nature and are assigned approximately 3 months in advance.

The production of hiPS lines of the current year is 49 lines (at the moment of writing, from January 19 lines have already been programmed and 30 remaining are on the waiting list).

Every 2 weeks, 2 to 4 lines start their programming. The numbers of requests for production are already in increase if compared with last year and the aim of the facility is that of increasing the capacity of throughput to 1000 lines per year.

2.5.4 Typology of requests

The lab is also planning to introduce a new typology of requests from other labs such as studies regarding the disease of skeleton muscles in patients with Parkinson, this typology of study integrates also the differentiation towards topogenic neurons. The fluidic system would work in the same way but during differentiation, the times of production would need to increase. Other commissions that are in the planning for the future are related to population studies, toxicology screening, and drug screening.

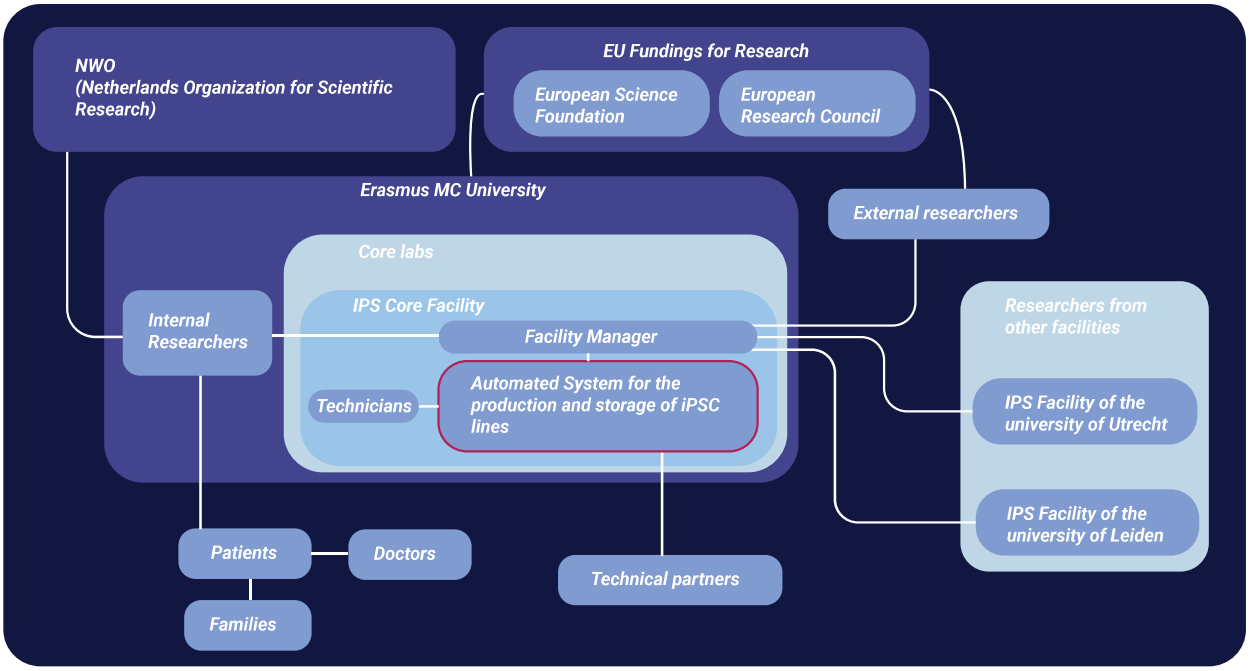
2.5.5 Current and future management and typology of the workforce

The number of technicians

At the IPS Core facility, the work of cell culture is executed by laboratory technicians which are biomedical researchers mostly with bachelor background studies. The current workforce is composed of 6 technicians, and they all work intermittently on all the lines taking care of the stages intermittently. In case of unexpected events, the work of a technician that goes on leave is replaced by the Facility manager, who usually has a role of supervision and contracting with researchers.

With an increase in the lines, also the

Figure 13: Stakeholders map



number of technicians that handle them has to increase accordingly. This has two downsides for the Facility: on one side the variability in the process of culturing increases and on the other side also the costs get higher.

The gross salary of a technician is very high and therefore the ability of the facility to be able to pay a new technician is reached only every new 15 lines.

The increase in the number of technicians also requires an increase in needed space and equipment (number of biological cabinets and incubators) which also increases the amount of rent and overhead costs.

In the case of automation, on the other side, the number of technicians would not need to increase with the same ratio and the background of the technician could also be of a less specialized level since the physical interactions should be simple such as prepare medium, filling the medium reservoir, preparing coated plates, DNA, RNA isolation.

More experienced technicians would be involved in performing FACS staining, RT-PCR, and some data analysis like SNP arrays.

In the future perspective, implementing robotics, there should be also personnel involved in programming and robotics.

Although the variability resulting from the work of different technicians on the cell culture would be reduced, there could still be consequences deriving from the organic behavior of the cells. Different solutions should be found to these problems from time to time, (sometimes it is repeating the procedure while some other times recalculation the splitting ratio..). A more experienced technician needs to be consulted in such a scenario.

Training

Every technician has to be trained for two to three months depending on their practicality and velocity of learning. Although it could be more logical to try to avoid this time of latency, the tendency is still that of hiring technicians with less experience because the elements and quality of the products they put attention to can still be shaped while the aim of technicians with research background would be more focused on one single aspect of the cell culture quality.

Technicians work every day of the week and, in turn, one weekend day per month,

which makes a total of 21 days per month, therefore they are presented with 42 to 63 steps to analyze in the period of training. However, the amount of data the technicians go through during the training is difficult to calculate with precision: they are exposed to the observation of different stages of the process per day so the number of examples they observe is higher.

The level of training of technicians is evaluated by their ability to participate in teamwork during daily work, the quality of the cells they leave the culture at after having taken care of them individually. The quality of the cell culture is evaluated by parameters of size, morphology and proliferation rate of the cells.

During training, attention is put on the stress factor. Technicians are asked to take care of the cells and understand their needs.

Having a wrong cell density or not refreshing the medium on time can make the medium poor of growth factors or accidentally change them which will then be expressed also in the genes of the cells.
Because of this, technicians are told to be careful of not growing artifacts and build an attention to the understanding of the culture needs.

Teamwork

Currently, information and communication between team members are fundamental for the evaluation of the stages and for allowing technicians to take care of the cultures intermittently. If a technician has had difficulties in the understanding of the morphology other technicians give support in this evaluation. Everyone evaluates the quality of the work of the others.

Budget for automation

The budgets for the process of automation in the coming years are 1 million euros of investment for the next 1 - 5 years.

The quantities of production are related to the fundings the facility is honored with. Researchers that are commissioning the fundings are the ones responsible for their applications to State or European consortiums. The possibility of getting fundings, which are yearly based, depends on the ability of the Facility to prove that the objectives can be reached along the timeline.

Commissioners and Network of the Lab

Most of the clients of the facility are internal and international researchers, the aim is to get more and more involved also with companies. Currently, there is an exchange of information with national and international contacts for the exchange of experience on new reprogramming methods and testing of material for the research community.

In terms of production and services each Facility works separately, one exception is that of the facility in Leiden who doesn't perform reprogramming of peripheral blood cells and has therefore commissioned the reprogramming order at the Erasmus MC Facility.

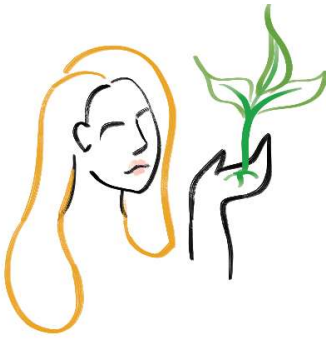


Figure 14: Interaction metaphor: During the culture of cells the technicians are told to pay attention to the needs of the cells, "talk to them" in order to "avoid artifacts".

RELEVANT INSIGHT

- The technitians inside the facility rely on team work in order ensure check up on each other's decisions.
- The budget of automation at the moment is of 1 million euros.
- There is not much collaboration between different Facilities
- A technician gets confidence and enough knowledge after a period of training of three months

2.5.6 Daily manual process

The process done by the technicians in the lab is performed daily and it can be semplified in a series of actions of liquid handling that treats the cells with different reagents.

In fact, the typology of activities performed on a daily basis in the lab by the technicians are of three kind of daily duties:

1. Visual check of the cultured cells with a microscope
2. Medium changes by use of a pipette
3. Passaging of cells by addition of reagents (bulk passaging) or picking of cells (mechanical passaging).

However, these basilar daily duties vary between day to day and their variety is dependant on the step of the culturing process to which they belong.

Therefore each step of the process can be analyzed in more detail, the outcome of the analysis allows to describe the machine components, the functions necessary for each step and therefore the necessary characteristics of the modules of the robot.

The process tree can be created starting from observations of the manual process and thinking on how to translate each step into a distinct automated action.

Observations of manual processes

Observations have been done over the method used during the daily activities of change of medium and passaging of cells. Due to unforseenable circumstances (the iPSC Facility being temporarely closed and restricted to one tecnician at a time due to Covid-19) the observation has been followed through a phone screen recording live and pointing towards the surfaces of the cabinet.

During the observation the manager of the iPS Core Facility Mehrnaz Ghazvini, who is also the practical coordinator of the work of the technicians within the structure, has been asked to elaborate and talk out loud about each step, the materials used and the reasons for which the step has been executed in a specific manner.

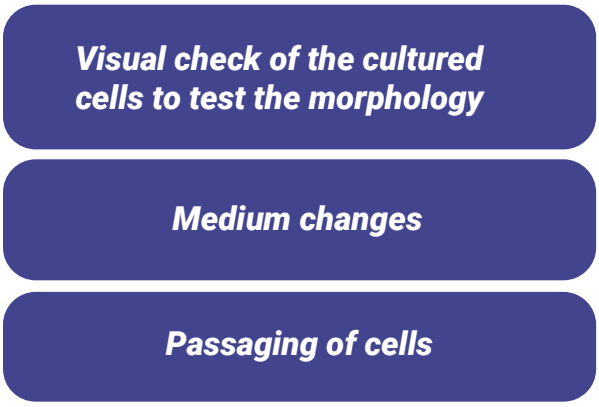


Figure 15: Daily activities

The observed actions and materials used have been noted and gave the basis material for the organization of a Focus Group session with five tecnicians of the laboratory, including the manager.

iPS Core Facility Focus Group

The aim of the focus group has been that of getting acquainted with the different figures within the facility and understanding which topics would spark conversation or debate within the group testing the differences in their way of working.

The partecipants, guided through a phone call, logged into a virtual environment, Mural, in which they could interactively add sticky notes and immages on a pre-prepared canvas.
A small ice breaker has been prepared to introduce them to the new platform and make the focus group more partecipatory, each member has been asked to introduce themselves shortly by saying which is their role, their background and the most and least liked thing about cell culture.

Except for the manager, all the other technicians at the iPS core Facility are

students or recently graduated students.They find the manuality and close observation of the process of cell culture interesting as well as the diversity of the process and the constant opportunity to learn from new different parts of it.
Only one complaint was about the burocracy of the process and the possibility to add artifacts.

The following step has been relative to the actions to do during bulk passaging for the method of RNA reprogramming.
The canvas has been preprepared with four columns to fill in following the steps reppresented graphically in the first column.

Gradually material used, machines involved and set up notes, intended as where could the variability of the step lie and what could be the risks of automation be, have been analysed by the technicians and the manager.

The nature of the discussion showed that all technicians had the same grade of confidence in their knowledge, but the leading word has been left to the Mehrnaz.

All the technicians agreed on the points discussed, although some little variables could be identified in the step of pipetting up and down at the end of the process: some technicians would pipette 2 times and then check with the microscope, while the majority would pipette directly three times and check only later. This can be caused by the higher amount of prudence in pipetting. This step, in fact, is responsable for spitting the cells into smaller dimensions, an effect that can not be reversed.

An other point of discussion was concerning the risks in the process of automation at the final stage of this passaging method: while in manual handling there could be the need for splitting the content of the cell in different wells in order to reduce the density of the cells in one well, this step could be skipped if the calculation of the correlation between well density and number of wells to split into is done correctly at the beginning of the step. However, the doubt may rise in if the automated system would be capable of establishing an estimation more precisely then the technicians and therefore avoid this recorrecting step.

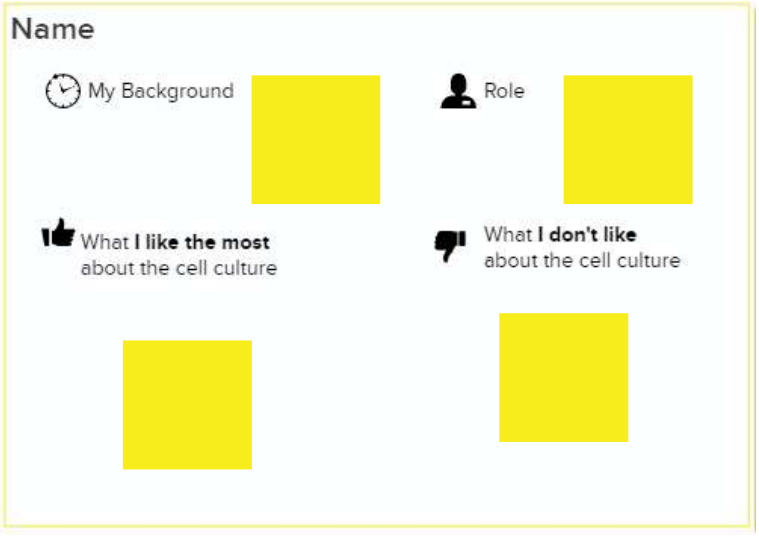


Figure 16: Example of space for interaction during the ice breaker activity

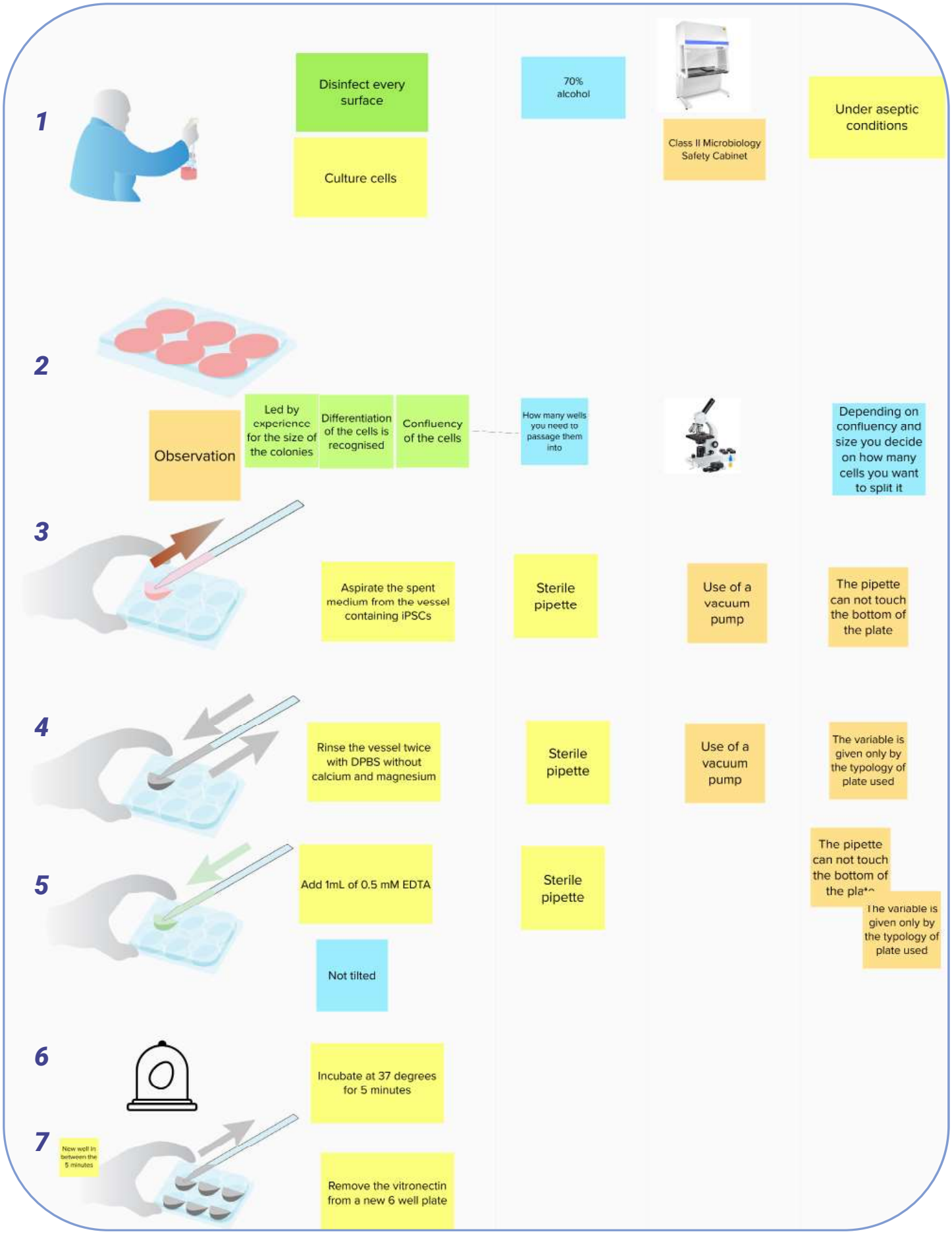


Figure 17: Focus group activity

8

Add an appropriate volume of pre warmed essential 8 medium to each well of vitronectin (at the end there should be 2mL of medium in each well)

Not tilted

The variable is given only by the typology of plate used and by the step you are into

9

Aspirate the solution

10

Not tilted

Add pre-warmed Essential 8 Medium to the vessel

Avoid air bubbles

Volume that you take up

Tip of the pipette should not get over the surface

Speed of pipetting

Having too much or too little splitting of the cells

11

Move the cells from the wells by squirting medium and pipetting the colonies up

5ml pipette

There could be cross contamination between the wells in case there could be slips over the edge of the well

Pippette 3 times up and down

12

collect cells

13

New well prepared during incubation

Microscope check

check if there is further need of pipetting up and down

Check of the density of the cells and the size

Automation would lead to less variablilty

14

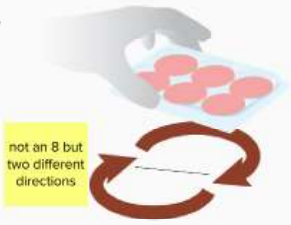
if the density is to high or the dimention is too big there is need to repipette

Depending on the current methodology but it can be can be avoided if the machine is tuned correctly

There could be the need of taking a quantity out

There could be the need of add more cells

15



not an 8 but two different directions

Move the vessel in several figure of 8 motions to disperse the cells across the surface of the vessel

"Don't shake too hard"

Should be adjusted by fine tuning

17

Place the plate in an incubator at 37 degrees 5 % Co2 incubator

Visual check of the cultured cells to test the morphology

Steps	***Necessities of the machine***
Step 1 - extract medium from fridge (4 °C) to warm up to room temperature, original temperature of the incubator for the cells is 37 °C. Step 2 - Activate the flow of air of the cabinet Step 3 - Spray all surfaces with 70% Ethanol alcohol Step 4 - Check the clones under a microscope inside the cabinet	• Warm up time depends on the quantity of the medium and its container • Aseptic conditions • Disinfected tools and working areas on a daily basis • Analyse wells one by one and decide on subsequent action by understanding the size of the colonies and the density

Medium changes

Step 5 - Turn on the flame of a sterilizer with a foot pedal Step 6 - Burn the tip of the needle Step 7 - Take out part of the medium with the pipette and distribute the medium in different wells Step 8 - Return the wells into the incubator	• Operate in aseptic conditions • Isolate area for the flame, only have a focused change of temperature • The distribution of the liquid inside the vells is not always of the same quantity, there is need for separated control of the channels

Passaging of cells: Bulk passaging and mechanical passaging

Step 9- Visual check of culture Step 10 - Remove media Step 11 - Wash cells once Step 12 - Add passaging solution once colony edges detach, Step 13 - remove passaging solution Step 14 - replace with fresh media with rock inhibitor	• Operate in aseptic conditions • Tilt the well of the plate in order to transfer liquid by contact and without integrating air bubbles • perform microscopic analysis to determine the right moment of passaging

Figure 18: Steps and notes deriving from the observations

Passaging of cells: Bulk passaging and mechanical passaging

Step 15 - Dissociate colonies by pipetting up and down
Step 16 - Distribute the resulting cluster in precoated cell culture plates supplemented with rock inhibitor
Step 17 - Incubate

- Pipette the liquid up and down with constant flow of liquid
- calculate the correct splitting ratio for passaging
- Perform a movement in all directions on a surface in order to distribute the cells eavenly on the well.

RELEVANT INSIGHT

- Asseptive conditions should be recreated
- The well plates are tilted only at the moment of suction
- Mediums also need to be stored i cooled positions
- Tip sterility should be mantained
- By pipetting up and down the size of cells is reduced, during the focus group session this became a moment of discussion, showing that the technitians can have different opinion on how much trust they would have in a system that would pipette cells up and down based on a calculation.

RELEVANT INSIGHT

- The final steps following passaging P6 to P7 are critical for high throughput and the most time and effort consuming in manual processes.
- The main activities executed by technitians are relative to visual check of the status of the cells and pipetting steps.
- Important parameters technitians have to pay extra attention through the daily procedures involve sterilization and control of flow of the fluid to the wells.
- Techicians inside the facility have the same knowledge between each other, their work and training method is based on assimilated experience through practice, discussions and peer assessment.

2.7 Instruments currently used

Laboratory equipment varies from analogical tools to automated and highly elaborated devices. A general description of the currently used tools is here given for the understanding of the components that are later also going to be taken into account for the design of the system.

For each instrument and tool there are specific attentions to be given by the operators during use, these are included in each description. Information has been collected during several interviews and discussion with the laboratory staff and selecting applicable information from documentation such as the Laboratory Manual for Biotechnology (Verma, 2014).

Manual pipetting

The current tools used in laboratories are syringes and microsyringes or pipettes. The first typology can be designed with manual or electronic holders to precisely control the piston displacement and assure accuracy. Pipettes can be single and multichannel, with the possibility of adjusting the tip spacing for transferring multiple samples between different tube rack and microplate configurations, fixed (for accuracy and precision) and adjustable-volume (for a larger scope of applications).

Rules regarding good practice for the actions for pipetting tasks are:

- pipettes must be calibrated on regular intervals so that results are consistent
- All pipettes should have (glass or plastic) should have cotton plugs to reduce chances of contamination of pipetting devices
- Air should never be blown through liquid
- Liquids should not be forcibly expelled from pipettes
- Mark to mark pipettes should be preferred to other types of pipette because they do not require complete expulsion of liquids
- Contaminated pipettes should be completely submerged in a suitable disinfectant in unbreakable container, they should be left in disinfectant for appropriate lenght of time before disposal.
- A discard container for pipettes should be placed inside biological safety cabinets and not outside it
- Syringes fitted with hypodermic needles must not be used for pippetting needs.
- Avoid the dispersion of infectious material dropped from pipette on the surface, this may be obtained by placing an absorbent material on the work area, it should be disposed off as infectious waste.

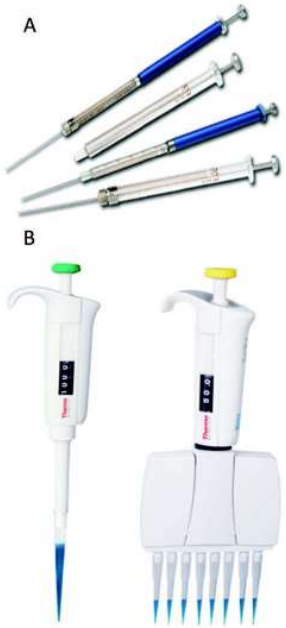


Figure 19: Siringes and single- and multi-channel pipettes

Autoclave
Autoclave functions based on the principle of sterilization through steam. In all biotechnology laboratories autoclave is used for preparation of media either for microbial culture or plant culture, however it is not usually used for the preparation of media for animal tissue culture but in these laboratories it is used for decontamination of laboratory waste, in particular biohazard one.

Centrifuges
In almost any biotechnology laboratory centrifuges are very common. Their function on the principle of isolation and separation of different components from a solution or suspension. They are used for cell isolation, preparative applications or washing of materials during experiments. There are different typologies of centrifuges depending on their size, type and functions. Differences between them are also regarding the capacity of rotors and their types and can be divided into 2 main categories: fixed angle rotors and swing-out rotors.

During use the technician has to pay attention to the following elements when using a centrifuge:

- Placement of the centrifuge on a sturdy and balanced platform with no shaking
- Use securely capped tubes for centrifugation
- use paired weight buckets when centrifuging

Incubators
Incubators' function is that of maintaining constant temperature, humidity and other conditions such as carbon dioxide (CO2) and Oxygen content of the atmosphere inside. Their purpose is to grow and maintain microbiological cultures or cell cultures. Two common incubators are CO2 incubators and 37 C incubators. and can be of three typologies: general incubators, bod incubators and shaking incubators.

The basic structure of an incubator is that of insulated boxes with an adjustable heater, typically going up to 60 to 65 C. Most incubators include a timer while more elaborate incubators can also include the ability to lower the temperature (via refrigeration), or the ability to control humidity or CO2 levels, or can be programmed to cycle through different temperatures, humidity levels, etc.

During the use of incubators, CO2 incubators in specific, technicians should pay attention to:

- Keeping an eye on the temperature of the incubator
- On regular intervals check water levels in the tray kept inside the incubator to maintain consistent humidity
- Check the level of the water inside the jacket of the incubator and fill only the recommended type of water inside the jacket
- Clean any media spillage on the surface of incubator chambers by 70% ethanol.
- Decontaminate the incubator with 70% ethanol if there is fungal growth.

Similar points of attention should be considered for incubator shakers.

Balances
Electronic balances are the majority of balances used in labs, these are cardinal instruments in the laboratory because weighting is the 1st step to start any experiment. They are very sophisticated and precise.

Precautions and good laboratory practice include steps to clean and preserve the balances as well as avoid the influence of other devices (such as centrifuges, vortex mixers or magnetic stirrers..) on the measurement of the balance.

Refrigerators and freezers
Biological material often has to be kept at low temperatures for storage. For general storage purposes refrigerators are used, while for long term storage -20 C, -80 freezers and liquid nitrogen tanks are used. In certain circumstances also dry-ice is used.

There are mainly two types of lab freezers (Long, 2010): chest freezers, more effective in cooling and maintaining the temperatures by retaining the cool air efficiently, and able to recover and go back to the original regulated temperature even when the door of the freezer is kept open for longer durations, and upright freezers, with smaller footprint but not compromising their storage capacity and giving more ease in locating the samples. Both typologies integrate alarms to detect change in temperature for a too long opening of the doors.

Refrigerators can use different typologies of refrigerants: hydrocarbon refrigerants have reduced energy consumption and can reduce the air conditioning requirement because they dissipate less heat to their surroundings. Green refrigerants R170 (Ethane) and R290 (Propane) are more efficient options; however, these highly efficient refrigerants are potentially flammable and their use is therefore often restricted.

Certain precautions need to be followed:

- Refrigerators need to be defrosted at regular intervals, this process can not be automatic because this cycle normally destroys the biological samples as it thaws and then refreezes the samples.

- liquid nitrogen tanks have to be checked for required levels of liquid nitrogen in the tank because of its evaporation with time.

- The door of the freezer should be kept open for minimal duration

- Any bio-hazardous material should be labeled clearly and the inventory of the freezer should be maintained and followed strictly

- Flammable solutions must not be stored in the refrigerator, unless container for storage is explosion proof

- Since cells are subject to abrupt change of temperatures a gradual shift to the freezing temperature should be maintained (common practice is to insert the plates and tubes inside slightly isolating boxes or, less officially, baby diapers).

- Room temperatures should not exceed 45 C and proper air circulation is very important for the functioning of refrigerators.

- Filling the room up with too many refrigerators would increase the heat emission and from the refrigerators and increase the overall temperature of the room putting pressure on the compressor.

- When setting up the freezer there is need to cool it down from room temperature, the pull-down time can go from 9 to 26 hours.

- ULT freezers can generate a considerable amount of heat and noise. As a result, adequate ventilation is critical. A minimum space of 203 mm on top and clearance of 127 mm on both sides and the back is recommended.

Biological safety cabinets

Biological safety cabinets are used to deal with material that can be infected or infectious.

They are ventilated enclosures offering protection to the user, the product and the environment from aerosols arising from the handling of potentially hazardous micro-organisms by discharging the continuous airflow to the atmosphere via a HEPA filter.

They come in different sizes and are of different types with different states of protection.

The typology needed the case of an iPSC culture practice are for a Biosafety level 2 typology A

Typology A means that it is suitable for microbiological research with the absence of any volatile or toxic chemicals since air is recirculated within the cabinet.

These cabinets provide personal, environmental and product protection and have 3 main features:

- A front opening with carefully maintained continuous inward airflow.
- HEPA-filtered, vertical, unidirectional airflow within the work area.
- HEPA-filtered exhaust air to the room or the exhaust connected to an external extract system.

Airflow is drawn into the work chamber via the front aperture, continues under the worktop and goes back, where 70 % is being recirculated through the main HEPA filter to provide down flow and 30 % exits out through the HEPA filter to exhaust. The vertical laminar flow Biological Safety Cabinet provides operator protection by means of inflow, product protection through down flow and environmental protection through of the filtered exhaust.

When using a biosafety cabinet the technicians should pay attention to the following elements:

- Air circulation at the rear side of biological safety cabinets should not be blocked
- Traffic behind operator should be minimized
- Air grills should not be blocked
- Surfaces of the biological safety cabinet should be wiped using appropriate disinfectant after work is completed
- The cabinet fan should be run for at least 5 minutes before the beginning of work and after the completion of work in the cabinet

Figure 20: Biosafety cabinet level 2

RELEVANT INSIGHT

- Insights of this chapter lay down several design restrictions to take into account in the phase of ideation or further later improvements.
- Each step of the process has rules that regulate the actions and movements executed by operators
- Tools used for liquid handling are syringes and microsyringes or pipettes, the rules involving their use mainly focuses on prevention of spillage, contamination and forming of bubbles within the pipette
- Containers in which used pipettes are placed should be umbreakable and placed inside the safety cabinet container.
- Sterilization is usually performed through autoclave or 70% alcohol
- Centrifuges should be placed on sturdy platforms
- Capping of tubes could be an element of risk during the use of centrifuges
- Incubators should be controlled for the levels of water available in their tanks
- There should be access to incubators in order to be able to clean its internal surfaces
- Preferred typology of freezers are upright freezers for a smaller footprint, while chest freezers are a better choice if there is no possibility of closing the door quickly.
- Freezers can not be used directly at set up an interval time of 9 to 26 hours of cooling should be considered.
- The system will need to be enclosed inside a Biosafety cabinet level 2 typology A
- The back of the safety cabinet should be left free for circulation of air.
- Frontal external sides of the safety cabinet should be left open.
- The safety cabinet needs a time of 5 minutes in order to start working

2.7 Process overview analysis

In order to analyze the requirements and functionalities of a robotic system adopted for this section of the process, the steps regarding mechanical passaging, expansion and RNA and DNA isolation need to be documented.

The information collected regards details relative to the consumables, the typology of mediums and the technologies used in each step, and the characteristics of the transitions between each step.

The collection of the data has been done during several observations and co-creation sessions with the Head of Erasmus MC iPS Core facility.

The first step of visualization has been done on a daily temporal map to understand the different destinations of the introduced wells, the subdivision of the organic material in the new wells, and the parallel flow of the processes.

The next phase of documentation zooms into each daily step and analyses with more detail the requirements for each given action in an effort to translate the manual activity in a robotical function.

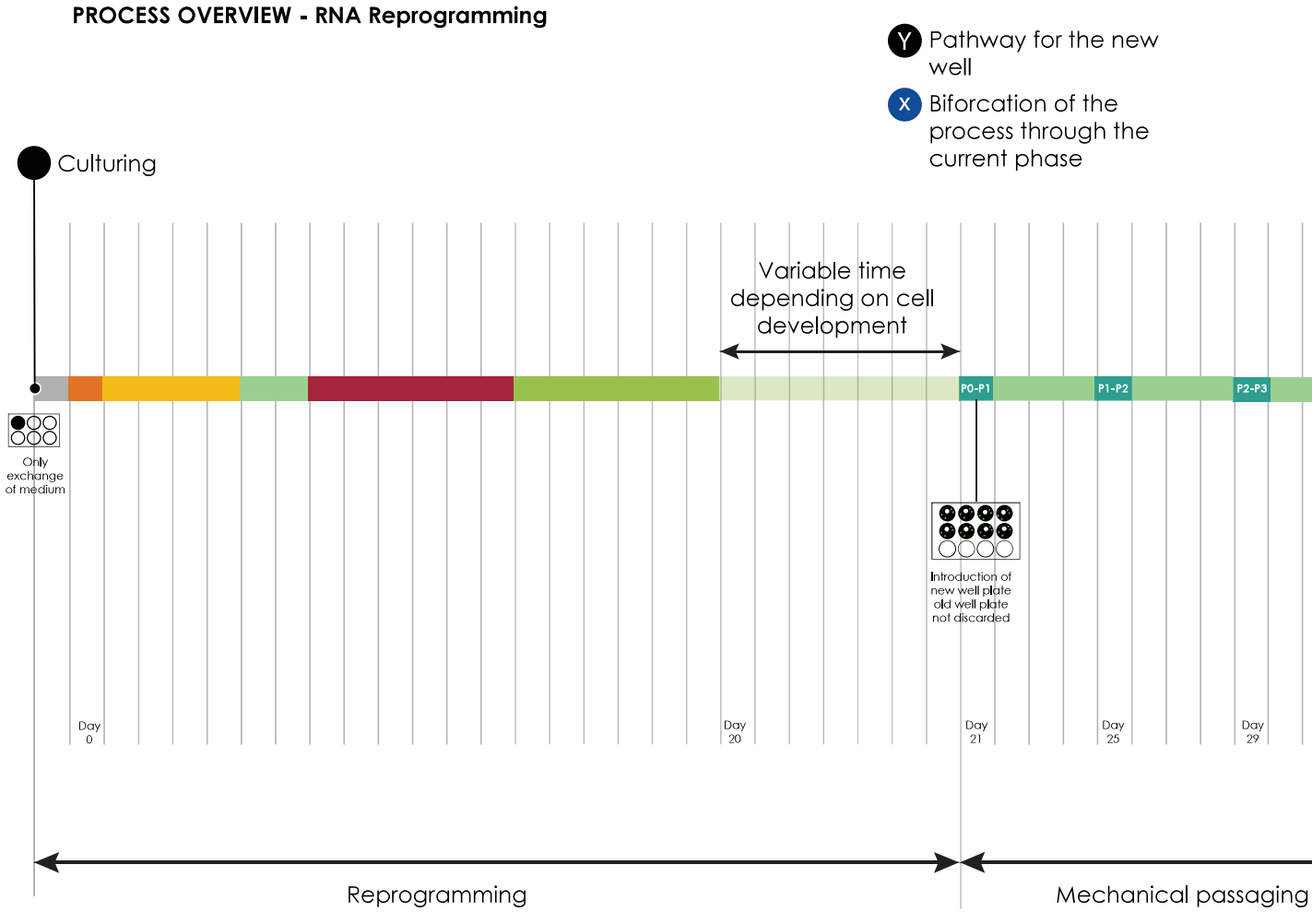


Figure 21: Overview of the process

Temporal Map

In the temporal map (Figure 21), each column represents one day of the culturing process.

For completeness of the overall culturing process, also the initial phase of RNA reprogramming is here reported although, as it follows standardized protocols, the specifics of this phase of the process are not detailed.

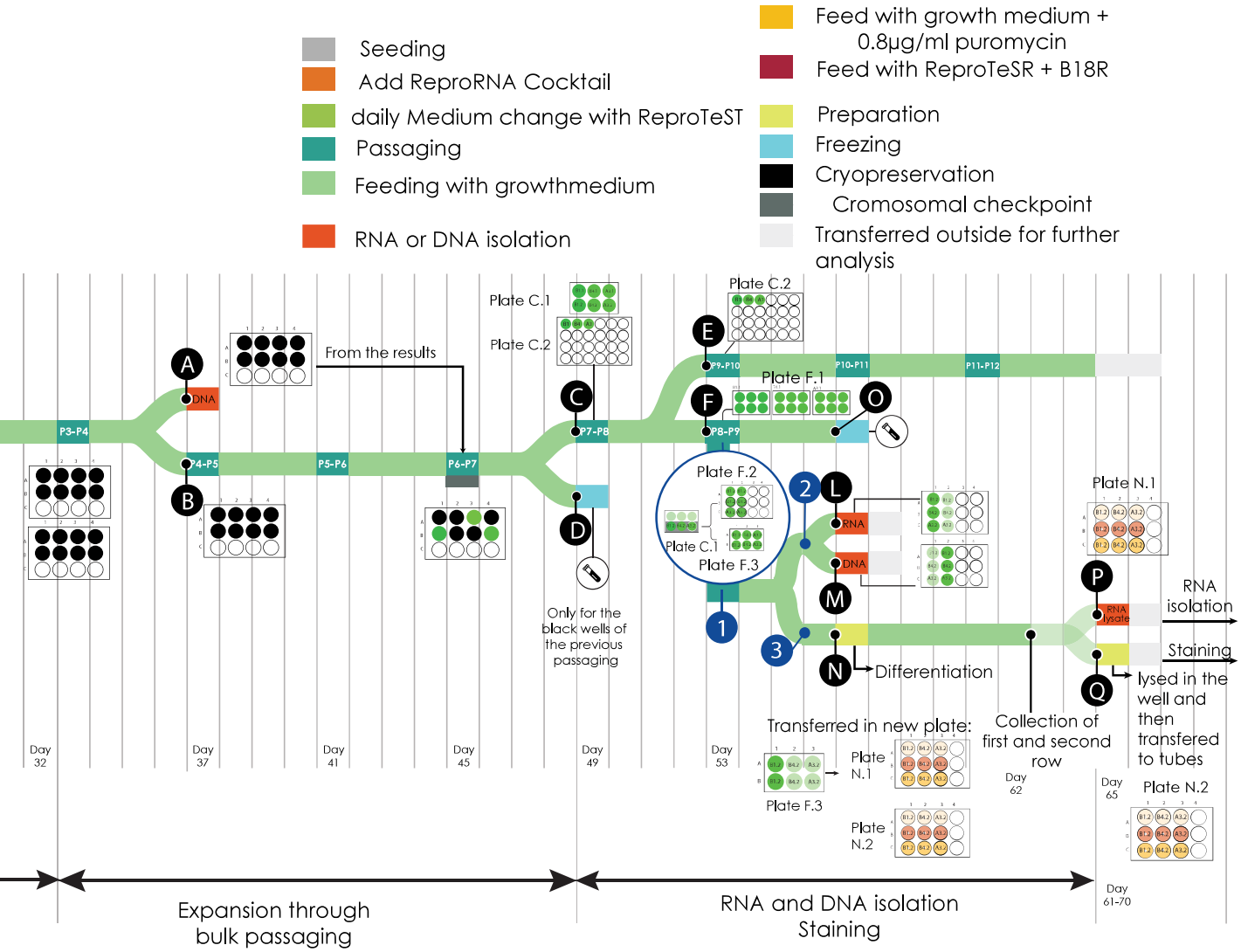
From a comprehensive point of view, it's possible to analyze how the process gets more complex as the cell material is directed to a new destination. The number of wells to be handled in which the original cells are subdivided into, in fact, raises subsequently from one well of a 6 well plate at the beginning of the reprogramming process to a total of 36 different wells handled at the same time around the 60th day of culture (N, M, L, O, and E are happening at the same time).

It's important to mention that since there are different variables in the process due to the organicity of the handled material the number of days can be slightly variable as well as the number of wells. In this case, the represented timing takes into account an optimal behaviour of the cells in all wells.

The culture process is composed by three main phases following the reprogramming phase:

Mechanical passaging phase: the steps that take place are relative to passaging and feeding of the cells.

Through three subsequential steps of



passaging all the untransfected and partially reprogrammed cells surrounding the iPSC colony are discarded and colonies are physically split in smaller fragments.

At each passaging step, new cultureware is introduced with a coated matrix with iPS cell maintenance medium.

In between each passaging, the feeding step takes place for approximately three days until the size of the cells is big enough to go to the next passage, which is determined through a microscope check.

Expansion through bulk passaging: Through this step, the splitting of the cells is done with the adoption of EDTA, a specific reagent that makes the colonies separate and split in a non-mechanical way. At this point, the cell size and their density are regulated through additional movements of pipetting up and down.

In between each phase of bulk passaging the cells are fed until their dimension and density are regulated and checked to be correct, more details on what escatly this term means will be explained later.

Freezing: Two steps of the process (D and O) are used to store the colonies for future backup through the process. During these steps, the cells are transferred to different vials and could be reintegrated in the process in case of contamination, cross-contamination between wells, or other unexpected problems.

Preparation for RNA and DNA isolation: As described previously, the processes of RNA and DNA isolation are used to check up the genome integrity of the cells. Because of this, they involve a point of contact with the technicians and researchers at their end in the form of delivery of the cells in tubes. The steps L and M happen simultaneously to the same well plate, this means that the pipetting channels for each of the two processes should be controlled independently and selectively for wells belonging to the same well plate. The main difference between RNA and DNA isolation is given by the different lysis reagent used. Since the RNA lysis reagent can not be stored long before the moment of use and it is created by a mixture of two different components this step, being simple and happening only one time at through the process and at the end, is still entrusted to the technician with a new moment of interaction.

Preparation for staining: After being transferred to a new well for the process of preparation for staining, single cells are lysed with a specific medium containing the three germ layers. The addition of these specific medium layers is done to the same plate directly by the technician, representing a new interaction point.

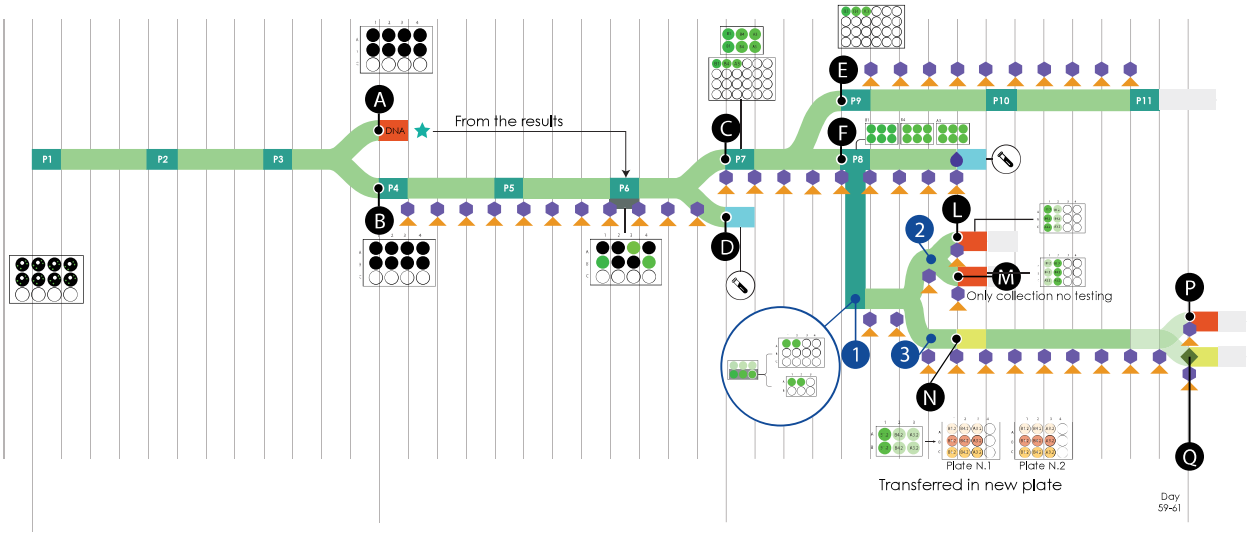
Through this overview, a different consideration can be taken:

The current manual process over the cells has also the functionality of constant monitoring of the status of the cells through the process. The daily microscopic checkups of the cell allow technicians to distinguish the following parameters, which lead also to important decision making elements.

1. **Quality control:** In this case, this term gives information on the contamination of the cells by pathogens

a. Contamination against bacteria and fungi is done by checking the turbidity of the medium every time the plates are taken outside the incubator, the clarity of the wells is checked through a microscope, in case of contamination, they are discarded, and the wells are sterilized. This control has to be done every day or every time the plates are taken out of the incubator.

b. Contamination from microplasma is given by checking 1 ml of medium in a cylinder before freezing. According to the iPSC facility directors' experience and



Check points

- Quality control, bacteria and fungi
- Quality control, microplasma
- Genome integrity
- Validation of the identity of the cell
- Correct functioning of the machine For having a record and train the machine learning process
- Observation that the cells are ready for splitting

What does it have to take in account?

- Turbidity of the medium
- DNA, done outside of the machine
- DNA/ RNA, done outside of the machine
- It is done through staining
- Cell morphology, cell density and cell size

Figure 22: Check- points of the process

opinion, the procedure of control against microplasma contamination should not be automated but executed outside by a technician.

2. Cell adhesion, size, and confluency: Optically monitored properties such as cell adhesion, size, and confluency give important information for the correct procedure of the process. Based on these parameters the technicians evaluate which is the further step to take or repeat.

3. An additional checkpoint should be considered in case of automation:

Correct functioning of the machine: the development of cell material can behave differently in each colony and, therefore, the decision making of the automated system should be able to take into account several interdependent variables: the size of the colonies, their distribution, and confluency in the well. In order to prevent mistakes and remedy any decision-making errors, an expert technician should monitor, control, and intervene in focal decision-points of the process.

By mapping all the quality-control and check-points defined through the process phases it is observed that the repetitiveness of the actions relative to monitoring is accountable mainly to quality control against bacteria and fungi and the correct functioning of the machine (Figure 22).

RELEVANT INSIGHT

- The process has a duration of 72 days
- At each passaging step, new culture-ware is introduced with a coated matrix with iPS cell maintenance medium.
- Checkpoints against bacteria and fungi are the elements checked the most through the process

2.8 Within the individual phases of the process

In order to assess the way of automating the process in a laboratory it is important to have a clear overview of all the sub- steps it comprehends, espetially when the process is specific for the laboratory and doesn't follow a prescribed protocol.
This can be done by creating a diagram that shows all the different components used, their actions, the materials introduced and used splitting points of each phase. The result of such analysis is a diagram map similar to the ones used in software flow-charts. These typology of maps, in fact, have been, also used in the description of biological processes with the aim of making unambiguous representations of biological systems (Roux-Rouquié et al. 2004).

The Diagram map created during the research phase has been based on the cronological follow up of the process steps and has been inspired for some of its elements by software flow chart elements:

- Subsequential order of the steps
- Direction of arrows to show the order of the sequence
- Arrows pointing inwards or outwards to indicate imput and output
- Use of AND/ OR logic for splitting points towards alternative paths.

During the documentation of the process a further step has been integrated: the manual actions done by hand are translated into the possibilities offered by technology. An example on how to translate manual processes into automated ones can be seen in the table at Figure 25 as provided by Opentrons, producer of liquid handling systems.

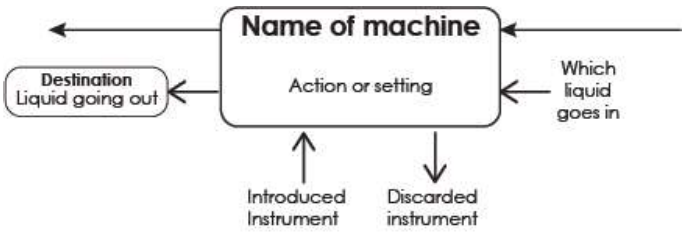


Figure 23: Explanatorion of the reppresentation of each substep

The complete Process Tree is presented in the Appendix I and is the result of the observations of the manual process.

By analysing and understanding the functional needs of each step throughout the process with a following Functional Analysis (that can be found in Appendix II) it is possible to identify the requirements of the overall system and the components that have to be involved for each step of the process.

Overall system
The system should be modular in order to be future-proof for changes in the reprogramming method that could be due to the constant discoveries within the field.

It should be enclosed fully in order to avoid risk of contamination. However, it should allow one technician to intervene in the supply of new consumables to the machine, check the correct functioning and instruct on changes in the workflow.

The system doesn't need to be moved in the facility and therefore its space footprint can be medium sized to big.

However, the modularity of the system should take into account also mantaining flexibility in the workflow and possible scale-up or scale-downs. For this reason, the size and weight of the system's modules should still allow the technicans to create different compositions by pulling the modules.

By visualyzing only the transportations of the

wells through different devices the devices needed for each step are identified.

The schematics in Figures 24a - 24b illustrate the devices necessary for each step.

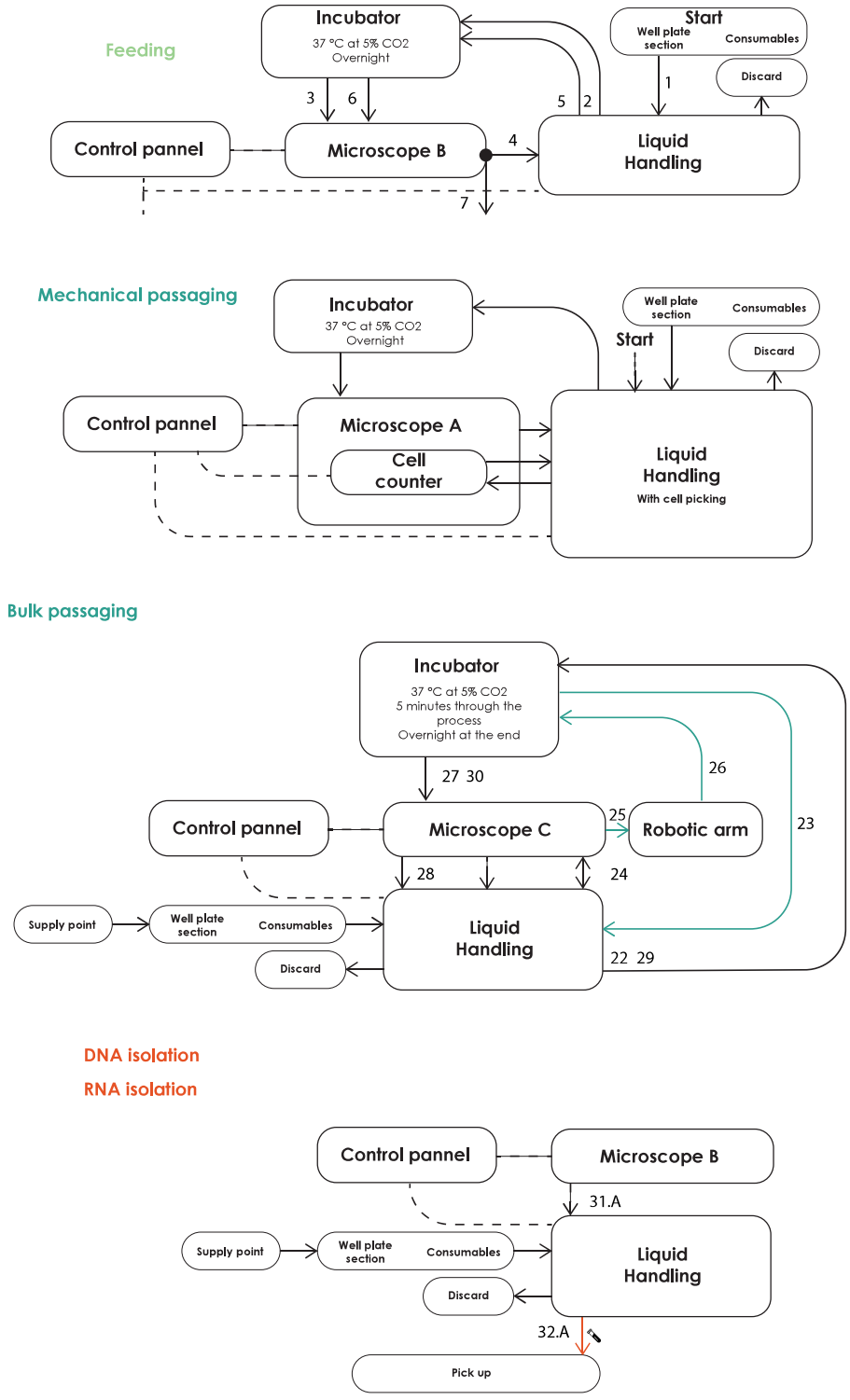


Figure 24a: Addition of components based on the level of coverage the system should provide at each process phase

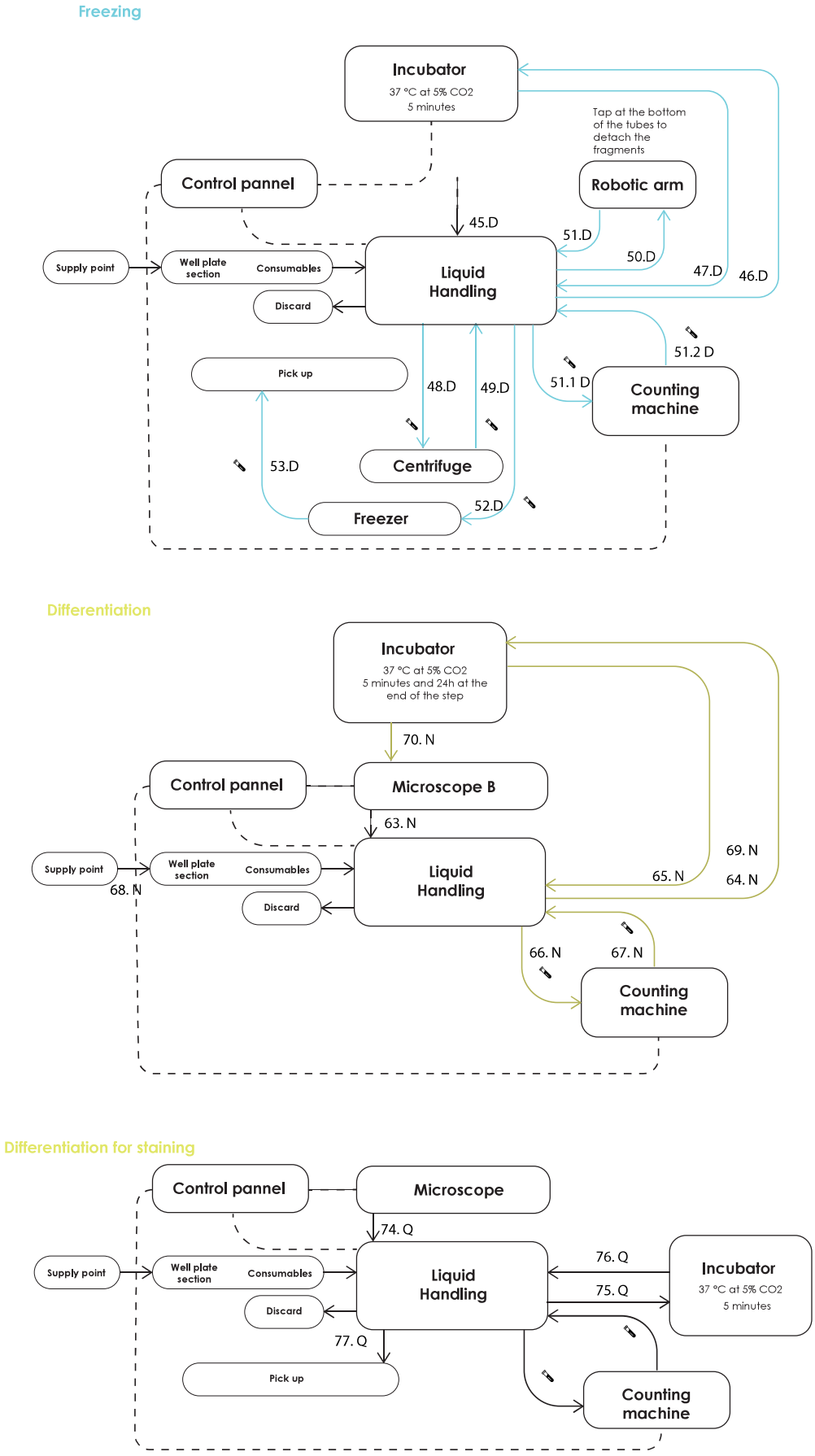


Figure 24b: Addition of components based on the level of coverage the system should provide at each process phase

PROCESS	MANUAL	AUTOMATION
Nucleic acid purification	Spin columns	Magnetic beads
Mixing	Vortex	Extensive pipette mix
Heating	Heat bath	Temperature module
Cooling	Ice bucket	Temperature module
Repeat dispense	Repeater pipette	Multi-dispense function

Figure 25: Translation of manual processes to automated ones, Source: opentrons.com

Other takeaways that can be derived from the functional analysis are the following:

- There are several steps in which it is necessary to have a second arm since at several steps there are movements that can happen at the same time, in parallel or in subsequential manner.
- The incubator is used either at the end of one step or in between liquid handling operations. Since at the end of each step plates are incubated for 24 hours before being checked through the microscope, the incubator should be placed near the microscopic section of the system and adjacent to the liquid handling robot.
- Some well plates are returned to the incubator in order to be stored for back-up, this incubator will not be used frequently, therefore one additional incubator could be considered for this spetial use.
- Once the process enters bulk passaging steps there are no additional decision ponts: all the wells that enter this step follow the same treatment.
- At the bulk passaging step the rack should accomodate one additional well plate in comparison to the previous reprogramming steps, therefore the liquid handling used from this step on should have double the capacity then the one used previously.
- During DNA and RNA isolation (steps L and M) steps take place at the same time, however, DNA isolation takes place without the intervention of a technitian, while the reagents used for RNA isolation have to be mixed and supplied to the machine by the tecnitian at the exact day of use.
- Mechanical passaging and feeding steps follow the same scheme of movements.

By analysing only the interaction points of the process it is possible to determine which are the interactions needed between the system and the technitians.

The interactions are of two different natures: control interactions are relative to assistance on decision making factors and management of movement of wells, collaborative interactions are relative to the pick up of the outcomes of the system in 1,5 ml plates, pick up of 12 well plates for chromosomal counting and later imput of data, imput of coated well plates, and resources, between which also the reagents to be mixed for RNA

reprogrammming.

By analysing the process on the side of liquid consumption and consumables it is possible to calculate the necessities of the system for one cell line, they are summarized in Figure 27a -27b.

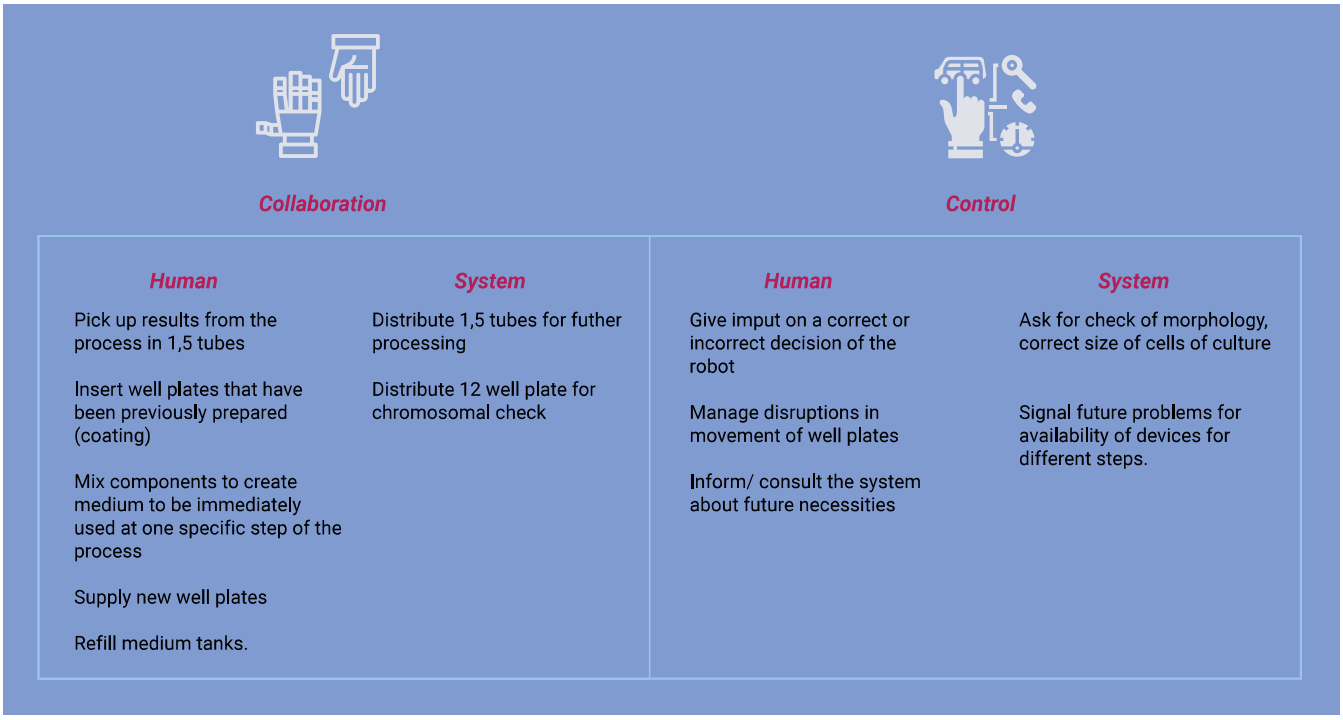


Figure 26: Human-machine interactions of the automated system.

Liquid supply per cell line						Current	Future
		times (wells)	quantity (ml)		Total ml	Per 50 lines (L)	Per 1000 lines (L)
Liquids	Fresh medium	207	1	207	529,5	26,475	529,5
		105	2	210			
		75	1,5	112,5			
	Fresh medium with ROCK inhibitor	16	1	16	80	4	80
		32	2	64			
	DPBS	160	2	320	320	16	320
	EDTA	80	0,5	40	40	2	40
	PBS	18	2	36	45	2,25	45
		9	1	9			
	DNA Lysis reagent	11	0,5	5,5	2,75	0,1375	2,75
	RNA Lysis reagent	12	0,25	3	0,75	0,0375	0,75
	Freezing medium	5	1	5	5	0,25	0,5
	Enzyme	2	0,5	1	0,5	0,025	0,05
	Germ layer- Endoderm	6	1	6	6	0,3	0,6
	Germ layer- Mesoderm	6	1	6	6	0,3	0,6
	Germ layer- Ectoderm	6	1	6	6	0,3	0,6
In the first phases of reprogramming	ReproRNA Cocktail				207	10,35	20,7
	Growth medium and puromycin				207	10,35	20,7
	ReproTeSR + B18R				207	10,35	20,7

Figure 27a: Data derived from the Process Tree analysis on the amounts of liquid needed

RELEVANT INSIGHTS:
Insights coming from the analysis of the process tree bring information that is relevant to the requirements of the automated system

- The system should be modular in order to be future-proof for changes in the reprogramming method that could be due to the constant discoveries within the field.
- The system doesn't need to be moved often in the facility and therefore its space footprint can be medium sized to big. Its size should allow to be transported by polling the module on wheels.
- The system should be provided of at least two robotic arms.
- After bulk passaging there are no additional decision point, all the wells follow the same paths (although variables are still present).
- The liquid handler used at the bulk passaging step should be of high capacity.

	Consumables	Quantity	**Total quantity per 1000 cell lines**
Pipettes	1 ml pipette	227	227000
	0,5 ml pipette	89	89000
	20 µL pipettor with filtered tip	8	8000
	22 - 25 Gauge needle or pulled glass pipette	8	8000
	2 mL pipette	463	463000
	250 micro liters pipette	24	24000
	500 micro liters pipette	6	6000
Plates/containers	6 well plate	5	5000
	12 well plate	11	11000
	24 well plate	4	4000
	1,5 ml tubes	29	29000
	cryo-vials	5	5000
	50 micro liter tube	1	1000

Figure 27b: Data derived from the Process Tree analysis on the consumables needed

2.9 Automated culture of hIPSC lines

As it has been seen in the fist chapter, automation has brought increase of efficiency in industrialization of commonly used products, however, there are more specific advantages and disadvantages that it can bring to biological processes because of their peculiarity.

2.9.1 Advantages of automation

In-process variation given by slight imprecisions between the execution of the protocols by the handlers (e.g. variation between the pipetting, slight imprecisions in incubation times etc.) is an additional reason for which automation brings to the field an advantage.

Automation can, in fact, perform a pipetting or mixing action consistently though a whole cell culture sub-process, process parameters, settings and timing can be exactly determined, saved and tracked, the employment of automated image acquisition and processing can give the possibility of implementation of adaptive processing based on objective and comprehensible criteria.

Additionally, automation can be adapted for increased throughput and parallelization.

The benefits of automation are also relative to the approach that is used:

- Automation of one step, these machines address the lack of consistency due to manual handling, are able to scale up the processing of larger volumes with a scaled-out approach and are programmed to imitate human actions.
- Integration of several steps in one machine (1st generation)
- Fully automated (2nd generation). By fully automated it is usually intended that, additionally to eliminating the need for manual operations for culturing of cells, all the need for manual transfer of materials from one-unit operation to another are avoided (Moutsatsou, 2019).

2.9.2 Disadvantages of Automation

Not all the laboratories decide to switch to automation but stick to manual labor, there are two examples that can be mentioned (Yandell, 2015):

One example is given by the iPSC core facility of the Harvard Stem Cell Institute that, although having tested the automated cell-picking system AVISO CellCelector (Automated Lav Solutions, have decided to still not adopt the technology: the automatically picked cells, in fact, were showing certain markers more consistently than manually picked cells but they were not differentiating more effectively.

Also the Coriell Institute for Medical Research in Camden, New Jersey, although collaborating with groups that have set up iPSC generation systems they run manual processes in-house. The reason is that in their experience lines made via automated methods are not immune to quality issues and a poorly done automation process could widespread consistent errors.

Cell manufacturing processes are highly laborious and often involve processes that are difficult to scale-up since they rely heavily on the operator's experience and judgment. Also because of this, however, they can include risks of contamination, batch loss, and batch to batch variability (Moutsatsou, 2019).

Already from the start of the process, since the iPSC production relies on the patient or donor cells as starting material for the manufacture, there is a significant batch to batch variation (Heathman et al, 2016).

Cell-based products are required to be produced in accordance with good manufacturing practices by minimizing variability and variation in cell quality. Unlike pharmaceutical processes that regulate a characterized strain and work without problems in repetition, cell and gene therapies require adaptive process strategies in order to succeed despite the variability of the living material (Moutsatsou, 2019).

A high level of constant control over process parameters can help to maintain product quality attributes. For this purpose extensive online process monitoring and integrated control are used and allow process characterization and detection and adaption of process changes (Cierpka et al., 2013, as cited in Moutsatsou, 2019).

However, basic knowledge of links between various parameters and process outcomes is not easy to define and described which makes control reproducibility and repeatability of this kind of bioprocesses challenging.

On the other hand, another difference with the pharmaceutical industry brings out the advantage in the automation of cell and gene therapy industry. In fact, post-processing quality control can be applied for the pharmaceutical industry, while cell and gene therapy industry are more suitable for quality by a design approach that takes more into account the documentation of the process real-time monitoring and control and incorporation of advanced automated systems (Lipsitz et al. 2016).

hIPSC - human induced pluripotent stem cells.

RELEVANT INSIGHTS:

1. Automation can be done for the whole process, parts of the process or only one step.
2. Advantages of automation are given by implementation of objectivity, limitations on variability and documentations
3. The main disadvantages are given by the fact that automation doesn't always reflect in better cell culture, the initiation of a mistake is more easily widestread to several cultures, and variability of biological production, which makes predictions difficult to achieve.

2.10 General description of automated systems

2.10.1 Generation 1 Automated platforms

1st generation of automated systems involve the use of robotic arms and pipetting robots, they are able to substitute human repetitive actions and allow to increase the productivity of the thoughput.

Most of the platforms of this kind are not able to support a process from the starting point until the end, and they have limited scalability.

The platforms Ambr15 and Ambr250 (Sartorius) have expanded the possibilities of such liquid handlers by introducing high-throughput options for cell culture while using single use, disposable bioreactor vessels using an automated liquid handler.

These platforms have been proven very useful for scale down studied and process development.

Generally speaking, the main issues with these platforms are their large footprints, their high costs both in initial investment and servicing and maintenance costs, the fact they often require manufacturer specific consumables, integration of additional equipment for covering the full workflow and performance highly dependand from the operator's programming skills.

The main advantages of these robotic platforms is in the minimization of the process variability and human error in manual actions.

A comparison between the most relevant platforms of this kind is shown in the next page.

2.10.2 Generation 2 Automated platforms

This is a cathegory of platforms still in the phase of development and embeddes all the characteristics of the wish list for automated platformsfor the cell and gene therapy industry.

Generation 2 automated platforms will, in fact:

- Allow for reduced manual handling because of the complete offer of automation on a sequence of operational units instead of only one at a time.
- Enable to go from donor tissue to a polished product ready for distribution.
- Provide continuous process validation and monitoring in order to enable recording and documentation and faster optimization.
- Reduce to the minimum interactions between humans and the full enclosure of the platform eliminating the contamination of the source material. Thanks to this, furthermore, the need for clean rooms would become obsolete and overall manufacturing costs would be, therefore, minimized.
- Be modular although fully integrated and therefore allow for flexibility for further developments in the cell and gene therapy manufacturing
- Could potentially include artificial intelligence and machine learning and therefore be used for process optimization and minimization of challenges (Moutsatsou, 2019).

An analysis of there platforms is provided in the following page.

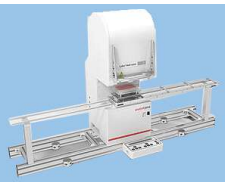
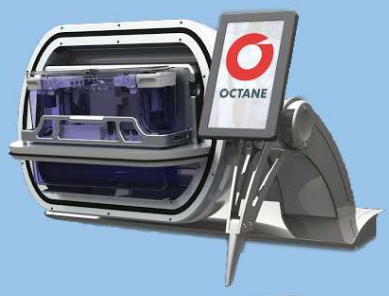
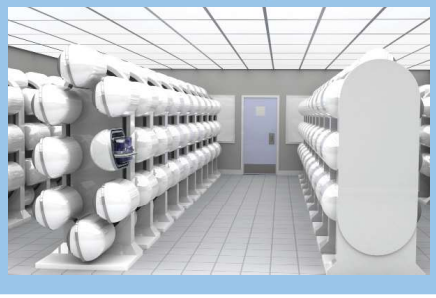
Model and producer	Advantages	Disadvantages
 **Freedom EVO Tecan**	• High precision • Effective speeding up of processing • Liquid detection	• Requires specific cunsumables and training • Requires programming for set up
 **STAR Hamilton**	• High precision at small volumes • Modular design and possibility of expansion	• Requires special consumables • Handles small volumes (< 5 mL) at a time
 **CompacT Select Sartorius**	• for adherent and suspension culture • Ability to process up to 90 flasks (T175) and 384 well plates • Runs subculture, cell counting and harvesting	• Requires additional pieces of equipment • Large footprint
 **Biomek 4000 Beckman Coulter**	• Provides accuracy at handling small volumes	• Requires specific consumables
 **RoboLector M2P labs**	• Includes preparation of media • Allows pH adjustments	• Volumes higher than 950 µL are pipetted in 2 steps
 **Cellmate TAP Biosystems**	• Uses both flasks and roller bottles	• Doesn't have automated harvesting
 **CyBio Analytik Jena**	• Includes full assay and preparation assay plates and measurements, cell seeding and incubator	• Only takes microplates • Requires specific consumables
 **Amb15/ Amb250 Sartorius**	• Proven scale down models • High throughput • Ability to run multiple conditions simultaneously • Suitavle for optimization studies	• Requires specific consumables • Limited agitation speed range

Figure 28: Generation 1 Main automated robots overview

Model and producer	Advantages	Disadvantages
CliniMACS Prodigy system ***Miltenyi Biotech*** 	• Integrates Centricult chamber for washing and culture, boundary layer detection, Cell culture microscope, temperature control system, atmosphere control (CO_2, N_2, Air) • Allows cell activation, transduction, amplification and harvesting • Allows to sampling without loosing sterilization • Modularity and flexible programming • Approved by the European Medicinal Agency for commercial manufacturing processes	• Only applicable to suspension cells and not jet tested on adherent cells • Maximal processed volume of 400 mL • To scale the production it would need multiple models to run simultaneously, the all-in-one architecture would require limiting the dose of the platform to one patient at a time and low efficiency in the time consuming steps of cell expansion
CocoonTM ***Octane Biotech Inc.***   	• Automated cell seeding, expansion, perfusion, difesting/harvesting concentration washing and formulation	• Limited to one typology of cells (CAR-T) and not tested for adherent cell culture • Still in development and not commercially available

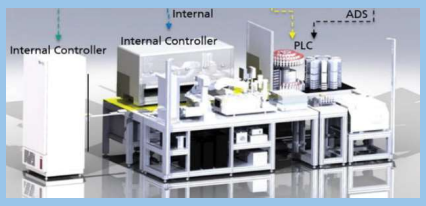
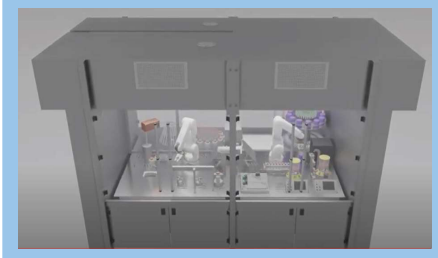
Model and producer	Advantages	Disadvantages
QuantumTM Terumo BCT 	• Embeds a bioreactor with single use cartridges	• Limited to cell expansion • Not adaptable to other bioprocesses
Stem Cell Factory 	• Fully automated production unit for reprogramming, cultivation and differentiation of iPSCs • Can develop 60 different iPSC lines in parallel	• Still in development phase and system composed by different suppliers and not commercially available
AUTOSTEM 	• Fully automated system embedding Bioreactors and bioreactor controllers, a pump module, robot assisted production, cryocontainer loading station, cassette loading station, cell counter, storages for disposals, -80 freezer, vial hatch, storages for disposales filling station, centrifuge, decappers, A to D connectors. All into an enclosed class II cabinet • Versatility of robotic arm grippers that allow for a big variety of culture vessels • Stores LOG files (files recording occurring events) creating quality management focumentation	• Still in development phase • Not commercially available • Big footprint

Figure28: Generation 2 Main automated robots overview

RELEVANT INSIGHT

- Automated platforms can be subdivided in two main cathegories: generation 1 that mostly coincides with the automated liquid handling systems, and generation 2 which is still in development and not commercially available
- Generation 2 systems can be taken as an example for their focus on flexibility, modularity and level of control over the automating process.

Components that make a system for the production of iPSC lines

The previous chapters have described the process of reprogramming of iPS cell lines, they have illustrated the devices that are currently necessary for this process and they have analyzed the offerings of the market. Additionally, informations regarding the advantages and disadvantages of automation have been analyzed making it possible to take into account several factors that allow understanding of the requisites of each component.

The present chapter merges these informations describing the general picture of the components to be integrated into the system for iPSC line reprogramming.

Components

- Liquid handling robot

The pipetting robot should ensure sterility per well and have the possibility of handling the channels separately from each other. The robot should be able to supply five different well plate and tube sizes, with adaptability of the configuration of the pipetting heads or with multible stable heads for each well plate size.
The well plates that should be satisfied are 6x well plates, 12x well plates, 24x well plates , tubes and cryovials.

- Material transport robots

The transport robots are responsable for the movement of the wells through the process and the execution of simple movements such as the movement in “figure of eight” for the distribution of the cells over the surface of the well, or tapping under the tubes in order to suspend cells in the tubes.

- Scanner or well plate tracking system

Each well and vial has to be tracked along the process, therefore a code-scanner or other method of tracking should be integrated. All the steps and their setting applied to the wells can be, in this way, sincronized with the location of the wells and the informations stored into the database of the system.

- Locks for storage

Solvents and consumables should be stored in determined compartments, all separated from each other but easily connected with the pipetting robots. A system of sensors should be integrated in order to show the compartment to refill to the technician.

- Cooling system and Freezers

Some plates are stored in compartments at freezing temperatures in order to serve as a backup until the end of the process. The recollection of these wells and vials should be managed in such a way that contact with the technician is minimized.

- Solvent waste and used disposables area

The process produces a large quantity of waste both in consumables and in solvents used for growth and washing of the IPS cells. The solvent waste should be discarded outside the machine with a connection with vacuum while the consumables should be stored in a closed environment without flow of air.

- Heating system and incubators

The system should be provided with an incubator that can be scheduled and optimizes the used space and cycles of the lines and the time of incubation of the wells.In fact, this parameter is very sensible in certain steps (i.e. during the period of reaction with EDTA) but can be more flexible during the 24 hours of time of incubation

between feeding days.

- Automated media refill system

In order to allow higher walk away time, media refil should be automated and managed so that the refill of the tank coincides with other necessary interaction of the technician.

- Cell counting device

Estimation of the number of cells is, along with other optical properties (explained later in this report), determinant for the decision making steps that dictate the sequence of steps. The integration of a cell counting device, already available on the market, therefore, is required and needs to be integrated with the other components of the machine.

- Microscope and imaging devices

Microscopic analysis of the wells is continuously done by the tecnicians throughout the entire process and at each of the wells. The integration of a microscope automated for the activities of checking the cells morphology, size and density in the wells and for storing relevant informations and pictures for reporting of the process is necessary in order to effectively increase the efficiency of the process and be able to relocate the workforce of the tecnicians to more useful functions.

- Biosafety cabinet

All the processes in which the well plates and tubes are transported without a lid and all the elements that come into contact with the well plate content have to be protected within a biosafety cabinet of safety level 2.

- Well sealers and well peelers

All wells that are added to the system are protected by seeling. Therefore, in order to avoid contamination, devices used for sealing and opening of well plates should be integrated into the system.

- Centrifuge

The final steps of freezing require the use of a centrifuge. This device should be able to store 15 ml tubes and cryovials.

In the Appendix it's possible to see a more detailed descriprion of the characteristics of the technologies available for liquid handling and microscopy while the outcomes of this part of the research has been integrated in the requirement description.

2.12 Existing system prototypes and concepts

As described previously, automation offers big advantages to manufacturing of pharmaceutical and biological material.

Because of this, several research centers have developped through the years automated system prototypes.

This chapter aims to give a general overview of the current analyzed state of the art. Additional non reported systems have been analyzed but are not included into this overview because no insight was comparable to the necessities of the IPS Core Facility at Erasmus MC.

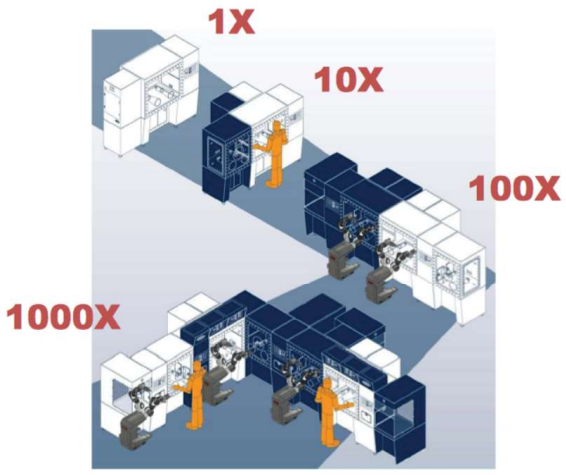


Figure 29: Man-mimetic arms learning movement for inductry

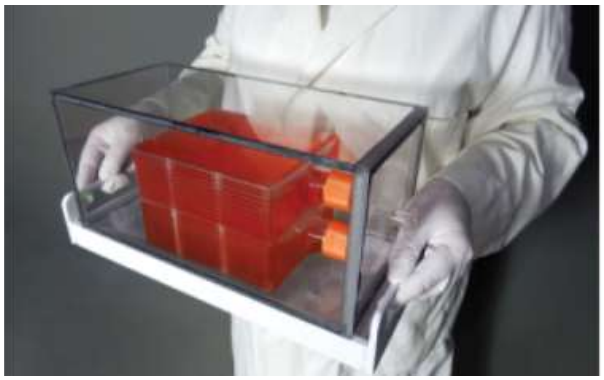


Figure 30: Concept of the asseptic transport between steps

2.12.1 Xvivo System model X2

The Xvivo system focuses on the process of automated by scaling up only the phase of incubation. This is the step that is recognized as the most rate limiting. The application is not clearly specified and the project seems to be only developped in a conceptual manner.

However, there are several sources of inspiration:

Man-mimetic industrial arms

The proposal is to have a transition to automation with the help of robotic arms that learn directly by observation of the human movements. In this way, in fact, the system would addapt directly yo the manual processes without the need for reassessment of the methodology.

Modules are stacked together and technicians are responsible for aseptic transportation between different compartments with methodology as in Figure 30. In this way, efficiency of scaling and flexibility could be achieved without any limit.

For the future, the researchers behind this concept envision distributed manufacturing of the same process in different locations around the world and with common central control.

2.12.2 The Tissue Factory

This system has been designed initially for manufacturing multilayered skeletal muscle myoblast sheets but it has been tested also with hIPSCs, although only for validation and not for the entire process of iPSC reprogramming.

The project focuses on the topic of modularity of the system but since it's application is not based originally on iPSC production the components of the system can not be taken as a starting point for the system.

The project, as in the previous case, can

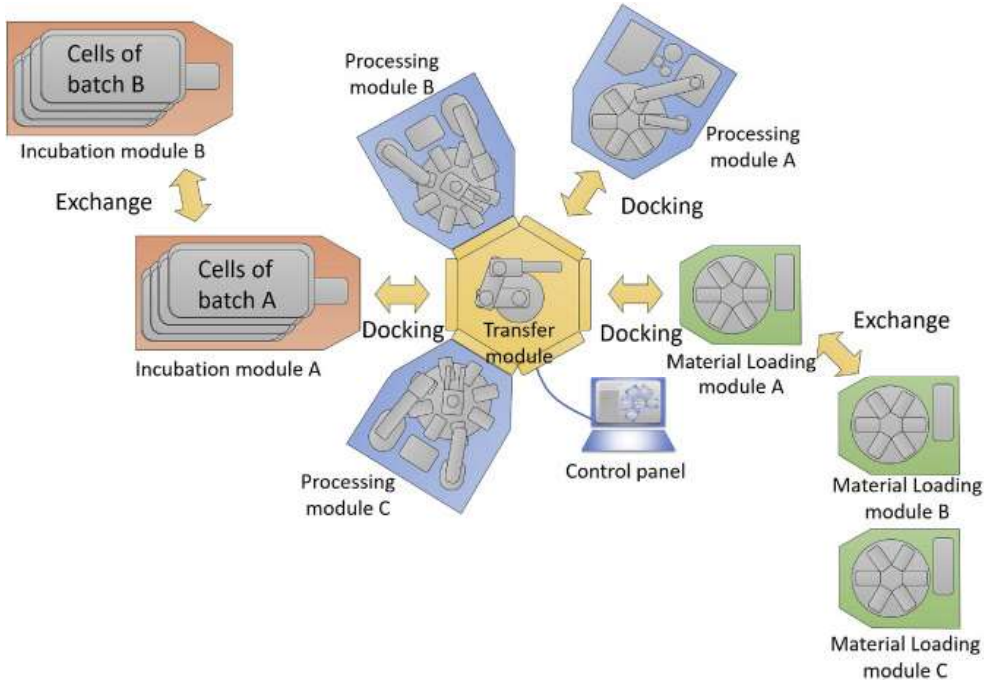


Figure 31: Modular design of the Tissue Factory

serve as a source of inspiration for the future phases of desigh:

The Tissue Factory is composed of a total of nine modules and a manual operation isolator, Figure 31. Each module conducts a relatively simple function but combining multiple modules via the Transfer Module allows to apply the system to a wide variety of cell culture processes. What this design possible is the standard interfaces used between each module.

Modules of the Tissue Factory are:

- Transfer module

- Cell processing module, contains a pipetting unit, enzymatic digestion unit, tissue shredding, centrifugal separation

- Seeding and Medium Change Module: Dispencing unit injecting element and aspiration unit

- Gelatin Gel Preparation Module: heating and steering unit, pipetting unit and cooling unit

- Cell Sheet Stacking Module

- Material Loading Module

- Robotic Incubation Module: Incubates up to 60 cell culture vessels

- Incubator Module: An incubator equipped with a phase contrast microscope which allows for the observation while culturing cells.

- Large Scale Culture Module: This module is a specially prepared incubator for culturing large amounts of cells at the order of 108 to 109 cells. Differently then from other incubation modules, this module is not connected to Transfer Module, but cells are delivered as a cell suspension via tubing and bags.

- Material Preparation Isolator: Used for preparing materials by hand before starting automatic processes.

RELEVANT INSIGHT

- From the comparison with already existent systems for biology production the Automated Cell Culture platform of the CEN Group at the University of Luxemburg has been identified as the system that comes the closest to the needs of the procedure followed by Erasmus MC

2.12.3 Autostem

A project started in 2017 and still in development the Autostem is designed for efficiency of the production of large numbers of therapeutic cells.

The system can be inspirational for the analysis of the method used for storing for diposables, which can be seen in Figure 33. The process for which the system is used, however, is completely differently then in the iPSC reprogramming methods of the Erasmus MC.

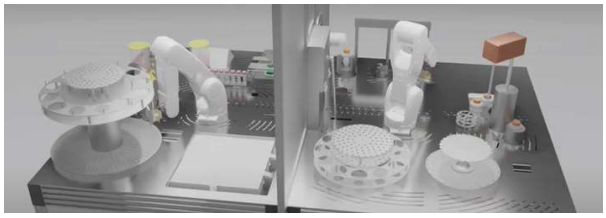


Figure 32: Devices used by the Autostem

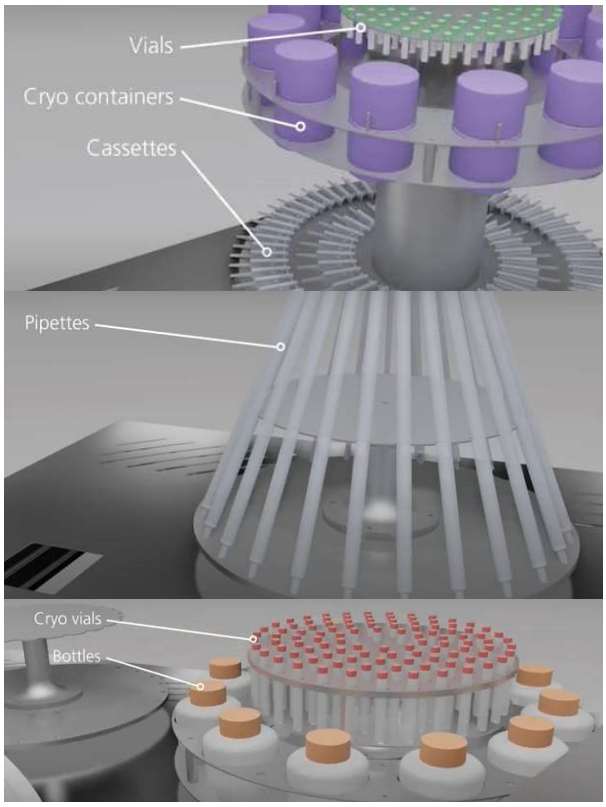


Figure 33: Devices used by the Autostem

2.12.4 Ai Cellhost

The Ai Cellhost is a system that has been designed espetially for two processes of iPSC cultivation: feeding and differentiation. Because of this reason, it can not be taken as an example for devices to be used by the system.

In the Ai Cellhost, generation of iPSCs was performed offline by nucleofection followed by selection of cells using a Miltenyi MultiMACS24 Separator (Figure 34). This device has already been used at the IPS Core facility at the Erasmus MC but it doesn't offer big advantages for cell picking because its results are not always consistant.

Furthermore, there are also other differences between this process and the process used at Erasmus MC:

- it makes use of separation filters that should not be used at Erasmus MC

- Cells are triturated threough the processes while this is not the methodology adopter at Eramus MC.

Figure 34: a Miltenyi MultiMACS24 Separator

2.12.5 Pellican - System for hIPSCs for deriving neuron cells

The Pellican system is applied for culturing processes on hIPSCs and is therefore more applicable to comparisons with the possible platform to be designed for Erasmus MC. This platform, however, can not be used as a basis of selecting components of the system

for the Erasmus MC facility because of four main reasons:

- It uses microfluidic technologies, which are adopted in order to increase the number of cell lines produced, and not the number of well plates produced, as in the case of the Erasmus MC Facility.
- It is focused on long term cell culture maintenance,
- It integrates an Organ-on-a-chip technology and design, which is an advanced technology applied to big population studies that can be considered for a future horizon but not necessary for the scope of the project.
- The used imaging system is semiautomatic.

This device configuration can be taken as an example for the use of camera tracking of different wells within the system.

2.12.6 Stem Cell Factory

The Stem Cell Factory (Figure 35) is one of the fully automated systems that integrate all the functionalities necessary also at Erasmus MC.

Its application and culture material also follow the same requirements (based on the use of 6, 12, and 24 well plates and using hIPSCs), however the throughput of the system seems to be higher then the one needed at Erasmus MC.

Therefore, only selected items of the Stem Cell Factory System have been taken into account:

- the ASL CellCelector, automated platform for the monitoring and picking of cells.
- The spectometer FLUO Star Omega for turbidity check.

2.12.7 Automated Cell Culture platform from the CEN group at the University of Luxemburg (2017)

From one of the conversations with the automated systems provider Beckman Coulter, it has been identified that the system developped at the Luxemburg University could be comparable to the needs and throughput of the IPS Core Facility at Erasmus MC.

The system, in fact, uses the same typology of labware, similar process, and similar typology of research that the hIPSC lines are used for later (between which differentiation of the cells into neurons for performing chemical or viral-vector-based genetic screenings).

The list of devices used in this system has been reviewed again and changes were done to it in order to come to a final components list (described in Chapter 04).

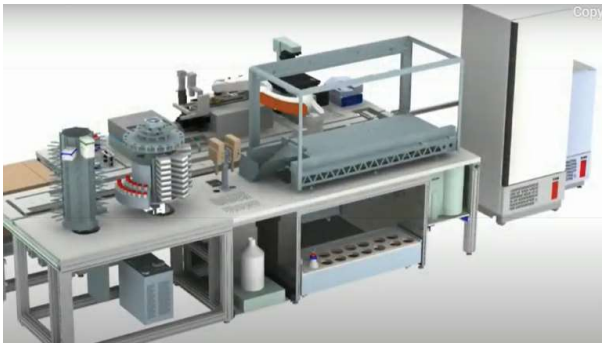


Figure 35: Graphical reppresentation of the Stem Cell Factory System

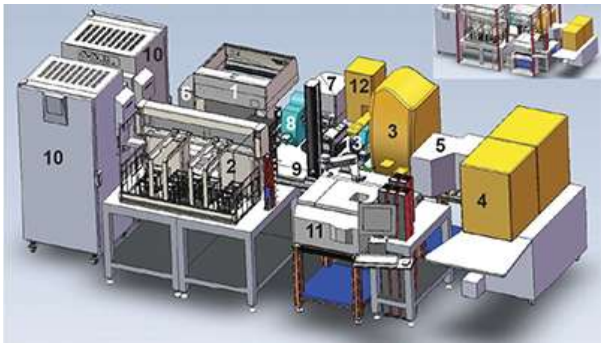


Figure 36: View of the components of the Automated Cell Culture platform used at the University of Luxemburg.

2.13 System software

The variability and dynamicity of the process used in adaptive cell processing influences the software necessities required for automated production platforms.

The challenges presented to the control softwares are relative to the ability of managing non-deterministic processes and the prevalent heterogeneity of device interfaces. These are the challenges that have been addressed by Sven Jung et al. in a service oriented approach to meet the demand for flexibility but giving control over data and devices.

In order to achieve the goal, hardware modules are integrated via agents into the control software with a “plug-and-produce” approach that allows to consider dynamic condition.

The characteristics of such a system (that has been developed in the course of the StemCellFactory, StemCellDiscovery and Autostem projects) are as follow:

- It uses the paradigm of Dynamically Interconnected Assembly Systems (DIAS), it decouples stations from each other considering them as individual units.
- It considers different products and variants in parallel on the same facility by generic and self contained interfaces.
- It uses conversion layers mediating between execution system and hardware, these layers have the task of providing a uniform way of communicating internally and hide the complexity of underlying systems.
- The central control software moderates the communication and the execution of processes.

The central control software is split into three modes: the manual mode allows running individual processes in order to test the single process step and allow maintenance; the automatic mode is used for efficient large-scale manufacturing processes, it autonomously runs sequences of services and coordinates their parallel execution; the virtual model is responsible for the management and recording of data types for devices, services, parameters, logics and materials. The virtual model allows parametrization and the build up of a virtual representation of the system from which the individual software modules can access needed data and form the basis for the modular architecture.

During the process of analysis of possibilities on the market two producers of automated systems have been contacted with the purpose of evaluating the differences between the softwares they offer. Both companies are also known producers of liquid handling systems, Beckman Coulter and Hamilton Company. The minutes coming from the interviews with these two companies can be found in Appendix XIII-XIV.

As a result of the confrontation it was possible to see that both companies use the three layered organization described by Sven Jung et al.

An additional interview has been conducted with a company providing a production planning and scheduling software, Access Orchestrate, minutes from this interview can be found in Appendix XV. Their service is directed to different industries between which also pharmaceutical ones.

The main outcome from this meeting has been that although Orchestrate is a very useful tool for understanding product capacities, due to its possibility of receiving input data only from a company ERP and not from devices it is not suitable for applications of automated system management.

Figure 37: The structure of the software used by automating companies such as Beckman & Couter

RELEVANT INSIGHT

- Perhalps the adoption of a software developped by liquid handling providers is going to have the characterictics needed for automated cell production.
- In fact, softwares used within the application of biological system automation are all trying to solve the same problems relative to the ability of managing non-deterministic processes and the prevalent heterogeneity of device interfaces
- Softwares able to achieve this goal decouple stations from each other considering them as individual units, consider different products in parallel on the same facility by generic and self contained interfaces and uses conversion layers mediating between execution system and hardware

Chapter 3

Requirements

This chapter provides an overview of the requirements of the automated system merging automation possibilities with the necessities of the culture procedure followed from Erasmus MC.

On these requirements the following chapter will diverge and explore solution possibilities. An overview of the interconnection between the requirements and the diverging phase is also provided in the second section of this chapter..

In this chapter

List of requirements

The Pugh's checklist of product requirements (Pugh,1990) is used for summarising the product characteristics.

This requirement list method defines the requirements step by step considering the product from different point of views: general requirements coming from the brief, its performance, environmental factors influencing the project and so on.

The level of definition at the conceptual stage is not equal for all the cathegories considered. This overview focuses mostly on product performance.

General brief requirements

R1 The product reduces the amount of actions done by the tecnician in order to reprogram and do required tests with the iPSC colonies

R2 The product reduces the amount of monitoring on the cells done by the tecnicians

R3 The product makes the process of cultivation of iPSC colonies independant from the precision of tecnicians

R4 The product is modular and allows the connection to different machines for flexibility of the methodology of programming

R5 Wish: The system should be modular with the possibility of using each module for different simultaneous functions done in the lab.

R6 The product takes advantage of existing platforms by connecting them physically

Performance

R7 The system allows to reach 1000 cell lines in throughput per year.

R8 The system is suitable for the culturing of iPSC cells in 2, 12, 24 well plates.

R9 The product integrates a pipetting robot, a microscope, a discarding section, an incubator, a cell calculation device, a freezer and cultureware storing section

R10 The product should initially not take too much space in the lab in order to allow tecnicians to work on the research, have access points to the device and continue with the steps of the process done manually

R11 The product should be provided by multi-channel pipets for the steps that don't require specific handling of the wells (such as feeding)

R12 The product should also be able to handle the channels of the pipettes individually and treat wells independently from each other in the same well plate (for enabling RNA and DNA isolation to happen in the same well plate)

R13 The product should record a detailed log and mapping files of processing steps with video recording

R14 Consumable and reagent consumption should be tracked in a database.

R15 The system should allow flexibility in throughput

Enviroment

R16 The product should mantain the internal environmental conditions constant and not be influenced by different levels of humidity/ temperature/exposition in the location where it is placed

R17 The product should allow to have a safe sample - without crosscontamination by preventing airflow movements

R18 The product should protect from contamination from the external environment by having a fully enclosed systems with positive pressure and filtering, laminar flow, HEPA filtration, positive air pressure and ultraviolet lamps

Life in service

R19 The product (robotic system) should be considered to be used for a period of time of 10 years at least

Maintenance

R20 The product modularity allows for easy repair and disinfection from eventual external contaminations

R21 All the surfaces can be cleaned on a regular basis by internal systems or by the access given to an operator

R22 It is easy to understand where there is a malfunction of the product, the module is directly closed off and the tecnician is instructed on how to procede

Target product cost

R23 The product development and costs are within the fundings of 1 million euro for the development of the robotic system (see interview notes with the facility manager)

Transport

R24 The tecnician can place the robot modules in different areas of the laboratory at the current stage.

Packaging

R25 The product should be protected from scretches or breakage during transportation of the device

Quantities

R26 The product is one of a kind and satisfies the specific needs of the iPS Facility at Erasmus MC, it should be developed as an open source project to contribute to projects of other facilities as well

Production facilities

R27 The system should be composed of off the shelf components with little adjustments

Size and weight

R28 The size and weight of the single modules allows technicians to move the modules by pulling it over the ground.

R29 The system can fit in a room of 26 square meters.

Appearence

R30 The product conveys a feeling of professionality

R31 The product conveys a feeling of trust and control by the technicians that use it

Materials

R32 The materials inside and outside the system allow cleaning with 70% alcohol

Ergonomics

R33 The comunication of states of the cell culture in progress is clear and avoids biases introduced by the control problem relative to automation.

R34 The system layout facilitates the workflow for the technicians.

Storage

R35 The system modules can be stored in different rooms of the facility

Societal and political implications

R36 The system can be trusted by the technician and the researchers that make use of it.

R37 The system preserves the privacy of the patients for whom the cell lines are developped.

Nr	Design section	Solution
	General brief requirements	
R0	Analysis of devices used in similar systems (Chapter 2.12)	Adoption of devices used in a system that handles production of IPSCs
R1	Choice of activities done by the technitian during discussions with the Head of Erasmus MC iPS Core facility	Choice of automating imaging check ups and only leave the activities related to doubt points to the technicians
R2	Choice of components	The technitian is involved only in the steps that are essential
R3	Choice of components, discussion with Beckman Coulter	The technitian is involved only in the steps that are essential and easy to handle. The digital platform allows to have peer review and comunication
R4	Considerations relative to modularity (Chapter 4.3.1)	Adoption of three separate modules
R5	Considerations relative to modularity (Chapter 4.3.1)	Adoption of three separate modules
R6	Choice of components	Chosen components are as much as possible mantained as they are of the shelf
	Performance	
R7	Choice of components (Chapter 4.4)	Adoption of Biomek i7 liquid handlers and Cytomat 10C incubators
R8	Choice of components (Chapter 4.4)	Adoption of Biomek i7 and Biomek i5 liquid handlers with Span-8 Pipetting head
R9	Considerations on modularity (Chapter 4.3.1)	Final concept design description
R10	Considerations on modularity (Chapter 4.3.1)	Adoption on a three modular design
R11	Choice of components (Chapter 4.4)	Adoption of a Multichannel pipetting head
R12	Choice of components (Chapter 4.4)	Adoption of a Span-8 Pipetting head
R13	Discussion with Beckman Coulter and Hamilton Company	Use of recording system currently present at the facility and integrated with plate and tube etichette printer. All chosen devices have imaging cameras embedded that allow the tracking of well plates.
R15	Considertions on throughput (4.3.1)	Adoption of medium sized devices (Final design description)
	Enviroment	
R16	Analysis of existing systems (Chapter 2.12)	Adoption of a safety cabinet enclosure containing all devices involved in the process
R17	Analysis of existing systems (Chapter 2.12)	Adoption of a safety cabinet enclosure containing all devices involved in the process
R18	Analysis of existing systems (Chapter 2.12)	Adoption of a safety cabinet enclosure containing all devices involved in the process
	Target product cost	
R23	Choice of components and contact with manufactureres	The product goes over the initially envision budget limitation.
	Production facilities	
R27	Choice of components (Chapter 4.4)	Choice of components and transportation devices is based mainly on their already integrated elements
R29	Ideation session on possible configurations	The final concept modules have a total footprint of 18,72 m2
	Appearence	
R30	Definition of the final concept	Personal proposal to be further tested
	Ergonomics	
R33	Envisioning product machine interactions during ideation sessions (Chapter 05)	Integration of certain interactions coming from the ideation session and Implementation of a routine control practice (Final design description)
R34	Ideation session on possible configurations (Chapter 05)	Modules organized in parallel disposition
	Storage	
R35	Measurements of the dimentions of the current Facility	Adoption of three distinct modules with overall longitudinal shapes
	Societal and political implications	
R36	Envisioning product machine interactions during ideation sessions (Chapter 05)	Horrizontal placement of devices and their visibility
R37	Discussion with Head of Erasmus MC iPS Core facility	Informations on patients are not accessible to the IPS Core facility

Methodologies used to satisfy the identified requirements

Figure 39 describes the methodologies used during the next phases of the design process to tackle the elements of the list of requirements.

As it can be noted not all the requirements are reppresented in this figure, in fact, out of the 37 requirements during this projec 27 requirements were successfully tackled.

More detail on what should be done in the future to match also the remaining requirements is described in the last chapter of this report.

Figure 39: Description on where to find the additional research used to tackle each requirement and the final solution adopted to satisfy it.

Chapter 4

Design

This chapter describes the main steps taken after the initial research phase in order to define the design of the system. It serves as a space for further research, reflection, and analysis and it gives further knowledge that is going to be used for the conceptualization (Chapter 5).

The topics analysed regard three main levels of detailing that all have consequences on the design.

Firstly a look into the future will be given by summarizing technology trends and placing the project within them.

Later on further background knowledge is given on modularity, throughput and configuration, functional choiches that have great impact on the system design layout.

To conclude this chapter a selection of the devices to be integrated into the system is made.

In this chapter:

4.1 Approach

A system is “a set of interrelated components functioning together toward some common objective(s) or pupose(s).” (Blanchard and Fabrycky, 2011, p.17)

Usually, the development of systems is done in teams in order to manage the necessity of dealing with several components that make the system. Starting from an identified issue the development process deals with increasingly more yet smaller details. Therefore, the initial iteration has to be more conceptual and overarching while subsequent definitions detail more and more smaller issues to solve, the problems to solve become smaller and the general picture more and more complex.

System design engineering wants to find a solution to an identified issue while considering its context, stakeholders, and the rest of the world. This means that at different levels of detail there is the need to define an issue, find solutions, and accept them before proceeding with the further step.

The scope of the present project is that of identifying the initial concept design for the automated iPSC System to be adopted at the Erasmus MC after which, as it has been described in the Roadmap in the introduction, further readjustments will have to take place.

For the definition of the concept two main levels of focus have been identified:

Context level:

Possible future developments of the system are taken into account in order to analyse additional possibilities given by technological developments.
In order to do so, further research is conduced on the future trends within the field of laboratory automation and cell production. This method facilitates a future driven development of the project.

Macro level:
This level concerns the modularity, throughput and configurations of the device. All three aspects influence the overall outcomes of the design in terms of the possibilities of development

Micro level:
This level regards the devices that are embedded into the system.

In order to avoid getting lost in the product offers on the market one of the systems analysed in Chapter 2.12 , following as closely as possible the needs of the iPS Core Facility, is taken as a basis for the devices selection. Readjustment and feedback has defined the final components selection.

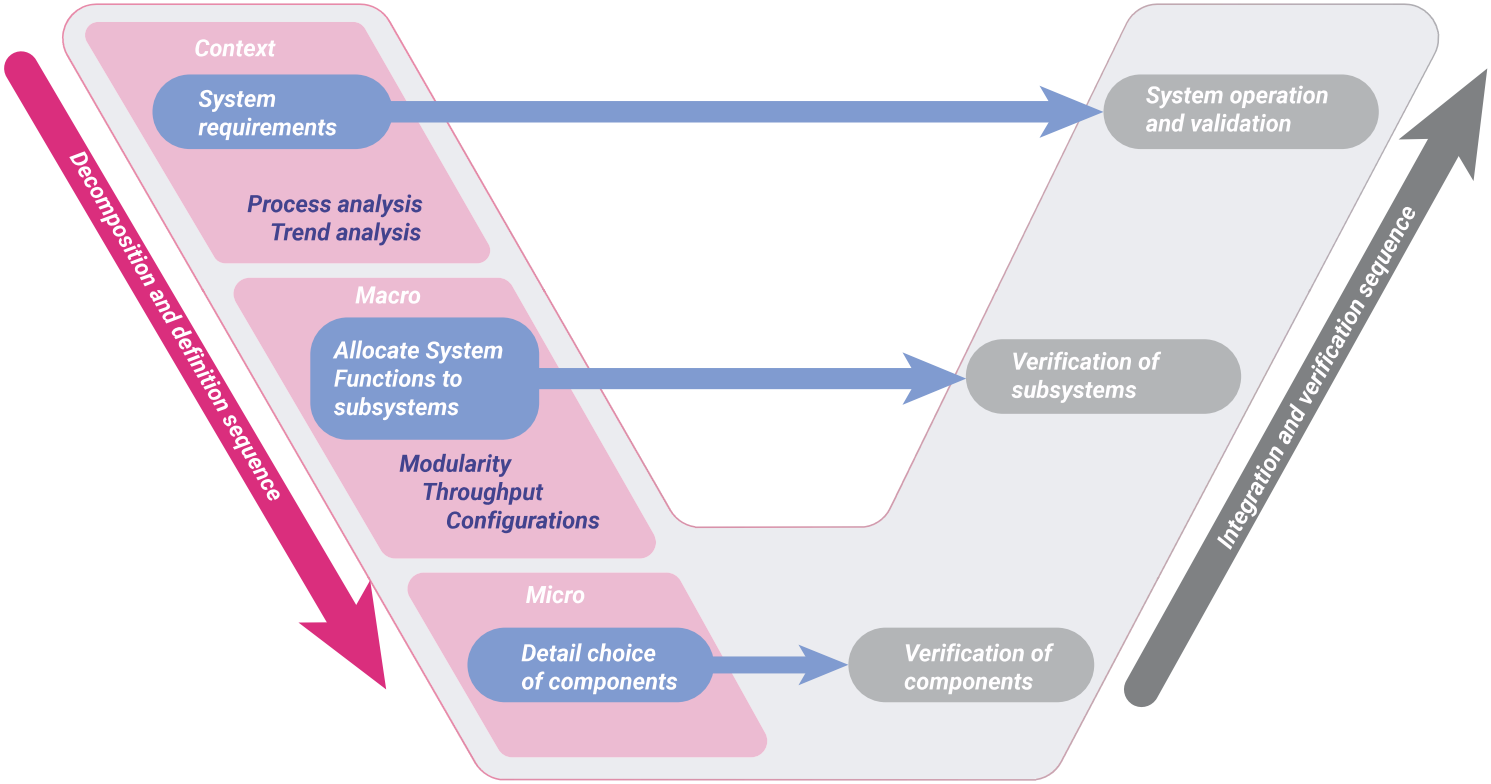


Figure 40: The process followed can be illustrated by the coloured section of the V Model, readapted here from the original one used by Bonnema (2008), a graphical representation of a system development lifecycle.
The left side indicates steps for the development of the system.
The right side indicates the steps that lead to verification, this side is out of the scope of this project.

4.2 Trends in the field and three horizons

The automated system has to be enclosed in a biosafety cabinet for avoidance of contamination of the cells. The enclosure, however, is not a very flexible structure and can not grow if the needs of the customer change.

Because of this, it is important, from the start, to have a clear picture of what are objectives also for the future in order to both make aims for future investments and requests of fundings and recognise where is necessary to make space for adaptation in the future. The current chapter aims at figuring out the possible innovations within the automated systems and the current innovation trends influencing the sector.

Innovation should not be linked to the present, but have a broad view going beyond the current perspectives, to reach this goal the current analysis has followed the Three Horizons Methodology (Curry and Hodgson, 2008). This methodology envisages different future possibilities and, in the connection between the current situation and the hoped one, it tries to reveal the possible breaks that might cause divergences from the desirable situation.

The methods starts by defining the status quo (Horizon 1), which is the actual, main, leading structure and the reasons behind a possible development and change in the future.

Horizon 2 is the step taken as a reachable innovation, but it is not derived by a consequential development of Horizon 1, but in fact it is derived by first evaluating Horizon 3, which is the ideal system to be desired, where innovation is no more seen as such because of the change acting also within the overall external environment in which the system is set and the normalization of certain technologies.

Horizon 2 is defined as a first step and prototype of the final desired outcome, a meeting point between the innovation as it is seen today and the innovation of the future.

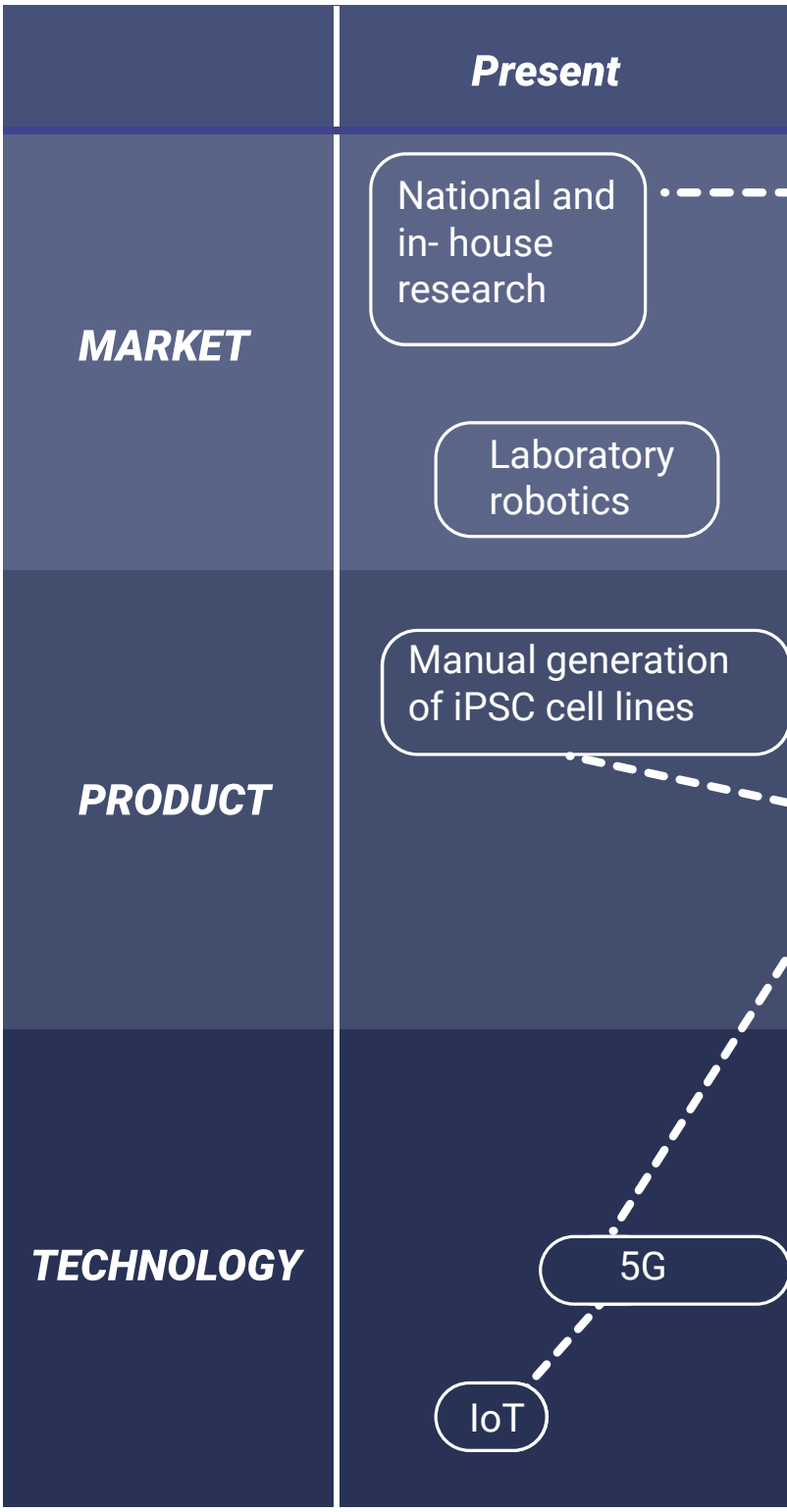
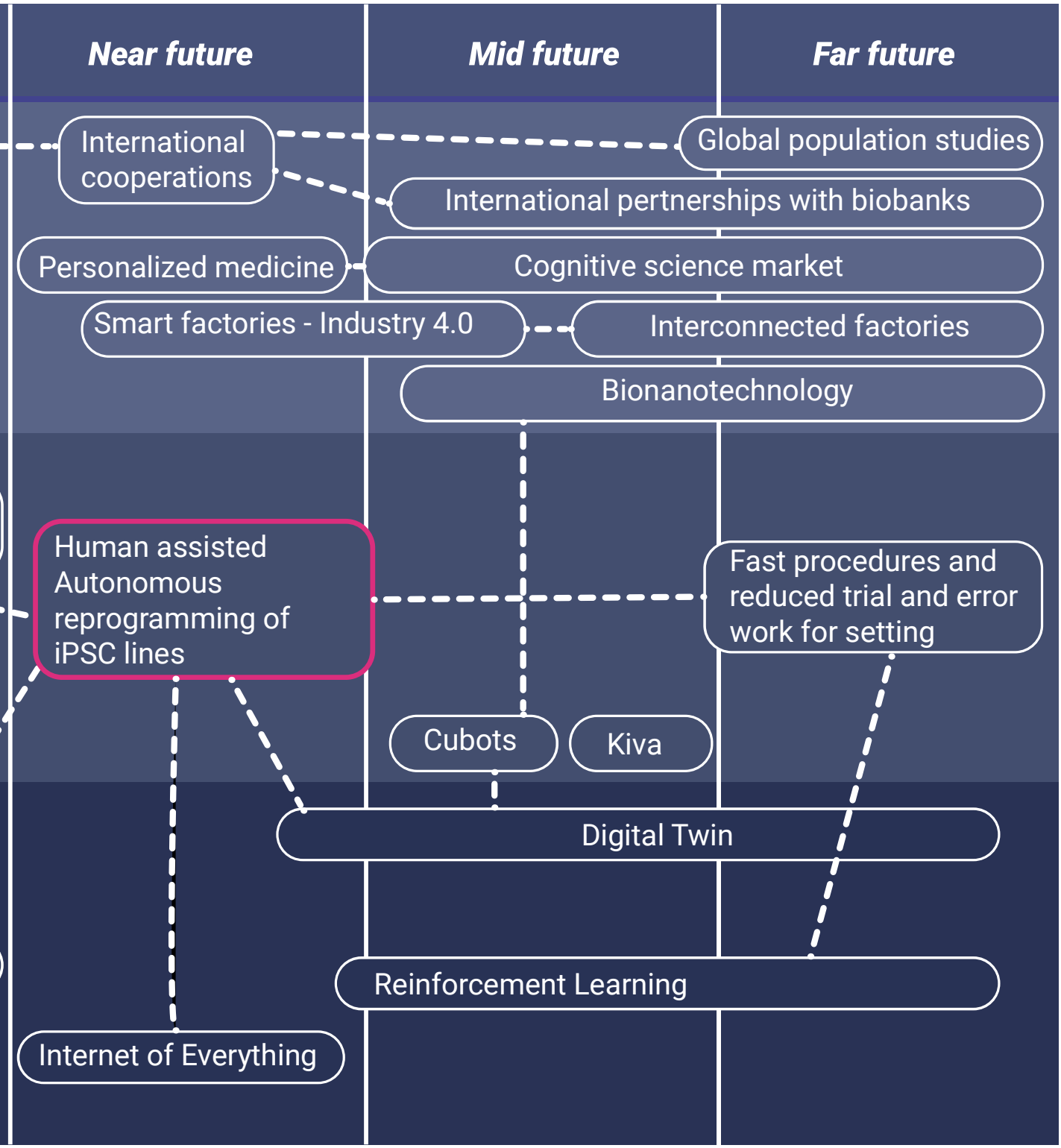


Figure 41: Horizons map



Definition of Horizon 1 in automated systems

The transition towards automation from manual processes is not a new topic: the bioprocessing industry has been addressing it already since thirty years and the first automated robotic arms were invented in the early 1980s by a group from the Kochi Medical School.

It is possible to say that the current status quo of a cell developing facility is that of transitioning the manual process into mechanical and automatable steps.

The technologies used nowadays and the products developed in the biomedical research industry and as the laboratory robotics using single devices have been deepened in the previous chapter.

Initially in fact automated cell culture systems were tested to legitimize their proficiency in reproducing actions primarily performed by human operators.

Since the outcome of these initial studies was successful and since there has been a rapid progress in the iPSC field, the institution large-scale archives of patient-specific iPSC lines derived both from disease-affected individuals and from healthy ones has been stimulated.

Nowadays the main aim of these banks is to grant the researchers with a sufficient resource of cells to support studies on various diseases, including diabetes, cardiovascular and neurodegenerative conditions.

These are the main exiting and developing biobanks (McKernan, 2013):

Large projects in the whole world aim at the generation of large numbers of iPSC lines into biobanks such as the The New York Stem Cell Foundation (NYSCF) aiming at 2500, the California Institute for Regenerative Medicine (CIRM) at 9000, the European Bank for induced pluripotent Stem Cells (EBiSC) at 10,000

Automation of the entire process is key in most of these programs, and there are others such as the StemCellFactory, and I-Stem which to the production join further specializations, the first one focuses on the following characterization and separation between neural and cardiac derivatives, the second on the screening of compounds with therapeutic potential and subsequent disease modeling using the CompacT SelecT platform. AUTOSTEM Consortium wants to further expand the automated platform developed for the StemCellFactory project and utilize it for the production and banking of therapeutic stromal cells. Another relevant project is that of the University of Melbourne, with focuses on patient-specific iPSCs and divides cells for ophthalmic research using a Tecan Freedom EVO platform (Schenk, 2013).

Summarizing it is possible to say that in Horizon 1 cell production is regulated and developed at a National scale and as a in-house research, mainly driven by laboratory robotics. It is characterized by a manual generation of iPSC cell lines but with the focus on automation and categorization in order to develop personalized medicine, specific to certain diseases or patient groups.

Trends within the Market

In order to foresee possible future market and technological shifts and to define the Horizon 3 and thus determine what are the aims to set in Horizon 2, it's necessary to analyze the coming trends within the various field involved: Atomization techniques, Machine learning techniques, Connectivity, robotic typology and variety, digitalization.

The urge to respond to specific needs is leading to increased international cooperation between researchers, which manifests not only with the exchange of ideas and know hows but also with the pooling of resources, intellectual and

material.

Biobanks are becoming shades, interconnected and interchanging, requiring the increase of productions, connectivity and precision.

Technology trends

Reinforcement Learning

Innovation in technologies include the use of Reinforcement Learning in cognitive science in robotics, which is currently happening with a gradual and powerful first wave (Botvinick et al., 2019).

Reinforcement learning (RL) is a set of methods for learning from rewards and punishments rather than from more explicit instructions. Instead of giving a set of rules the machine has to follow in the situations it operates, it tries to create a behavioral policy able to translate the analysis of situations into consequent logic movement by maximizing cumulative long term reward given by experience. For complex settings it has to be developed in multilayer neural networks, called “deep”.

This technology applied to life sciences promises the reduction of time wasted in trial and error happening in a chemical lab by giving supporting the work of research technicians (2020 Trends in robotics, AI, and healthcare innovation). In fact, developing methods such as episodic deep RL and Meta-RL are going to be able to increase the sample efficiency of deep RL and therefore mitigate the original demands of huge amounts of training data.

5G Network

Operations within Bio-nanotechnology are facilitated by the developing and rising introduction of 5G: this technology provides connectivity at higher data rates and at shorter distances and allows network slicing. The use of 5G could provide to computing and AI needed in the labs with the devotion of an independent part of the network for that specific service. Furthermore, also the extreme ultraviolet lithography, technology used in labs, is going to gain more use in this better connectivity (Gazit et al., 2013).

Cobots

Cobots are another coming trend, although being slower than traditional system robots they have the advantage of not needing highly skilled engineers and expensive safety equipment to operate in an integrated system with humans, giving the possibility to these facilities to become more common and versatile.

Autonomous Mobile Robots (AMR) are expected to expand in use in industrial automation improving logistics, as it can be seen by the example of Amazon introducing new robots such as Kiva.

Digitalization and automation in management of laboratory practices

Experimental records are documented in a diverse way in all labs.

Electronic laboratory tools have definitive advantages over traditional notebooks, but often find reluctance from scientists because of the costs of implementation, the activation energy required to change work habits and the number of options to choose from.

Professors and accademics use management systems such as Labguru to achieve the goal of having digital databases of resources. This system is also able to streamline ordering by putting product vendors and current orders in one place, easing research of items.

Development and implementation of informatics have also encouraged electronic documentation.

Systems can offer streamlining of process management, secure and permanent storing of data while rapidly retrieving needed information. An ideal system would even find “dark data”—previous work that could answer

current research questions but is buried in disorganized files.

Also on the side of laboratory softwares it's possible to recognise the trend of globalization and multisite collaboration. In this horizon softwares will be able to integrate multisite collaborative projects and standardize heterogeneous data. An example of software that is moving in this direction is the LabKey Software. In the close future vision, there will be no need for manual entry in the system but scientists would simply do their work while an automated tracking system simultaneously keeps records.

Virtualization

In life science automation laboratories, virtualization systems have played an important innovative role since the last decade: as further explained in "Virtualization System for Life Science Automation Laboratory" by Yanfei Li, developed at the university of Rostock. Already in 2014 there was the emerging trend of creating real time virtualization using Four-dimensional (4D) technology, uniting time information to the traditionally static 3D model.

Integration of time related informations, in fact, allows virtualization to happen in real-time through the combination of three components: dynamic model simulation, virtual reality simulation for 3D graphics, and an interface to the controller and input hardware devices.

This foreseen trend has not appeared yet, but since VR technology is increasingly used for personnel training and has become more user friendly, 4D Virtualization could finally become an option for interfaces between operators and the life science automation systems in the near future. This would put in relation the process of automation and the computation of real time data, bringing advantages of a more coherent planning and control of the system.

The amount of elements described until now aimes of giving an idea of the complexity of the considerations to take into account. Even though probably partial, the analysis of the innovations foreseen or predicted leads to the definition of the current innovation found at Horizon 2, on which this project is focusing and which will be further discussed in the system requirements chapter.

RELEVANT INSIGHT

- Three horizons can be visualized by the analysis of trends, shifts in the market and product typologies:
  Horizon 1 - the present
  Charactrized by a manual generation of iPSC cell lines with the focus on automation and categorization in order to develop personalized medicine. Development is still based on a national level.
  Horizon 2 - the near future
  Characterized by virtualization, with integration of time related informations Development of 5G networks and improvement in documentation.
  Horizon 3 - the further future
  Characterized by reinforcement learning that promises the reduction of time wasted in trial and error happening in a chemical lab, new technologies in robotic automation such as completely independet robotic collaborative platforms and the possibility of rethriving quickly necessary data. In this scenario collaborations have reached a global level.
- Within these three Horizons the current project of automation can be placed in the second horizon.

4.3 Modularity

One of the requirements of the system, coming from the analysis of the process of RNA reprogramming and from the knowledge on the other processes carried out at the iPS Facility, is that the system has to be modular, this defines other sub requirements depending on how this concept is understood.

What is a module

The Cambridge Dictionary reports several definition for the word module: it can be both "one of a set of separate parts that, when combined form a complete whole and that can operate independently one of the others when separate", and "one of a set of separate parts that can be joined together to form a larger object".

In the first definition of modularity puts the stress on the segmentation of the process. This gives the advantage of being able to apply one module of the system to different processes or upscaling one specific module in comparison to the others.

Within the second definition of the word, attention is put more on the possibility modularity gives to use the same module in multiple configurations, enabling a large variety of designs without using many component types. This highlights the importance of having a standard interface between modules and gives advantages especially when the scale and the scope of the project are large.

Modular design

Modular design comprehends both definitions in the design approach that first subdivides a system into smaller parts (the modules) and then coordinates the modules in different configurations. Modules have to be scalable and reusable, they have to have well-defined modular interfaces and make use of industry standards for interfaces.

Advantages of modularity

Modular design allows customization and easier upgrading, repair and reuse in contrast with the need of replacing an entire unit. Because of this it is often used in a culture of do-it-yourself manufacturing.

Disadvantages of modularity

Non-module design has often commercial advantages for the manufacturer as it pushes people to replace units that can not be easily upgraded.

Historically, non-module design has been preferred because of the complex interfaces. For example, initial concepts of personal computers were modular in order to be able to upgrade memory, however, in addition to the economical advantages of the resulting product obsolescence, the interfaces never worked well and required technical skills (Spacey, 2016).

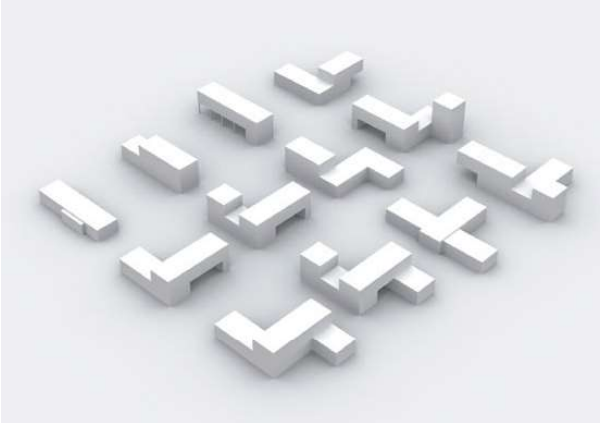


Figure 42: Modular architecture design http://www.archicentral.com

The use of modularity can be applied to the iPSC process in two ways:

Modularity of the entire process
If the production throughput increases the capacity of the system can grow proportionately with several modules each of them able to perform all the steps and phases of the process.

In this case, each module would result of the combination of the devices needed to perform all the functions within the process. The devices within the system would need to have low capacity requirements and be organized in such a way that their stacking is efficient once several modules are combined between each other for higher throughput.

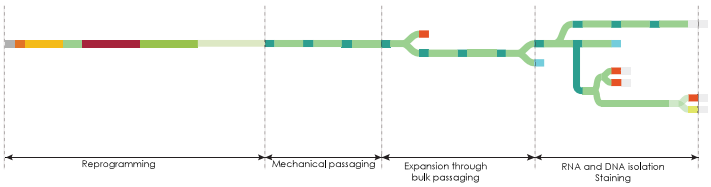


Figure 43: Each module would cover the entire process

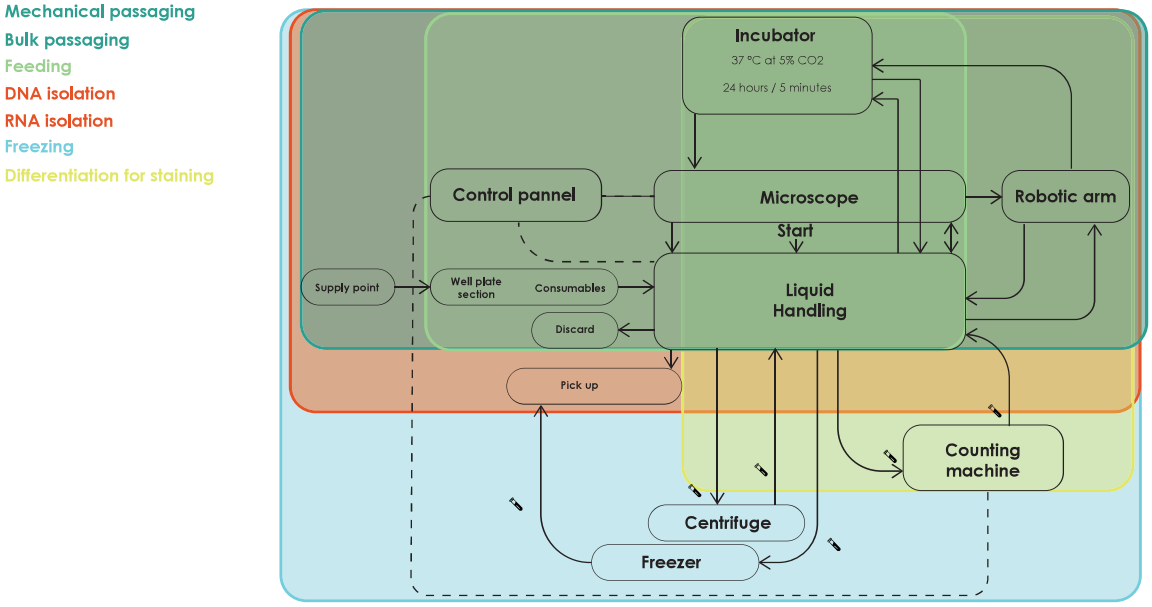


Figure 44: Analysis of the element necessary for the flow of well plates through the entire process, areas of interest for the steps are highlighted with the step specific colours.

Splitting the process into modules grouping steps:

In the previous chapter, the process has been analysed in terms of necessities for each phase and step.

In collaboration with the facility manager, who has background knowledge also on the similarities with the process with other not analysed processes, the main following typologies of modules have been identified:

- ***Reprogramming Module***

This module takes care of the initial feeding steps in the reprogramming phase and the mechanical passaging and feeding steps in the mechanical passaging phase.

The only interaction with the technician is in inserting the plates inside the system at the start of the process and check up of the splitting stage of the cells through the immaging devices.

- ***Expansion Module***

In this module the steps of bulk passaging and feeding of the Expansion phase take place, as well as the processes relative to the RNA and DNA isolation and staining phase.

The interactions relative to this module are of pick up of the plates, insertion of coated plates and check of the state of the cells throught immaging.

- ***Freezing Module***

This module takes care only of the freezing steps both relative to the Expansion phase and of the RNA and DNA isolation and staining phase. It has smaller capacity needs and less frequent run time of the liquid handling robot, centrifuge and incubator.

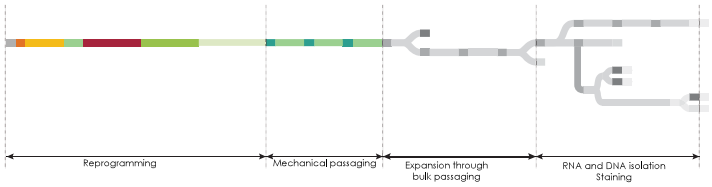


Figure 45: Highlight of the section of the process that would be covered by there reprogramming unit.

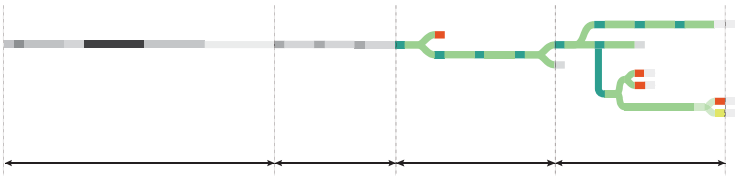


Figure 46: Highlight of the section of the process that would be covered by there expansion unit.

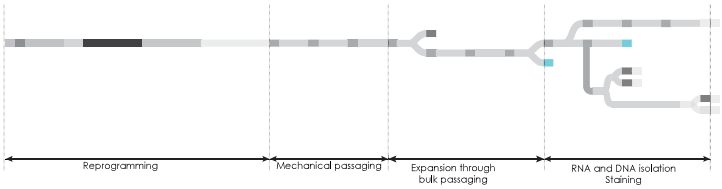


Figure 47: Highlight of the section of the process that would be covered by there freezing unit.

RELEVANT INSIGHT

- Modularity can be considered in three main aspects: each module is functional to a single step, each module can deliver the full automation of the process and the module combines only partial steps.

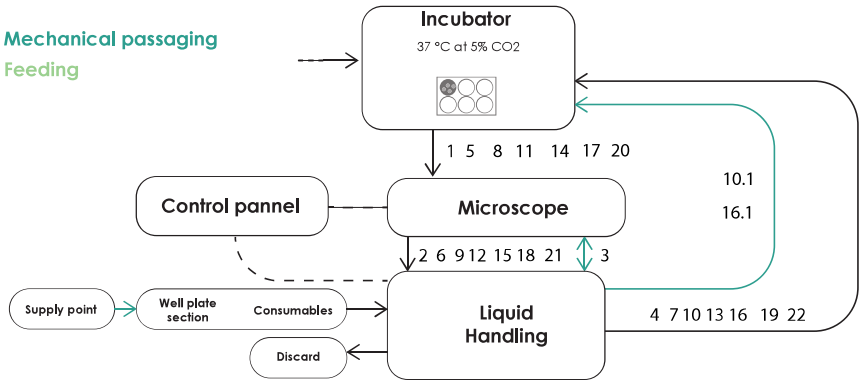


Figure 48: The elements and flows relative to the feeding and mechanical passaging are combined together

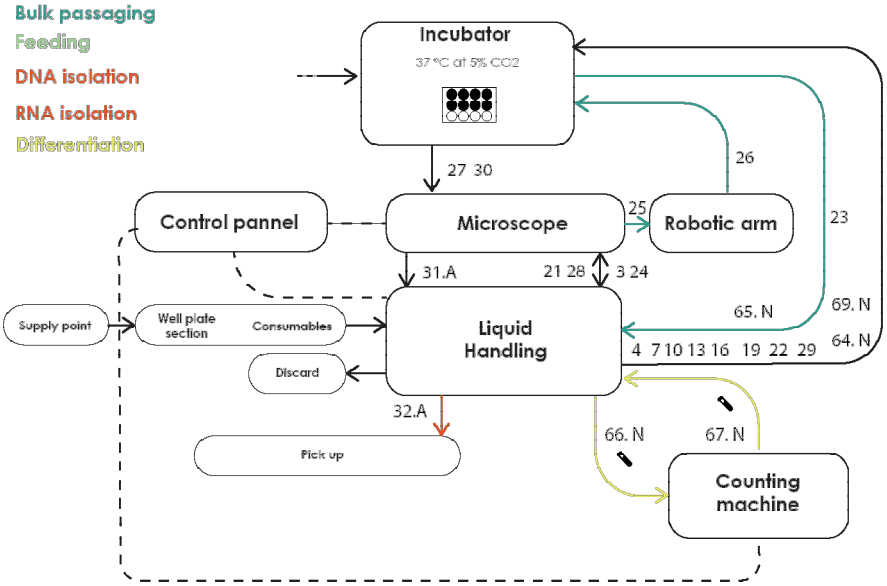


Figure 49: Devices and flows relative to the different typologies of steps taking place in the expansion unit.

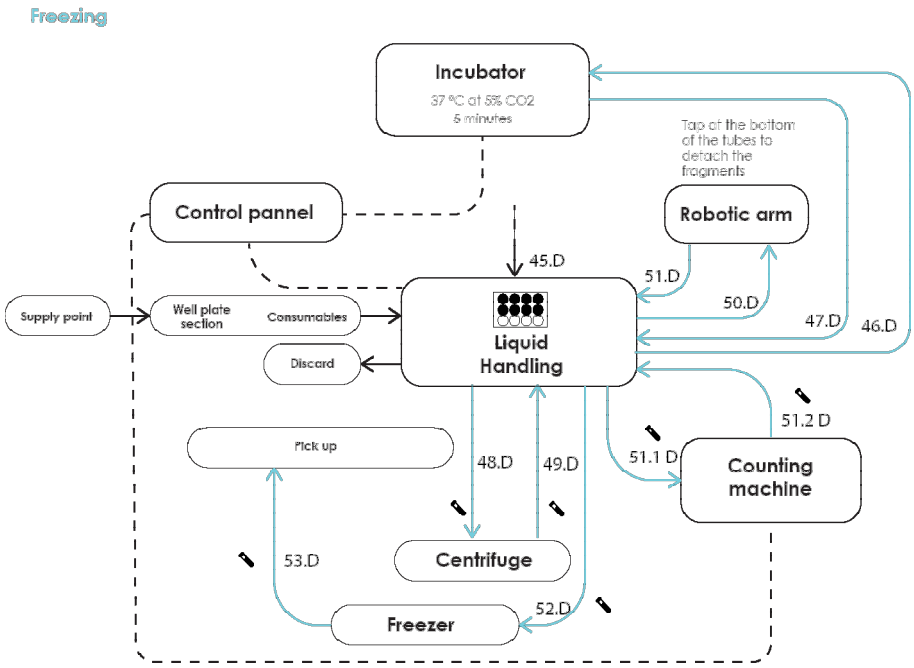


Figure 50: Devices and flows relative to the freezing unit.

Legend

- Dashed lines refer to the decision making data that passes from the device to the control pannel.
- Arrows highlighted in a specific colour indicate that the specific transfer is relative only to the process step of the same colour.
- Black arrows indicate transportation to a next device through the process steps
- Unless specified with the tube icon the transport regards well plates of 6, 21 or 24 well plates.
- The numbers indicated on the arrows refer to the transfer steps analyzed in the process tree workflow (Appendix I).

4.4 Considerations relative to different strategy for throughput

The aim of the facility at the moment is to get to the production of 1000 cell lines in a year.

Currently, only 50 lines are produced in this time frame, which means that it is necessary to increase the throughput of 20 times.

The production of cell lines follows the timing necessary for wells to react with the reagents, absorb the nutrients, and grow in relation to the needs. Therefore, the increase of throughput is going to depend on how many lines in parallel the system is going to handle.

In a commercial product scenario, the start of the production should be limited within the year so that the last batch of production could be handled in time, which would mean that at the last 66 days (the average time needed for one cell line to be produced) of the year no new production should be sent. This is, in fact, the time necessary for the development of each line.

However, given the nature of biological production, as attested by the facility manager, Joost Gribnau, this yearly limit doesn't have to be followed strictly but it should be used as an index factor: if the last batch crosses over with the production into the new year it could still be executed. The value of maximum 1000 lines per year should be considered as an extreme scenario that would happen only in the future.

Researchers don't know with absolute certainty if they are going to benefit from grants for their research so the contact with the facility is reached and the production is confirmed usually with short notice, mainly three months in advance.

Likewise, since the Facility is still at the beginning of the transition towards automation,it is difficult to foresee how the development of requests is going to be in the future.

Ideally the machine would need to adapt and manage starting days of cell lines so that their bottleneck periods of would not be overlapping between each other and the workload is evenly distributed through the production.

At the same time, it is important to have an idea of the possible numbers needed for the capacity of the system in edge cases and scenarios since from this factor will depend also the size and the costs of the devices.

Considering parallel production, it is possible to identify two main directions:

1. ***All lines in parallel: All the production lines start at the same time.***

This is an extreme scenario that would most probably never have to take place, especially in the first period when new researchers will still need to get in contact with the iPS Core facility.

Having all the lines start at the same time is not necessary for the facility.

In fact, the production would take only the first 2,2 months of the year leaving the remaining 4 periods of the year unused.

This scenario could materialize only in three cases:

1. in a later future when the machine modules and devices should be used for other purposes for the remaining time of the year;
2. if the research for which the lines are commissioned for is highly urgent
3. if the yearly needed production increases

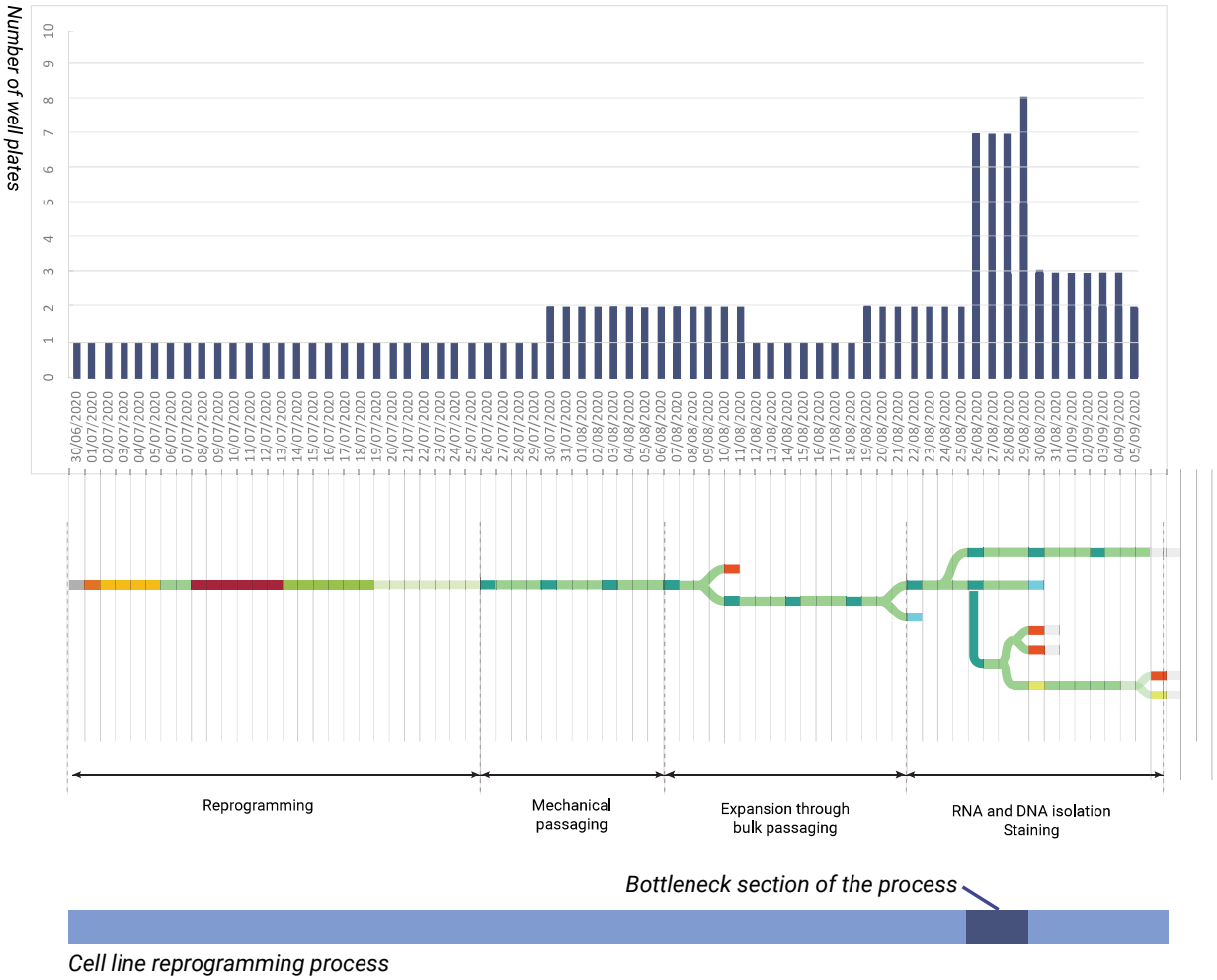


Figure 51: Each cell line has more or less the same frame of days that are most intensive in terms of requirements of capacity, i.e. when the highest number of well plates is required (day 53 to 56 of the protocol). In the following pages the process has been reppresented by the lightblue line while the bottleneck days are highlighted in dark blue.

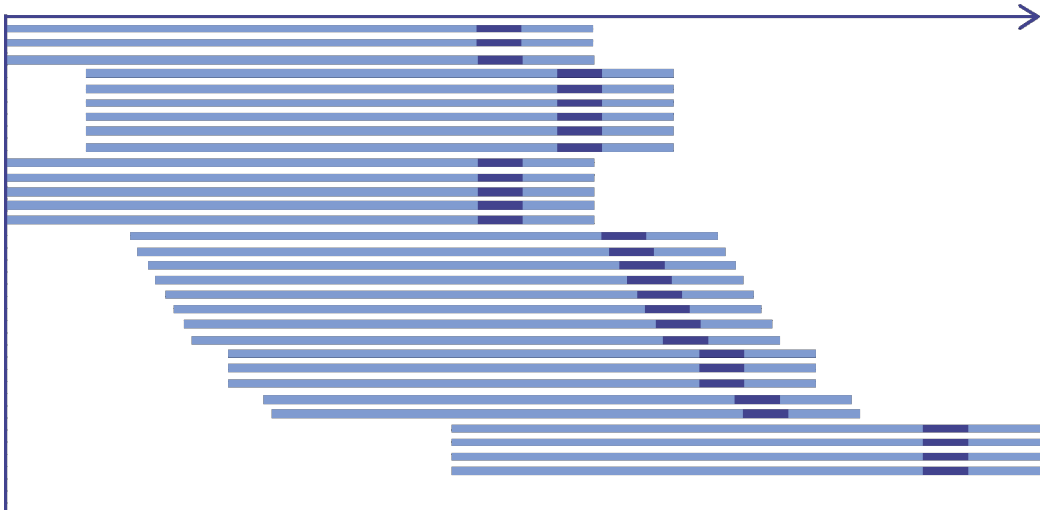


Figure 52: Reppresentation of the ideal scenario of autonomous management of the lines start and duration in order to optimise the resources of the system and manage the bottle neck periods.

4 times the present aim of 1000 lines per year.

There is a high probability that all the above-mentioned cases never take place in the Erasmus MC facility in Rotterdam.

Therefore, also having it in the scope of future readjustment, towards which to prepare or be flexible for, this situation is not needed.

2. In batches

A more likely scenario could be that of having several batches of lines running in parallel but shifted between each other of a determined number of days so that the workload for the system is spread through the year and provides work to the technicians of the lab in a distributed manner.

The two variables that determine the needed capacity of the devices in the system are:

The number of lines running simultaneously in one batch (line number, ℓ) and the number of days in between each batch start (interval number, i). These two parameters, in fact, determine the overlap of the bottleneck days (days that require higher handling of number of lines).

In order to run without complications also

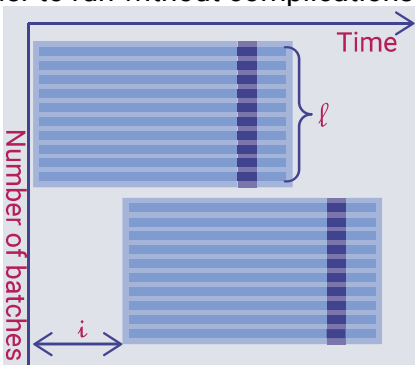


Figure 53: variables of identification of the parallelization methodology.

through the bottleneck sections, devices should be able to host at least 8 * ℓ *y number of plates, where y is the number of batches with bottleneck sections overlapping between each other.

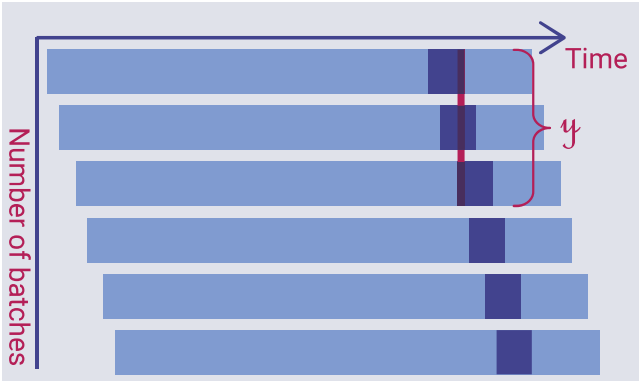


Figure 54: Overlap of bottleneck sections of the process

Example of combination of variables:

Having every day one new line: Each production line would start at its own date, in batches.

Having one single new product line starting daily on a regular basis would produce only 366 lines per year, which, although being an increased throughput if compared to the current manual process, would still not be enough for the aimed throughput.

For this reason, the scenario of constant input of lines in the machine on a daily basis will need to be of several lines contemporarily.

If new lines start being produced every day the maximum throughput of 1000 lines per year would require 3 (for 200 days) to 4 lines (during the remaining 100 days) to start in parallel every day.

The parallelization of the lines would follow the reppresentation in Figure 55.

As it's possible to see the bottleneck days would overlap between the different lines for five lines contemporary.

In this case, with ℓ equal to 4 (higher value) lines and y equal to 5 lines, the necessary capacity of devices in order to be able to host the necessary number of plates going through the bottleneck phases is of 160 plates simultaneously.

The number of combinations is very high and it would take programming time and effort in

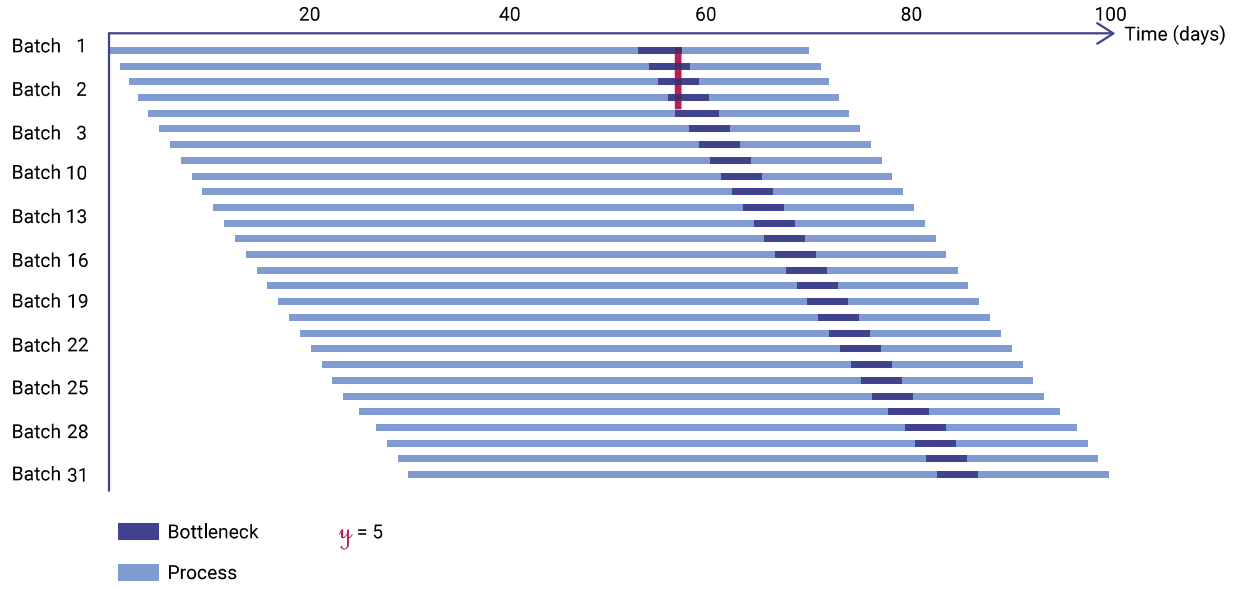


Figure 55: One new batch starts each day

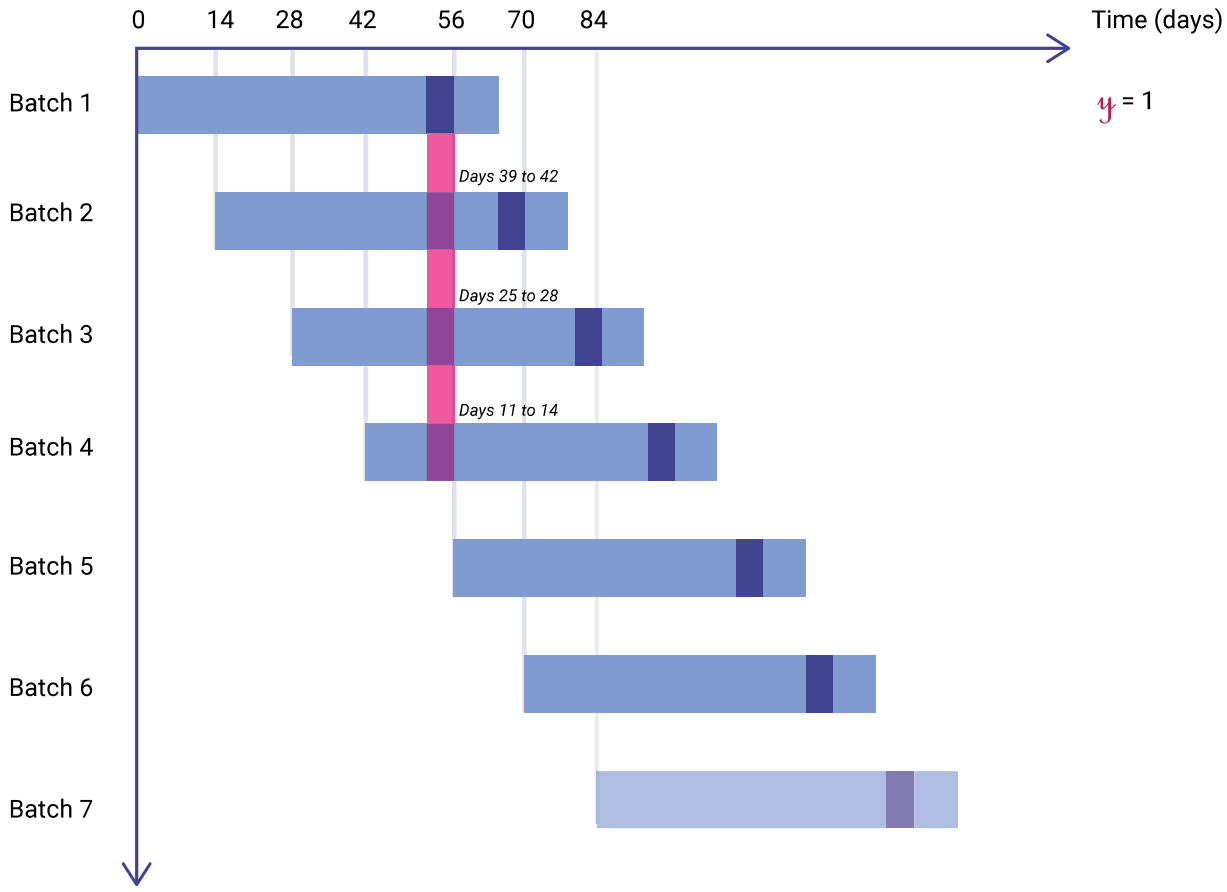


Figure 56: Schematic of the allignment of periods of the process in the lower limit scenario

order to model a simulator that would be able to determine the ideal batch size and interval, which is not in the scope of this project.

However, an overall estimation of the minimal and maximal capacity of the system can be calculated.

Two extreme scenarios can be taken into account:

Lower limit scenario
On the lower side: the automated system will need to support at least the current laboratory work organization of the Core Facility: 50 lines per year, in which more or less 3 to 4 lines start every 2 weeks.

As its possible to see in the Figure 56 resulting from the simulation, this will mean that four lines will be processed by the system constantly.

The capacity for this scenario can be calculated as following:

The bottleneck of the process for one line, although being the only bottleneck at that moment. coincides with 4 other periods in the remaining lines processed at the same time by the machine. Since the number of coinciding lines is low, it is possible to analyse better the characteristics of these periods in the other lines in order to be precise on the necessary capacity:

- For the line immediately subsequent, the second entering the process, it will coincide with days 39 to 42 that need the handling of one well plate per cell line per day.
- For the third cell line batch coming into the system, the bottleneck of the first cell line batch will be happening at days 25 to 28 needing the handling of one well plate per cell line each day.
- For the fourth cell line batch, the bottleneck of the initially inserted cell line batch will happen in parallel to the days 11 to 14, still in the reprogramming phase, also needing the handling of one well plate per cell line.

The same logic repeats itself with all the batches.

If the calculation is relative to the number of lines composing each batch at the current production intervals and throughput of the facility, the needed capacity for liquid handling and incubation should be of 44 well plates per day.

Highest limit scenario:
The timespan of production of one line takes 66 days, approximately 3 months, the higher extreme scenario would be that of high intensity batches with the aim of having all the 1000 cell lines within the year (therefore having the last batch of lines staring before the last 66 days). This would mean having 4 possibilities of starting a batch in a year. In this extreme scenario, therefore, the two variables (ℓ, i) could be of 250 lines which run together in each batch every 66 days.

At the very best intensity, if the system is going to produce 250 lines per batch, since the needed capacity of the system for one cell line during the bottleneck time frame (days 53 to 57) is of 8 well-plates per line, the unit should be able to handle 2000 plates contemporarily.

Variant of the first lower limit scenario

An other scenario could be that of having the interval between batches maintained the same as it is currently (every two weeks) in order to have the same typology of task distribution between the technicians, but to increase the number of lines per batch to meet the throughput of more or less 1000 cell lines per year. In this case the number of lines in each batch would have to be of 38 lines, [1000/ (365/14)]. With the same methodology used for the calculation of the capacity at the lower scenario, it is possible to calculate that the needed capacity for the

Parallelization scenarios			
Parallelization	*Needed daily capacity*	*Evaluation*	*Total lines p.a.*
In parallel			
A ***All lines*** start simultaneously	Not calculated	Unlikely event	1000 - 4000
In batches at intervals of time			
B1 **Mantaining the state of the art** 3-4 lines every 2 weeks	44 well plates	At immediate transition	78-104
B2 **New batches each day for a throughput of 1000 lines p.a.** 3-4 lines every day	160 well plates	Could be adopted at times while increasing throughput.	1000
B3 **Increased throughput (1000 p.a.) at current interval** 38 lines every 2 weeks	1330 well plates	Maximum limit to consider. The possibility this scenario could take place is relative to big projects happening with short notice.	1000
B4 **System at highest stress** Four batches of 250 lines	2000 well plates	Unlikely event	1000

Figure 57: Identified scenarios of parallelization

system should be of 1330 plates per day.

Conclusions

Four scenarios of throughput and relative parallelization have been identified, a summary is presented in figure 57, along a brief evaluation.

Scenarios B1, B2 and B3 can be taken into account for further evaluation of devices based on their capacity.

RELEVANT INSIGHT

- There are several ways in which management of throughput can be done. This is mainly related to what strategy is used in order to run the lines in parallel.
- Different parallelization scenarios have been envisioned, between these the three scenarios that can be taken into account are relative to mantainance of the status quo of the production within the facility, sudden increases of request and full year of productivity needs.
- Each parallelization scenario necessitates a different capacity within the system. the minimum and maximum limits for the devices considered further are relative to parallelization scenarios B1 and B3

4.5 Configurations and Layout planning

The design of a system consisting of modular elements is built taking into account a scheme for the disposition of it's elements that reflects the future scalability goals but still maintains possibility of variability in results (Correia, 2019), for this aim it is possible to adopt the method of layout planning.

Layout planning refers to the physical configuration of the production system in its context or facility. It studies the spatial boundaries, the capability specifications and the characteristics of the results of the system.

The layout of the system is an important factor since in production management material handling costs are 75% of total manufacturing costs of a facility and the rearrangement of an existing production facility can be later more expensive in resources (Sule, 1994).

In order to design the layout one method used is the Simplified Systematic Layout Planning (Muther e al, 1994), it is based on the study of three fundamental elements, (relationships, space and arrangement) and it consists of the following phases:

1. Identify the location and its needs
2. Identifying the interrelation between elements of the production system and advantages given but their respective proximity.
3. Represent graphically the findings of the first two steps by emphasizing the relations of proximity
4. Laying out the elements of the production system inside the real facility considering the outcomes of the third step
5. Creation of different layout proposals for different priorities and evaluation of the so created options
6. Develop in more detail the chosen plan

Given the concept exploration nature of the scope of this project the location for the system has been left initially undefined until a later stage of the layout design. Therefore, it was chosen to make changes to the method, integrating the forth step into the evaluation of identified options and in the development of a more detailed plan.

Identify location and needs

The layout planning design is embedded into the requirements of the system and therefore connected to other chapters of this report. Following the methodology of Systematic layout planning the key features to be considered when doing the layout planning are summarized in based in the following picture, through the PQRST key elements:

P - Product - what are the goods produced by the company, the starting materials both raw and purchased, the treated parts, the results and the service items used through the process.

Q - Quantity - the amount of goods produced, supplied and used.

R - Routing - the process, the equipment, operations and sequence which are previously documented in process sheets, flow sheets, operation and equipment lists.

S - Supporting Services - The backing elements that are necessary for the system to be operative. For manufacturing layout planning in this category are included elements such as repair, receiving areas, shipping areas, locker rooms, .. elements of the iPS Core FAcility that are not in the scope of the project, and therefore are not going to be analysed.

P - PRODUCT	goods produced	iPSC lines
	starting materials	Fibroblasts and other cell types derived from patients
	treated parts	Reprogrammed hIPSC for further analysis, delivered in 1,5 ml tubes
	results	Reprogrammed hIPSC for further analysis, delivered in 1,5 ml tubes
Q - QUANTITY	service items	Scenarios B1, B2 and B3. Described in Chapter 3.3.2
R- ROUTING	The process, the equipment, operations	Described in Chapter 2.2.4 and 2.3.3
S - SUPPORTING SERVICES	Supporting Services	Chromosome validation for colony selection
T - TIME	Cycle time	The cycle time is not known

Figure 58: The key elements to start with the layout planning

T - Time - in this case the parameter considered is the cycle time, resulting from the time available divided by average demand rate. It indicates the maximum time useful to satisfy the production needs. This is directly linked to the quantity of a piece of machinery or stations required which rules how much space is required, the necessities in term of operators and management of activities. Urgency is also described in this section since it is codependent from the run timing and the supporting services organised around it.

Identifying interrelationship between elements

Based on the information gathered during the definition of the PQRTS factors a list of required elements within the system can be written

During this phase the identified elements in the process can be challenged with the following questions:

1. Exclude –can it be erased or is the activity needed?

2. Merge – Can it be several operation be incorporated into each other?

3. Modify – Can these be adjusted differently?

4. Improve details –Is there a design for improvement of this method adopted?

In this way the resulting elements are skimmed to essential elements necessary for the design of the overall relationships.

Once the identification of elements is performed and the identification of elements done, the relationship between them can be detailed through the relationship chart.

This is a cross-section that records the activities and allows to show in a whole the link between activities, and the importance of their closeness.

While creating the chart displayed in the next page the following operations and elements have been merged:

Line 1 - Direction and Management office: this block unifies the roles of the department director and the facility manager: their role of supervision on the process can be placed in the same location since the operations they do are interrelated, instruments such as remote system control and printer/ copy machine are also implied in the same section.

Line 2 - Contact point and genetic analysis point: This block reppresents the researchers area, technical support and genome-integrity check station: Researchers are the customers of the facility, they use the services of the devices hosted within the Lab but could potentially need to interact with the system or the technicians of the Facility for consultation, delivery of results or directives. Also the technical support to devices is probably not going to be needed on a constant basis but mainly for management of problems or during a new configuration of the devices, change of protocols and alike. In the same way, the genome integrity check is a event that needs several days of processing and is related to the decisions done for continuation of the process, therefore it could need interaction with the other two elements of the group.

Line 4 - Some liquids used by the liquid handling systems need heating/cooling to room temperature before being supplied to the wells. This can happen more quickly adopting a cooling or heating device, however this is ignored in this section of the analysis in order to simplify the schematization.

Line 6 - Sample preparation, storage for material used by technicians and coating of well plates are united into one element because of logical positioning

Line 9 - Liquid reservoirs for the liquid handling are here considered together with hydrogen tanks

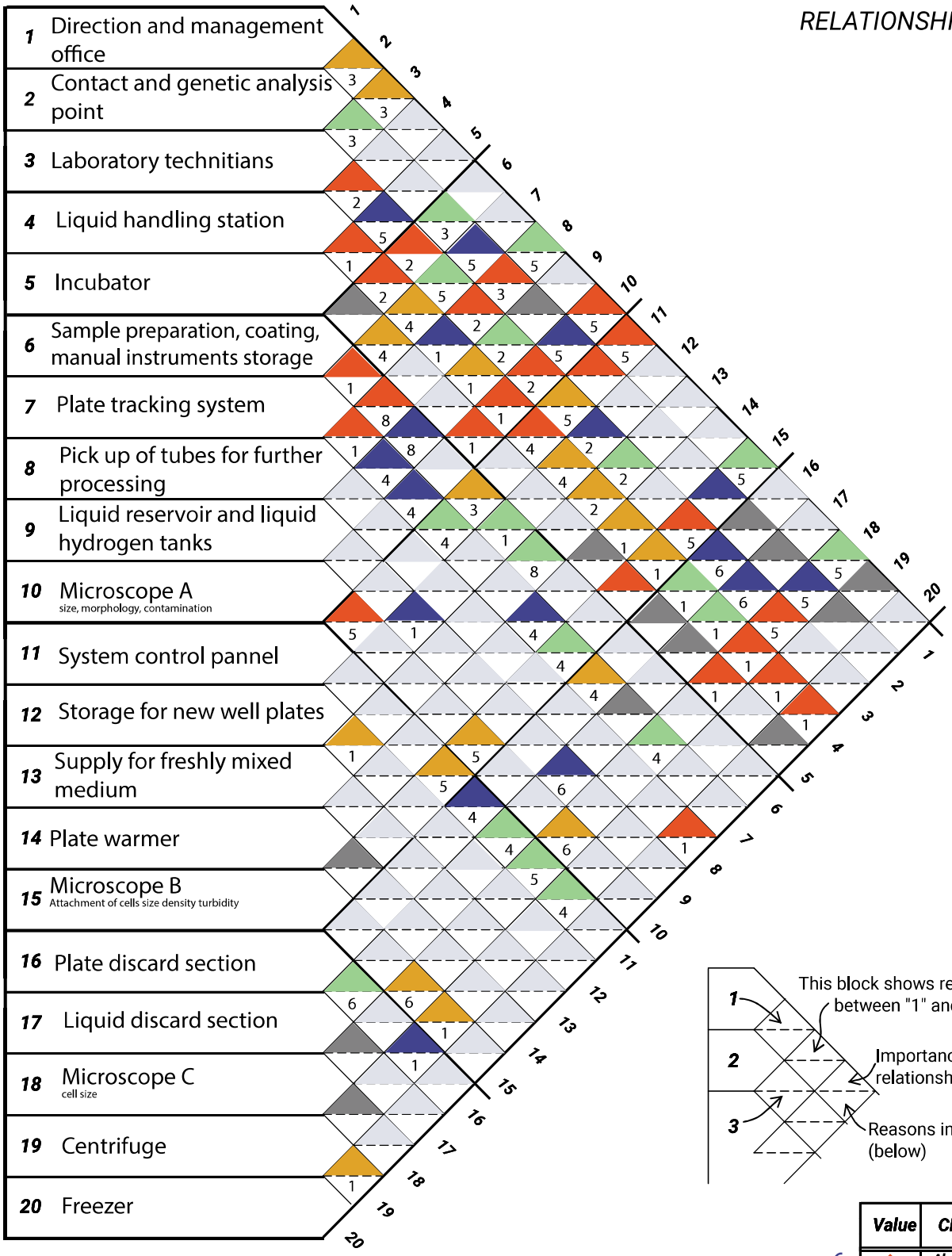
Line 15 - Since the microscope B also has the function of detecting the density of the cells in the well plate, it merges with the functions of a cell counter, these two elements are considered together.

Not present - A robotic arm used for the transportations between the sections of a system could be able to perform the functions of a robotic arm assigned to the “movement of 8” (in a two axis direction) and “tapp under tubes to suspend the cells” actions. Because of this these elements are not present in the chart.

The graphical reppresentation of findings coming out of the relationship chart can be seen in Figure 61.

It is going to be used forward in the process in order to position elements within the possibilities of layouts.

Figure 59: Relationship chart



Reasons behind the "Closeness" Value

Code	REASON
1	Flow of materials
2	Needed interaction
3	Share personel
4	Share data/ records
5	Supervision and control
6	Use same utilities
7	Management desire
8	Reduce walking time between interactions

"Closeness" Rating

Value	CLOSENESS	No. of Ratings
A	Absolutely Necessary	25
E	Especially Important	19
I	Important	19
O	Ordinary Closeness OK	17
U	Unimportant	16
X	Notdesirable	93
Total =	$\frac{N \times (N-1)}{2}$ =	190

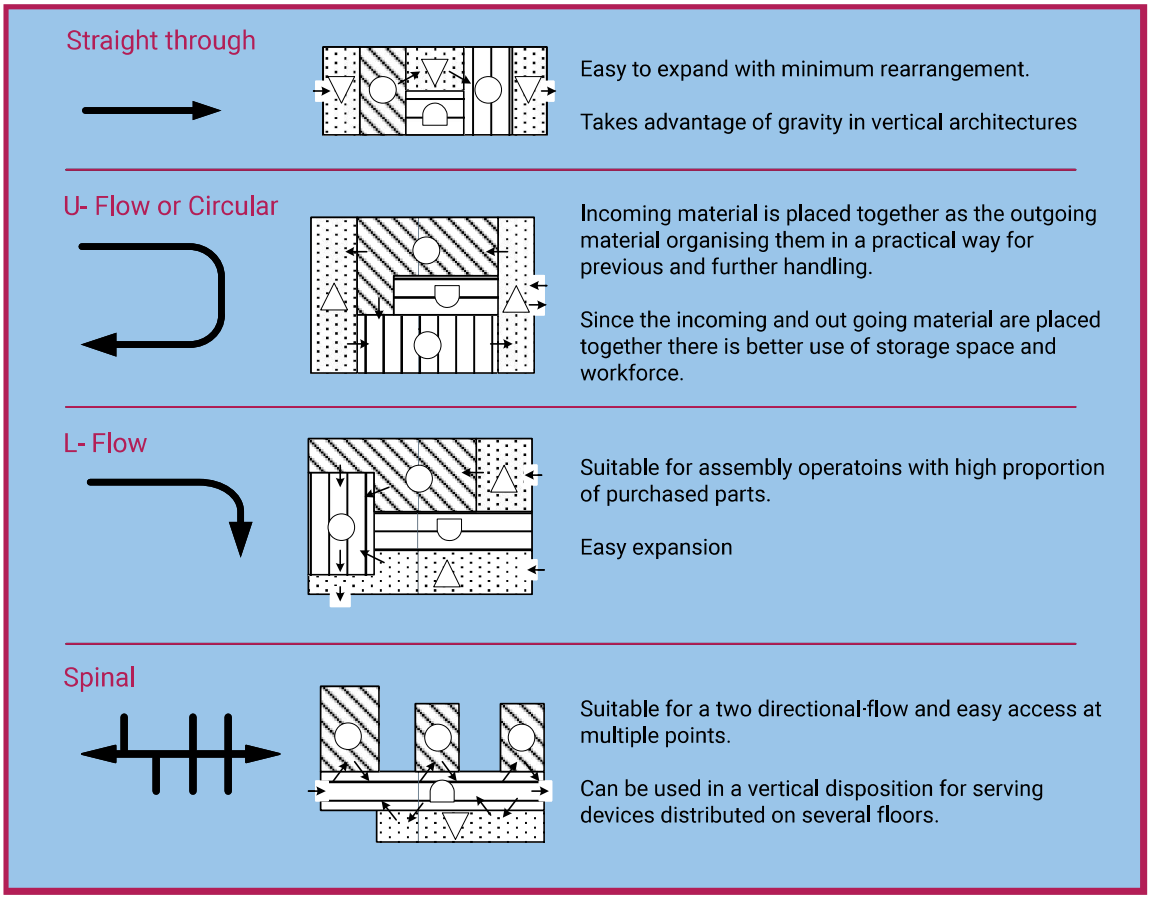


Figure 60: Four flow patters that can be experimented in different layouts.
The original observations from Muther et al (1994) , Page 154, Figure 9 - 1 for manufacturing systems are readapted for the current application in a smaller size system

Developing alternative layouts:

As reported by Muther et al (1994) one of the fastest ways of developing different layouts is that of challenging teams of people and then compare their outcomes.

There can be several alternatives but the main outcomes are generally exploring the following general standards:

1 - Different flow patterns and flow directions through the layout: Straight through, U-flow, L-flow slightly varied of coupled. Figure 60 represents the four basic flow patterns in the plant layout.

2 - Variants of the dock, input, and output positionings.

3 - Layout and pattern of main aisles: number of, locations, and orientations.

4 - Keeping a highly-fixed area in place, or allowing it to move. In rearrangement projects, it is good practice to rate the “fixity” of activity-areas

5- Varying the space available – its structure or positioning.

6 - Centralizing or decentralizing support areas consistent with their relationships. Not combining perhaps, but “pushing them together” into a block or cluster

7 - Combining or splitting key activity-areas by making experimental variations to the rules derived from the relationships.

8 - Mirroring or rotating an alternative within the space available.

An initial ideation based on the methodologies here described brought to the options described in Figure 63. The methodology is brought to further development in the Ideation phase, Chapter 05.

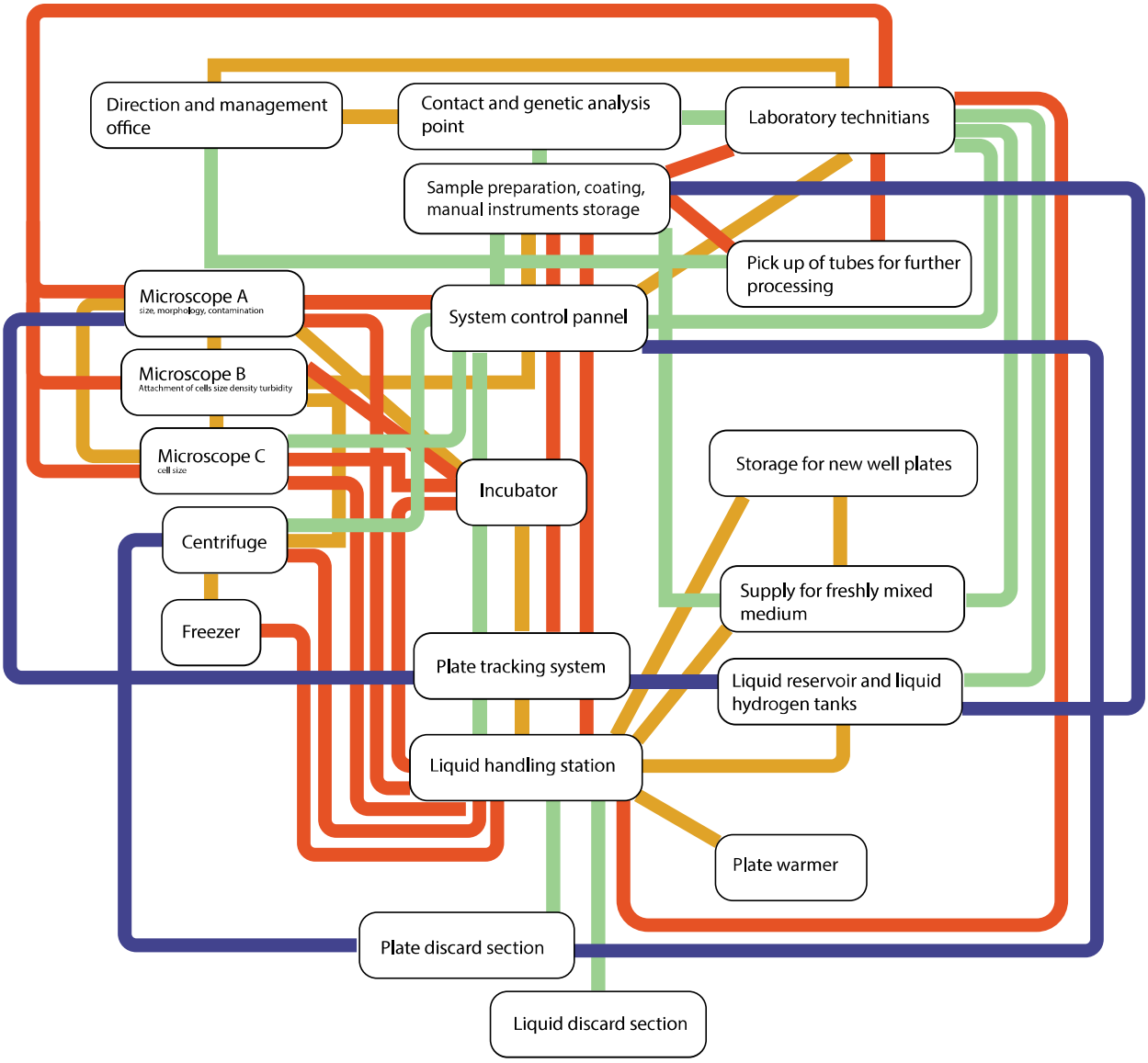


Figure 61: Graphical reppresentation of the outcomes of the relationship analysis

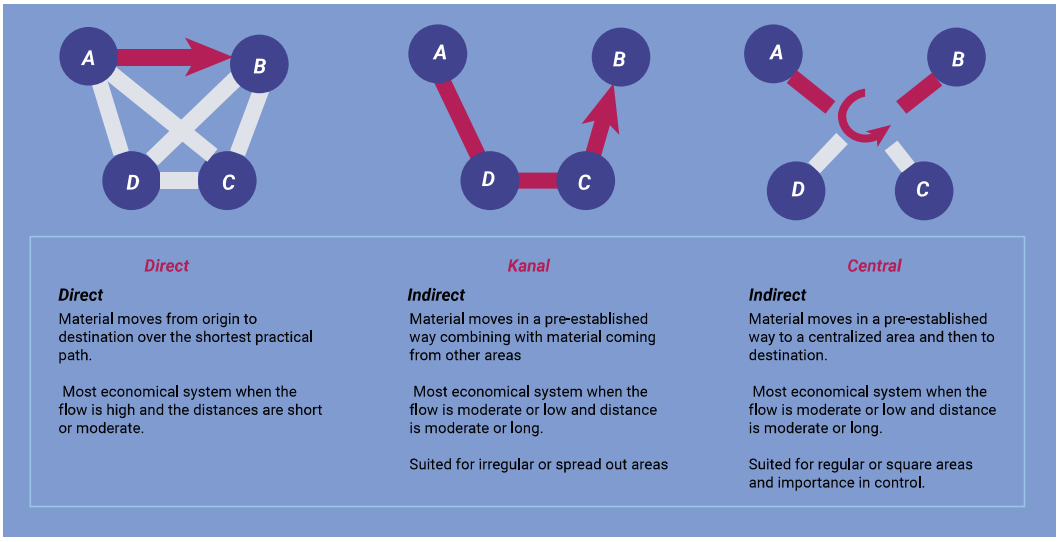


Figure 62: Advantages of different indirect flows in comparison to the fastest way.
Muther et al (1994) , Simplified Systematic Layout Planning. 3rd Edition, Page 158 Figure 9-5

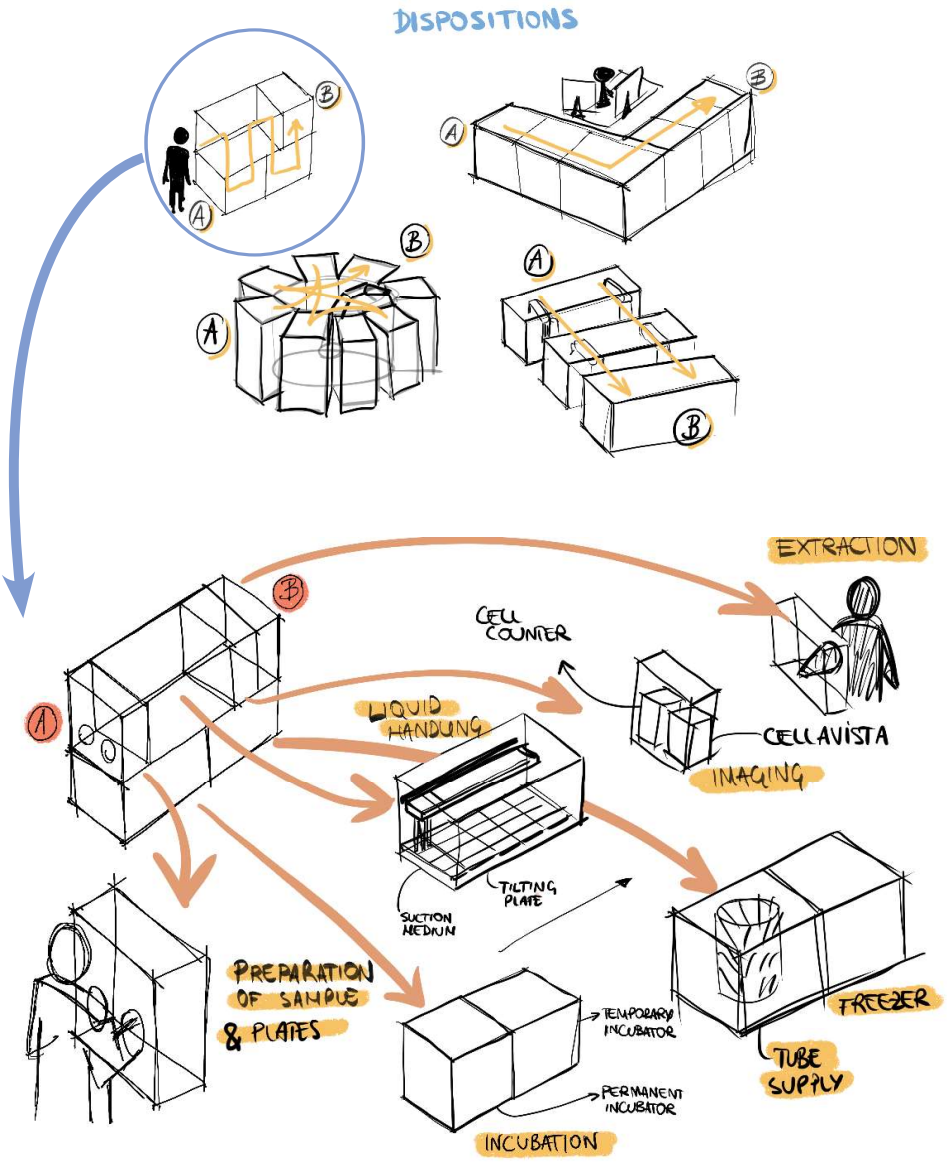


Figure 63: Initial different layout options

RELEVANT INSIGHT

- When designing the layout of the modules it is important to take into account both internal elements of the system, and therefore its internal flow, and the external stakeholders and elements relative to the context.
- The direct connection of devices is not always the best option to choose, a centralysed and indirect flow can be more efficient for putting attention on control.

4.6 Choosing components

In the previous chapters, the process of production of iPSC lines has been analyzed, the components necessary for an automated system have been identified and automated systems for cell culturing and preservation analysed.

In this chapter, the final list of components is going to be presented. This list is the result of the analisys of the components used in the comparative system at the University of Luxemburg, developped by the automation system company Beckman Coulter, and the requirements of process used at the iPS Core Facility.

Figure 66, represents the final list of components and their relative sizes, Figure 67 also indicates the weights and estimated prices.

Components mantained from the Luxemburg model:

Liquid handlers
BioMek FX

The BioMek Fx is used for automated cultivation of iPSCs in the phases of reprogramming and feeding.

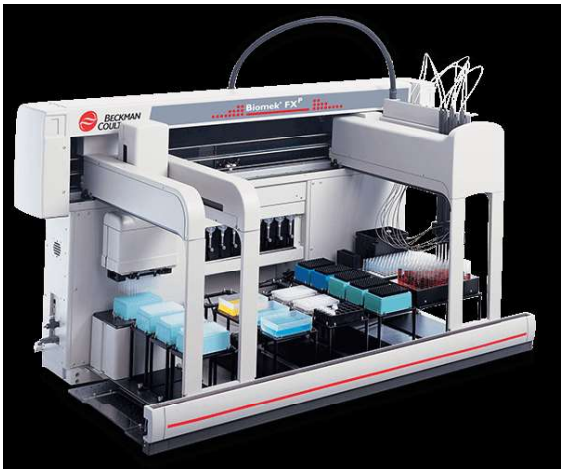


Figure:64: The BioMek Fx

- The BioMek FX's efficiency can be increased from one to two pipetting pods and with its deck capacity provided with automated labware positioners it has, without any extendions, 24 deck positions.
- It has a built in gripper that allows to move labware on the deck and lid and delid the plates.
- It has a built in barcode tracker that allows plate tracking.
- Because of the open architecture it can integrate well with labware storage devices and robotic transport systems.

BioMek NX
The Biomek NX is used for the phases of cell culture

Figure:65: The BioMek Nx

- with the Span-8 configuration it is able to have well access for tube-based operations.
- It has 12 Deck positions and can be therefore also be used for the steps

Component	Function	Dimention
BioMek FX	Screening liquid handler	152 cm x 82 cm x 140 cm
BioMekNX	Cell culture liquid handler	79 cm x 91 cm x 86.4 cm
FLUOstar Omega	Spectrometer based microplate reader against turbidity	44 x 48 x 30 cm
Celigo Imaging Cytometer	Cell counting	49.5cm x 40cm x 61cm
X-Peel	well Peeler , closing or opening wells	37,3 x 64,5 x 37,3 cm
ALPS 3000	Well sealer	185 x 414 x 350cm
Sigma 6K	Centrifuge	79,0 x 71,1 x 48,5 cm
Cap-it All	Capper decapper	32.8 x 36.0 x 56.0cm
Sci-Print	Etichette printer	67 x 35,5 x 84,3 cm
Freezer		
Incubator Cytomat (210pcs) (two)	Incubator	830 x 822 x 915,4 mm (10C) 927 x 894 x 1858 mm (24 C)
Cell Celector	Cell picking and cell morphology and size Each pick takes approx. 25 seconds	970 x 634 x 641 cm
Biosafety Cabinet	Sterility	on request
SCARA Robotic Arm		on request

Figure:66 Function and dimention of components

Component	Weight(kg)	Price (Euros)
BioMek FX	124,7	19000 *based on used and refurbished models
BioMekNX	86	4000 *based on used and refurbished models
FLUOstar Omega	28	11.000 - 17.000
Celigo Imaging Cytometer	53	100 000
X-Peel	35	5000*based on other similar devices
ALPS 3000	80*based on dimentions	34000*based on other similar devices
Sigma 6K	50*	125*based on other similar devices
Cap-it All	35*	-
Sci-Print	41	-
Freezer		-
Incubator Cytomat (210pcs) (two)	65*	-
Cell Celector	52	-
Biosafety Cabinet	100*	-
SCARA Robotic Arm	100*	-

Figure:67 Weight and price of the components

relative to Freezing and DNA or RNA isolation.

Since the Biomek FX and Biomek NX have an etichette reader integrated in their frame, the etichette printer Sci-Print was integrated.

Some devices didn't have to satisfy too many requirements. For example the Well peeler, X-Peel, and well seeler, ALPS 3000, used for closing the well plates before pick up from the device, and the Capper/ decapper Ca-pit All IS only had to satisfy the possibility of being integratable within an automated system:

- they can all be integrated into automated interfaces through Serial RS232 remote interface
- Have a capacity of 200 plate openings per hour (X-Peel) and 600 well sealings per hour (ALPS 3000)
- The Capper/ decapper Ca-pit All takes Approximately 15 seconds per cycle (cap or decap)

Centrifuge

From the component list of the comparative system only the brand of the centrifuge could be derived. By contacting the vendor it was possible to derive more details about the model:

- Can be integrated in automatable systems through Serial 232 RS232 remote interface
- The centrifuge is part of the Sigma 6-16KRL line which is usually implemented in automated systems.
- The rotational force to be used is of 200 G, therefore, a swing bucket rotor (used in medical and research laboratories) with forces of maximum 6000 x g will suffice the task.

Incubators

Incubators of the line Cytomat C are integratable for automation and have an already embedded rack system.

- The two models Cytomat 10C and Cytomat 24 C are selected for their capacity, respectively 210 well plates and 504 well plates.
- The Cytomat 24 C incubator can be grouped as one incubator with a twin Cytomat 24C for future flexibility if internal capacity of 1008 well plates becomes necessary.

Figure:68: Details of the incubators

Modifications to the initial list of devices:

The Acoustic Dispenser Fluostar Echo 550 was not selected from the list. This technology, in fact, although interesting for the avoidance of contamination, is used for low-volume applications and is not suitable for volumes above 5 microliters.

All the devices for quality control have been replaced.
In the following scheme a reppresentation of the devices used for each Microscopy function can be found:

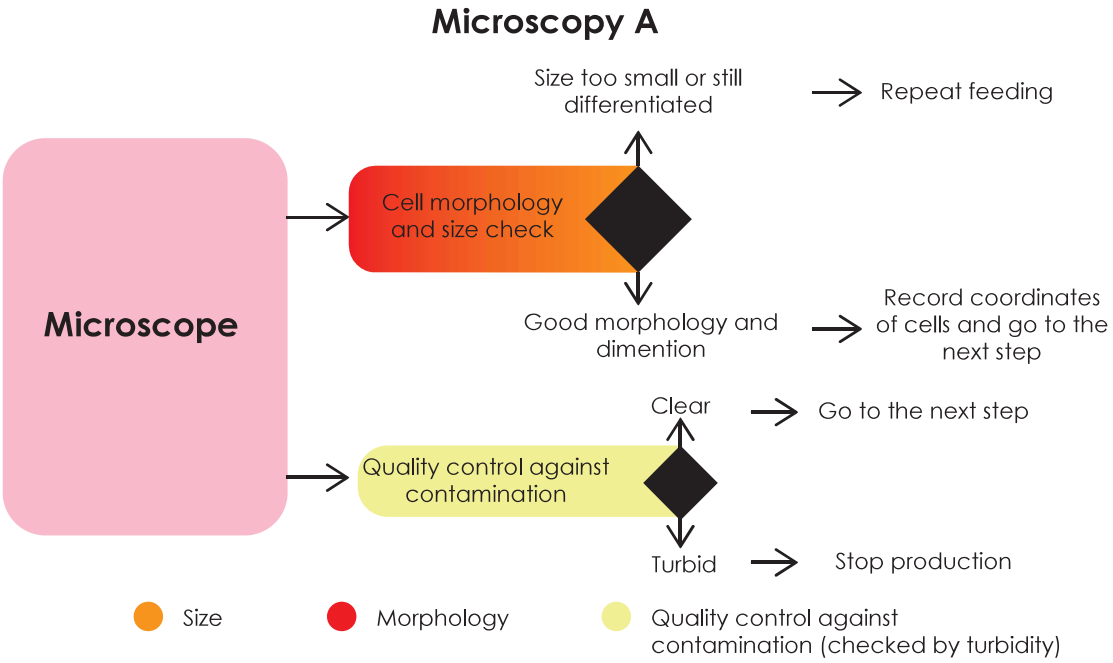


Figure 69: Fluostar OMEGA for cell turbidity

Figure 70: ASL Cell Celector

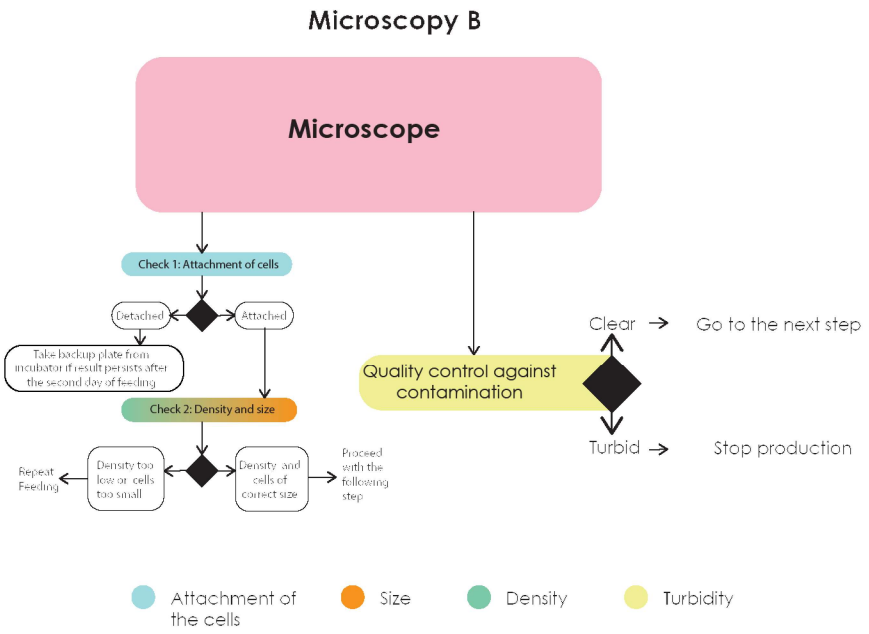
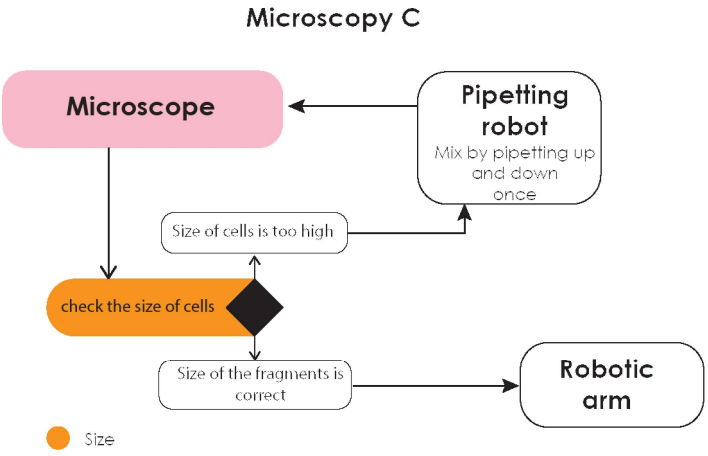


Figure 71 Fluostar OMEGA for cell turbidity

Figure 72: Nikon Eclipse TE 2000-U

Figure:73 Celligo Automated Imager



For the freezing module the chosed device is the Hamilton SAM HD.
This device, in fact, is one of the only freezing devices suitable for -80 degrees that also integrates automation and has the possibility to host 1,5 ml tubes, which are used in the process of IPSC production.

However, this device has capacity which is 50 times the one necessary for the process and troughput of the Facility. Further investment of time could lead to the selection of a better suiting freezer.

RELEVANT INSIGHT

- Devices coming from the starting comparative system have been reassessed considering the different needs of the procedure used at the IPS Core facility at Erasmus MC.
- The process of confirmation and evaluation of devices is an iterative process. The final and most complete list of devices considered within the project can be found at Appendix VIII along the cost price evaluation details.

Chapter 5

Conceptualization

This chapter describes the process adopted to go from the requirements of the assignment and design process outcomes described previously towards the realization of four concepts that are going to be evaluated in the following chapter as basis for future reccomendations and detailing.

Initially the design approach is going to be described explaining the methodology of ideation and combination of ideas.

An overview of the resulting four concepts is going to follow.

To conclude, a selection of the continuing concept is given.

In this chapter:

5.1 Approach

The process of ideation, visualized in Figure 74 is subdivided into five phases.

Phase 1: Idea generation
During the previous context- and process-analysis, several design opportunities, coming from the system requirements are identified. It is possible to subdivide the requirements in component-dependant, relative to the characteristics and properties of the devices chosen to be integrated into the system, and component-independent, regarding characteristics of the system on the macro-level.

The brainstorming sessions taking place in this phase of the project regarded the macro-level requirements, clustered in three categories: interactions, variety of dispositions and build of trust, and connection between devices.

Participants were students from different studies and received selected background knowledge. Also, the methodology used, although mainly focusing on the How Might We Method, was slightly changed to address the different topics of the sessions. Figure 75 synthesizes the procedure used to carry out the sessions.

Phase 2: Morphological chart and combination with trend and horizon research
From the first phase, generated ideas regarded the practical shaping of the system and did not include considerations coming from system design analysis on modularity, parallelization and quantities of throughput, and from trend analysis, and the three horizons method (Chapter 3). By stepping back and combining the outcomes of the two typologies of analysis (Figure 77) it has been possible to create four main principles to be used as the basis of the concepts. The next step has been to develop a morphological

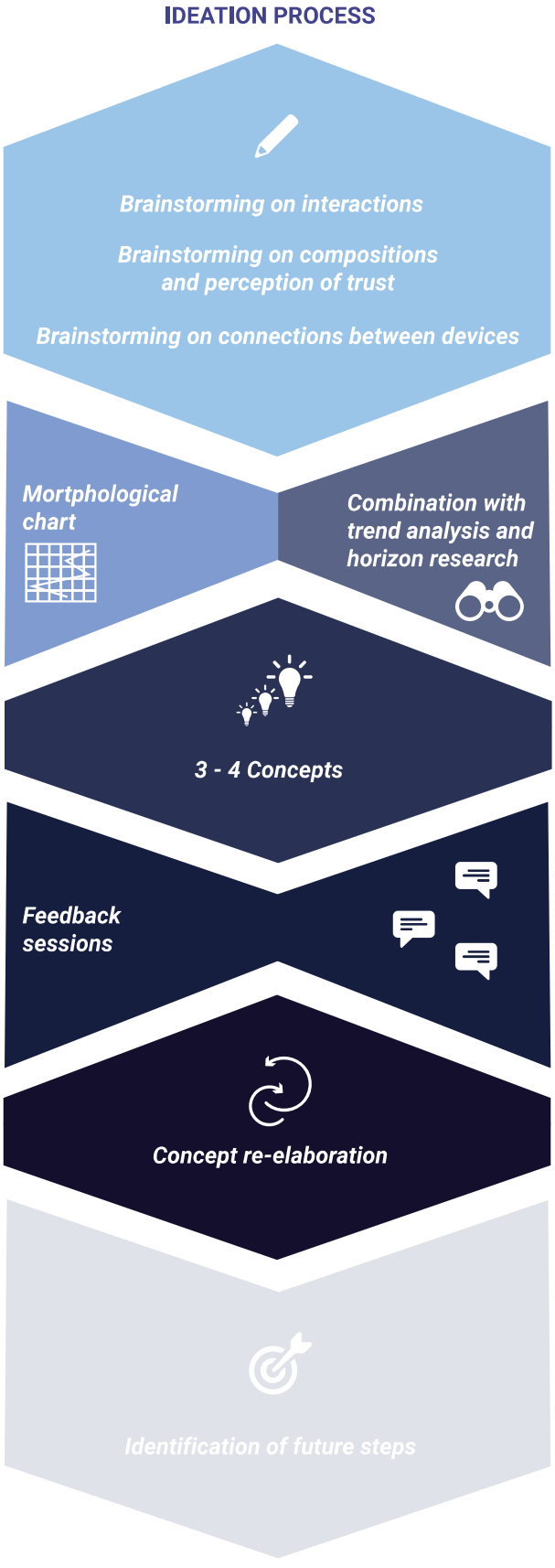


Figure 74: Ideation process

Brainstorming on interactions

Preparatory background	Participants	Methodology and results
A preparatory introduction document (Appendix X) was sent to the participants of the session introducing iPSC reprogramming and the functions of the devices of the system. At the beginning of the session, a presentation was given to bring everyone on the same page.	Who: Partecipants had previous experience in design of integrated complex products, with the focus on medical devices, and system design. Mainly students or graduates from Integrated product design master course. Number of partecipants: 7	Design methodology: • How Might We method The session was held virtually. Tools used: Zoom and Power Point Results: Ideas on new device connection technologies to explore and integrate.

Brainstorming on connections between elements

Preparatory background	Participants	Methodology and results
The presentation given to the partecipants was focused on the background knowledge of the control problem.	Who: Partecipants had previous experience in design of interactions with products. Mainly students or graduates from the Design for Interaction master course. Number of partecipants: 6	Design methodology: • Brainstorming on the topic of Human-machine interactions • How Might We method The session was held virtually. Tools used: Zoom and MURAL Results: Conceptual ideas on ways of visualizing interactions with the technicians

Brainstorming on compositions and perception of trust

Preparatory background	Participants	Methodology and results
Given the various backgrounds of the parteciants the presentation given to the partecipants focused on the definition of robots and automated system to clarify the subject of the session. The main rules of creative brainstorming were introduced. The devices integrated in the system have been described only as building blocks.	Who: Partecipants from various master courses that have had experience with working in a laboratory and in organization of team work Students or graduates from master studies in Food Technology, Biomedical Engineering, Architecture and Management. Number of partecipants: 5	Design methodology • Brainstorming and brain-drawing • Methaphors and analogies • How Might We method The session was held virtually. Tools used: Zoom and Power point Results: Insights on factors stimulating trust in automated products; general principles to consider for dispositions of elements of the system

Figure 75: Details on the different ideation sessions

chart summarizing the ideas coming from the sessions.

The following activity consisted of combining the ideas of the matrix in order to realize the generated three principles.

Phase 3: Concept creation
Coming from the second phase, ideas were combined in three main concepts regarding main points and ways of interaction, technologies used, and final objective for throughput and modularity.

This phase also considered the components of the system. In fact, after modeling the overall footprint of the devices in CAD (with the dimensions declared from the producers) their relative proportions were used through the process of sketching the identified concepts.

Phase 4: Collecting feedback
Two feedback sessions were organized first with laboratory technicians and then with the facility head manager and the head of the department.

In the first session, technicians were asked to brainstorm on what risk factors they could see going through the process in the different presented concepts.

In the second session, the concepts were evaluated on a functional level observing technical advantages and disadvantages and corrections to be made in each concept. Through the discussion, it was possible to take decisions on the number of technicians, their specialization, and on the quantities of production.

5.2 Final concept creation

Outcomes of the previous analysis and of the ideation sessions have been combined in order to propose different concepts. The initial concept principles were four and are summarized in Figure 78. The morphological chart (in Appendix VI) connects ideas and concepts based on their familiarity with the concepts principle.

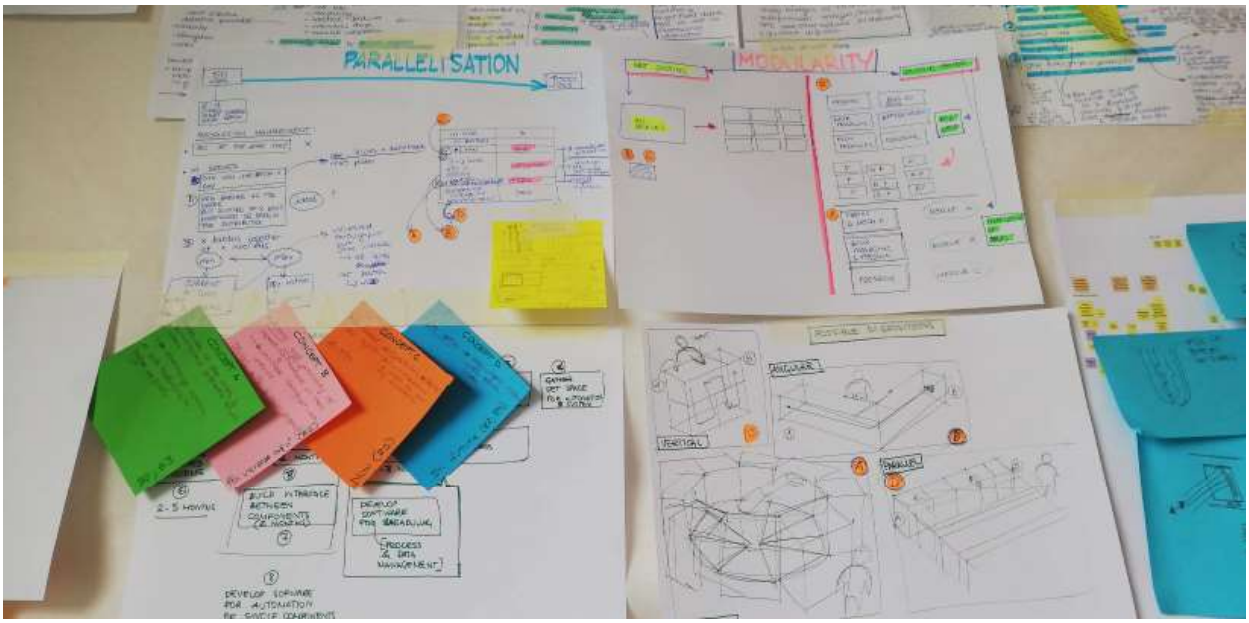


Figure 76: Key elements coming from the previous phases combined to form the concept principles.

Figure 77: Process of combination and development of the concepts

	CONCEPT A	CONCEPT B	CONCEPT C	CONCEPT D
Principle	***Ability to combine differently specified modules and increase the modules relative to bottleneck operations***	***Easier placement of several modules, in the same room***	***Vertical disposition of elements so that the devices take little floor space***	***Lines in series, the system would take up a whole room devices can be placed around the room in such a way that they take up very little space.***
Scenario	Increase of throughput and same intensity but higher throughput	Increase of number of lines	Current throughput and during increase of throughput	High intensity throughput and future extream scenarios
Roadmap placement	Present	Mid future	Near future and mid future	Far future
Parallelization method	*B2 and B3*	*B2*	*B1*	*B3 and B4*
Bottleneck well plate capacity	160 well plates ; 1330 well plates	160 well plates	44 well plates	1330 well plates ; 2000 well plates
Composition	Central	Angular	Vertical	Parallel
Level of automation	Process either fully automated or with transitioning between modules done by the technician	Process fully automated with	Process fully automated	Process either fully automated or with transitioning between modules done by the technician
Modularity and use	*Splitting modularity in a combination of steps* The process is split and different steps are combined between each other, 4 different modules to be interconnected and combined between each other	*Not splitting modularity* The system integrates within itself all the devices necessary for the system; If the modularity has to be increased the devices within the system would need to increase in size or the entire system should be replicated	*Plitting modularity per step:* The system integrates within itself all the devices necessary for the system; If the modularity has to be increased the devices within the system would be increased in number	*Splitting modularity per step:* Each section is a combination of the modules, the increase of steps is obtained by an increase of singular modules

Figure 78: Combination of principles for four different concepts

The possible concepts

Four different concepts were the result of the process of combination of ideation and principles (Figures 76 - 77). The development of the concepts was to a level of detail that would allow to visualize possibility of volume incrementation, mode of use and interactions.

Since the combinations of ideas was forced through the morphological chart some proposals of slightly unrealistical detail were intentionally integrated in order to allow discussion and critical evaluation with the technitians and experts of the laboratory also on out-of-the-box ideas.

In this way, even if creative ideas didn't originate from the partecipants, it was possible to collect their view and optinion on these elements.

In order to create the concepts efficiently, the development mainly came from sketching and comparison of relative dimensions of components in CAD modelling.

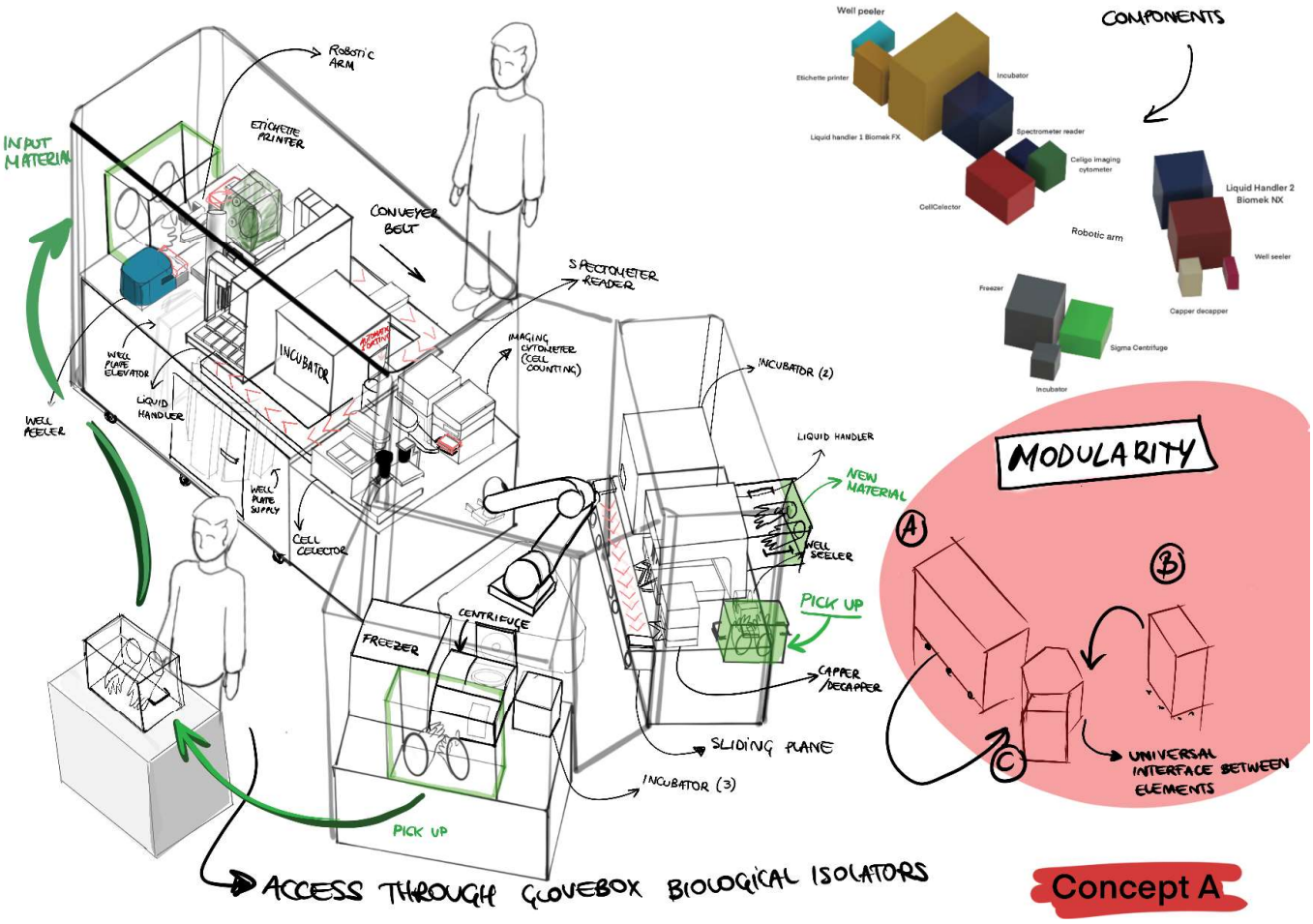


Figure 79: Concept A, focus on modularity

Concept A

The sistem consists of three modules, organized by combining different steps together: the modules use the same interface between each other in order to be potentially rearranged with an additional fourth module that is able to connect the overall process flow.
The technitian performs the first step of the process in a separate area of the laboratory, coating the wells and preparing the patient derived cells for treatment, and later supplies it to the system through biological isolators. These isolators, once attached to the machine enclosure, open automatically towards the module.
Each module is completely separated from the rest of the system, if there is contamination of the wells in one module this can be isolated and cleaned without spreading the contamination to other parts of the system.
The flow of the process is positioned on the higher level of the machine, at arms reach, while the storage compatment is situated below the devices.
Two small robotic arms and a conveyor belt in the reprogramming module; one bigger robotic arm in the merging module, also used for transportation of wells in the freezing module and one sliding plane in the expansion module, allow the transportation of the well plates through the process.
The techitian can monitor the process by looking the pictures that are sent and recorded on the office screen. The sides of the device are transparent, allowing the technitian to look inside the system.

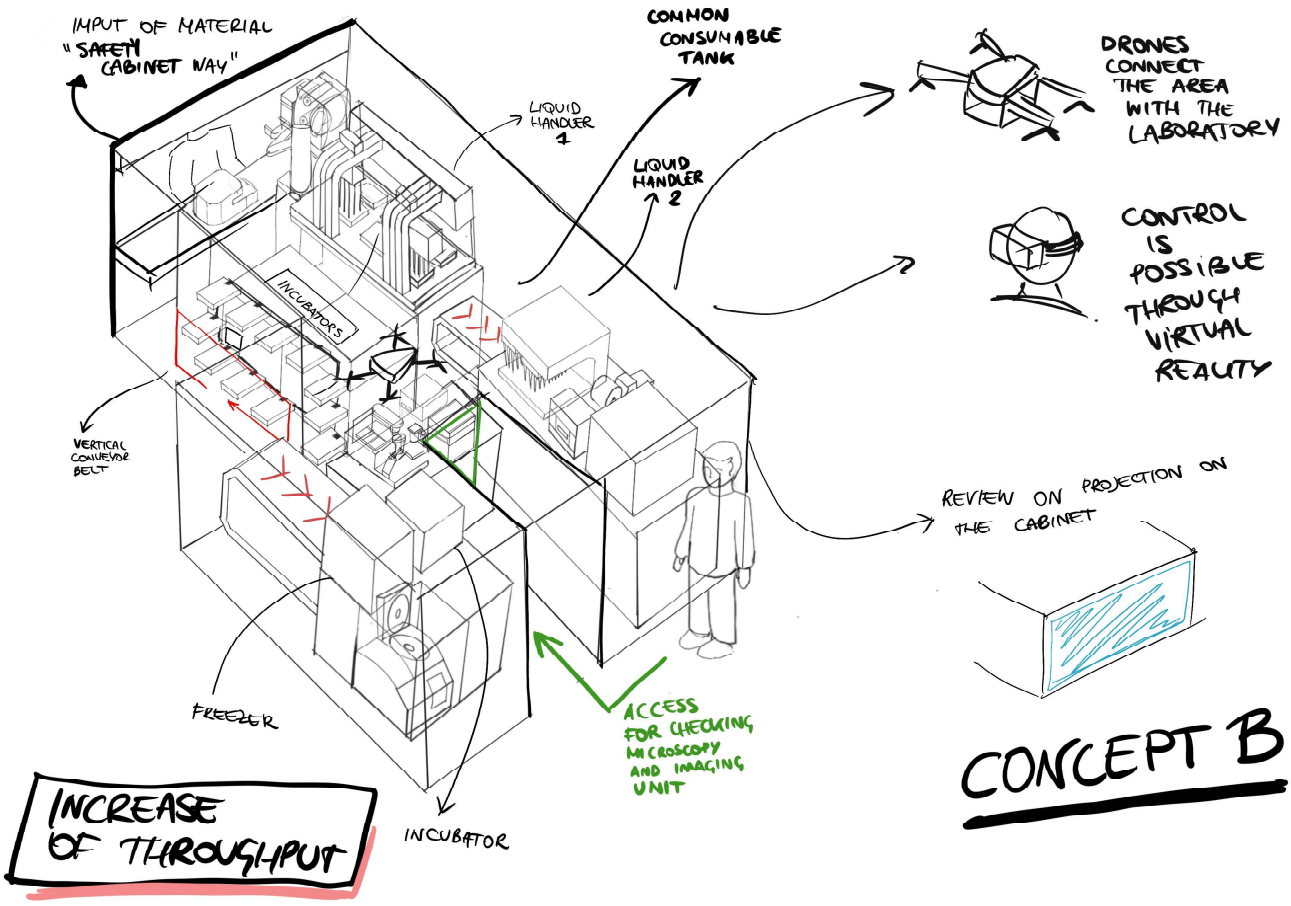


Figure 80: Concept B, focus on possibility to increase throughput for the mid future

Concept B

All the devices are placed into one module that combines the necessary devices for the entire reprogramming process.

The technitian can prepare and insert the well plates inside the device through an entrance that resembles the same functioning of a safety cabinet. From here on the wells are transported by a robotic arm first, a carousel system transports the well plates from the liquid handling and the incubator sections. The imaging section is central to the system, since it has to be close to all liquid handling devices and incubators. A small drone continues the flow of the well plates to the freezing or expansion sections of the module.

The technitian can access the imaging section through a corridor created by the separation between the freezing and the expansion sections of the module.

Data regarding the process is projected onto the side of the module, in this way an overview of the current status of the process is allowed also while being distant from the machine in the newighbouring rooms of the Lab. Control and schedling is possible through Virtual Reality, in this way 4D virtualization and the embodiment of digital twin technologies allow to foresee, with necessary margins, the future quantities of production and adjust the techitian's workload and supply accordingly.

This disposition allows the use of common incubators and liquid handlings through the wprkflow, enabling optimization of spaces and reduction of costs.

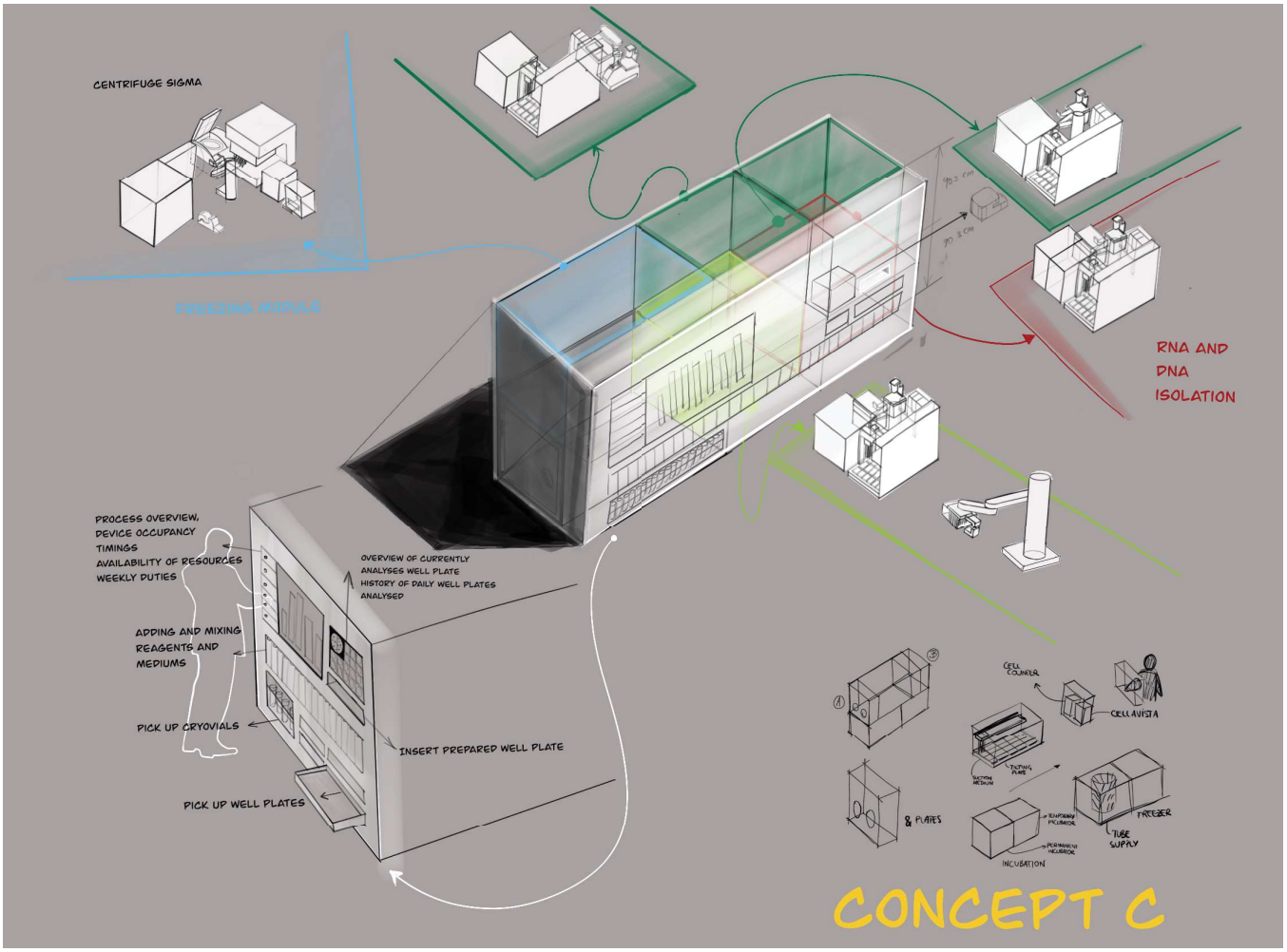


Figure 81: Concept C focus on reducing footprint

Concept C

This concept is based on the reduction of the overall footprint by stacking several modules, embedding each all the necessary devices for each step, onto each other.
Transportation of the well plates is allowed by one robotic arm in each module, for the transportations to be done for each step, while by one well plate elevator and two well shuttles for the transportation of plates through the different steps.
All the interactions with the system are allowed through the frontal pannel which integrates: A pick up section, a overal system control pannel, a well pick up tray and a well insertion shelf.
The preparation of the well plates to be inserted happens in the neighbouring laboratory rooms that are supplied also with biosafety cabinets. Continuous air flow separates the internal areas of the robot from the external environment at the opening for well plate insertion.
A well peeler is positioned close to the entrance of well plates.
Since each module is able to do one step of the process independently it is possible to increase the number of modules gradually and automatize the progess in a gradual manner still using mostly the manual methods initially.

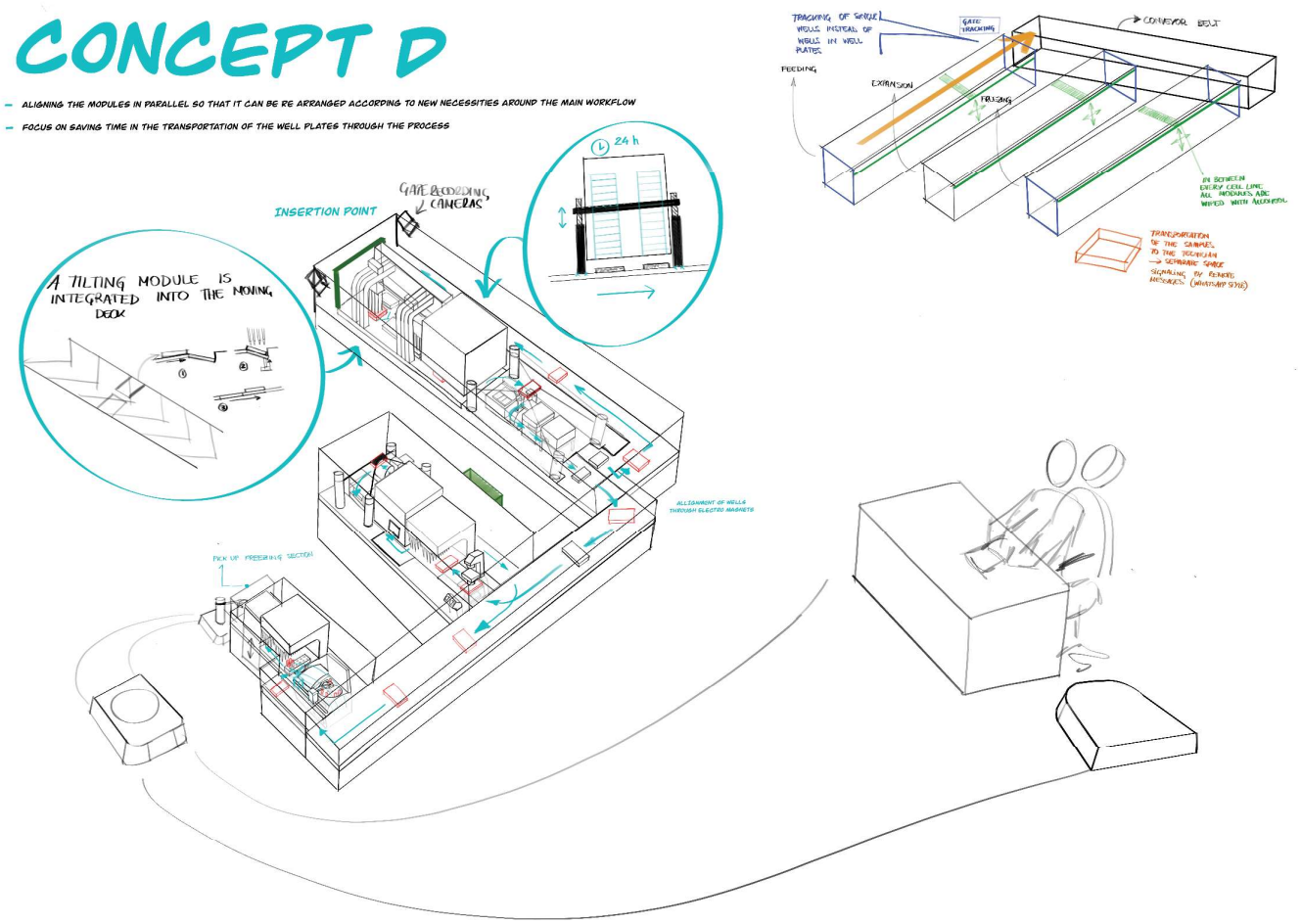


Figure 82: Concept D focus on increase of throughput for the far future

Concept D

Modules are the result of combined steps of the process and they are positioned in parallel, all connected by a bidirectional central carriadge line, on which the directioning of the well plates is organized by electromagnets.
The transfer of well plates from one device to the other is allowed by modification of the devices in order to make their functioning compatible with the conveyor belt: the incubator releases the wanted well plate in a vertical direction, the deck of the liquid handling device is a conveyor belt itself speeding up the process. The cartesian method of directioning of material (the same used in 3-d printers) is used for the transportation of well plates and tubes to imaging devices and to centrifuges and freezers.
Material and consumables necessary for the steps are contained in the space underneath the devices.
Cobots and automated robots bring the final results directly at the table of the laboratpry technitians that do not have to visit the system room but can continue with their work.

5.3 Evaluation and correction

This chapter explains the outcome of the evaluation of the initial concepts previously described through feedback coming from the laboratory technitians and the facility manager and director.
After gathering feedback the concepts were evaluated through the method of weighted criteria and the advantages of several concepts were merged within the better scoring one in order to bring it further in development and detailing.

Feedback from the technitians
One creative session has been organized with the technitians of the laboratory (Figure 83). During the session, to which three technitians partecipated, after giving an introduction to the project and each of the concepts the technitians rated the level of risk that each point of the process has, compared with the current situaion of manual processing. In this case, risk meant diminished control or trust in the correct handling of the cell cultivation by the machine. At each identified risk a way of solving or mimimising it was discussed. Technitians presented to each other different ideas and opinions which gave the trigger to new qualitative evaluation and discussion.

Due to the busy skedules of the technitians and of the restrictions to the number of people working at the same time at the facility at the moment of the session only two of the concepts were discussed and analysed (concepts A and B). The observations made on these two concepts can be extended in some aspects to the other two concepts as well.

Feedback from experts
A later on consultation done this time on concepts (A, B and D) first with the Facility manager and then also with the department director gave additional notes on adjustments to be done on a functional level and on further needs of the Facility. in terms of people operating the system, their possibility of management of the system and which parallelization option should be considered at this stage of the development of the project.

Takeaways from the feedback sessions:

Functional aspects:

1. Thanks to her additional background knowledge the Facily manager was able to evaluate that the devices embedded in the expansion module in Concept A could be also used for the process of differentiation (different from the differentiation *step* described in the process of iPSC reprogramming). Because of this, each of the elements of the system could be more usefull not only if their modularity would allow a different disposition each time, but also if modules are completely independant between each other, allowing use also for other practices of the facility other then iPSC reprogramming. Therefore the expansion module of Concept A should integrate also an additional inverted microscope and the freezing module should have an additional small liquid handling system.
2. An other tool that can be used instead of the incubator for the quick incubating time of the freezing, bulk passaging and differentiating steps is a plate warmer, Figure 84, which will allow reduction of costs and can still achieve the same culture quality. For such a short period of time, in fact, the different CO2 percentage doesn't have great impact on the cells.
3. A biological isolator, althrough providing maximal avoidance of contamination,

Figure 83: Creative imput session with technitians

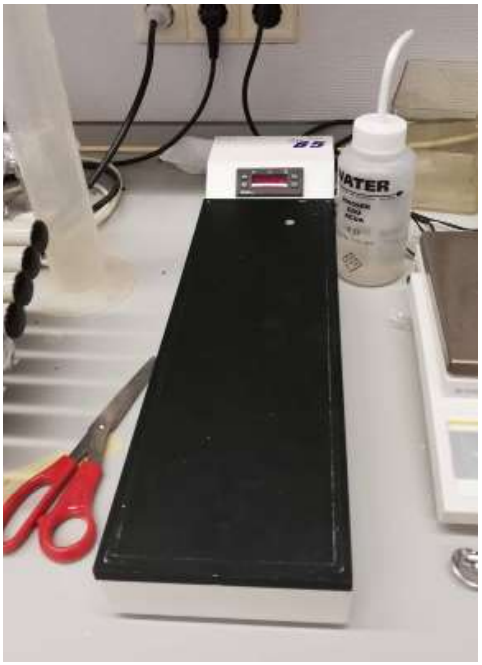


Figure 84: Plate warmer

is not necessary in this case. Trying to integrate the level of isolation given from a safety cabinet can be considered enough.

4. In order to minimize the space occupied by the system Concept A is also preferred because it allows to have one module per different room of the Facility.
5. Full automation of the system is still not required because one technitian is envisioned to be transporting plates from one module to the next.
6. The physical check on the wells through the integrated inverted microscope of ALS Cellavista is a whish.
7. considering that every element that enters the system is clean, there should not be cleaning of surfaces, (no need for wiping as described by Concept D). However, liquids present through the tubing of the system should be discarded and the tubing should be washed after each cleaning.

Usability, risk avoidance and control aspects

1. Before inserting a new line in the machine, there should be a screen showing an overview of the disponibility of each section of the system. In this way the cell line could be postponed by the technitian directly and not wait inside the incubators of the system, causing further delay.
2. An additional lentrance to the system could be added, close to the imaging section. In some cases it could be handy to have an additional imaging step before starting the process.
3. Glovebox biological isolators could be implemented as a way of sterilization of the tools used in the automated system.
4. As a way of ensuring at least a daily check of the system, this should be an obligated step for technitians to be able to visualize their following daily tasks.
5. Mobile notifications should be used for request of intervention.
6. Transport systems such as robotic arms and conveyer belts are preferred over the sliding planes or methods that could cause more internal movement of air

(such as drones). In fact, even if all the material entering the system is sterilized, the risk of crosscontamination between wells through spillage of medium seemed to worry slightly the technitians.

7. In order to keep the technitians aware of the current steps for each cell line and the quality of the process there could be necessary physical steps.
8. The possibility of sharing tanks for consumables, incubators and liquid handlers could reduce the costs of the devices, this would bring an over the modularity for combined steps.
9. Tracking through the system doesn't have to be well based but only relative to the plates. if one plate is contaminated the entire plate should be revised by the technitian before going further.

Evaluation

Method

From the discussions and observations, Concept A seems more in line with the wishes and needs of the iPS Core Facility. In order to confirm this theory, the "Weighted Objectives" (Roozenburg, 1995) graiding method was used to compare them.

The list of requirements of the system is mostly relative to the functions of the chosen components, which are quite fixed as well as their usage.

At the same time, the concepts have been designed following also the requirements derived from the Process Analysis of the reprogramming method. Therefore, the concepts don't differ much in their ability to satify general brief and preformance requirements.

The criteria according to which the decision is made are coming from the following points of interest:

A. The degree to which the system concept allows feasibly of implementation of automation in the iPS Core facility. This is the main requirement for this project to exist and is composed by the possibility of the system to fit in the iPS Core Facility spaces, and by the possibility of use of off-the-shelf components with minimal changes.
B. The initial reasons stated as motivations for the iPS Core Facility to engage into automation (Chapter 1.2.1)
C. The elements deciding uppon the system's layout (Chapter 3.4)
D. The elements allowing the identified human-machine interaction (derived from the Process Analysis, Chapter 2.2.4)
E. The degree to which the concept allows flexibility and implementation of new tecnologies (Chapter 3.3)

Different weights were assigned to the points of interest unevenly for a total weight score of 100. The Concepts were then evaluated for each point giving a grade from 1 to 5, in which 5 was the maximum score.

During evaluation of concepts it has been decided to create an additional variation to Concept A, Concept A2, in which the modules would appear to be separated between each other instead of connected.

This, in fact could lead to better placement and management of the modules within the facility and allow to section the module in different operative units. When one unit is not used at a given time of the process, it could be possible to use it for other similar processes.

	Criteria	Weight	Concept A1	Concept A2	Concept B	Concept C	Concept D
IPS Facility reasons to invest in automation	The system facilitates the possibilities of *future increase of throughput* for the production of iPSC lines within the facility	15	75	75	75	75	75
	The system facilitates the possibility of having *assay reproducibility, facilitating documentation*	10	50	50	50	50	50
	The system facilitates *time efficiency* on cell line production	10	40	20	**50**	30	50
	The system allows to reduce the work load of the technitians for derivation of IPS lines and therefore refuce the ratio between number of technitians and number of lines	15	75	**75**	30	60	45
Usability	The system takes measures to *avoid the control problem*	15	60	60	**75**	45	15
	The system facilitates the *integration of automated and non automated steps* of the process,enabling a clear cooperation between technitians and machine	8	32	32	**40**	24	16
	The system enables to have *control over disruptions* due to parallelization	2	8	**10**	6	6	4
Connection between integrated devices	In the connection between devices the system allows to have the *lowest level of contamination* from contact with the external environment and between well plates	1	**5**	3	**5**	**5**	**5**
	The system allows for *efficient transportation of well plates*	1	3	1	4	5	2
The systems structure facilitates the implementation of automation at the iPS Core Facility in the present (costs and space)		15	45	**75**	75	45	15
The system facilitates flexibility for future horizons and adoption of new methods		8	32	**40**	24	32	40
		100	**425**	**441**	**434**	**377**	**317**

Figure 85: scoring of the different concepts

Results

The concept to develop in higher detail is the A2, merging the benefits of concept B (Physical access to the microscope and possible implementation of 4D virtualization in the future) and C (one pannel for all the informations on the process).

Chapter 6

Final design

This chapter describes the final concept proposed for the IPS Facility at Erasmus MC. This concept proposes the subdivision of the process in three different modules that are detached from one another. The connection between the modules would be done by a laboratory technician that is also responsible for the maintenance of the machine, its supply of materials, and control.

The control over the process is allowed by converging the points of input, and storage of data through the main control software. The data collected will allow in the future to implement 4D virtualization.

The system considers the possible problem of the detachment of responsibility for the process by the technicians in a fully automated system and, therefore, stimulates inter-collaboration of technicians for easier and quicker steps of the process.

The modules consist of a maintenance module used during the initial reprogramming phases and mechanical passaging; an expansion unit that takes over the steps relative to bulk passaging, and its feeding steps, and the final unit used for freezing. The following chapter goes into further detail on the characteristics of each module (Steps, Flow organization, interactions with the machine), overview on their embedded devices.

In this chapter:

6.1 Additional improvements on the final concept

The evaluation of the proposed four concepts identified several aspects that needed further development and modification. The design was, therefore, further improved and adjusted upon check-up with device producers, the facility management, and technicians, for giving a better fit to the day to day device's users.

Liquid handlers

Following correspondence with the manufacturing company Beckman Coulter, the liquid handlers FX and NX initially identified, have been replaced with their corresponding newest versions Biomek i7 and Biomek i5. The main advantage of these devices is a spatial open-platform design that enables access from all sides and therefore integration with adjacent-to-deck and off-deck processing devices.

These models also have the possibility of integrating a status light bar that signals the instruments current mode from a distance, it integrates an internal LED light shining on the deck of the instrument for better monitoring of the workspace status and rotating grippers that can, therefore, access in an optimized way all the areas of the deck. Multichannel pipetting heads of the new model are suitable also for large volume liquid handling.

Medium storage

Initially, each module was identified to have several mediums for liquid supply. However, by further discussion with the process experts, it has been pointed out that the reagents that are supplied through the process are too precious and expensive for being stored in a liquid tank: the ratio between the amounts that would need to be supplied and the amounts that would need to lay in the tubing interconnections with the tanks makes it preferable to avoid this typology of supply.

The steps of feeding with expensive reagents would, therefore, need to be done manually while the supply of the general growth medium is allowed through a tank with 5 liters of capacity.

Figure X describes the different liquids that are needed for each unit, however, each unit will be supplied only of two tanks, one for the general growth feeder and one for the waste.

Control panel

Both chosen concepts saw the integration of displays inside the frontal panel of the device. From the initial observation, it was visible that, although being quick and easy steps, the process of plate preparation is usually done sitting behind the safety cabinet on slightly higher chairs or stools. For this reason, the control panel could be readapted to be used in a sitting position and it was merged with an operator's position, offering also a laptop-interaction with the software.

This would both align with the current interface, resulting in a natural transition, as well as avoid needing additional costs.

One only insertion point

During the development of the concept, a suggestion was made from the technicians on the possibility of adding a lateral insertion point to the reprogramming module, in order to be able to check with the inverted microscope integrated into the ASL-CellCelector device the initial state of the harvested cells before initiating the process.

However, upon contact with the ASL-CellCelector producer, it was checked that enabling the inverted microscope to be used also by the technicians physically would need an additional eyepiece to be added to the system and it would obstruct the typically automated components around the microscope and would, therefore, create

additional issues for integration. Because of this and because of the additional possibility for placement of the module against a wall, the integration of the eyepiece was omitted in the further development of the concept. In order to check the status of the plate, the technician would need to still insert the well plate at the working bench and select from the control panel to skip all the liquid handling steps, bringing the well plate to the ASL-CellCelector and, only after this step is confirmed, continue further including the liquid handling steps.

Refinement on the choice of the freezing module and the centrifuge

The automated freezer selected through the initial concept design phase was suitable in terms of capacity, however not for what concerns automation. Additional systems for tracking would be necessary as well and the door of the freezer would need to be redesigned in order to enable retrieval of tubes. A further investigation of the freezing systems was done, this time focusing on automated freezers. The Hamilton SAM HD system was selected from this further research since it follows the temperature requirements of the process (-80 °C). However, this system should be taken as an example because of its higher capacity. This point will be further discussed in the further challenges chapter.

Pick up points at the reprogramming module

The reprogramming module liquid handler can host up to 24 deck positions, therefore the initial proposition had 24 release points for the pick up of the well plates. Upon review with the facility manager, 12 pick up points seemed to be sufficient, for the initial phases of transition to automation. Reducing the number of pick up points would allow having a more clear interface.

Choice of centrifuge

Upon contact with Sigma-zentrifugen and their vendors in the Netherlands (Salm en Kipp), the selection of the centrifuge became more specific, the final selection came to the centrifuge Sigma 4-5KRL (91309) with buckets 91319, 91318 rotor, custom adapter and sticks for the integration of the adapter to the robot. This was possible by sharing with the company the process overview and details on the transfers of the content of the plates in Eppendorf tubes (with permission from Erasmus MC).

The loading of the centrifuge can be done through single loading of the tubes in the centrifuge as well as by fitting the tubes in the adapter initially and then loading the adapter directly in the centrifuge all together. The choice of modality influences the way the robot is designed in order to interact with the tubes and the centrifuge, which will

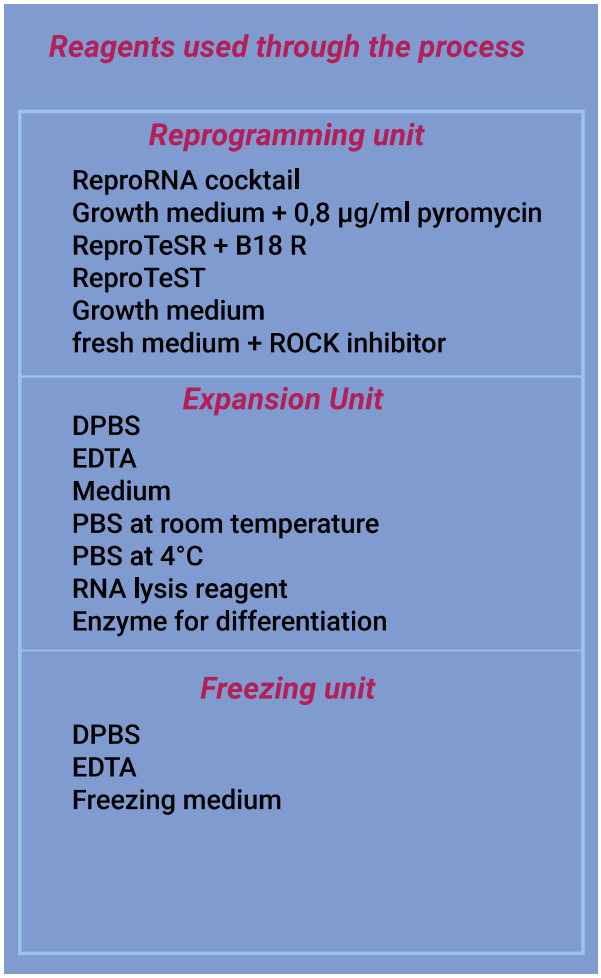


Figure 86: In each module different reagents would be used, onlu the growth medium or generically called medium or fresh medium is stored in the tanks.

be discussed again in the future challenges chapter.

Correction of the imaging devices used
As previously described, during the evaluation of concepts, it was decided to take into account also a second variation of Concept A in which the three modules are separated between each other.

While in the previous concepts the units were connected and could, therefore, share between each other the imaging devices, in this other alternative, functionalities of the microscopy sections would need to be independent.

For this reason, further analysis of the imaging needs for each unit was conducted. During this phase, the initially chosen devices have been reviewed once again.

Cell control for the reprogramming module:

The reprogramming module contains the needs of Microscopy A (for size, morphology, and turbidity control; used during mechanical passaging) and Microscopy B (checking attachment of cells, size, confluency, and turbidity; used at the end and the start of feeding).

For these functions, the chosen devices have been the Cell Celector, for size and morphology, the Fluostar Omega, for turbidity, and the Celigo imaging Cytometer automated imager for cell attachment of cells and density. All the devices seemed still to fulfill the functions but, with respect to confluency, further discussion is done. In fact, this property is quite specific and should not be confused with density:

Confluency: In the language used in the biological context, confluency is the measurement of the percentage of the surface of the well-plate that is covered by cells. This term should be preferred to the term “density”, which may lead to miss-understandings.

In the opinion of Mehrnaz Ghazvini, at the moment of automation, the measurement of confluency should be done differently than how it is done nowadays (through estimation by the experience of the technician). Several options can be explored:

1. The device used for the estimation of confluency should be able to take several images of the well plate and connect them all together to be able to count the colonies (and not single cells). Counting single cells, in fact, would give a wrong estimation and would need to be practiced through suspension of the cells, which is not done at this stage of the process because of the need to have the cells organized in colonies. The counting of colonies takes place in the later phase of trypsinization, which consists of a method of detaching the cells from the wells, done through the enzymes added at the steps of differentiation (Step N and Q of the process overview.
2. The device could, perhaps, compare different areas of the well plate and detect the measurement of confluency, by comparing the contrast found in the different areas of the well plate, by translating the information of contrast between the pictures into a measurement of the percentage of the surface of the well plate covered by cells.

The Cell Celector can be used for the measurement of both morphology and confluency, however, although it would be necessary to adopt this typology of the device for the function of picking of cells during mechanical passaging, its is a very expensive machine for the measurement of confluency, therefore it is advised to have a separate device for this purpose as well as for the detection of turbidity. For the purpose of the measurement of confluency, the Celigo Cytometer was initially selected because of

its possibility of detecting the density of the material present in the well plate. However, as here described, density could mean a different indication than confluency since it could indicate the measurement of the density of single cells instead of colonies. The ability of the Celigo Cytometer to perform confluency check as here described should be further investigated with the manufacturers and was assumed for the moment to be implementable into the device.

Cell control for the expansion module:

In the expansion module, the functions to be checked are regarding microscopy B (see above) and microscopy C (checking the size of cells during the steps of bulk passaging)as well as counting of cells. From Mehrnaz Ghazvini's input, at this stage all the above-mentioned functions can be done through a high speed inverted microscope with the ability to determine confluency, morphology of differenciated cells and good cell condition. In fact, at this stage, the counting of cells is of single cells and not colonies. The inverted microscope Nikon Ti2-E microscope has been chosen for this purpose. In fact, as described by Schenk F. W. et al (2016) in *High-speed microscopy of continuously moving cell culture vessels*, this high-speed phase contrast and bright field microscope can be used for scanning and analysis of also larger cell culture dishes without compromisation of image quality and it is used also in the Stem Cell Foundation automated system.

Cell control for the freezing module:

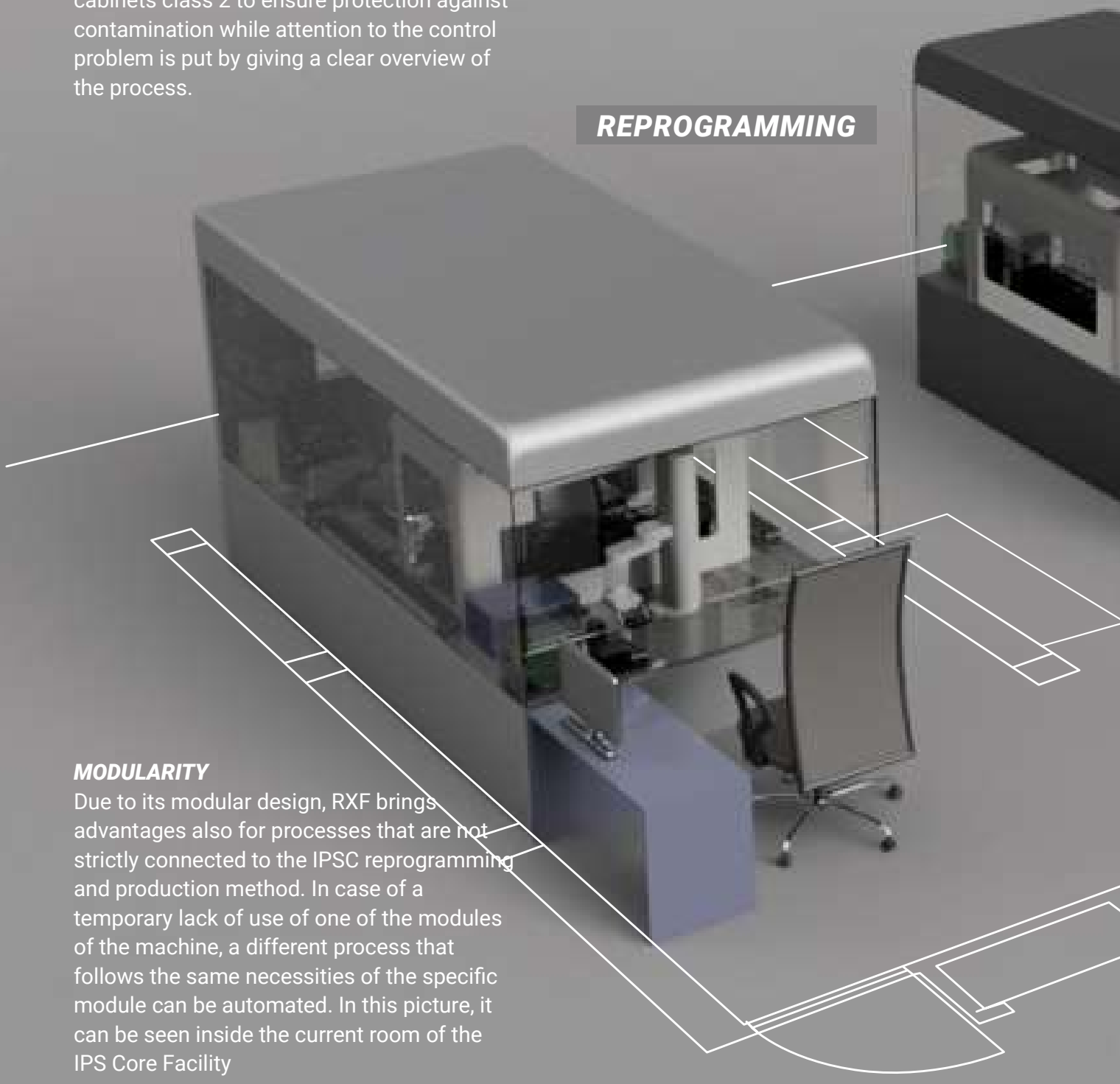
At the steps relative to freezing the cells do not need additional control, therefore no additional device for cell control needs to be added to this module.

Final proposal: The RXF System

RXF is an automated system able to enhance the necessary steps of production of IPSC lines and enable to reach and sustain the production of a total of 1000 lines, the maximum envisioned yearly throughput of the facility. The system is composed of the combination of three modules, each of which is responsible for taking over one segment of the reprogramming process of production.

All the steps are carried out within the safety cabinets class 2 to ensure protection against contamination while attention to the control problem is put by giving a clear overview of the process.

REPROGRAMMING

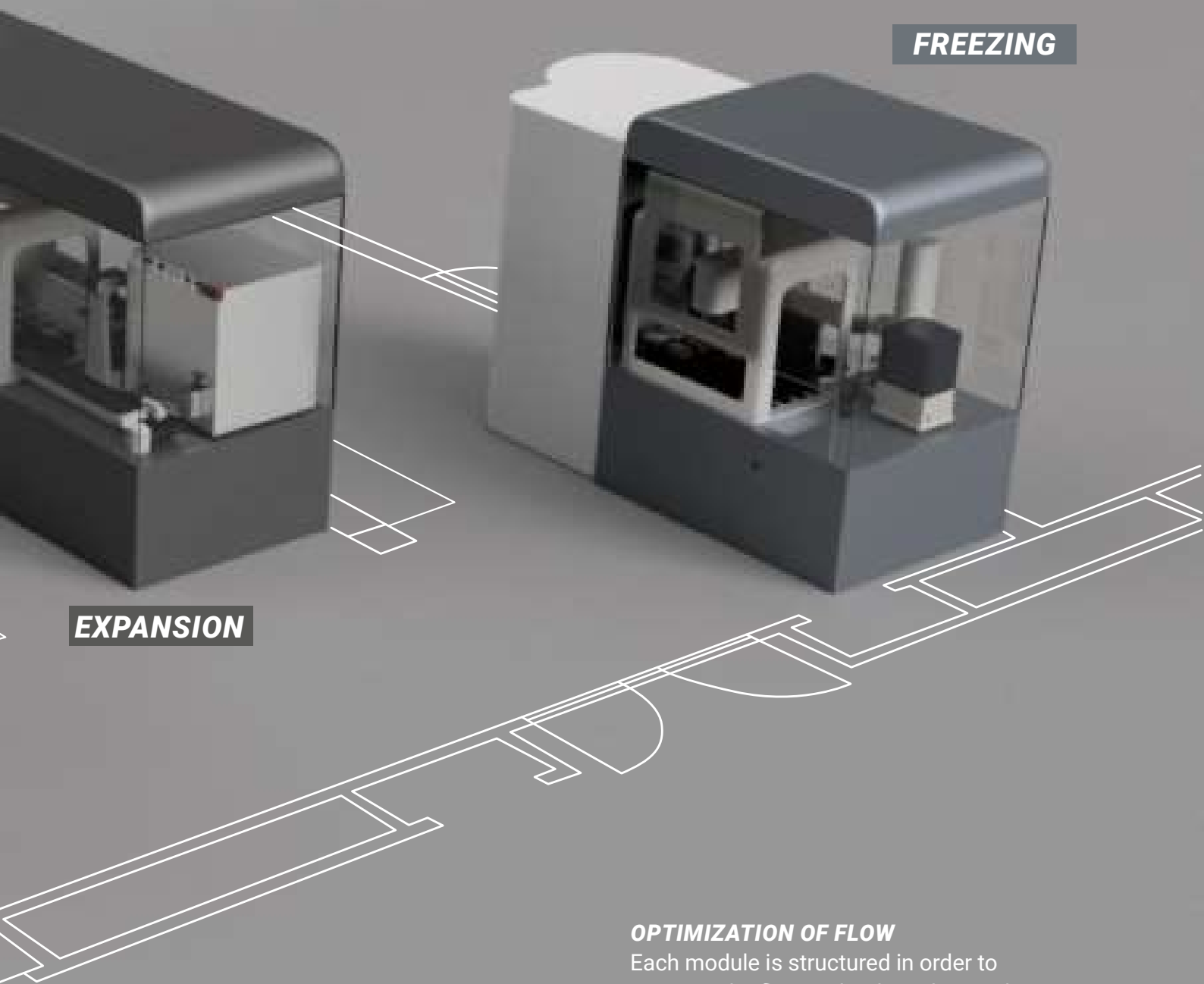


MODULARITY

Due to its modular design, RXF brings advantages also for processes that are not strictly connected to the IPSC reprogramming and production method. In case of a temporary lack of use of one of the modules of the machine, a different process that follows the same necessities of the specific module can be automated. In this picture, it can be seen inside the current room of the IPS Core Facility

ADAPTABILITY TO SPACES AND INCREASE OF THROUGHPUT

Due to the small footprint of each individual module, the system can easily be integrated into the spread architecture of the iPS Facility, allowing for a quick transition towards automation and facilitating future scale-up.



OPTIMIZATION OF FLOW

Each module is structured in order to optimize the flow and reduce the need for additional changes to original devices already present on the market and, therefore, reduce the impact on costs.

The interactions with the RFX system allow efficiency, low effort tasks in a high throughput system and control manintainance.

EFFICIENCY
The RXF system allows to have an overview of the functioning of the modules in one centralized control pannel point, which is also the starting point of the process.
In the X and F modules, the interaction happens through a mobile application, giving the possibility to the technicians of having a quick, standing interaction.

AUTOMATION ONLY OF NECESSARY TASKS
RXF allows to have balance between walk-away time, for technicians to dedicate their time to the analysis of results, and increase of throughput of the facility. In fact, RXF takes over only time consuming tasks while fast steps, such as coating of well plates, that are easily and effortlessly done manually, are still done by the technicians as part of the tasks to do for material supply.

CONTROL
The RXF System allows to have high throughput and still enables the mantainment of control over the process through constant monitoring and interactions.
The system is independent in nearly all the steps of the IPSC production. However, due to the modularity of the design, technicians need to approach the system in order to transport well plates from one module to the other. During this quick and easy step, technicians have the opportunity to gain better track of the lines they are following.
Doubts of evaluation of decision making steps are prompted to the technicians before the next step takes place.

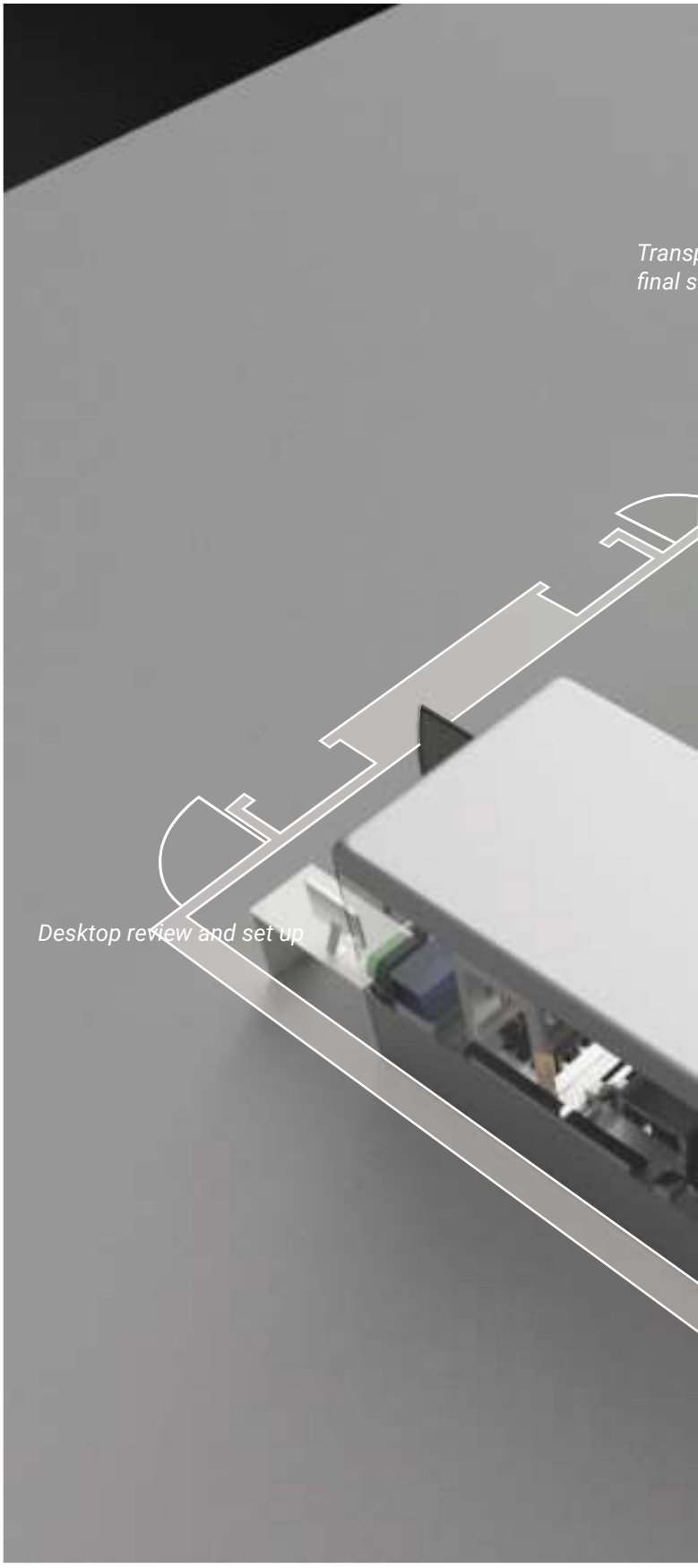
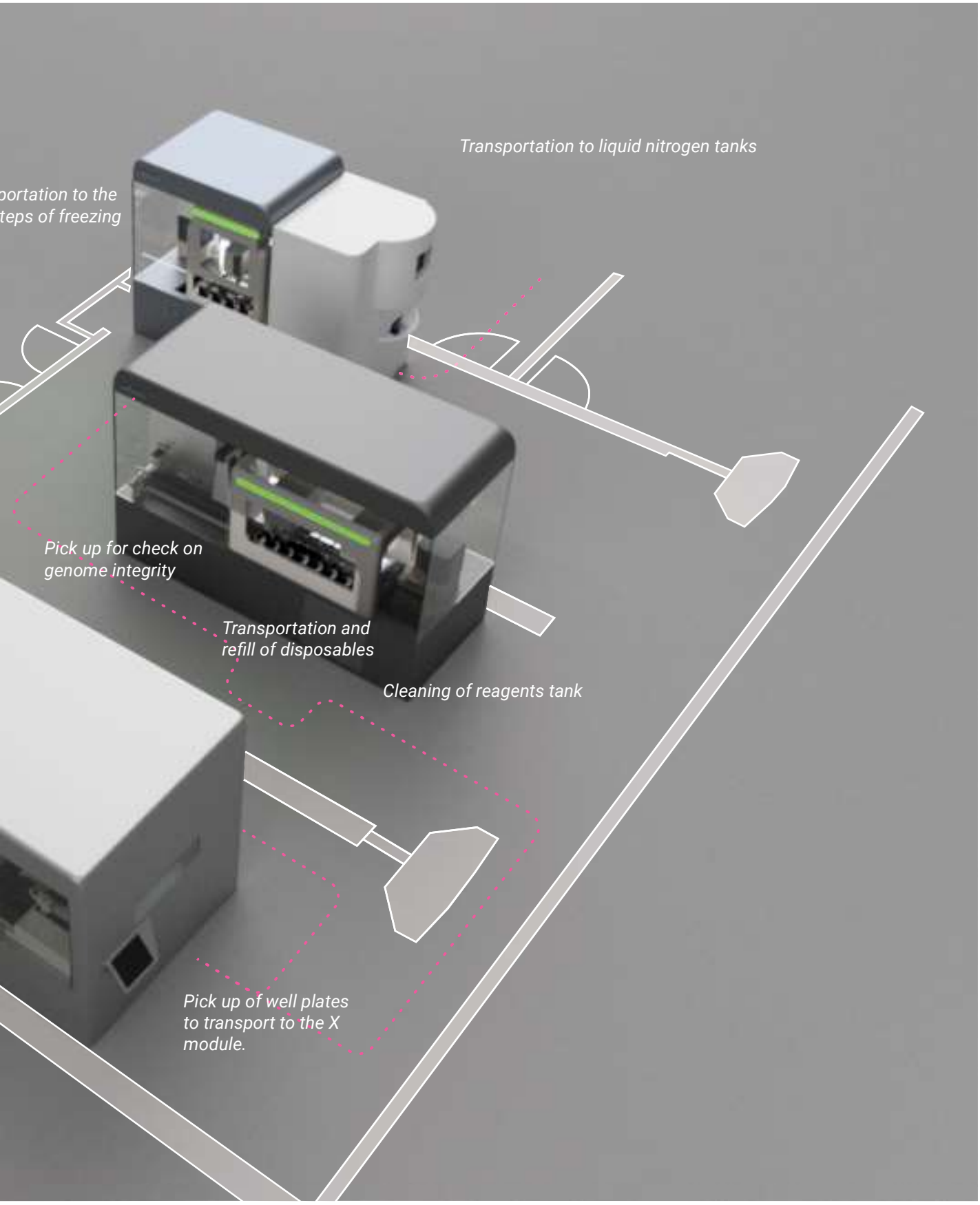


Figure 87: The reprogramming module


portation to the
teps of freezing
Transportation to liquid nitrogen tanks
Pick up for check on
genome integrity
Transportation and
refill of disposables
Cleaning of reagents tank
Pick up of well plates
to transport to the X
module.

The R - Reprogramming module

General description of the R module:
The first module is where all the initial steps of initial reprogramming for the creation of IPSCs and mechanical passaging take place.

Since it is the starting point, all the initial steps of set up of the production are done at this module, and uses, therefore, the intercade of a desktop device.

The module presents three entry points: two in contact with the working desk, at the front and at the rear side of the module, and on the lower side of the module:

- The front side is used to insert the initial biological material and the new coated well plates.
- The rear side is accessed for pick up of the well plates before transportation to the next module or manual intervention of the technician. This second side is also provided with a screen that is used to open and close the double sided doors of the machine, to confirm the state of the cell culture, to show capacities of the next module, supply requests of all three modules and gives the possibility to the operator to check again the tasks to do.
- The lower opening, on the longer side of the module, is used to load material for the liquid handler and pick up and clean the disposable waste trash.

The pick up of the well plates could be done also through the frontal opening of the module but the decision to open the rear side of the module has been taken in order to facilitate possible combination with the other modules in the future.

Due to the disposition of devices in this configuration access to the liquid handler is mediated with a robotic arm, which transports material first to the etichette printer and then to the liquid handler.

The capacities of the devices integrated inside the unit is calculated taking into account that this module is responsable for the steps regarding half of the production process (from day 0 to day 39). Because of this, altough each line uses only one well plate at a time through the steps of this module, the parallelization of the lines still requires medium sized liquid handlers and incubators.

In order to facilitate keeping an eye on the flow and having an overview of the process, devices are placed in a horizontal disposition.

In order to access the internal devices the enclosure will need to be designed with possibility to open its sides.

Figure 88: The reprogramming module

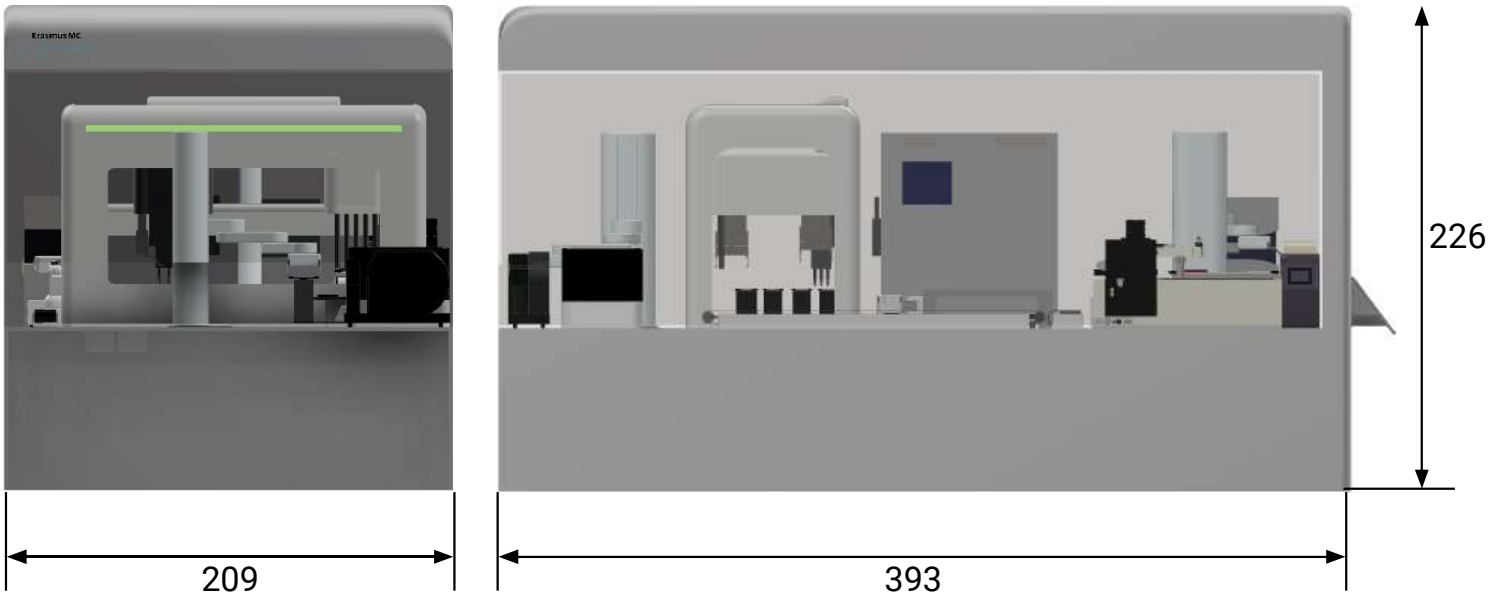
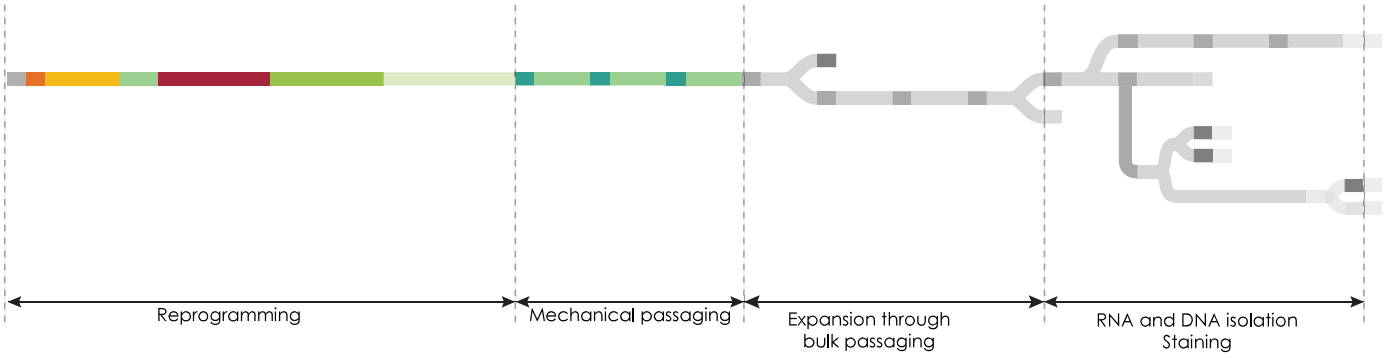


Figure 89: Overall dimentions of the reprogramming module.



Organization of the flow in the reprogramming module.
The disposition of devices within the reprogramming unit has been organized in order to have an optimized flow as described in the following paragraph.

The transportation flow of the steps of feeding and mechanical passaging, taking place in the reprogramming module are reorganized in Figure 90 in order to highlight the connection between the culture control elements (Microscopy A and B), the liquid handling for feeding, and the liquid handling used for picking.

As it can be observed already from this scheme the flow is best organised in a circular manner interconnecting the feeding and the mechanical passaging process without complication of the flow. Mantaining control over this phase of the process, in fact, is crucial, since this is the phase in which the IPSC colonies are formed. As described in Figure 60 of Chapter 04 , the best disposition able to mantain control is the central indirect disposition. Having the culture control elements as necessary steps within the loop will, therefore maximise the flow.

In Figure 91a the previous schematic is simplified by grouping transportations together, while in Figure 91b this can be visualized in the final proposal for the reprogramming module.

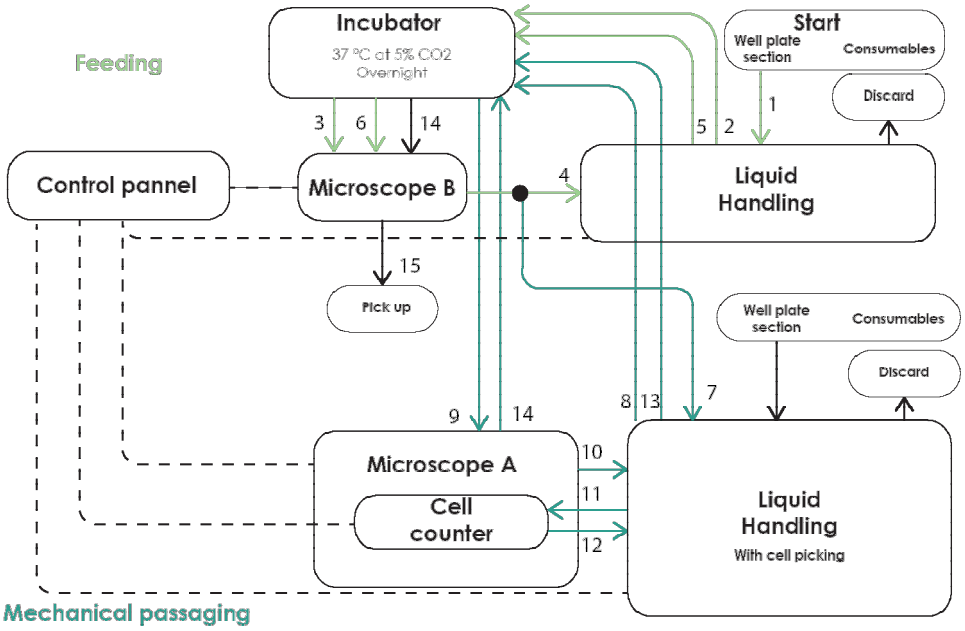


Figure 90: Overall dimentions of the reprogramming module.

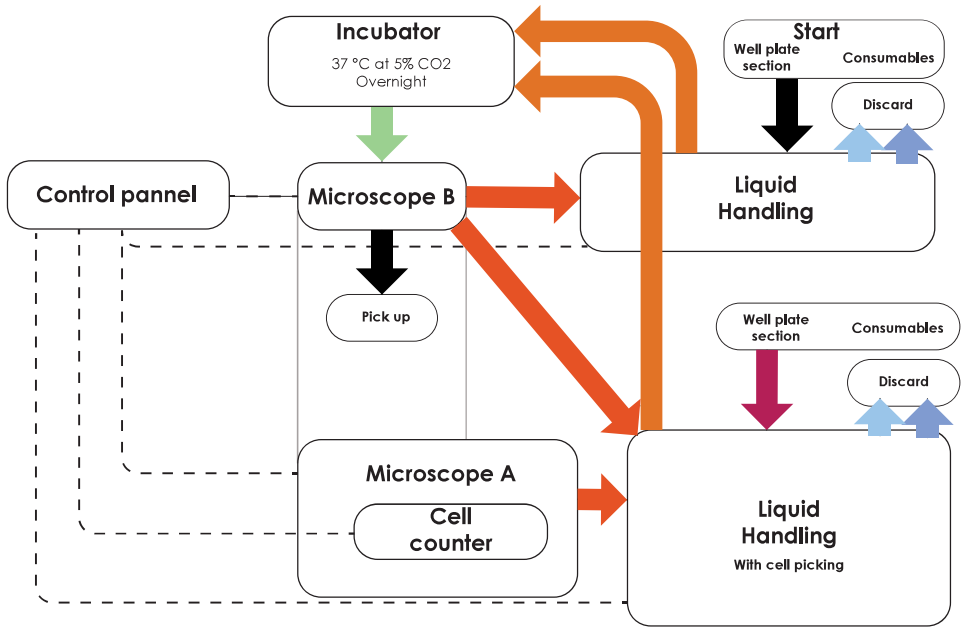


Figure 91a: Grouping of flows

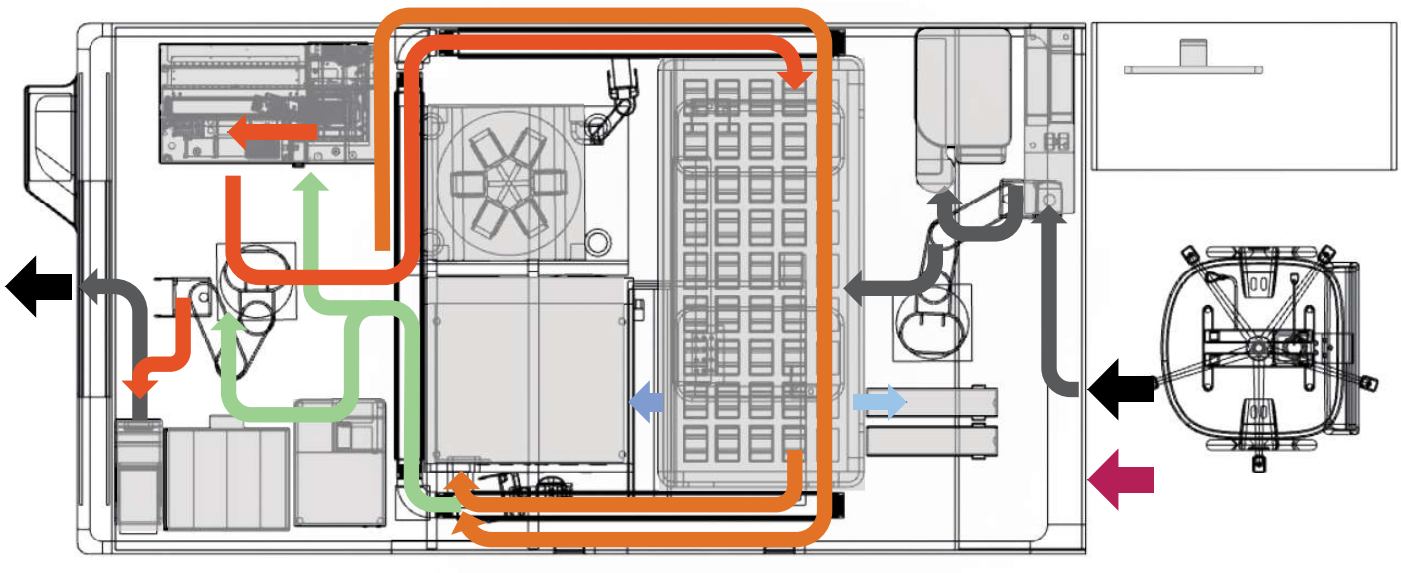
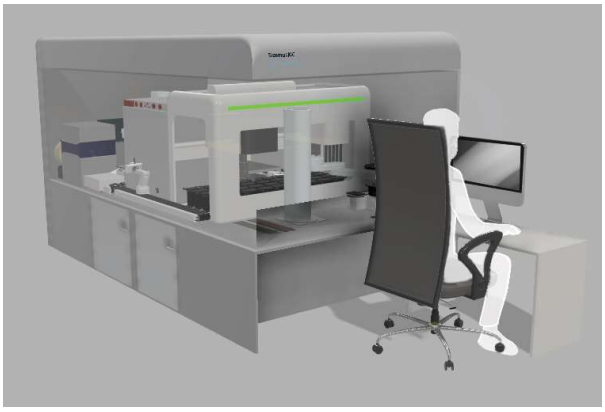
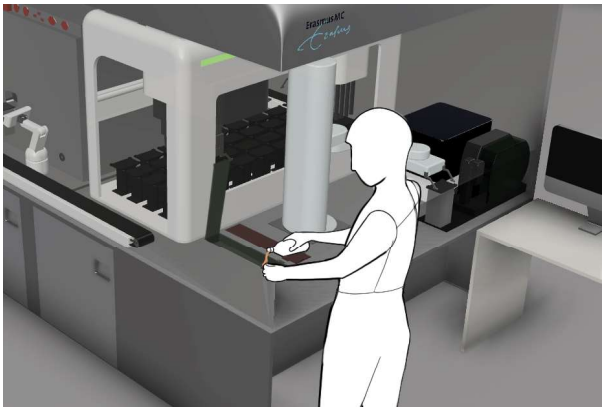


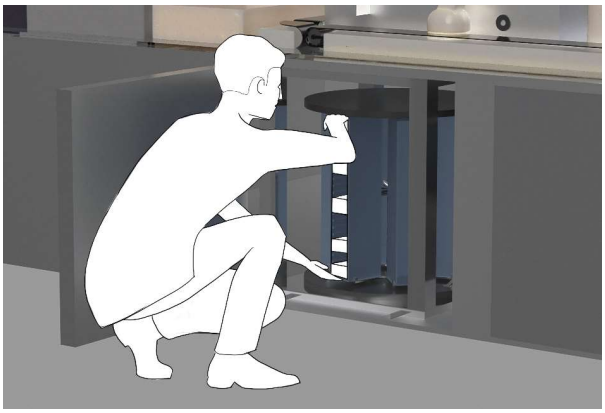
Figure 91b: Overall dimentions of the reprogramming module.



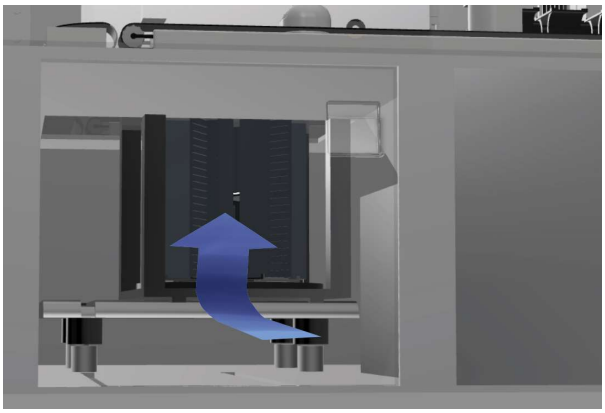
1 ***Set up of the process:***
The production of IPSC lines is done at the computer screen of the control unit, here the laboratory manager sets the needs of the production and receives information on the needs of the automated system.



2 ***Loading feeding reagent***
Once the needs of the machine are given the technician can refill the feeding reagent tank at the front of the module.

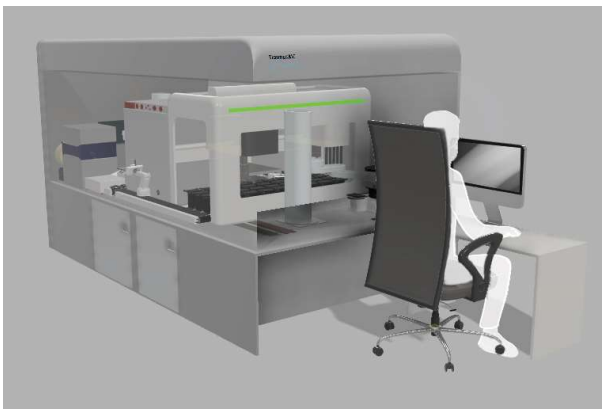


3 ***Add new disposables***
New disposables needed for the process are directly added to the machine through the lateral opening.

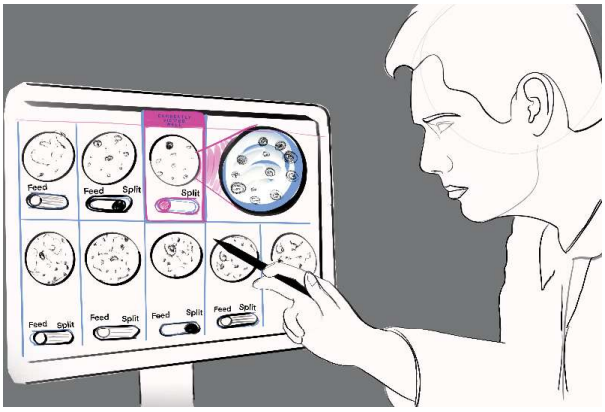


4 ***Automated transport of disposable to loading deck position***
At the closing of the door the automated storing hotel transitions backwards to the initial position, ready to supply the liquid handler.

5 ***Supply of coated well plate***
New disposables needed for the process are directly added to the machine through the lateral opening



6 ***Check up on decision points***
The technician is asked to give instruction on which decision to take regarding the continuation of feeding or transition to mechanical splitting each time the system faces a difficulty in classification.



7 ***Control of currently processed lines***
The technician can also decide to set a daily control of all the immaged wells and set a continuation of the process only when all the decisions are confirmed.

8 ***Management of bacteria infected well plates***
Bacteria infected plates are picked up by the technician who cleans it up manually. At the end of this process, the well plate can be given back to the system.

9 ***Safe pick up of the well plates***
At the moment of pick up, the sealed well plates are left inside the pick up chamber. This prevents the possibility of contaminating factors to enter the enclosure.

10 ***The pick-up***
The technician can interact with the module through the touchscreen at the back of the enclosure. After checking the status of wells, he can open the chamber and pick up the plates.

11 ***Cleaning of the disposable waste***
By access on the side the disposable waste can be taken out and emptied.

12 ***Cleaning of the reagents waste***
At the end of the process the tank for the disposed reagent can be extracted from its position and cleaned.

The X - Expansion module

General description of the X module
The expansion module is responsable for the second half of the production process, except for the steps of freezing.

The steps this module is used for are relative to feeding, bulk passaging, steps in preparation to RNA and DNA isolation and differentiation.

Due to the variety of steps for this section of the process, the number of well plates per line increases, as analysed in Chapter 04. As a consequence of this, the bottleneck of the whole process takes place in the expansion module.
Therefore, although the process is slightly shorter then the one happening in the reprogramming module, the incubator and the liquid handling system are medium sized.

The overall longitudinal architecture of the module facilitates a possible future expansion of the module, in the case in which the current capacity of the system results being tight.

Inside of the structure, the devices are organized between each other in order to take advantage of the principle of the U-Flow, suitable for organizing in a practical manner previous and further handling (Fig. 60, Chapter 04).

With access to the module on one side, and due to its longitudinal shape, the X module is suitable to be placed also in corridors of the Facility. In this case, an additional protection should be added to the safety cabinet's opening.

One important aspect of difference to point out is regarding the liquid handler.
Even if both R module and X module use the same liquid handler with two pipetting heads and medium-high capacity, the Biomek i7, the X module has a different head for one of its arms, the Span 8 pipette head, which allows it to handle liquid handling actions with tubes. and cryo-vials.

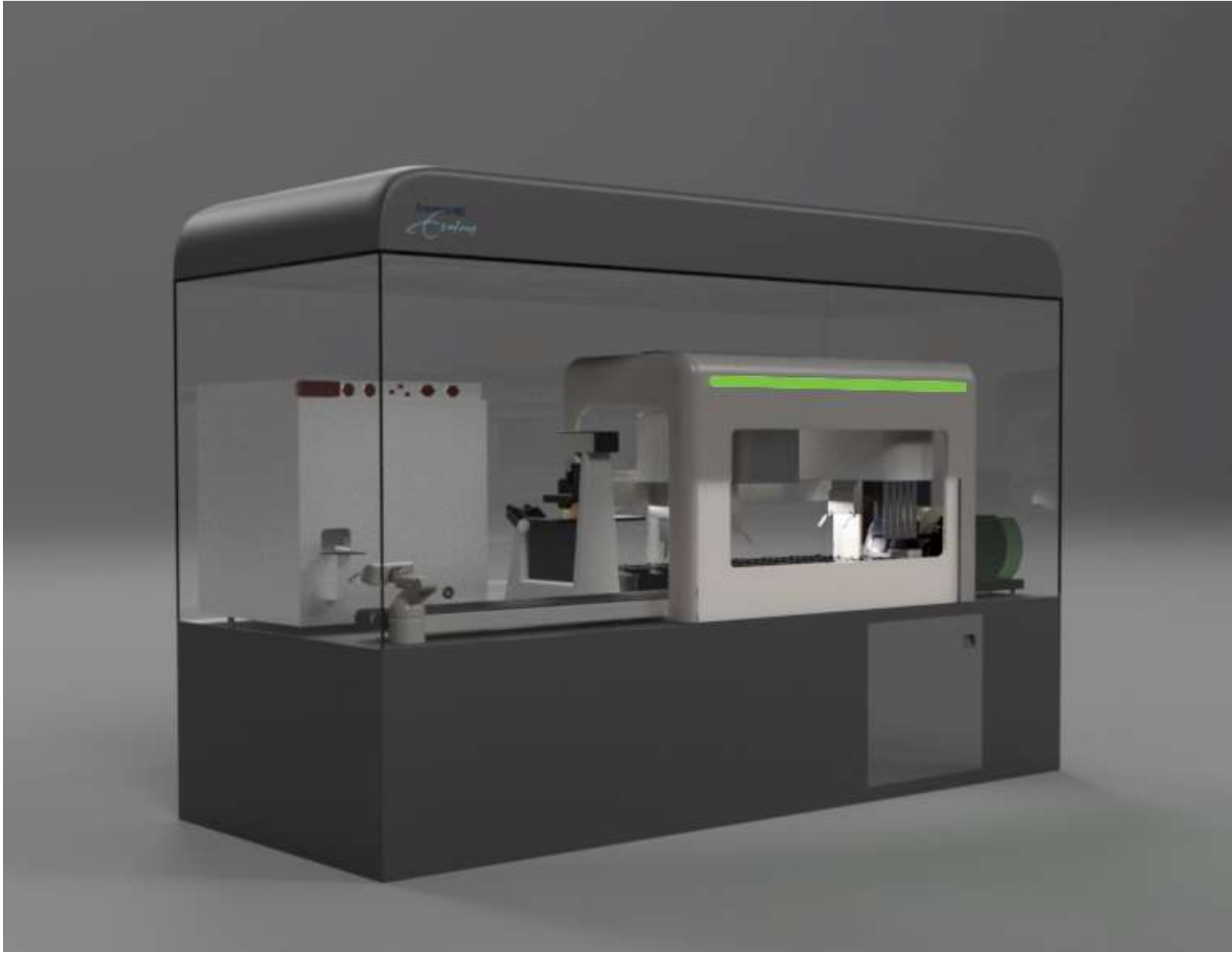


Figure 92: The expansion unit

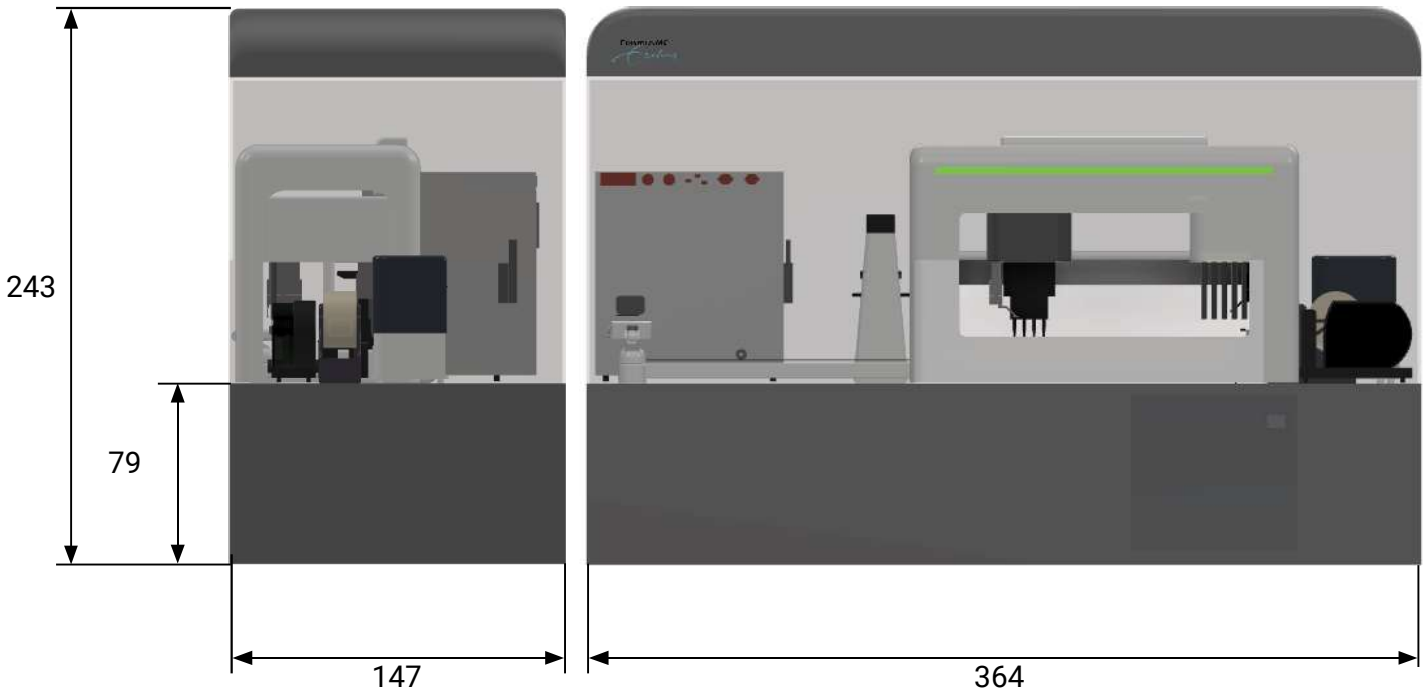


Figure 93: Dimentions of the expansion unit.

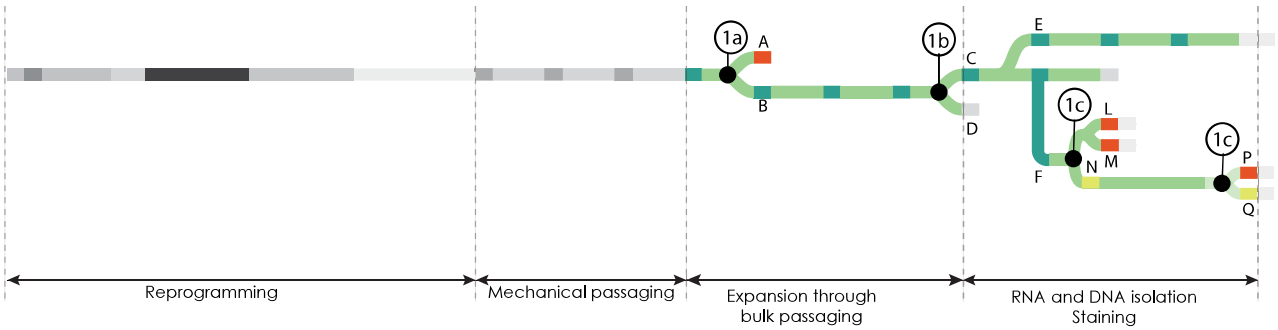


Figure 94: Steps of transition taking place in the expansion unit

Organization of the flow in the X module

The flow chart from Chapter 03 has been rearranged in Figure 95 in order to highlight the transition of well plates from one device to the next specifically for each phase and the process splitting points. In this version of the flowchart, the steps of incubation that last only for 5 minutes have been substituted with a transition on a warming plate.

The path has still an overall circular direction but the different transportations interconnect between each other having as a central point the liquid handling system.

Therefore, considering the disposition of devices in the module in a chronological way could lead to a complicated set up, which, in case of high throughput, could cause to internal jams of the liquid handler or delay in transportation.

As described by Figure 96 the transportation between devices has been , therefore, grouped in transition paths.

Each path is responsible for transportations in the same direction or to the same device: one for transportations towards the incubator (light orange), one for transportation towards the Microscope (green), towards the elements positioned on the deck of the liquid handler (dark orange). Additional flows are added for supply of well plates (black and dark blue) or disposables and for pick up (light blues).

As a result, the X module has a disposition of devices around the deck and in the enclosure which minimizes the transportations to 10 main movements.

The key factor of this solution is the subdivision of the transportations on the deck in two cycles: one for the right liquid handler arm and the other for the left liquid handling arm, each organized as much as possible in circular flows (Figure 97).

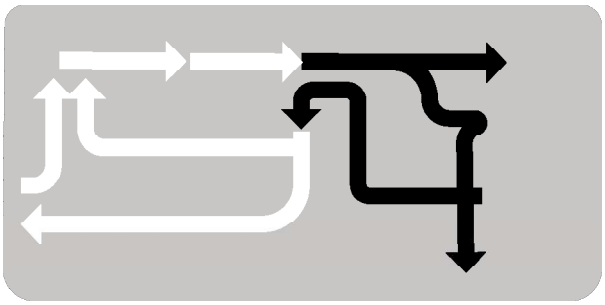


Figure 97: Visualization of the circular flows for each of the grippers in theliquid handler of the expansion module.

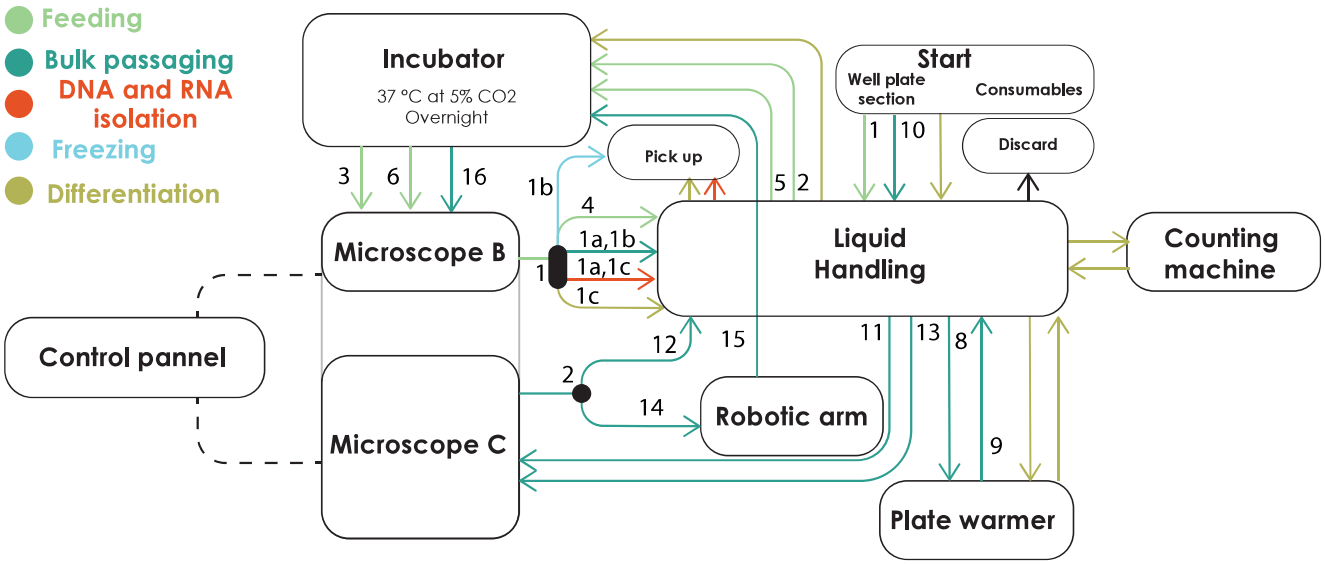


Figure 95 : Steps of transition taking place in the expansion unit

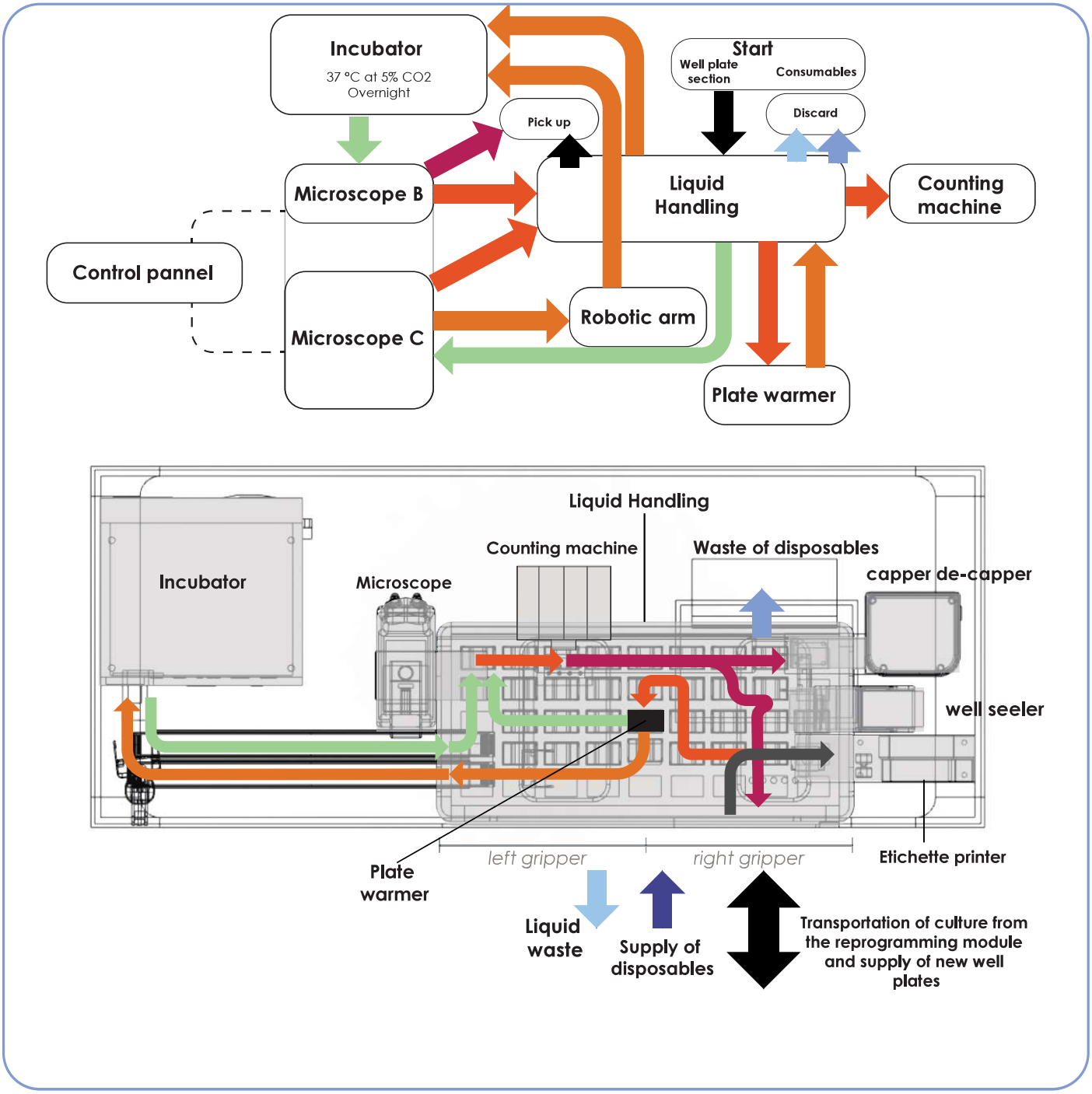


Figure 96: Correspondance between the transportation of steps and flowchart diagram and the unit design.

1 ***Transportation of well plates to the expansion unit***
The technician takes the well plates and transports them to the X module by using wheeled transport table.

2 ***Confirmation of the transport through phone application***
When in front of the machine, the technician can confirm on the app the transport in order to go to the next stage.

3 ***Insertion of well plates and disposables inside of the deck***
The operator can now follow the instructions on the screen in order to load materials inside the deck in the right order.

4 ***Loading of disposables and well plates with cultured cells from the R module***
Once the deck is prepared, the technician can confirm the step and leave the laboratory.

5 ***Control and intervention***
Immaging control is allowed also through the application on the technician's app which also allows for remote control.

6 ***Pick up for genome integrity control***
At the fifth day, well plates are collected for control on genome integrity. The liquid handler temporarely stops and highlights the plates to pick up.

7 ***Inserting results on genome integrity***
Through the application on the phone, or from the central control system platform, the operator can insert the results of the RNA isolation (genome integrity).

8 ***Insertion of new coated well plates***
New coated well plates are inserted directly through the deck of the liquid handler.

9 ***Notification on pick up of well plates***
When well plates have to be picked up light at the top of the liquid handler lights up and blinks. This is notified also in the application.

10 ***Pick up of well plates***
The technician picks up highlighted plates. Destinations are colour coded: blue for freezing, red for RNA and DNA isolation and yellow for differentiation.

11 ***Cleaning of the disposable waste***
By access on the side the disposable waste can be taken out and emptied.

12 ***Cleaning of the reagents waste***
Disposed liquid reagents are placed in a tank in one area of the deck. after picking up the well plates also this tank is replaced with an empty one.

The F - Freezing module

General description of the F module

The freezing module is used only during the freezing steps, which take place for each cell line on two days (step D, on average at day 47, and step O, on average at day 56).

This stage is the final of the process and takes place directly before the material is transported to liquid nitrogen tanks.

Compared to the other two modules the freezing module is a slightly simpler one. In addition to being independant from cell control devices and incubators, its flow of material is one directional.

The main difference with the other modules, furthermore, is that the device doesn't necessitate interactions of technicians additional to machine maintenance and culture transport and pick-up. In fact, the only necessity this module would request help from the technicians for is for eventual disruptions due to malfunctioning.

Since the module is used only for two days of the production process, the capacity needs of the freezing module are smaller then for the previous two modules: for each cell line the liquid handler would need to host only one well plate and two typology of tubes, firstly 15 ml tubes and then cryo-vials. Due to the many days of distance between the two freezing days there can not be difficulties for the capacity of the freezing module when considering the same line. However, In case of the high throughput scenario of starting batches of 3 to 4 lines every day, parallelization scenario B2 (Chapter 04), the overlapping days of freezing could be between lines that started the production 9 days apart from each other: the freezing day at step O of the first production batch and the freezing day at step D of the nineth production batch could overlap. In addition to this, it should be taken into account that for all the steps previous in the process there could be variability in number of feeding days needed between passaging. This means that there could be the possibility of other overlapping between freezing steps of different batches.

Given the capacity of the devices chosen, this possibility of overlap are easily handled by the F module, also due to its ability to predict disruptions and inform the operators.

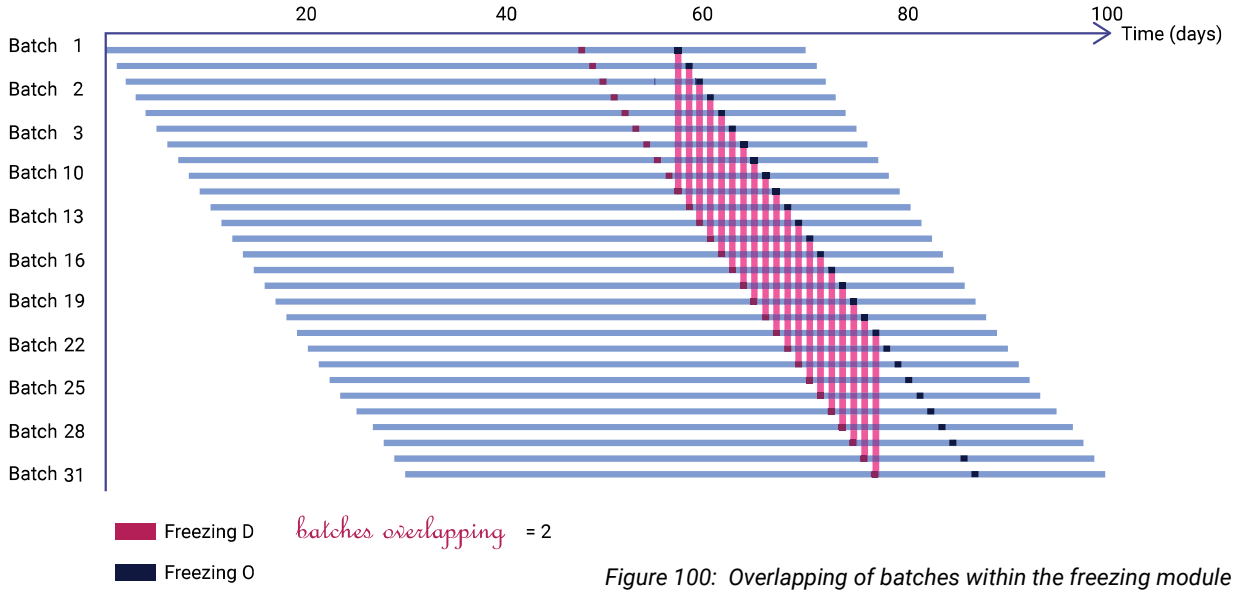


Figure 100: Overlapping of batches within the freezing module

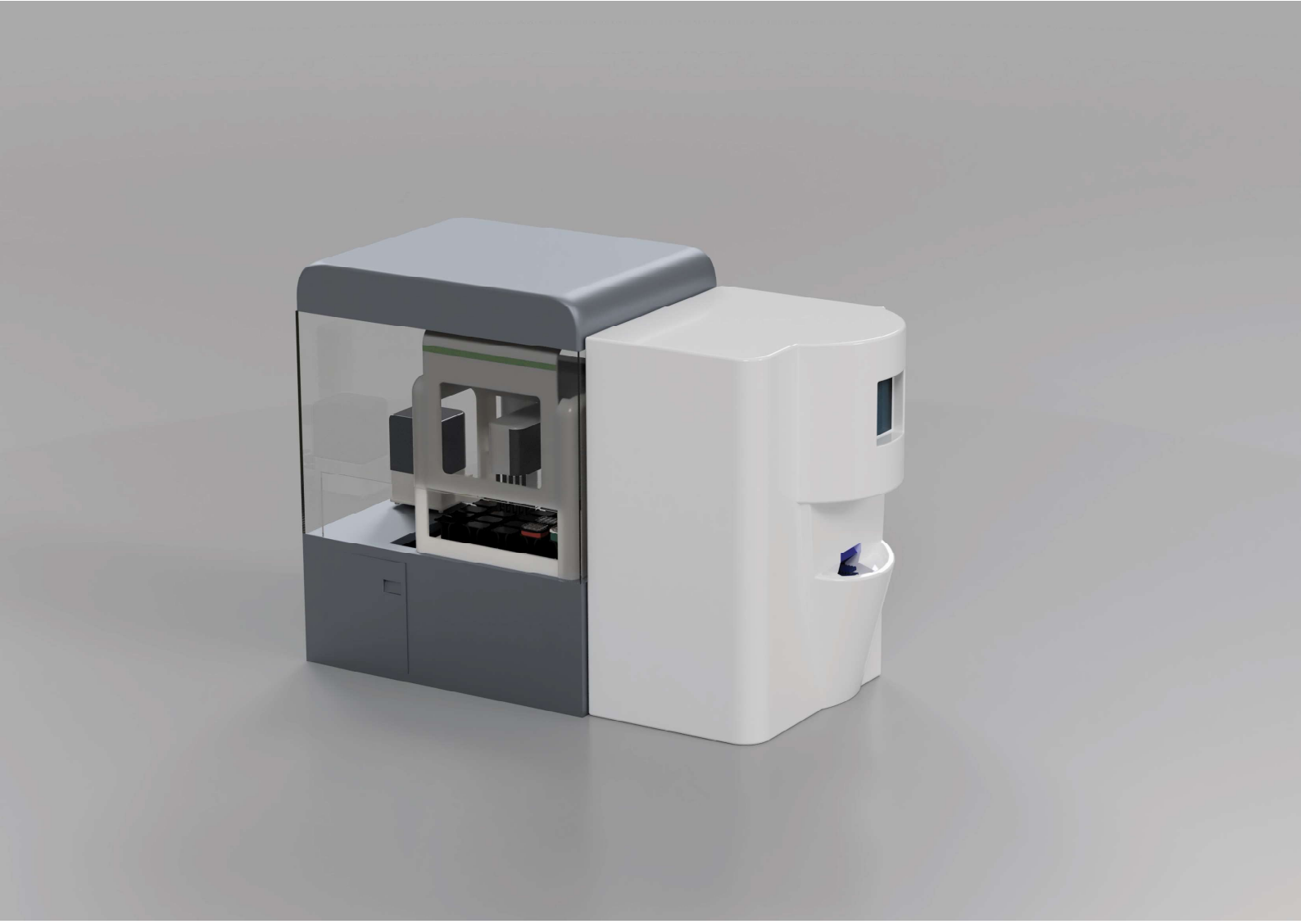


Figure 98: The freezing module

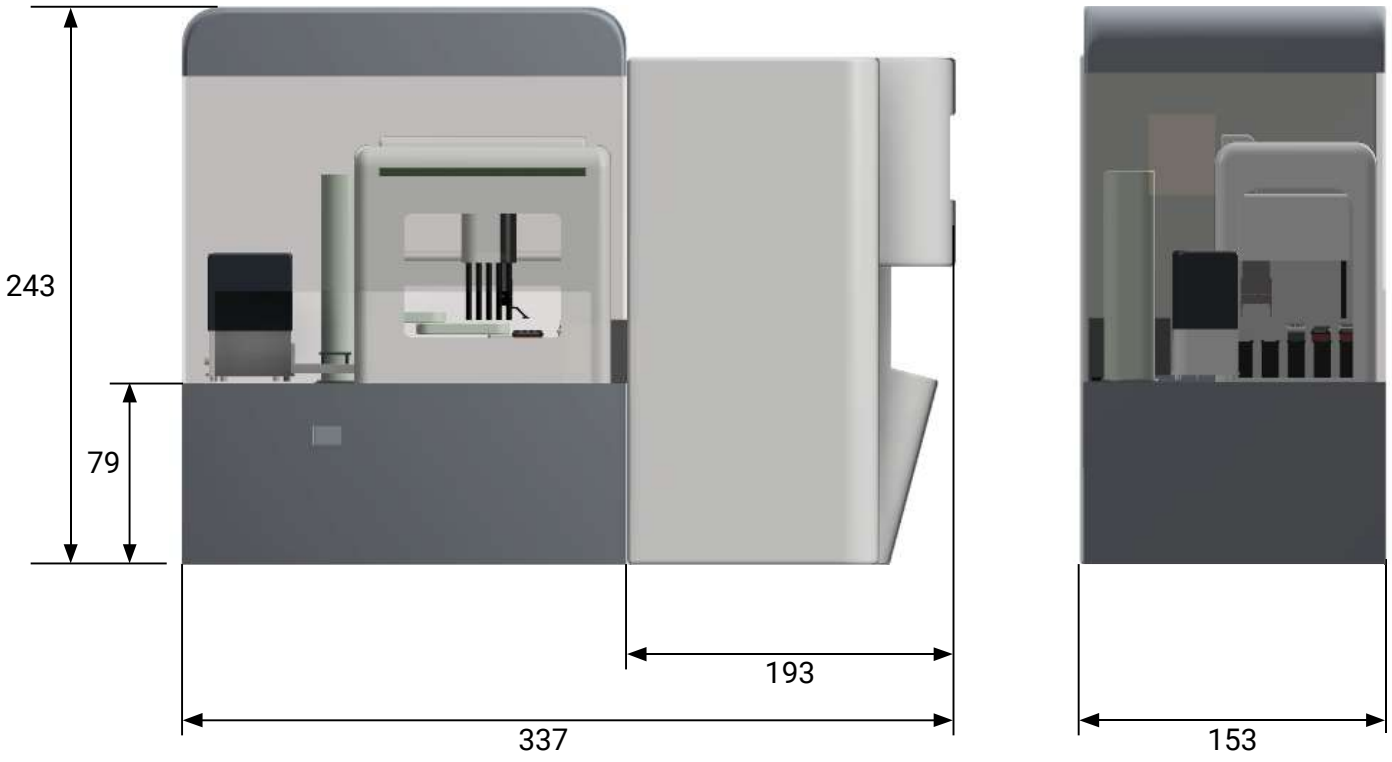


Figure 99: Overall dimention of the freezing module.

Organization of the flow in the F module
In Figure 101 the Process tree schematic regarding freezing is organized to highlight the subsequency of steps.

It is possible to see how the flow is this time very linear, and strictly centered around the liquid handling system.

As seen in the previous modules, transportations can be grouped together, although in this case the analysis doesn't change the initial observations. As a result, in fact the only conclusion that can be taken is that the transportation towards the the robotic arm for tapping on the bottom of tubes and the plate warmers are happening over the deck.

The F module reflects the analysed outcomes in its design, placing all the elements around the liquid handler.

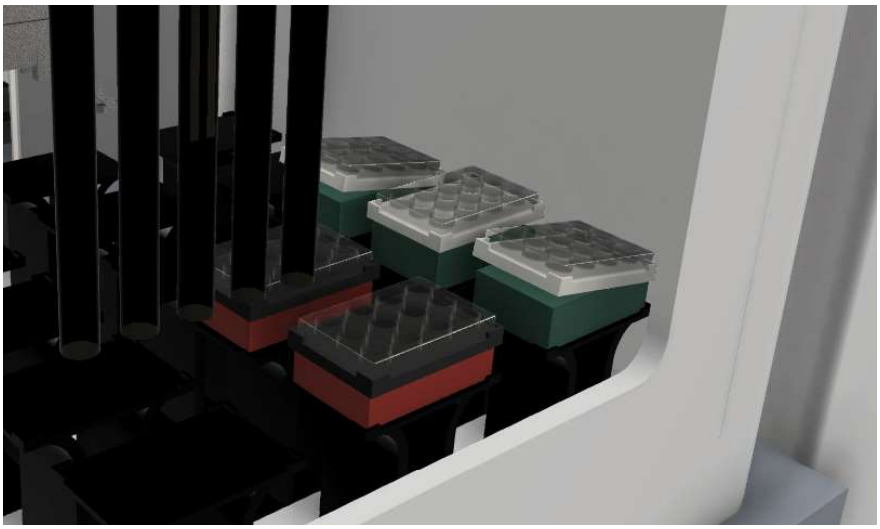


Figure102: Detail of the on deck warming plates and tilting plates.

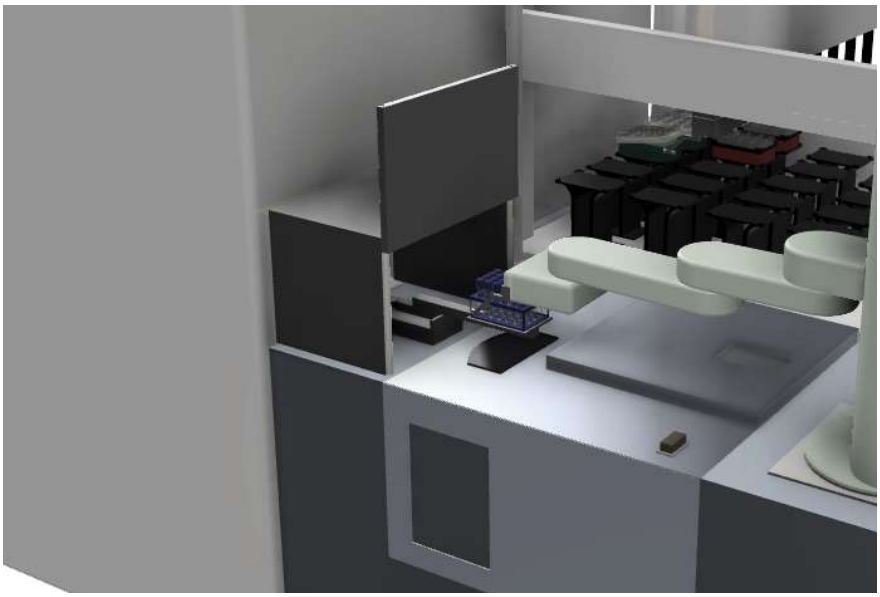


Figure103: Detail of the transportation into the freezing module.

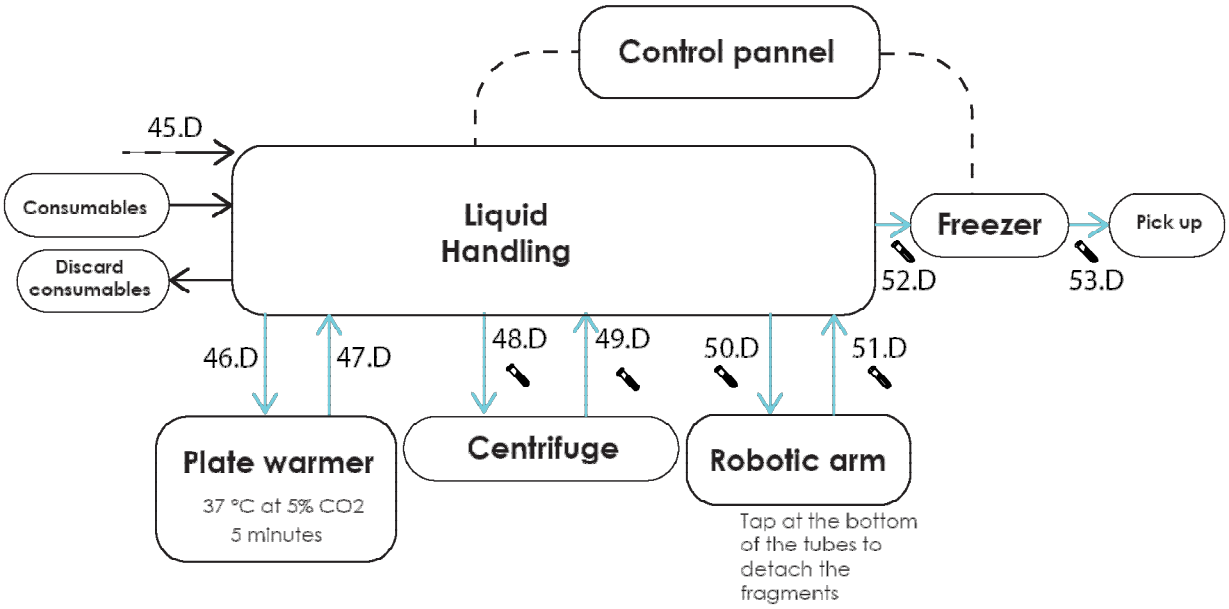
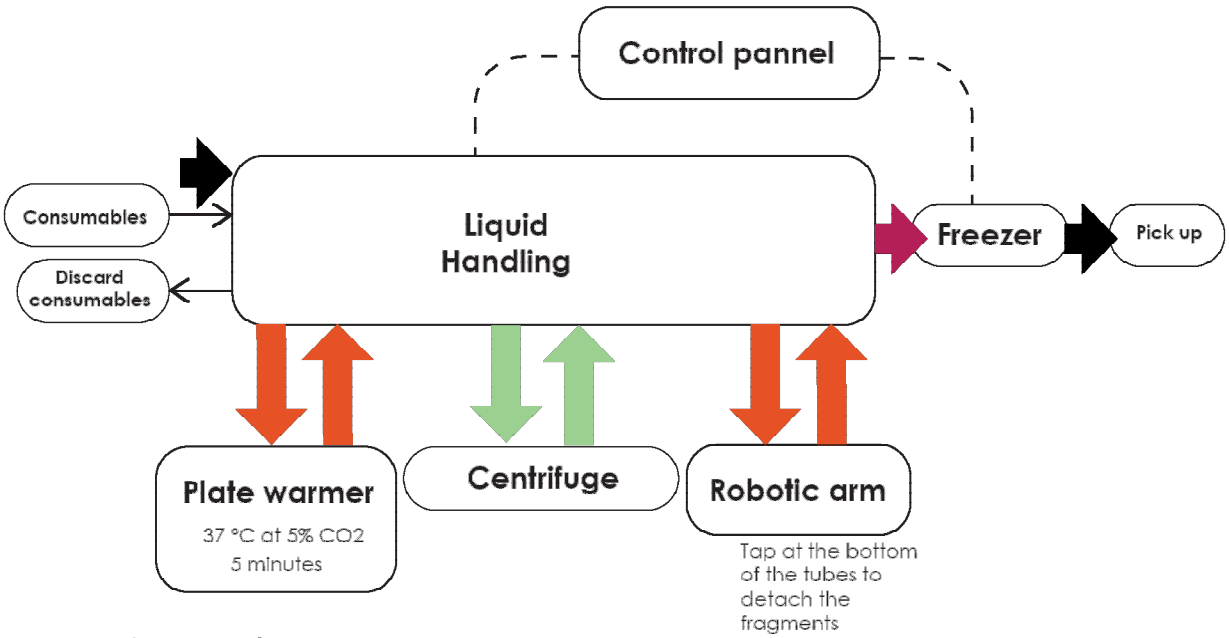


Figure 101: Transportation steps taking place during freezing. The numbers refer to the substeps of the process tree (Appendix I)



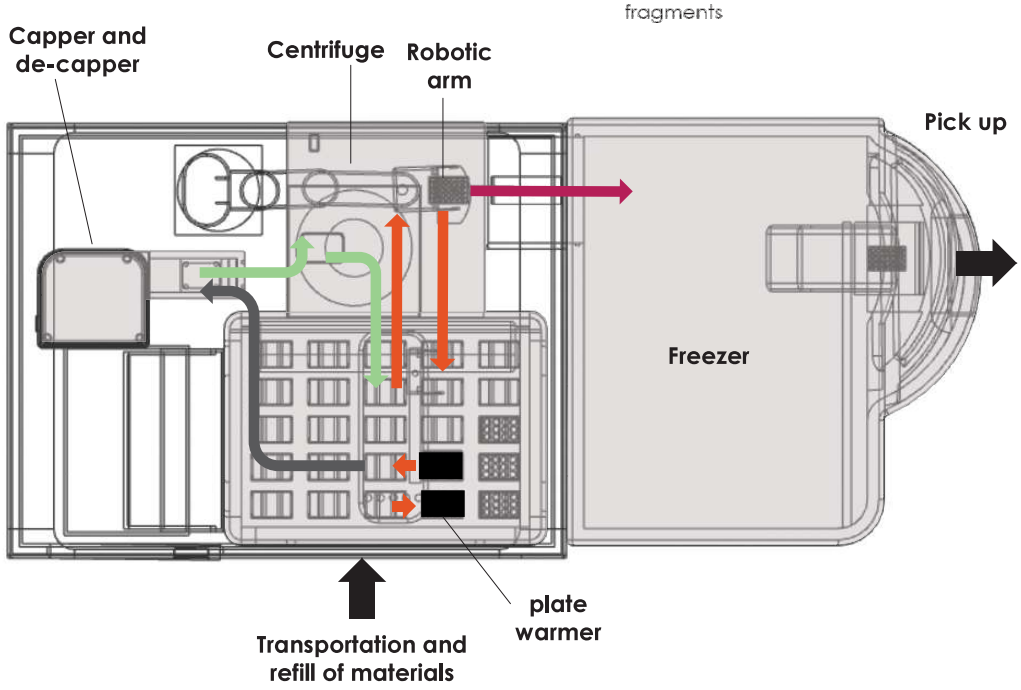
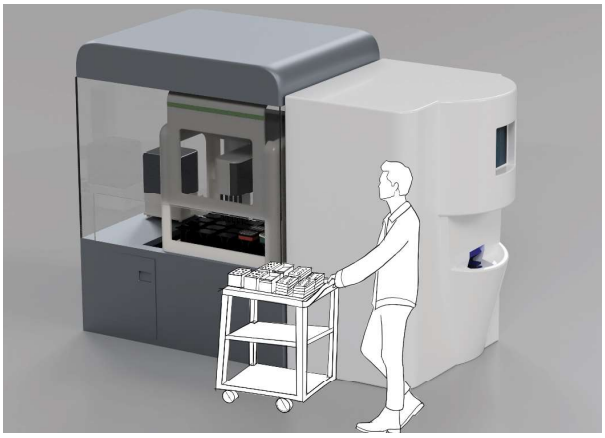
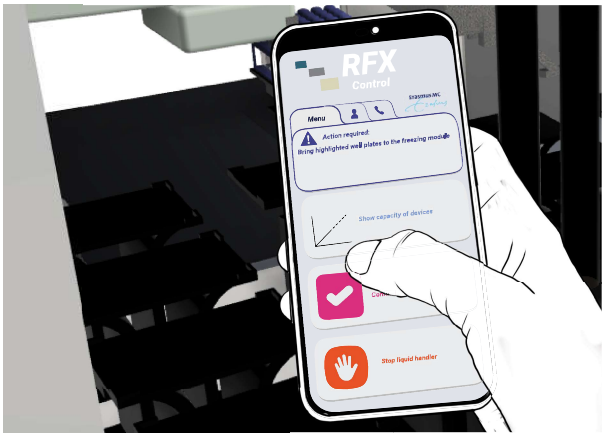


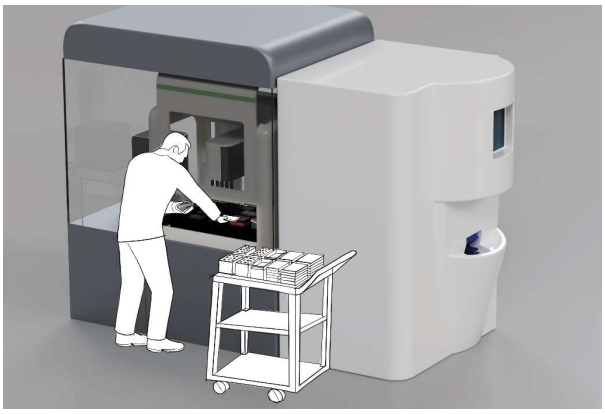
Figure104: Substeps taking place during freezing



1 ***Transportation of well plates to the freezing unit***
The technician takes the well plates and transports them to the X module by using wheeled transport table.

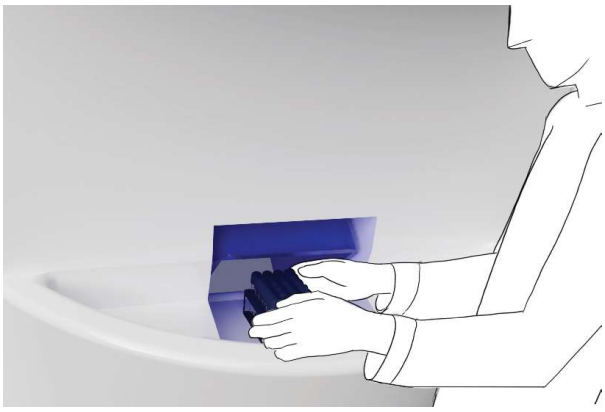


2 ***Confirmation of the transport through phone application***
When in front of the machine, the technician can confirm on the app the transport in order to go to the next stage.



3 ***Insertion of well plates and disposables inside of the deck***
The operator can now follow the instructions on the screen in order to load materials inside the deck in the right order.

4 ***Loading of disposables and well plates with cultured cells from the X module***
Once the deck is prepared, the technician can confirm the step and leave the laboratory.



5 ***Extraction of tubes from the freezer***
Tubes are scanned and organized into racks, no confirmation is needed from the technitian for the automatic slider to close uppon removal of the tubes.

Figure: Steps of interaction with the Freezing module. The steps of cleaning are not reppresented because they follow the same procedure as in the X unit.

Chapter 7

Evaluation, future steps and conclusions

This last section of the report describes the activities that have been done in order to evaluate the final design.

Initially, the evaluation of the proposed concept design points out, through risk analysis, possible hazards present in the design and therefore risks that require immediate action in order to be prevented.

Secondly, a general cost evaluation presents future elements of further investigation and a base for future decision making and action activities.

Thirdly, recommendations given by feedback on feasibility from an enclosure builder, and elements noted during consultation with some of the producers of the chosen devices are reported.

The chapter concludes with an evaluation of the project from the perspectives of its limitations, assumptions and tackled research objectives. These elements are then converged into the envisioned future challenges.

Finally, a personal reflection on the project is given.

In this chapter:

Risk analysis

As an evaluation of the presented final concept, a risk analysis can be made.

Guidelines over Risk Management are provided in ISO 31000 by the International Organization of Standardization to give structure to the process of identification, prevention, and handling of risks. The main principle of these guidelines is relative to the iterative nature of risk management and the importance of considering the stakeholders within the framework.

The iterative process of risk management is described in Figure 105: Initially, the context of the activity is identified, analyzing all the possible stakeholders and the possible involved parties. Some of the initial stakeholders are already described in Chapter 2.5 *Organization of Erasmus MC* while the overall picture can be extended further including also the stakeholders involved in the design process as in Figure 106.

A complete usability engineering program plan would take in consideration also phases of the product journey previous to the use phase such as pre-use and post-use. In this case, also hazards that involve the extended stakeholders would need to be analyzed. However, since the current project is related only to the use of the machine during cultivation of IPSC lines, usability sides are considered only when regarding the use phase.

The envisioned interaction overview (Figure 107) has been analyzed with the main management stakeholders by pointing out the possible risks which could obstruct the process, cause harm to operators or cause a failure of the culture. Due to the stakeholders involved, the identified risks were mainly relative to the cultured cell lines, more sfavorable events could be identified by repeating the process with other stakeholders.

The negative situations have been reviewed afterward in a matter of probability of occurrence and impact of the consequences (Appendix VII). Those with higher risk and probability are the risk factors that should be tackled in the next design phases.

Method

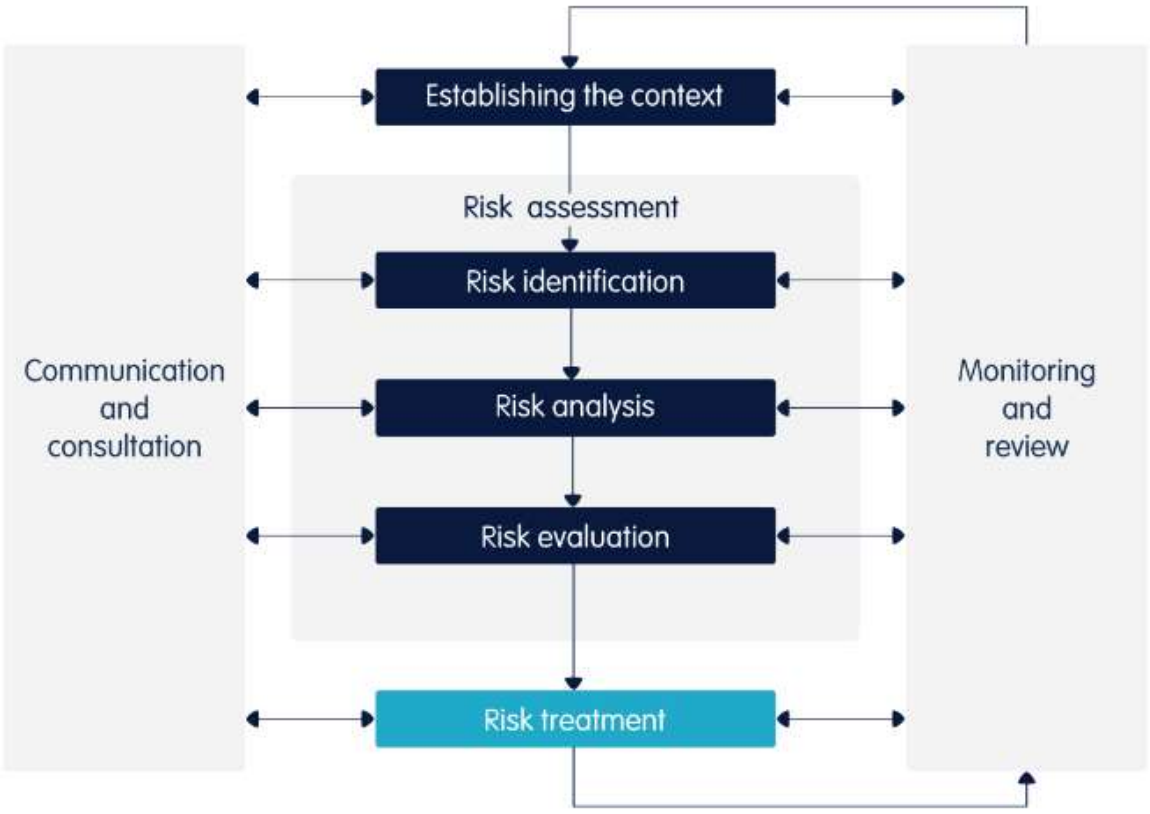
The Bow-Tie Method has been used on specifically chosen risk events in order to propose a possible way of prevention or limitation of their consequences.

The Bow-Tie Method is a diagram that enables to visualize the risk that is tackled in one easy to understand picture by giving an overview of multiple plausible scenarios in a single picture. The situations tackled with this method are the ones scoring highly in the hazard Matrix (Figure 108).

The Bow-Tie Method is performed by identifying, in the following order, these elements (Pilkington, 2017):

1. Hazard: It reppresents something that could possibly cause a harm, or a negative consequence.The hazard is presented as something neutral while the rest of the BowTie is focuses on keeping the normal but hazardous aspect from turning into an unwanted negative factor.
2. Top event: this describes the context in which the control is lost. Although the situation has still not turned negatively this element describes the exact situation in which the damage is can happen.
3. Threats: Any element that can cause a top event. It should be as specific as possible identifying the person that

Figure105: Risk analysis method



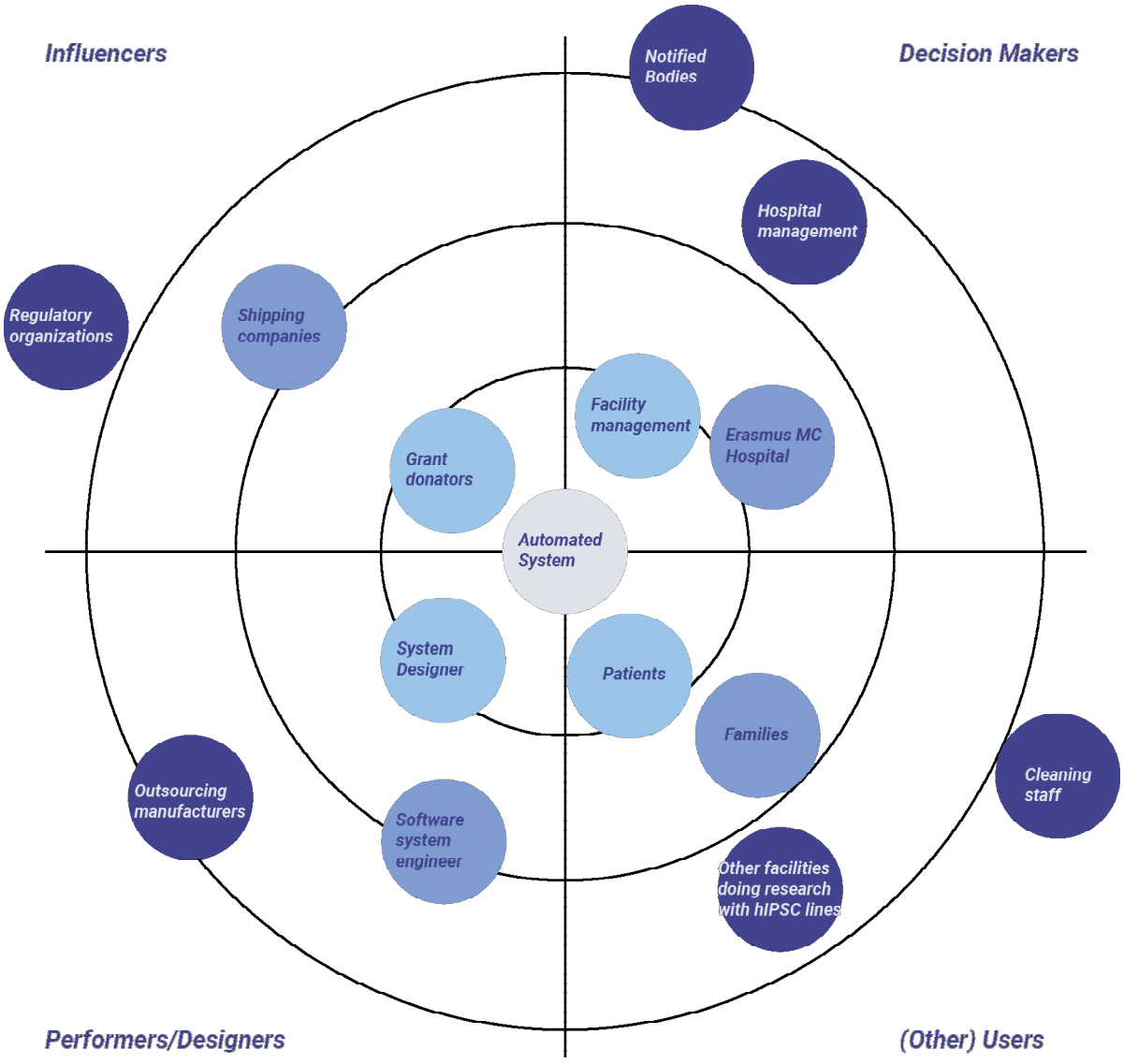


Figure106: Stakeholders

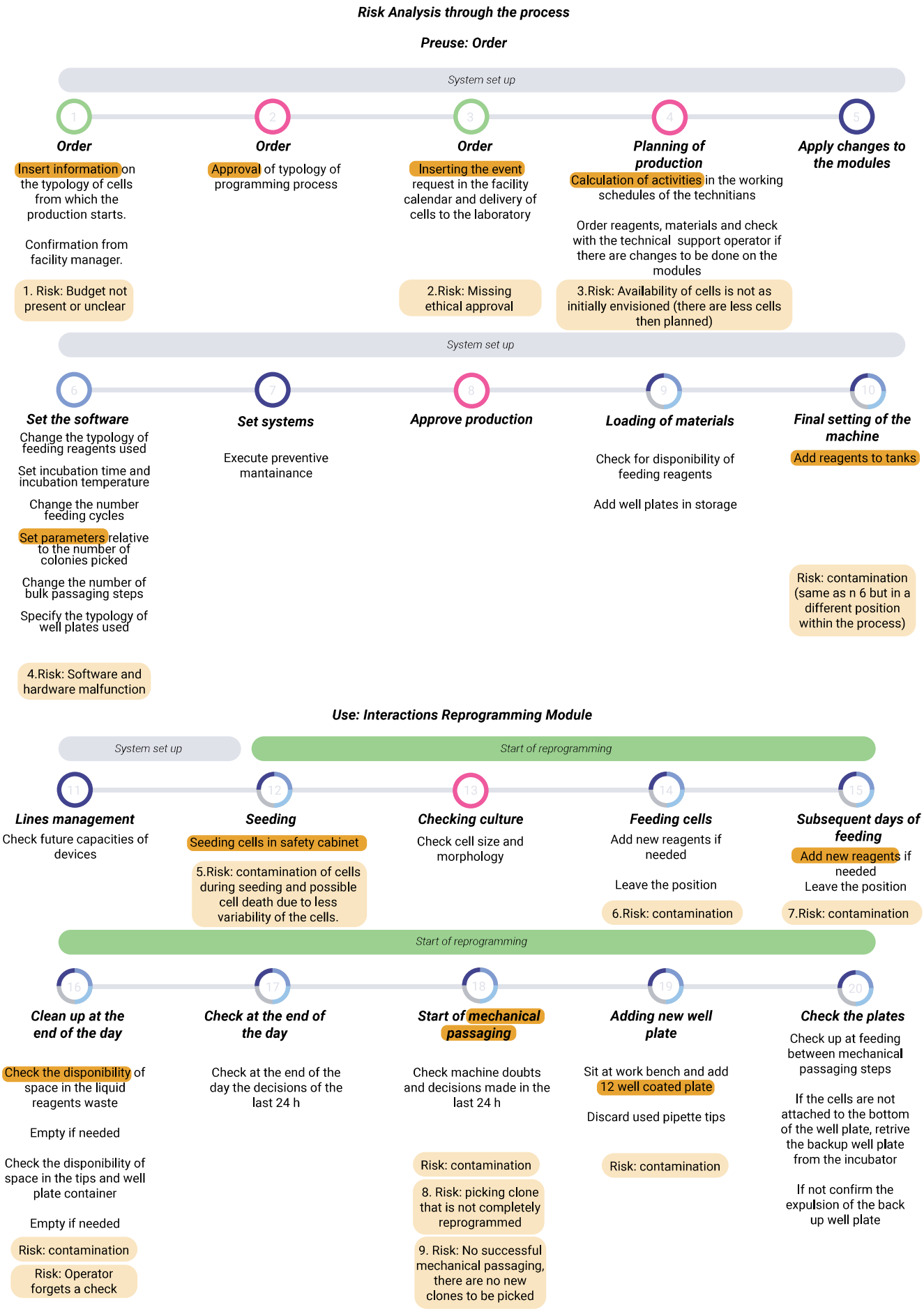
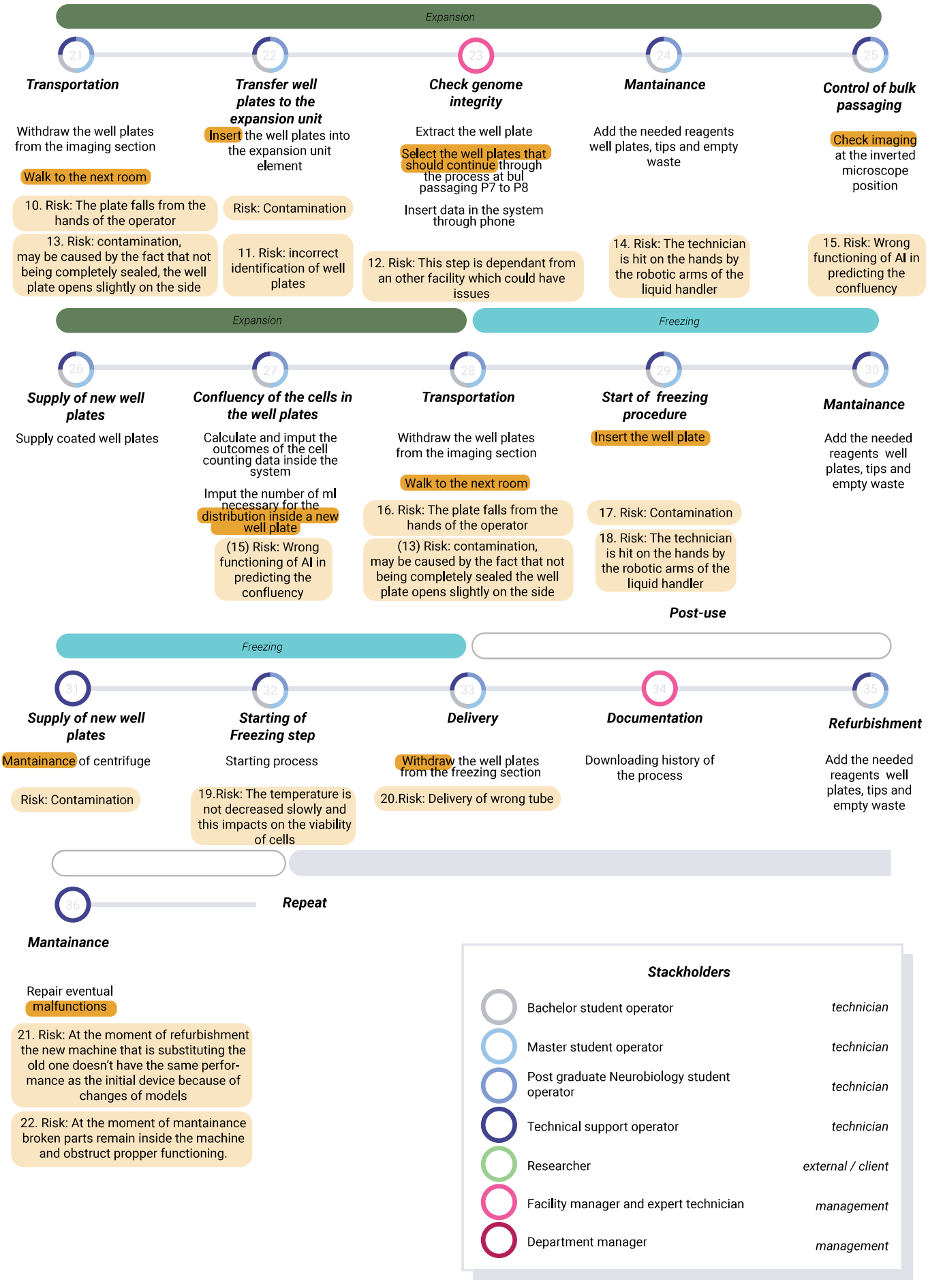


Figure 107: Interaction overview


Expansion
21
Transportation
Withdraw the well plates from the imaging section
Walk to the next room
10. Risk: The plate falls from the hands of the operator
13. Risk: contamination, may be caused by the fact that not being completely sealed, the well plate opens slightly on the side
22
Transfer well plates to the expansion unit
Insert the well plates into the expansion unit element
Risk: Contamination
11. Risk: incorrect identification of well plates
23
Check genome integrity
Extract the well plate
Select the well plates that should continue through the process at bul passaging P7 to P8
Insert data in the system through phone
12. Risk: This step is dependant from an other facility which could have issues
24
Mantainance
Add the needed reagents well plates, tips and empty waste
14. Risk: The technician is hit on the hands by the robotic arms of the liquid handler
25
Control of bulk passaging
Check imaging at the inverted microscope position
15. Risk: Wrong functioning of AI in predicting the confluency
Expansion
Freezing
26
Supply of new well plates
Supply coated well plates
27
Confluency of the cells in the well plates
Calculate and imput the outcomes of the cell counting data inside the system
Imput the number of ml necessary for the distribution inside a new well plate
(15) Risk: Wrong functioning of AI in predicting the confluency
28
Transportation
Withdraw the well plates from the imaging section
Walk to the next room
16. Risk: The plate falls from the hands of the operator
(13) Risk: contamination, may be caused by the fact that not being completely sealed the well plate opens slightly on the side
29
Start of freezing procedure
Insert the well plate
17. Risk: Contamination
18. Risk: The technician is hit on the hands by the robotic arms of the liquid handler
30
Mantainance
Add the needed reagents well plates, tips and empty waste
Post-use
Freezing
31
Supply of new well plates
Mantainance of centrifuge
Risk: Contamination
32
Starting of Freezing step
Starting process
19.Risk: The temperature is not decreased slowly and this impacts on the viability of cells
33
Delivery
Withdraw the well plates from the freezing section
20.Risk: Delivery of wrong tube
34
Documentation
Downloading history of the process
35
Refurbishment
Add the needed reagents well plates, tips and empty waste
36
Mantainance
Repair eventual malfunctions
21. Risk: At the moment of refurbishment the new machine that is substituting the old one doesn't have the same perfor-mance as the initial device because of changes of models
22. Risk: At the moment of mantainance broken parts remain inside the machine and obstruct propper functioning.
Repeat
Stackholders
Bachelor student operator
technician
Master student operator
technician
Post graduate Neurobiology student operator
technician
Technical support operator
technician
Researcher
external / client
Facility manager and expert technician
management
Department manager
management

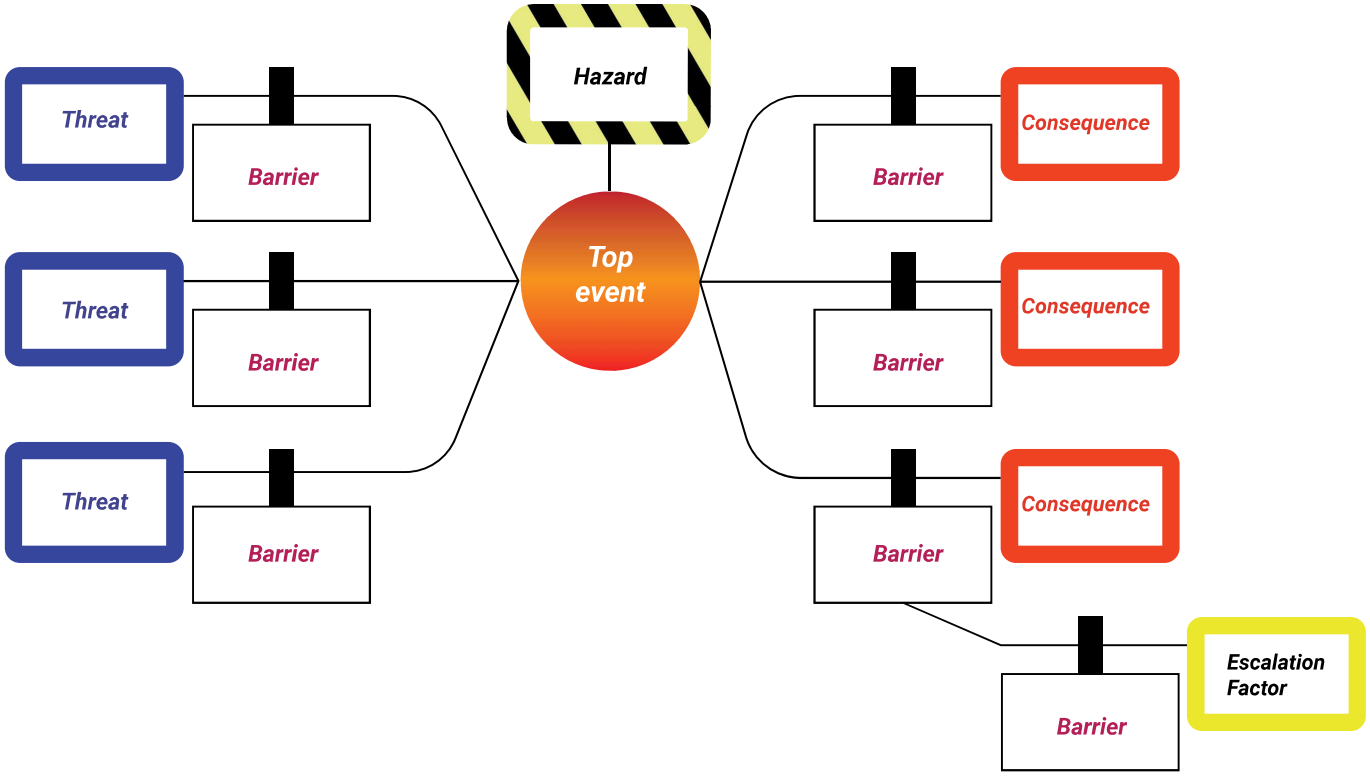


Figure109: Visual reppresentation of the Bow-Tie method, readapted from www.cgerisk.com

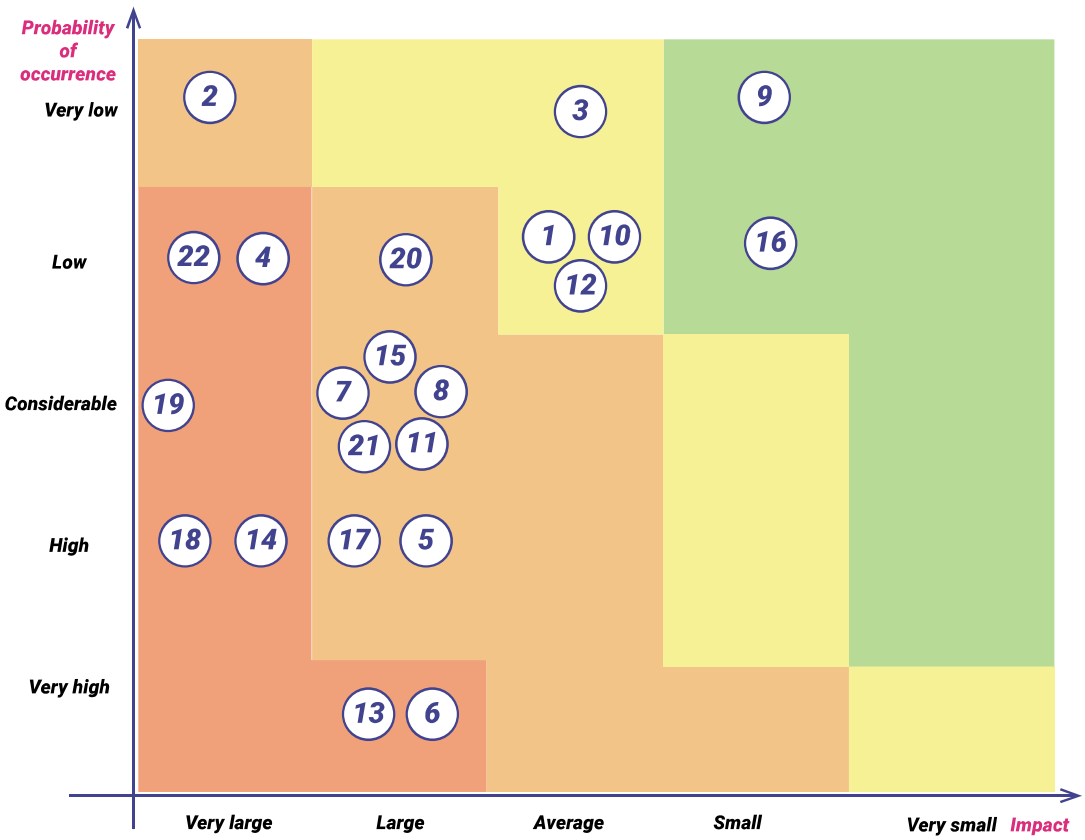
causes the top event, the equipment that fails, what external conditions cause the event or what are the external conditions that cause the event.

4. Consequences: the different specific consequences deriving from the top event are described in the last step of the analysis phase of the Bow-tie.
5. Barriers: Barriers are ways in which the unwanted scenarios can be prevented. Barriers can also be assigned to a specific person or event within the project
6. Escalation Factor: Escalation factors describe the cause that could make the barrier fail and they are used in order to understand why the barrier could fail. Whereas barriers are elements that can be achieved and indicate possible solutions, escalation factors represent other challenges to take into account.

During the analysis phase the risk events have been pointed out. Within the Bow-tie workframe the hazards relative to each risk can be highlighted (words in orange in Figure 108).

The results of bow-ties (Appendix VII) done for the seven risks identified as extremely high are integrated into the risk descriptions in the Chart at Figure 110.

It is possible to note that similar risks (e.g. contamination, falling of plates from the hands of technitians, possible harm to the hands of technitians when operating the deck) have scored differently in the matrix. This is due to the fact that their incidence or probability of occurrence are different depending on the phase these events could take place along the process. For example, the probability of occurrence of harm to hands of technicians is higher in the freezing module due to the smaller width of the deck of the Biomek i5 liquid handler.



Extremely high risk: Act immediately to mitigate risk.

High risk: Mitigate risk, if control measures are not available set a timeframe for implementation

Medium risk: Take reasonable measures to mitigate the risk with temporary lower level controls

Low risk: Take reasonable measures to mitigate and monitor the risk. Controls can be of administrative nature

Figure 108: Risk Acceptability Matrix

1. Budget not present at the moment of order of iPSC production.
2. Missing ethical approval from patients
3. Availability of cells not as initially envisioned, there are less cells then planned.
4. ***Software or hardware malfunction at the set up of the machine***

Risk mitigation: design a set up workflow that checks each detail of the software and each device in a semi automated manner.

5. Contamination of cells during seeding, possible cell death due to less variability
6. ***Contamination of well from external factors***

Risk mitigation: execute a regular weekend sterilization of the cabinets, also in areas for storage of reagents

7. Cross contamination of well
8. Picking a clone that is not completely reprogrammed
9. unsuccessful mechanical passaging, no new clones to pick
10. Plate falls from the hands of the operator during transportation
11. The system has problems in recognizing the well plate
12. Failed RNA isolation, problematic dependant from an other facility
13. ***External contamination***

Risk mitigation: Implement additional sensors to ensure well plates are properly closed during transportation steps

14. ***The technician is hit by the robotic arms while loading the liquid handler***

Risk prevention: Implement a sensor that warns the technitian on when he is crossing safety zones while loading new disposables

15. Wrong functioning of the AI system in prediction of confluency
16. The plates fall from the hands of the operator during transport
17. Contamination
18. ***Loading the liquid handler the technician is hit by the gripper***

Risk prevention: Implement a locking system for the slider of the liquid handler

19. ***Abrupt insertion of cells inside the freezer***

Risk prevention: Limit the intensity of the production in order to respect temperature transition times

20. Delivery of wrong tubes
21. New refurbished machine doesn't have the same performance as the initial device because of changes of models
22. ***At mantainance, broken parts remain inside the machine and obstruct functioning.***

Risk prevention: improve accessibility of devices for mantainance

Figure 110: Risk descriptions

RELEVANT INSIGHTS

- RXF still holds possible risks for very important factors to IPSC cultivation such as contamination of wells from external factors and cross contamination internal to the system, further changes to the design should be considered for prevention of these occasions to happen.
- The design of the RFX system has not considered yet difficulties relative to mantainance, the design should be further improved integrating aspects relative to accessibility of components.
- The software used by the system should mantain flexibility and adaptability to future integration of new devices, a possible solution for ensuring the desired grade of freedom could be starting an internal project of software development. This possibility could lead to longer times for the overall automation project.
- Further design iterations should focus further on the safety factors for human-machine interactions: the operation of the liquid handlers should be stopped when the operator is setting up the deck of the liquid handler
- Follow up on device upgrades should be ensured from the start of discussions with future vendors

Cost price evaluation

Aim of the cost evaluation
Further evaluation of the presented concept can be done through cost price evaluation.

There are two ways of using the cost price evaluation method: in a qualitative way, in order to compare the design proposal to existing products that are already on the market, or in a quantitative way, by adding up cost factors and margins in order to build and sell the product (Buiting-Csikós, 2012). In this project, however, cost price evaluation is not used with the same purpose, since the cost of other IPSC production systems are not published and, on the other side, there is no will for the facility to sell the product.

The reason why the cost price estimation is done for the RXF system is to understand future challenges to tackle, help the judgement of feasibility of the transition to automation of the entire process, and serve as a tool for considerations on partial or full process automation.

Methodology used for the cost evaluation
Cost evaluation has been calculated both through bench marking and, where possible, through direct contact with the producers of the chosen devices. Figure 111 describes the general overview of the costs for each module, the full calculation can be found in Appendix VIII.
This synthesys of the cost estimation outcomes highlights unknown factors that could make the final value vary, and the elements influencing the most the outcome of the final budget.

When analyzing the results of the cost price evaluation also a second scenario implementing a fictitious freezer of smaller capacity and therefore lower cost, instead of the chosen SAM HD automated freezer, was taken into account. This is the reason why for the F module two values are reported.

Limitations of the cost price evaluation
It is important to mention that at the stage of concept development relative to this project, there are still many undefined variables that can influence the calculation of costs greatly. In fact, although being chosen to align to the requirements of the process, each device implemented into the system has to be slightly changed or readapted in order to be integrated. Therefore, it would be necessary to consider also possible additional costs of engineering that are defined further on in detailing iterations, usually done with more contacts with device manufacturers and other stakeholders.

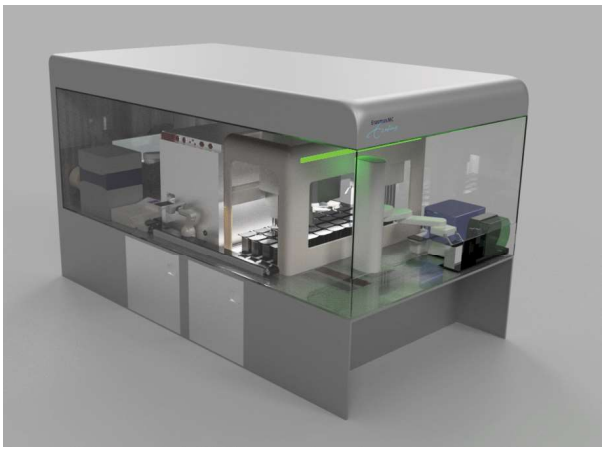
Furthermore, in the current level of development of the project, devices have been selected in relation to the functions that they perform within the process, without considering possible additional costs of assistive devices such as pumps, cabling, tubing or pipette racks for liquid handlers. All these details are considered in later stages.

As a result, currently known prices of devices can vary in the several iterations and discussions that need to take place in the future.

Taking only this cost estimation, which is done within the limitations of the present project (Chapter 07) as a parameter for future decisions is not recommended.

In fact, as seen in Chapter 01, (paragraph 1.2), one of the possible drivers for a Facility to transition towards automation is the reduction of costs by cutting on waste and labor tasks.

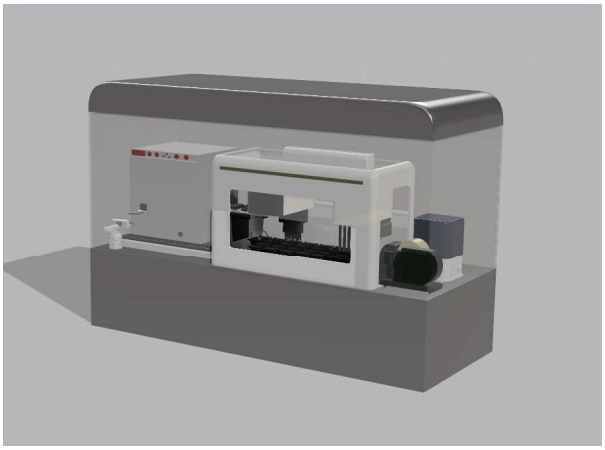
In order to evaluate the saved costs that the facility would have in the future, it is necessary to know if the costs of investment of automation would be absorbed by making a return on investment analysis.



Reprogramming Module

Cost of internal devices	€ 544.078,00
Total cost	€ 744.078,00

Unknown factors: Devices that assist the functioning of the liquid handler, needs of adaptation of the storing hotel for the supply of disposables for the liquid handler, software development related costs.
Most expensive devices: ASL Cell Celector, Celigo imaging Cytometer and the Liquid handler Biomek i7 (dual multichannel) liquid handler.



Expansion Module

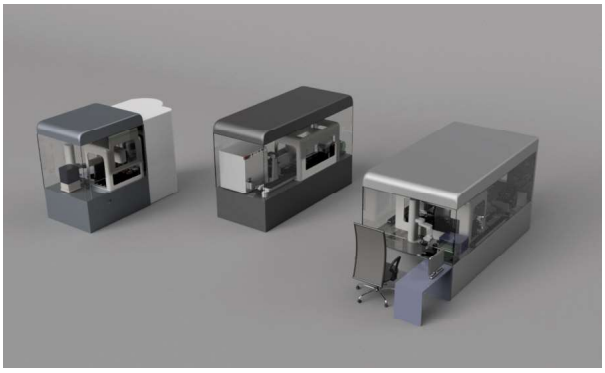
Cost of internal devices	€ 338.693,00
Total cost	€ 538.693,00

Unknown factors: Devices that assist the functioning of the liquid handler, number of platforms present on the deck for plate shaking and medium warming and cooling, software development related costs.
Most expensive devices: Biomek i7 Hybrid (MC + Span-8) liquid handler, high speed inverted microscope Ti2-E microscope.

Freezing Module

Cost of internal devices	€ 317.973 - € 517.973
Total cost	€ 367.973 - € 567.973

Unknown factors: Cost of an automated freezer (-80 °C) of more suitable capacities, software development related costs.
Most expensive devices: Liquid handler Biomek i5 Span-8 without enclosure, Celigo imaging Cytometer



RXF System

Cost of internal devices	€ 1.400.744,00 - € 1.200.744,00
Total cost	€ 1.650.744,00 - €1.850.744,00

Unknown factors: Overhead costs and adaptation of the facility for the integration of the system.
Most expensive elements: Safety cabinet enclosures and liquid handlers.

Figure 111 Overview of identified costs for the single modules and for the overall system

Based on the number of variables present and the present unknows relative to the recurring and non-recurring costs of the manual and automated-scenario, it is possible to say that no such analysis is feasible within the timeframe of this project.

Nonetheless, the information's gathered from this research can be used for future analysis, discussions, and considerations with stakeholders.

Results

The initial budget to request for the development of a concept such as the one reppresented by the RXF is of approximately 1.5 billion euros, which is slightly higher then the initial expectations of the management of the IPSC Facility. However, this result can still be readapted and further decisions can lead to significant reduction of cost when the elements optimized are regarding the used liquid handlers and immaging devices.

When these relevant devices are put into comparison, in fact, it is possible to see how the most exspensive liquid handler is the Biomek i7 Hybrid (MC + Span-8) liquid handler because of the integration of the Span 8 pipetting head in order to be able to execute pipetting of fluids inside tubes. This same function is also done by the one pipetting head of the Biomek i5, therefore an integration of the X and the F module could be taken into consideration.

Since the IPS Facility wants to have a gradual transition towards automation, the final decision of the facility could be of investment of modules in order of increasing investment, so that along the way, new learnings are gathered and less risks are taken with the more expencive modules.

In this scenario the order of investment would probably first involve the F module, then the E module and lastly the R module.

Overall the most exspensive device is the ASL CellCelector.

RELEVANT INSIGHTS

- Cost price estimation should be repeated further on in the process extending it with gathered informations from producers, installers and other stakeholders.
- The initial budget for the development of the proposed concept is of approximately 1.5 billion euros. Which is a cost higher then the envisioned budget of the IPS Facility.
- In order to reduce costs two strategies can be considered: start with the investment on cheaper modules and, once further budgets are approved, invest in the next modules or optimize the system further by the combination of two modules (the X and the F module) together.

Evaluation from enclosure builder and device producers

One of the core principles of the RXF concept is the embodiment of all devices within a laboratory safety cabinet of class II in order to protect the culture from contamination.

In order to validate this core element of the design, a consultation with Gregor Holdampf, account manager at CleanAir by Baker, producer of safety cabinets, was organized.

The model of the concept was then presented through a tour of the CAD model and assumptions were discussed.

The full interview report can be found at Appendix.

The main result coming from the evaluation is that there are elements to be improved in future stages of development of the project, but solutions can be found.

The models showed are a good starting point because they give an indication of all the elements necessary to go further. In fact, in order to continue with the project, it is necessary to have clear what are:

- The devices to be integrated into the system and their dimensions

- The areas that are necessary to reach

- What are the elements important for the maintenance

- A flow showing the sequence of use.

The main challenges for the future are going to be related to the inclusion of a return duct, to circulate in the air in the upper direction. Due to the big dimensions of the units, the airflow would need to be of very big amounts (more or less 10.000.000 liters/ hour of air supply). The bigger the safety cabinet gets the higher would be the needs of air supply.

However, also projects of bigger dimensions are done and can be feasable. The only main aspect is the impact is of the engeneering needed for the design of such custom safety cabinets and transportation costs.

There are no real ways of containing the budget because of need for investment in engineering and transportation, except for the fact that the freezing module could reduce the costs by being readapted to a standard safety cabinet.

An other main aspect to be further discussed will be the integration of the devices inside the cabinet. In fact, their volumes create an obstruction to the air flow and could therefore need to be readjusted, for example by adding feet underneath the devices to rebalance the airflow.

Other device producers have been contacted throughout the process in order to gather insights on feasibility.

Except for the main insight on the costs, used then in the cost estimation, and further definition of the choiches, the main insight that can be taken from these connections is that further engineering is very probably going to be needed.

As an example it is possible to mention that there is going to be need of engineering in order to create a custom adapter responsible for the loading and unloading of the centrifuge. This will also depend on details such as the decision on weather or not the robot will load/ unload the tubes invididually.

Going into these details is, however, out of the scope of this project.

RELEVANT INSIGHTS

- The concept of integrating devices within the safety cabinet is possible.
- There are corrections to be made in order to ensure the possibility of including a return duct, used within Safety cabinets class II.
- Changes in the architecture of the disposition of internal devices may be necessary in order to ensure air flow.
- Further design and engineering is probably needed for handling transportation of plates between devices.

Limitations and assumptions taken during the project

The focus of the present project has been on the envisioning of the automated system design. Considering that usually in projects of system design a team of several experts arrives at an initial concept design in a slightly longer period of time, the present concept, in order to be executed, has been realized taking some assumptions and within limitations.

Limitations:
The limitations of the project concern mainly the level of depth to which the project has been developed and its testing.

Design aspects that fell out of the scope
The design concerned mainly the overall aspects of the system: the analysis of possible ways of satisfaction of the functional needs of the process and the choice of devices to be implemented, the grouping and modularity of the system, the organization of the flow of the process within the system and the design of interactions with technicians.

Aspects that are on a more specific micro level, such as specific intercommunication technologies used between devices, material choices, detailed design of robotic arms, or structural design of the enclosures, did not partake to the same level of decision making, and would not add additional information to this storyline. Although being important aspects of the design to tackle in further stages, they were not considered within the scope of this project.

All these aspects can be tackled concretely in the next stages of this project once the boundaries, risk factors, and general requirements are already visible and expressed.

With less time spent in research, assumptions should be further tested
Additional limitations, namely time restriction (due to the timings of graduation projects), safety restrictions (due to social distancing due to Covid-19), and the complexity and length of the reprogramming process limited the possibilities regard physical and virtual testing of the system. As a result, the presented concept has been evaluated mainly through comments on visualizations and through a review of CAD models.

A possible way of improving the testing, with more time in disposal, is to simulate the process tree flow into a Simulink State Flow simulation through the Matlab Software for analysis of bottlenecks, while an overall AR prototype could be built in order to physically identify interaction problems in the identified interactions (Figure 112-113).

The final decision on devices may need further refinement
The devices taken into account in the current final concept design proposal follow the requirements coming from the analysis of the reprogramming process. During the process of ordering devices, there is going to be a better refinement of these product specifics which may lead to a different final choice. The process of acquisition of the devices could, therefore, need further iterations which would have taken further time and would not have added to the scope of this project.

Budget estimation may vary
Originally one of the aims of this project was to identify the final budget for the transition to automation, however, from the start of contacts with device suppliers it became clear that each variable can cause a very big shift in prices, also due to engineering costs,

therefore, the final budget is very difficult to identify clearly and the proposed one should be taken only as a starting validating point on which to base future developments and decisions.

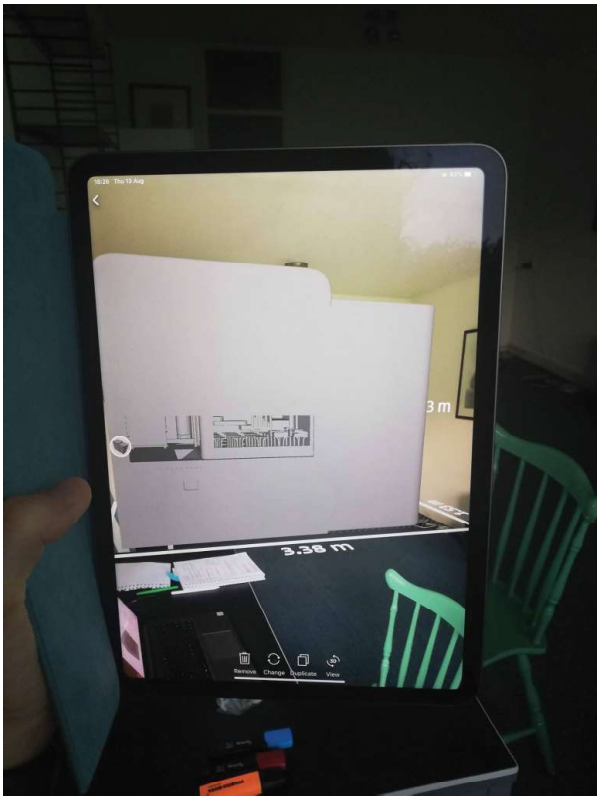


Figure 112: Viewing of the freezing module inside of an empty room through the application Augment, without materials applied. This typology of testing, repeated in the facility, could help ideate on other aspects of the architecture of the rooms to be adapter for the integration of the modules (where to connect power supplies, how to provide ventilation...).

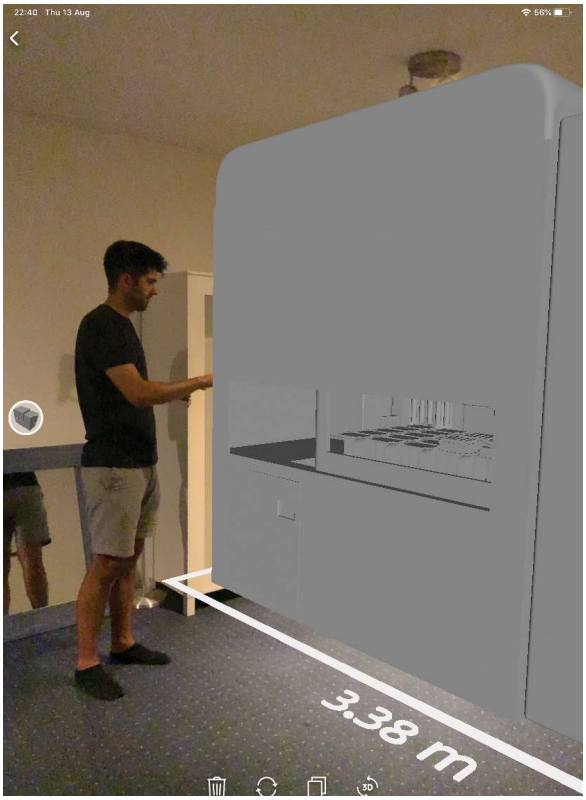


Figure113: Example of testing that could be done in order to validate decisions on interaction. Ergonomical testing is not sufficiently accurate and would be difficult to simulate since the image of the model is always overlayed on the screen. However, this viewing can be helpful in the estimation of proportions and interaction flow preferences.

Nr.	Assumption	Source/ Reasoning
1	The manual action of pipetting up and down can be substituted by shaking of the well plate, the possibility of different needs between different wells in the same well plate can be omitted	Shaking plates are used in exiting liquid handling devices, the disadvantage could be that while a technician can decide to pipette up and down the content of the well plates differently for each well of the same plate, this specificity can not be reached with the adoption of shaking techniques. For the level of study of this project this detail has been omitted.
2	An inverted microscope can perform the quality control to be executed during the steps of bulk passaging, feeding, and differentiation	Discussion with Mehrnaz Ghazvini, see Chapter 6.1, section "*Correction of the imaging devices used*"
3	Work of the machine can be scheduled so that no technician has to be present at night at the facility, the system can postpone the judgement on disruption at the start of the next working day time.	Conversation with Beckman Coulter
4	In the expansion unit there is need to supply with new well plates and this can be done with materials such as tips and pipette tips subministred throgh a storing hotel	Assumption taken due to elements of the functioning of the Biotek Liquid handler not known and not reppresented in the product manual
5	The steps of incubation of 5 minutes can be substituted with steps that use a plate warmer	Discussion with Mehrnaz Ghazvini
6	If liquid handlers are not easily reachable at closed enclosure, the refill of the tips can happen through tanks positioned closer to the reach of technicians.	Conversation with Beckman Coulter
7	New tips can be supplied reaching directly to the deck when liquidid handlers are positioned close enough to the deck opening	Observation of promotional videos of the liquid handlers on Biomek's website
8	The Celigo Cytometer is able to perform confluency control	Ability to measure cell phases for adherent cells is described at the vendor site Nexcelom.com as described at Chapter 6.1 this should be confirmed for counting of cell colonies as well.

Figure 114: Assumptions taken

Nr.	Assumption	Source/ Reasoning
9	The safety cabinet can stay opened to one side if the continuous down stream airflow is provided	Initially none, later confirmed by the enclosure builder at CleanAir by Baker, Gregor Holdampf, see Further Improvements Chapter
10	Movement of air due to transportation of devices through the system is negligible	None, further discussed with enclosure builder at CleanAir by Baker, Gregor Holdampf, see Further Improvements Chapter
11	There should be only one opening of the safety cabinet.	This assumption has been taken to limit the number of possible sources of contamination, from the discussion with the safety cabinet builder it was evident that possible other openings can be acceptable
12	The safety cabinets can still provide protection even if in big dimentions and with objects moving inside	Later confirmed by the enclosure builder at CleanAir by Baker Gregor Holdampf, see Further Improvements Chapter
13	Integrating incubators and freezers in the system doesn't change significantly the temperature of the internal environment.	In order to facilitate continuation of the project, relative changes of temperature should be considered in future detailing
14	The transportation of well plates can be done by converyor belts and robotic arms at a fast enough speed to not cause significant delay in the process	Assumption taken in order to facilitate continuation of the project, relative changes of temperature should be considered in future detailing
15	The downflow of the safety cabinet can be provided by small columns on the side, at the vertixes of the enclosure	Assumption taken in order to facilitate continuation of the project, later on proved wrong in confrontation with the enclosure builder. Action in order to change this assumption should be taken

Figure115: Assumptions taken

Assumptions

The process of reprogramming of hIPSC lines (human induced pluripotent stem cell lines is a complex procedure that requires both understanding on the biological side, system design and the interdependance between the two.

Because of the time limitations of the project several elements were based uppon assumptions and would need to be checked on in the future before continuing with the process of transition towards automation.

The main assumptions are presented in the tables. They are grouped based on their main subject: the overall process of cell culture (Figure 114), specific devices (Figure 115) or safety cabinets (Figure 116).

Following the second section of the V model (Figure 40) it is reccomended to validate the assumptions in this order.

Nr.	Assumption	Source/ Reasoning
16	The production of hIPSCs can be automated and disruptions can be calculated by a specifically developped software	Initial assumption of the Brief, later confirmed by the existence of other products able to perform this function also for hIPSCs.
17	The times of production of hIPSCs is related only to the timing needs of the cells and not on the speed of operation of technicians.	The process involves stationing of well plates inside an incubator over night before continuation, even if the timing of production is quick the limitations of speed of the process would be relative to incubation times, which can not be speeded up
18	There is need to provide both product and person protection since the material handled, although not being hazardous because of chemicals it can be hazardous for possible development of bacteria and viruses, therefore the material should be separated by a class II safety cabinet	Informations relative to safety cabinet used at the Luxemburg University system, which also produces hISC lines.
19	The system used by the Luxemburg University can be taken as an example for starting point devices of the IPSC production at Erasmus MC	Reasoning explained at Chapter 2.12.7

Figure 116: Assumptions taken

Reflections on the project

Reflections on the research objectives
This chapter compares the final result of the project with the initial research questions in order to evaluate if the RXF has provided answers to them and which elements should be explored further.

MRO Define and test the configuration and interactions of an automated system at the IPSC Core facility.

When comparing with the main research objective, the project has covered fully the research regarding definition of the aspects necessary for an automated system to take place within the IPSC Core facility. As pointed out in the limitations section, however, testing has been conducted only in a scenario based manner and further testing should be conducted to prove the identification of the correct bottle necks for the process, capacity needs and user interfaces.

RO. 1 Identifying design aspects that most influence the transition of human-controlled processes towards automation.

In Chapter 2.9 Automated culture of hIPSC lines, the main advantages and disadvantages on automation of cell line production have been analysed, as points to take in consideration in the further design phases. These aspects have been later on embedded within the ideation sessions that followed in order to bring forward these insights in the proposed solution.

In the validation section of the project important stakeholders of the Facility, both in the management and the technicians have analysed the proposed solutions and offered their remarks over possible risks, also regarding elements previously mentioned as difficulties encountered for automation (control of batch to batch variations need for extensive online control over process parameters an improved possibilities for process documentation).

It is possible to conclude, therefore, that the current project has explored which are the critical aspects for the transition towards automation and that the future steps of iteration should try to bring these insights further.

RO. 2 Determine the roadmap towards automation of the process of iPSC culture to be adopted by the iPSC Lab Facility at Erasmus MC.

Through the process the consultation with several stakeholders usually involved in the system design development for automation of cell culture has allowed to identify the initial Roadmap (Figure 3). This project's scope has been limited to the second milestone of the overall automation Roadmap. However, along the definition of the proposed layout plan, relevant elements to be used further for discussions and decisions have been initiated. Also this research objective results being covered by the final outcomes of the project.

RO. 3 Evaluate the state of the art of technologies and different product architectures for automation of iPSC culture.

The state of the art regarding both liquid handlers and full automated systems has been evaluated respectively in Chapters 2.10 and 2.12. One product architecture, the one used by the Automated Cell Culture platform from the CEN group at the University of Luxemburg has been selected and used as a reference example during the creation of the final proposed design.

RO. 4 Identify the design opportunities for the automation of decision-making processes.

Decision making elements to be considered

within a project involving automation has been described in Chapter 2.1.3, when discussing the control problem, and in Chapter 4.2 when analyzing the opportunities given by the development of technologies. However, it can be argued that due to the initial stage of concept development only the idea of the integration of these technologies has passed onto the final design. The outcomes relative to this research objective could, therefore be evaluated in future testing of the project.

RO. 5 Identify risk factors which might obstruct automation of iPSC culture production in case of increase of throughput.

The initial evaluation stages of this chapter, finally, have identified the current risks and limits that could obstruct or prevent further investment in automation, in the future steps it will be necessary to tackle these aspects first in order to ensure the viability of the overall project.

Reflections on the results of the project

The RXF system can be checked against the initial list of list of requirements in order to validate that the RXF is a solution to consider for this project. Taking into account Figure 39 at the Chapter 5 it is possible to see that several aspects of the general list of requirements have been covered. However, others were not met, partially or fully.

R17: The product should allow to have a safe sample - without crosscontamination by preventing airflow movements. The RXF system safeguards from cross contaminantion by using disposable pipetting tips at each step and by sealing the well plates during transportation However, as analyzed by the enclosure builder, changes should be implemented in order to ensure that air flow caused by moving objects is minimized

R19 - R22: Requirements relative to lifetime expectation and mantainance needs better understanding of the specific details of chosen devices.

R24 -R28: The size and weight of the single modules allows technicians to move the modules by pulling it over the ground. This requirement resulted evidently not reachable given the weight of the liquid handlers for medium- hight throughput (225 kg).

R30 - R31: The product conveys a feeling of professionality, trust and control by the technicians that use it.
Aspects of product aesthetics were considered just in the final stages of the design, therefore there is space for further improvement.

R32: The materials inside and outside the system allow cleaning with 70% alcohol.

The choice of materials for the external enclosure should be chosed in collaboration with a manufacturer, while fullfilment of this requirement should be checked with each device producer.

R36: The system should be trusted by the technitians: This aspect should be embedded better also through the development of the software and in further testing.

Further challenges

Further challenges

Summarysing the insights gathered in this last chapter (elements of improvement, assumptions or limitation to validate, and un-covered research objectives) it is possible to identify future challenges of the project. These aspects are presented in focus areas:

A) ***Ensure and check the objective of transition towards automation***

1. *Validate the assumptions:*
   The assumptions described in this project should be initially verified.
2. *Validate and readjust the concept in relation to the disponibility of budgets:*
   Given the initial cost price estimation initiate further discussion with stakeholders and validate the decisions to make for continuation. Segmantation of the investments could be considered initially, as well as a different combination of the proposed modules with each other.

B) ***Refinement of the final concept design***

1. *Refine with device manufacturers the elements of design to be improved*
2. *Correct the overall design* by integration of additional needs of the devices identified through the previous step.

C) ***Set up of a software platform***

1. Information gathered from interviews with automation platform providers (Chapter 2.13) can be used for taking a decision on whether or not to adopt an existent software or develop a software in house.
2. Gather data for the development of machine learning
   Through the initial stages of research of this project factors important for the automation of imaging control have been identified (Chapter 2.3.2). The process of gathering pictures for the development in the future of a machine learning device, specifically tailored for the laboratory needs should be initiated.

D) ***Initiate a slow transition to automation***

It is recommended to test initially the system on the cells by understanding details of the automated system and try to manually mimic the culturing conditions on the cells before ordering a system.

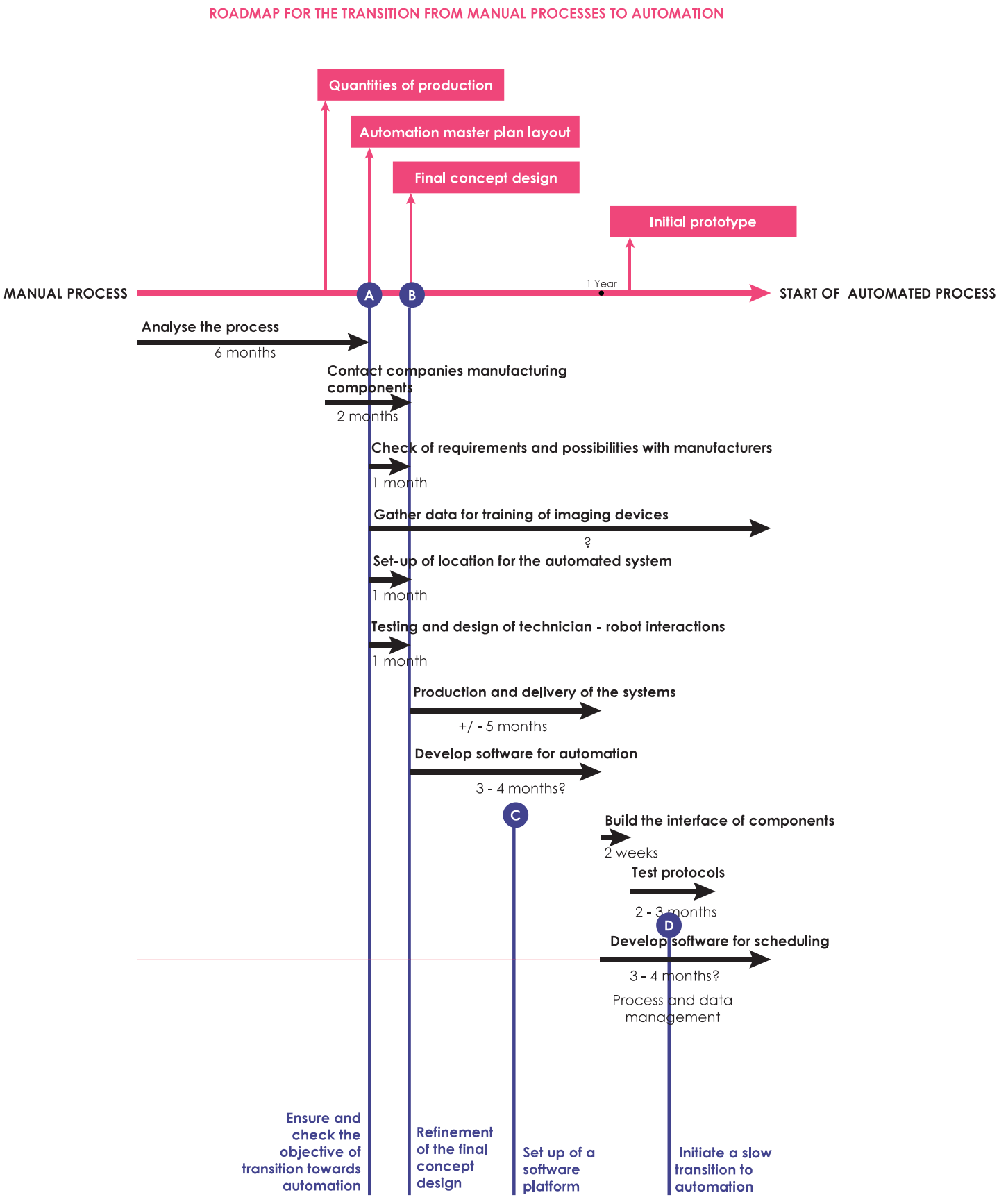


Figure117: Updated Roadmap for future challenges

Personal reflections

Reflections about the process

Considering my background, prior formation and the initial brief that was set, the study and developpement of this master thesis project was an articulated path.

As an initial briefing I had the idea of trying to reach a very practical outcome and design stage by roughly evaluating the autonomation process of production of OPSC lines within the IPSC Facility at Erasmus MC, but mainly by focusing on the imaging devices involved within this process.

After the initial research period, I discovered a the complexity I didn't take into account and that made me re-evalue the goal set at the beginning.

The initial research disclosed that the process of automation was actually considered still at a very early stage within the faculty implementation, so that other, broader aspects needed to be understood first.

Within Erasmus MC the production processes rely mainly on the experience and know-how of the technitians that operate within it, but considering the will of automation, these processes needed firstly an extensive esamination, tracking and documentation. Each step needed to be understood for its meaning and for its relation to the previous and subsequent ones in the chain. This tracking and documentation was regarded as prioritary activity also in view of the need to provide at least an initial database for the developpement of an imaging machine learning software, crucial to automation and not jet present.

Since my background doesn't include such a extensive knowledge of imaging devices and a specific undestanding of biological characteristics of cell culture, these fields were not seen as a possible contribution of mine, but more elements that needed to be researched and at times assumed in order for my contribution to be adequate the the context it is useful to.

Once the production flow was undestood, benchmarking of biological laboratory equipment and comparison with other abstracted similar systems gave me the set of tools to pass on the actual ideation steps and concept development.

I realized that my strength in this very specific situation could be actually being a figure able to combine processes of analysis and abstraction.

I decided to focus my research on the flow of production and on the overall coposition design by using methods of evaluation and comparison in the decision making moments.

System architecture design knowledge gave me the possibility to approach the design of complex systems even when my knowledge of them was not so specific, an example is the field of programming and machine learning: altough being one interest this project triggered in me and that I hope to develop further on in the future, for the scope and time logistics of the thesis project, I couldn't explore it to an advanced level, but I still managed to understand it's functioning within the process and accomodate it's needs in the concept design through the application of a great number of methods, some new and some assimilated through the masters program.

The change of the final goal of my project from beginning to end of the thesis work tought me there are limitation I could not undestand fully or influence and that at this stage, it was necessary to take some

assumptions, I took the caution to try to reach the smallest degree of possible variation by grounding them on as more scientific methods of comparison and evaluation as possible.

Personal reflections

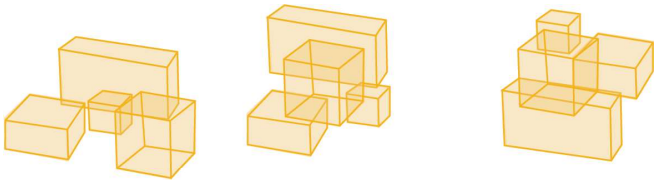
From a working environment point of view, the project gave me the possibility to experiment the dynamics of an individual work on a complex matter within the context of a company and consideting the possibility of counting on the collaborative interactions happening among the people of the same department. I saw the benefits of being part of an organization also from a motivational point of view, since it gives clarity, scope and continuation of my contribution and joins the various professionals by having one shared interest.

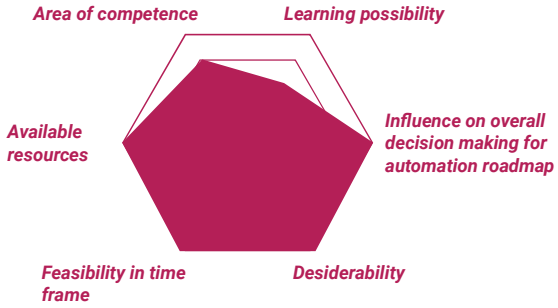
In the working process I have also learned to embrace some aspects of my nature that I have previously considered as weakneses. One example is my tendency to get attracted by details: although sometimes it is something that leads me to the investment of a higher amount of time then accounted, I believe it is one of the aspects that also allowed me to bring this project to a quite detailed design of a complex sistem used in a field completely distant from my reality.

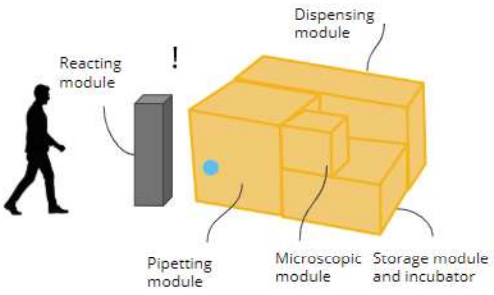
This master thesis concludes my specialization in a medical design, a field I happily found myself passionate about.

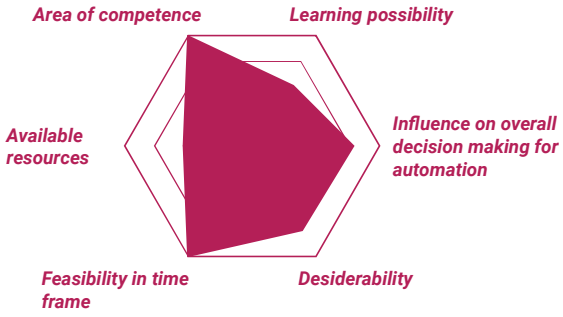
Appendix

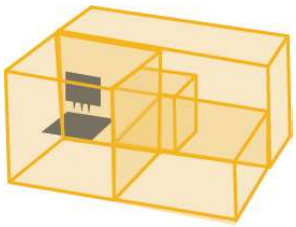
Appendix I - Functional Analysis Table

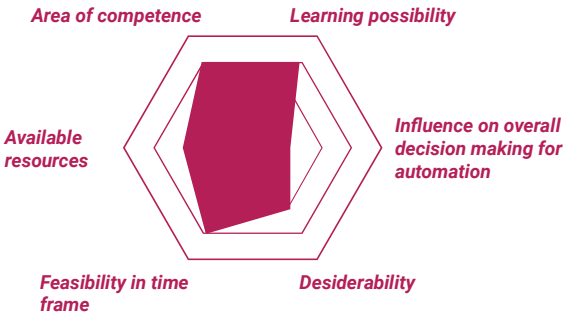


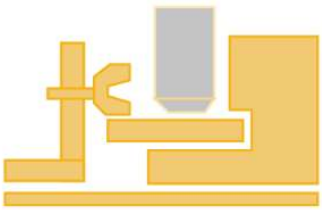


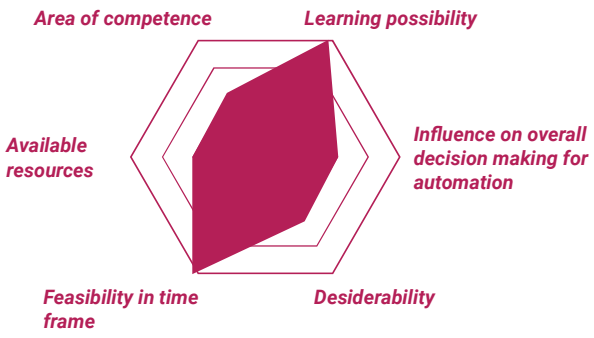


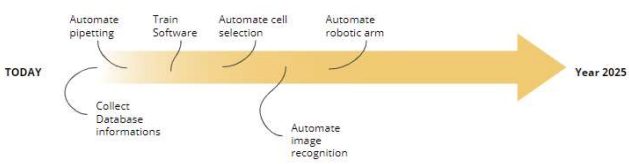


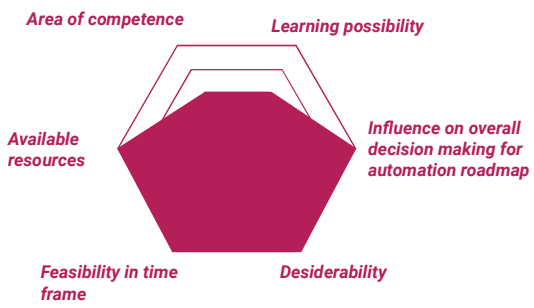












Design the overall architecture of the machine by orchestrating the interactions between components and the necessary interactions with the technician.
Conceptual approach with more focus on the parallelization of the production lines

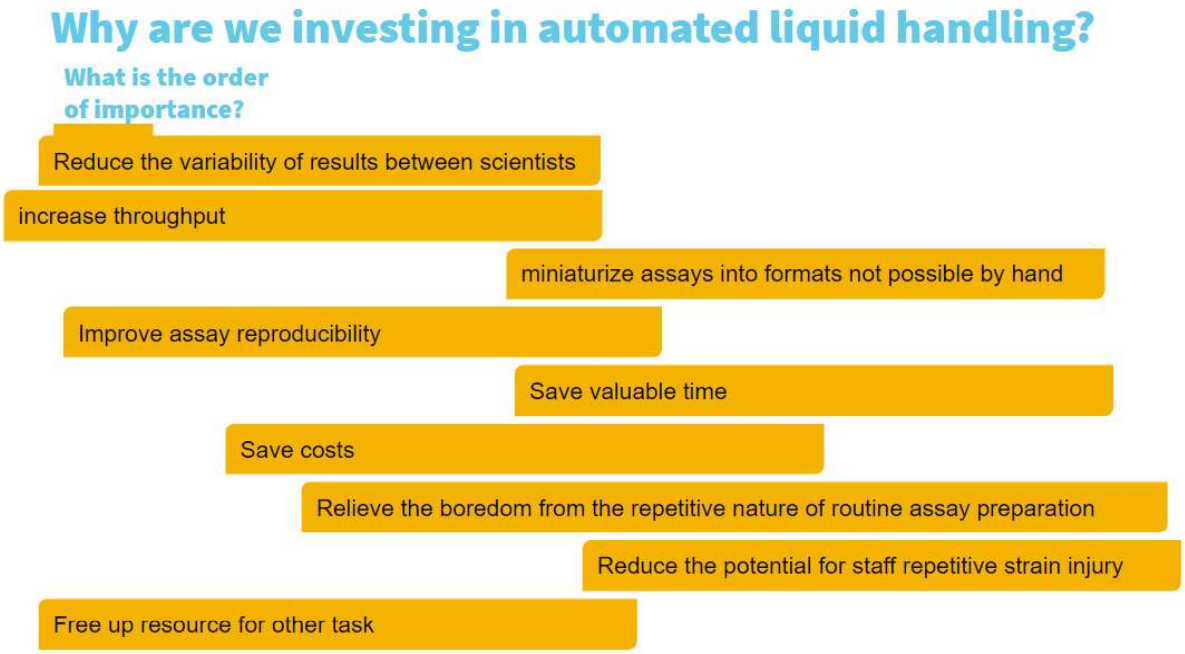
Design the system that will allow to react when the machine commits a mistake along the process
Conceptual design, focuses more on testing the accessibility, interaction and clarity, easiness of use

Design one specific component inside the machine:
Embodiment design for a specific functionality within the system that will be independent from the overall product architecture taking into consideration the necessary flexibility of the system.
A component candidate for this could be the robotic arm, the challenge is that of translating the movement of the wells to a robotic handling that can be able to adapt to different wells and sizes.

Design for the microscopy module:
- Design and prototype the connecting device that will allow the transfer of the plates from the incubator to the microscopy module and to the pipetting robot.
- Design the components of the microscopic element so that different microscopic functionalities are combined.
(Not concerning the training of the machine learning algorithm)

Identify the Roadmap that will lead the facility to the integration of an automated system.
In this roadmap, design a product that will be able to assist in the creation of a database of images for the better training and supervision of the correct decision making of the machine.
Focused on the details of the benchmarking side and of strategic nature

Appendix II - Priorities for Automation



Appendix III - Functional Analysis Table

	Compartments involved		Nr (per line at best scenario)
1	----------	**P0 - P1**	
	Incubator	Incubator standard setting, 1 x 6 well plate stored and 12 well plate	
		Timer for 24 hours	
	Microscope A		
	Actions:	Checks **morphology** and **size** of the cells	
		Understands the **coordinates of the colonies** that can be picked, transfers this information to the next step of the process	
	Liquid handling Robot		
	Should have both multi-channel (providing fresh medium in the newly added well) and single channel pipetting (cutting and seeding wells)		
	Should be connected with the sterilizing compartment in order to sterilize the filtered tips before they get used		
	Should handle the pipettes individually, it goes from one well only from one plate to an other one with different size of wells		
	Should be close to a discarding container for the used liquids or have a safe connection to the discarding container		
	The plating should be able to detect the concentration of the liquid of 10 µM plating		
	Its movements should be regulated in such a way that it can cut the cells and aspirate them according to the coordinates of the colony disposition		
	Pipetting actions:	Suction, Addition, Cutting, Aspiration, Plating	
	Movements of the rack	Tilting of the well plate for propper suction without creating bubbles inside of the well	
	Consumables:	1 ml pipette	x1
		20 µL pipettor with filtered tip	x1
		22 - 25 Gauge needle or pulled glass pipette	x 8 or more
		2 mL pipettor	x1
		6 well plate	x1
		12 well plate	x1
	Liquids	1 ml Fresh medium	x 8 wells
		1 ml Fresh medium with ROCK inhibitor	x 8 wells
	Cell counter	Detects the amount of colonies that are already plated	
	Robotic arm or Transportation mechanism	Two robotic arms are needed: One brings the old well to the pipetting robot and the second one brings the new well directly to the incubator. Or there is a subsequential transportation	

Decision point	Interaction Point	Variables	Movements	Environment	Data transfer to the control unit
			Incubator to Microscope		
If to go foreward in the process or go back to an other day of feeding	Imput on the selsction of cells				
		Location of the selected colonies			
			Microscope to Liquid Handler		
To repeat the pipetting robot's actions or pick up the old well plate from the incubator if the attachmen of the chells doesn't improve with time			Liquid handler to cell counter		
			From cell counter to liquid handler From cell counter to Incubator		

2	----------	**Feeding**	
	Incubator	Incubator standard setting, 1 well plate stored	
		Timer for 24 hours	
	Microscope B		
	Actions	Checks **attachment of cells**	
		Checks **turbidity of the medium**	
		Checks cell **density** and **size**	
	Liquid handling Robot		
	Multi channel pipetting, several wells of the same well plate will be feeded at the same time		
	Actions	Transfer by suction and trashing old medium	
		adding new medium	
	Consumables	2 mL Pipette	x 16
	Liquids	2 ml Fresh medium	x 8 wells
	Robotic arm or Transportation mechanism	Two robotic arms are needed: One brings the old well to the pipetting robot and the second one could need to be used in order to reintegrate in the process old wells because of unattachment or turbidity Or there is subsequential transportation	

			From incubator to Microscopic analysis		
Wheather to step back and take plate from incubator	In the eventuality of lack of attachment of the cells after the second day the technician should get a prompt on the action to make, rethrive the old well plate/...	Which wells should be still be considered in the further steps?			
Wheather to step back and take plate from incubator					
Weather to repeat the step or go to the following step					
			From Microscope to liquid handling		
			From liquid handling to incubator		

3	----------	**P1 - P2 Mechanical passaging**	
	Liquid Handling		
	Multi channel pipetting, several wells of the same well plate will be feeded at the same time, but it can also happen that some wells in the same well plate need an extra feeding		
	Actions	Transfer by suction, add, discard, cutting, aspirate only liquid on the top of the well	
	Consumables	New 12 well plate	x1
		22 - 25 Gauge needle	x8
		2 mL pipette	x8
		1 mL pipette	x8
	Liquids	2 ml Medium with Rock inhibitor	x 8 wells
	Incubator	Incubator standard setting, Old and new well plate stored	
		Timer for 24 hours	
	Robotic arm	Only one robotic arm, all the wells that enter this step are following the same treatment.	

	Add the precoated well to the pipetting robot module				

7	----------	**P3 - P4 Bulk passaging**		
	Liquid Handling			
	One additional well plate is inserted therefore the rack should have more space for this step and the number of wells to treat doubles			
	Actions	Transfer by suction and trashing old medium		
		Adding reaction liquids		
		Seed cells in the new wells so that cells are 70 % confluent in each well		
	Consumables	1 mL pipettes	x16	
		0,5 mL pipettes	x8	
		12 well plate	x2	
	Liquids	1,5 ml Medium	x 8 wells	
		1 ml DPBS	x 16 wells	
		0,5 ml EDTA	x 8 wells	
	Incubator	Incubator standard setting, 1 well plate stored		
		Timer in between steps of pipetting 5 minutes		
	Microscope C	Checks the size of the cells		Re act
	Robotic arm or Transportation method	Move the plate in a figure of 8 movement		

	Insert two coated new 12 x well plates		Microscope to Liquid handling		Seeding of wells is done according to the volume decided at the microscopic density estimation
		The volume of seeding of the wells depends on the numbers decided initially by the informations given from the microscopic analysis			
				The EDTA has to be prewarmed to room temperature level before inclusion	
			Liq Handling to Incubator	The EDTA has to be prewarmed to room temperature level before inclusion also when integrating the new well plate	
			From Incubator to Liq Handling		
epeat a pipetting tion		Number of times the pipetting should be repeated	From Liq Handling to Microscope		
			Movement in a figure of 8 and into incubator		

	----------	**DNA isolation**	
	Liquid Handling		
	Multichannel pipette doing the same steps for all the well plates		
	Actions	Transfer by suction	
	Consumables		
	Liquids	2 ml PBS	x 8 wells
		0,5 ml DNA lysis reagent	x 8 wells
	----------	**Freezing**	
	Liquid handling robot		
	Only the selected wells are transferred, the pipetting robot should be provided with multichannel pipette		
	Should be able to handle three different typologies of containers: well plates, tubes and cryo-vials		
	Actions	Transfer by suction, Adding medium, Transfer to tubes with longer tip,	
	Consumables	2 ml pipette	25
		0,5 ml pipette	10
		15 mL Tubes	5
		1 ml pipette	10
		cryovials	5
	Liquids	2 ml DPBS	x 10 wells
		0,5 ml EDTA	x 5 wells
		1 ml Freezing medium	x 5 wells
	Compartments involved		Nr (per line at best scenario)
		15 mL Tubes	9
		1 ml pipette	27
		cryovials	9
	Liquids	2 ml DPBS	x 18 wells
		0,5 ml EDTA	x 9 wells
		1 ml Freezing medium	x 9 wells
		1 ml Medium	x 9 wells

			From microscope to pipetting robot		
				PBS is stored at room temperature (between 20 and 22 Celsius degrees)	
	Collect the 1,5 ml tubes for further analysis		From microscope to collection area	DNA lysis reagent is stored at room temperature (between 20 and 22 Celsius degrees)	
			From Microscope to pipetting robot		
ecision point	Interaction Point	Variables	Movements	Environment	Data transfer to the control unit

----------	**Preparation for staining**	
Pipetting robot		
Actions	Transfer by suction, mixing pipetting up and down, transfer into tubes, transfer of sample for counting machine	
Consumables	2 ml pipette	3
	0,5 ml pipette	2
	15 mL Tubes	1
	1 ml pipette	4
	50 micro liter tube	1
Liquids	2 ml PBS	x 2 wells
	0,5 ml Enzyme	x 2 wells
	1 ml Medium	x 4 wells
	Germ layer- Endoderm	x 6 wells
	Germ layer- Mesoderm	x 6 wells
	Germ layer- Ectoderm	x 6 wells
Incubator	5 minutes and overnight before going towards the feeding step	
Counting machine		
Actions	Gives the number of cells that are needed for seeding two plates with two wells containing 800 000 cells (for Endoderm and Ectoderm) and one well of 200 000 cells (Mesoderm)	

			From microscope to liquid handling robot		
			From liquid handling robot to incubator		
			From incubator to liquid handling robot		
	Insert numbers from cell counter to control unit for giving directions on how many ml of volume need to be plated in each well		From liquid handling robot to counting machine		Informations on the cell density is used to calculate the volumes used afterwards by the pipetting robot to seed the new plate
	Coating of new well plate with correct germ layer for each column		From counting machine to liquid handling robot		
			From counting machine to incubator		

	----------	**RNA isolation**	
		As previously described for each of the first three columns of the 12 well plate, quantities change accordingly. Two of the three colums are collected separately at the 5th day of feeding	
	Pipetting Robot		
	Actions	Suction, addition and mixing by pipetting up and down	
	Consumables	1 ml pipette	x18
		250 micro liters pipette	x18
		1,5 ml tubes	x9
	Liquids	1 ml PBS	x 9 wells
		1 ml Fresh Medium	x 9 wells
		250 microliters RNA lysis reagent	x 9 wells
	----------	**Preparation for staining**	
	Pipetting robot	**Counting of cells not needed**	
	Actions	Transfer by suction, mixing pipetting up and down, transfer into tubes, transfer of sample for counting machine	
	Consumables	2 ml pipette	3
		0,5 ml pipette	2
		15 mL Tubes	1
		1 ml pipette	4
		50 micro liter tube	1
	Liquids	2 ml PBS	x 2 wells
		0,5 ml Enzyme	x 9 wells
		1 ml Medium	x 9 wells
	Incubator	5 minutes timer, 37 degrees celsius at 5% CO2	

			From microscope to liquid handling robot		
			From liquid handling robot to incubator		
			From incubator to liquid handling robot		
			From liquid handling robot to pick-up section		
	Pick up the i,5 ml tubes from the machine				

Appendix IV- Technologies for liquid handling

Different typologies of liquid dispensing

Liquid handling has a pivotal role in the process of iPSC line generation expansion and storing. Because of the tediousness, time consumption and difficulty in precision of the steps if executed by hand, and the increase interest in the field in recent years, laboratory automation has attracted attention and fueled the development of robotic technologies.

The difficulty that liquid dispensing technologies have to face is relative to overcoming surface adhesion and dispensing of droplets from dispensing tools due to the high surface adhesion happening at micro and nano levels.For this reason different methods of liquid dispensing have been deevloped and can be classified in two main cathegories:

Contact dispensing: where a touch-off is necessary to complete the liquid dispensing and a drag-back action is done to overcome the surface tension between liquid and tip. This method is prefered for samples of small volume because of simplicity, reliability and low cost. Its drawbacks are the need to wash or replace tips for avoiding cross-contamination and risk of damage of the dispenser tip by colliding with the container.

Noncontact dispensing: Developped to avoid the problematics connected to crosscontamination this method avoids contact between liquid and surface container using a method in which the liquid is ejected from an orifice. Noncontact dispensing makes use of different technologies: solenoid, piezoloctric, acoustic dispensing, thermal inkjet printing, gas drive, electrostatic forces. Driving the liquid through non contact methods has been explored always further developping unconventional methods such as superparamagnetic

beads, surface tension of the liquid, light, and a spatial light modulator for processing a large array of droplets (Kong, 2012).

The current tools used in laboratories are syringes and microsyringes or pipettes. The first typology can be designed with manual

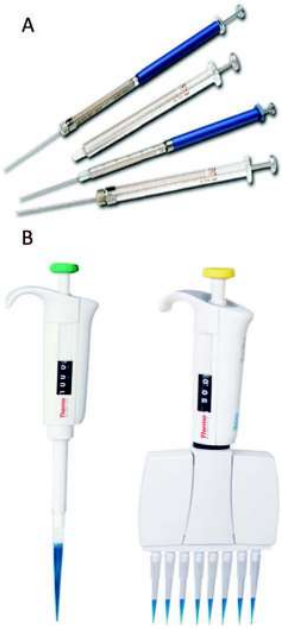


Figure: Siringes and single- and multi-channel pipettes

Figure: In-development, microfluidic robots developped at Volta Labs integrating elecrowetting.

or electronic holders to precisely control the piston displacement and assure accuracy. Pipettes can be single and multichannel, with the possibility of adjusting the tip spacing for transferring multiple samples between different tube rack and microplate configurations, fixed (for accuracy and precision) and adjustable-volume (for a larger scope of applications).

Robotic automated liquid handlers have such a variety in the scope:

- Focused on the liquid handling and leaving all the rest to human, usually of a bench scale, working in sincle- or multi-channel mode, sometimes embedding a

washing station

- Integrating plate washers, plate readers, plate sealers
- Integrating also components for DNA expression, delivery of results and interpretation.

The generic system architecture for liquid handling workstations is composed of a control center, that controls a robot, which moves between the dispensing part and a washing station, used to clean the dispensing head. Sensors are incorporated to monitor the status of the dispensing part and provide feedback control.

The most important component of the station is the robot manipulator.

The liquid-handling robot usually has a gantry structure for rigidity and stability. For smaller workstations a cantilever structure can be used to save cost. The positioning precision of the robot usually is of 10 to 100 microns.

Two models are defined dependent from the movability of the substrate:

Fixed substrate as the Freedom Evo (see at the figure in the following page), it saves space and it is commonly because of the benefit of good stiffness.

Movable structures need a larger movement space but they facilitate substrate replacement processes in high throughput.

Other system need higher degrees of freedom to extend the volume of the workspace (change plates, adding covers to the plates...) and therefore integrate matured industrial robots such as six-axis articulated robots with intelligent plate grippers (Kong, 2012).

The options for a laboratory are either in designing their own dispensing tools and components or to build their own workstation. Most laboratories prefer

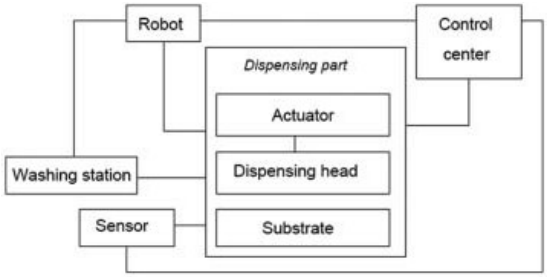


Figure: Generic architecture of liquid handlers
Source: Kong 2012, Journal of Laboratory Automation

commercially available workstations because they are easily integrating with other components and an increasing number of products in the field are able to deal with different numbers of well plates and dispensing tips.

The major downside of this option is the difficulty in system integration because no manifacturer provides yet all the components needed in the workstation and software integration is often a challenge also in the effort of providing a graphically consistent and intuitive software interface.

Differences and qualities in robotic Liquid handlers

The market offers a wide variety of liquid handlers. The production and design of such machines focuses on several aspects in order to provide a correct functioning and performance.

Performance of liquid-handling workstations is measured through precision and accuracy. Precision is meant as repeatable dispensed volume without reduction of accuracy, accuracy indicates the coincidence between the actual dispenced volume and the selected volume.

Periodical calibration of the workstations ensures their precision and accuracy with standards such as DIN12560 and ISO8655 (Kong, 2012). Other parameters to measure performance relate between the dispensing volume and speed.

Performance of liquid handling devices is susceptible to the following challenges:

- Evaporation Control:

This is more of an issue on the level of the nanoliter scale, for which different methodologies are used between which are used either to monitor the conditions of the environment on a high level and reduce evaporation by mantaining precise humidity and temperature, or to predict the evaporation percentage and adding extra volume of liquid.

- Handling the viscousity

Biomaterials can be highly viscous and therefore be challenging with nanoliter volumes. For this parameter the most important characteristic is relative to the dispensing distance (the height of the needle tip over the well), mathematical models are used for the assessment and guiding in the setting of the correct height.

- Evaluation and calibration

Environment conditions can vary the performance of the liquid handling system and therefore calibration and parameter related adjustments are needed. Automated methods with real-time adjustments have been developed but often require also a large amount of computation.

- Clogging detection and air bubble dissolution

Sensor methods are introduced also in this case for detection of air bubbles but there is no dissolution method available yet that can be applied for longer times since ultrasonic cleaning, which is normally used for this purpuse, can damage the dispensing head.

DIY Approach

The other option for Laboratories going on the road of lab automation, if they have a specific need that the market can not address or the budgets of available sistems is too high is that of creating for themselves a roadmap towards automation and design the system along the way.

Aspects to take into consideration are (Opentrons, 2019):

Liquid Volumes:
What are the liquid volumes that are most frequently used in the workflow:

For a range of liquid volumes that goes from 1µl and 1000µl, there is more offer of options to use. Robots get more expensive with smaller or larger volumes. Transfers of volume below 1µl get more expensive and complicated because of a different behaviour of surface tension and capillary action.

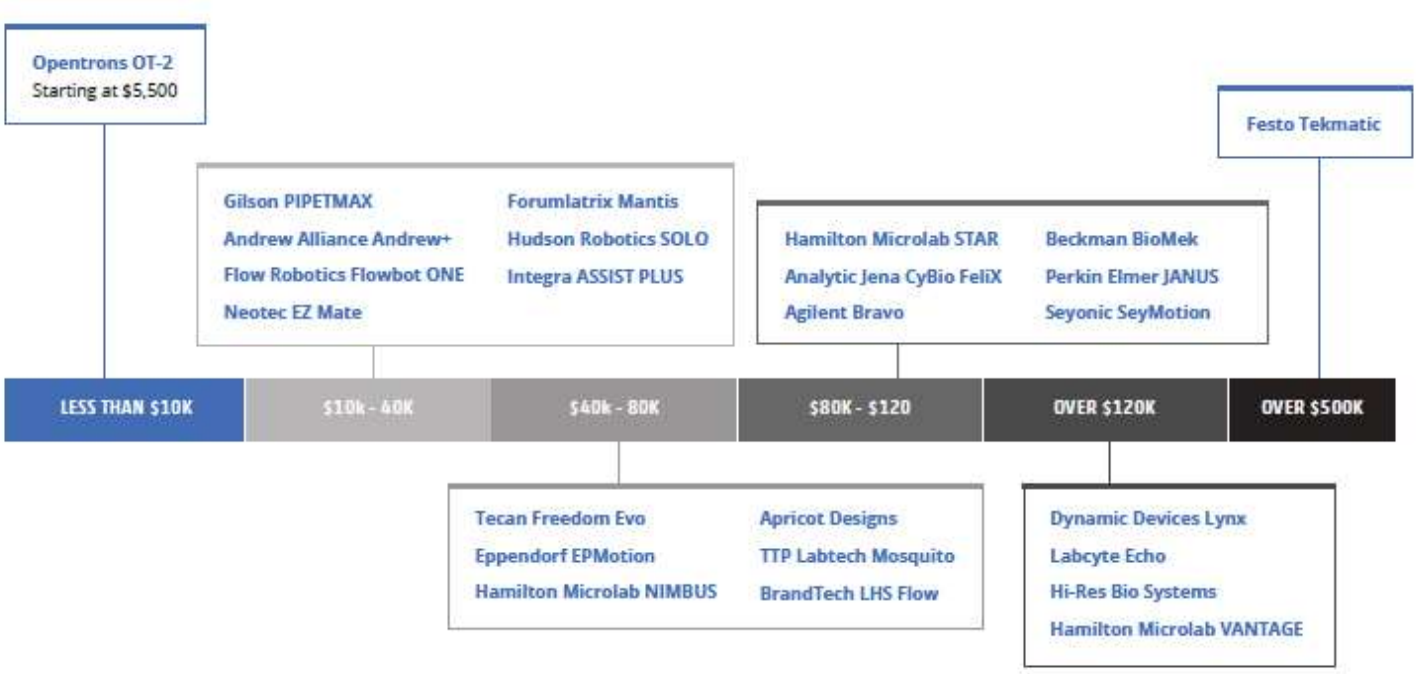
Need of ease:
What are the processes that are troublesome, time-consuming, error-prone and monotonous: these are the ones to which is most important to apply automation to.

Frequency:
The automation workflow should be ran often enough to justify automation, the more often the automation is run the quickest there is return on investment.

Independance:
The workflow for which to find and automation solution should allow the technician and researcher to walk away from the machine.

Precision:
One of the elements that highly affects the price of liquid handling solutions is the precision it has to allow. The workflow should be satisfied with a 5% precision, robots that allow for a higher precision have higher budgets (75k $ -100k $).

Throughput
Number of samples what are the volumes of production needed in the system to process



NOTE: These prices are estimated because most manufacturers do not publish their pricing openly—except for Opentrons.

at once: low, medium, or high throughput. For each of these the outcomes of typology of robot is different.

- ***Low Throughput (<1,000 samples/ week):*** Robot that runs a single-channel pipette, or has limited multi-channel capability.This system can process tens or hundreds of samples a week and can carry out most simple liquid-handling steps.

- ***Medium-throughput (<10,000 samples / week)*** Robot that works with an 8-channel pipetting head or more expensive robots that use 96- or 384-channel pipettes to do whole plates all at once.

- ***High-throughput (10,000+ samples / week)*** Robotic liquid handlers that take up entire rooms of space

Sterility

Related to the possibilities of contamination

- No separation, in which all the protection is reliant on the clean room capabilities
- Simple glass or plastic barriers to contain airflow between samples
- Fully enclosed systems with positive pressure and filtering
- Biosafety cabinets
- In this case everything that is running the workflow or is associated equipment has to enter a hood and leave enough access as needed

Size

Having a completely automatized workflow would need a bigger robot, which influences its movability.

If the robot should be movable in the lab and be placed in different parts of it to be connected to external workflows the decision on which section of the process needs to be automated and which not should be addressed.

Appendix V- Automated microscopy

What is automated microscopy

Technicians operate traditional microscopes entirely manually by regulating the examination of the slides, the selection of the right lens to use, or how information is visualized and interpreted.

The automation of microscopy has risen in importance by facilitating many of the most laborious tasks in the laboratory environment.

The automation of a microscope replaces many components of the device such as shutters, filter wheels, stages, light sources, and focus control by electronic components controlled by intelligent imaging software. By doing so the microscope only requires the user's input once the images are produced.

Areas of action for automated microscopy

Automation in microscopy can affect several aspects of the process of image production:

Focus and stage control: This form of automation is used on fine focus transmission gears, allowing advanced image acquisition software for autofocusing.

Wavelength selection: Motorized rotating filter wheels allow for high-speed wavelength selection, allowing different experiments to be run even when the user is not present and prevent the slow process of manual selection of filters, beam splitting units, monochromators and acousto-optic tunable filters (AOTFs)

Automation of mode selection: Shutters, illumination sources, environmental control, and image acquisition can all be automated for more productive and more effective microscope applications.

Products and technologies used for automated microscopy

Automated microscope and image analysis systems feature a wide scale of products going from high throughput fluorescence screening devices up to fully automated confocal high-end fluorescence microscopes.

Products such as the **IncuCyte** acquires and analyzes images on the cell status continuously and it has the ability to automatically identify regions of interest via masks.

Microscopy check-ups to automate:
As seen in the previous process analysis the microscopes used during the process of cellular reprogramming are used at each day of the process to monitor the level of development of the cells and detect eventual contaminations. Based on these elements decisions are taken over repetition of the feeding, need of sterilization of the wells, or continuing the process by further passaging or preparation for staining, RNA and DNA isolation or freezing. In the processes of mechanical passaging, where cells of desired morphology and size need to be picked the information of coordinates of the cells needs to be detected and registered by the microscope.

Functions
In different moments of time in the process, different aspects appear to be relevant and give input for other machinery in the robot.

Therefore the microscope should have the following typology of behaviors with different functions:

Microscopy A
Function: This step of microscopy should collect information regarding the cell size, the cell shape, and the brightness of cell edges.

It should also check against fungi contamination through the recording of the liquid's turbidity.

An image of the well-plate should be recorded and mapped in order to gain information on where the colonies of cells that can be picked are located.

This information will also control the pipetting robot.

Interaction: Send report and image of the well to the researcher, wait for the approval on continuing to the next step, which would control the transition to the next step through the robotic arm.

One image of the well is stored in a database.

Stage: It is used during the stages of mechanical passaging

Checked properties:

- Cell size: The size by which the cell aggregates should be picked goes from approximately 50 μm to 200 μm.

- Cell morphology: Stem cells have a specific morphology, their shape is circular and the borders of the cell start glowing in a specific way when observed at the microscope, the automated microscope should be trained to recognize the good morphology that characterizes somatic cells.

Microscopy B
Function: This step of microscopy should collect subsequently two pieces of information:
Check 1: regarding the attachment of the cells to the bottom of the plate, if the cells are not attached on the second day of feeding the process should be repeated with another plate that is better attached.

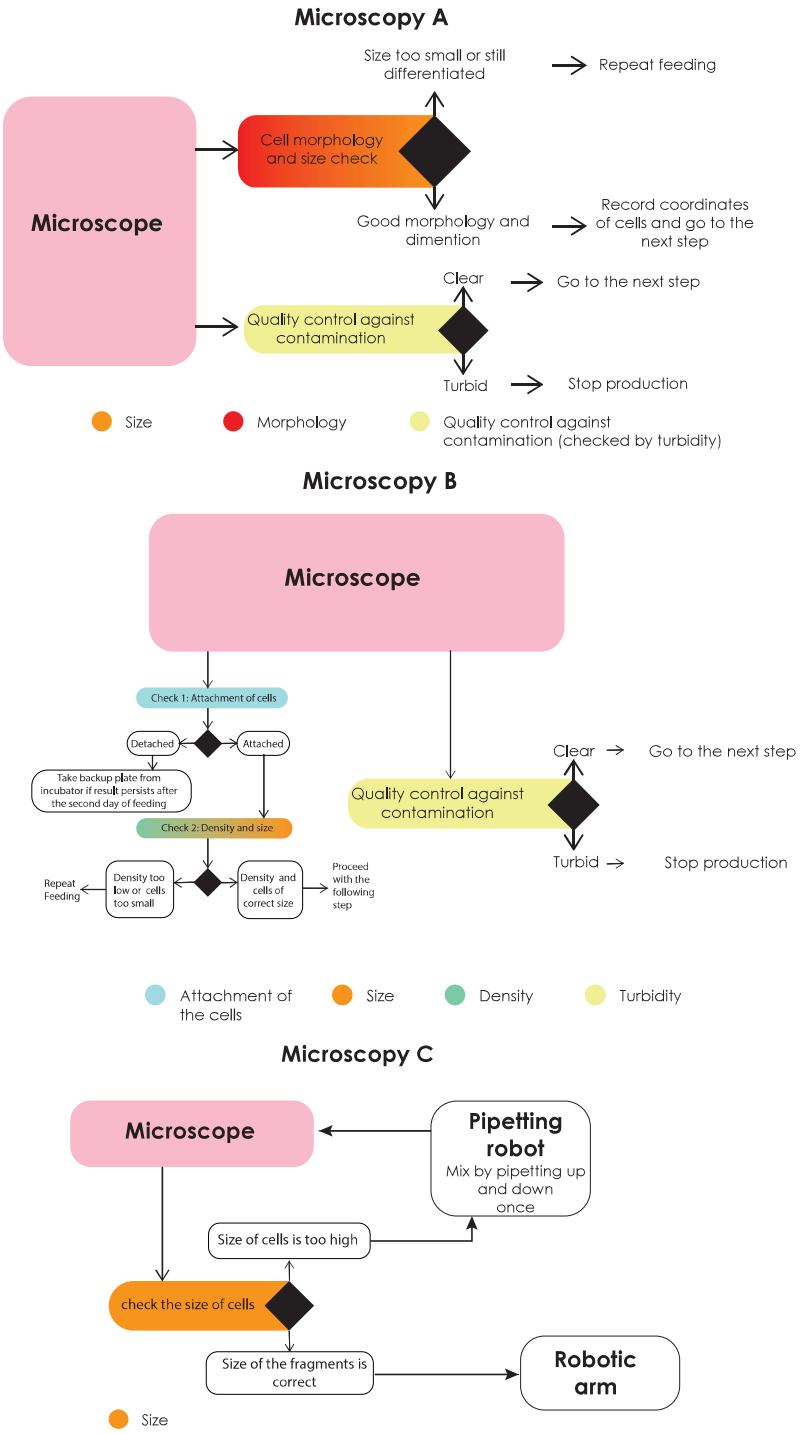
Check 2: should give information on the cell density and their size and either lead to the repetition of the previous feeding day or, in case the cell density and morphology result correct, the next Bulk passaging step, DNA isolation, RNA isolation, freezing or preparation for staining.

At the same time also the turbidity of the medium should be checked in order to stop bacteria-contaminated wells from going further through the process.

Interactions: The microscope should send to the technician only an image regarding check 2, regarding the dimension of the cells.

If the cells result not attached to the bottom of the well the technician has to reprogram the machine to start the program again with the remaining old well-plate.

Stage: this functionality of the microscope takes place at the end and at the start of the feeding.



Checked properties:

- Adhesion to the bottom of the well is checked by analysis of the hight of suspension of the cells in the well.
- The confluence of the cells and their size.

Microscopy C

Functionality: Determines if the pipetting up and down should be repeated or not to reduce the dimension of the cells

Interaction: During the initial stages of training of the machine learning algorithm, in case of a dubious outcome, the microscope should avoid mistakes by sending an image regarding the dimension of the cells to the technician, wait for approval on the decision to take by the technician and improve its training capability.

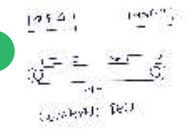
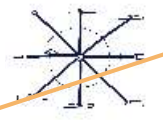
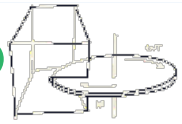
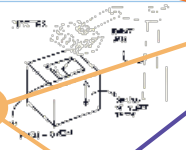
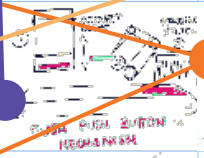
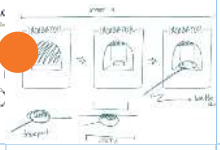
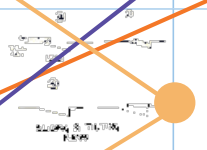
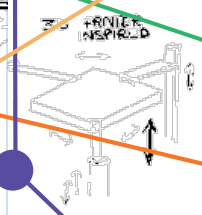
Stage: This functionality of the microscope takes place at the steps of bulk passaging.

Checked properties:

- Size of the cells.

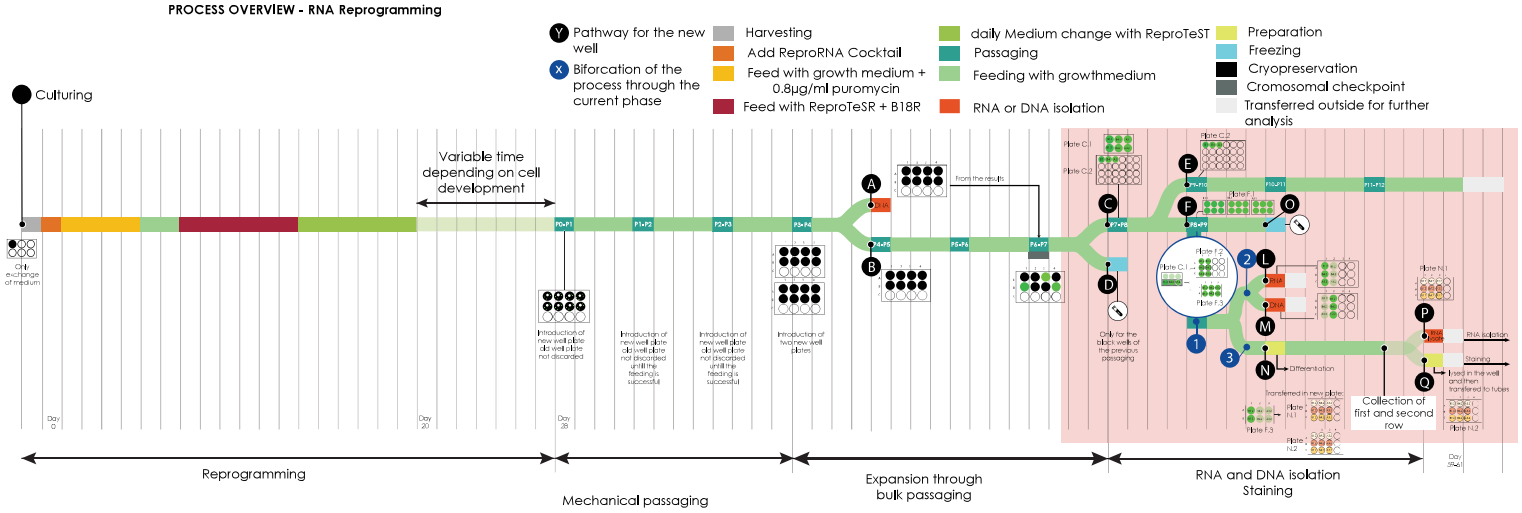
Appendix VI - Morphological Chart

Concept A Concept B Concept C Concept D

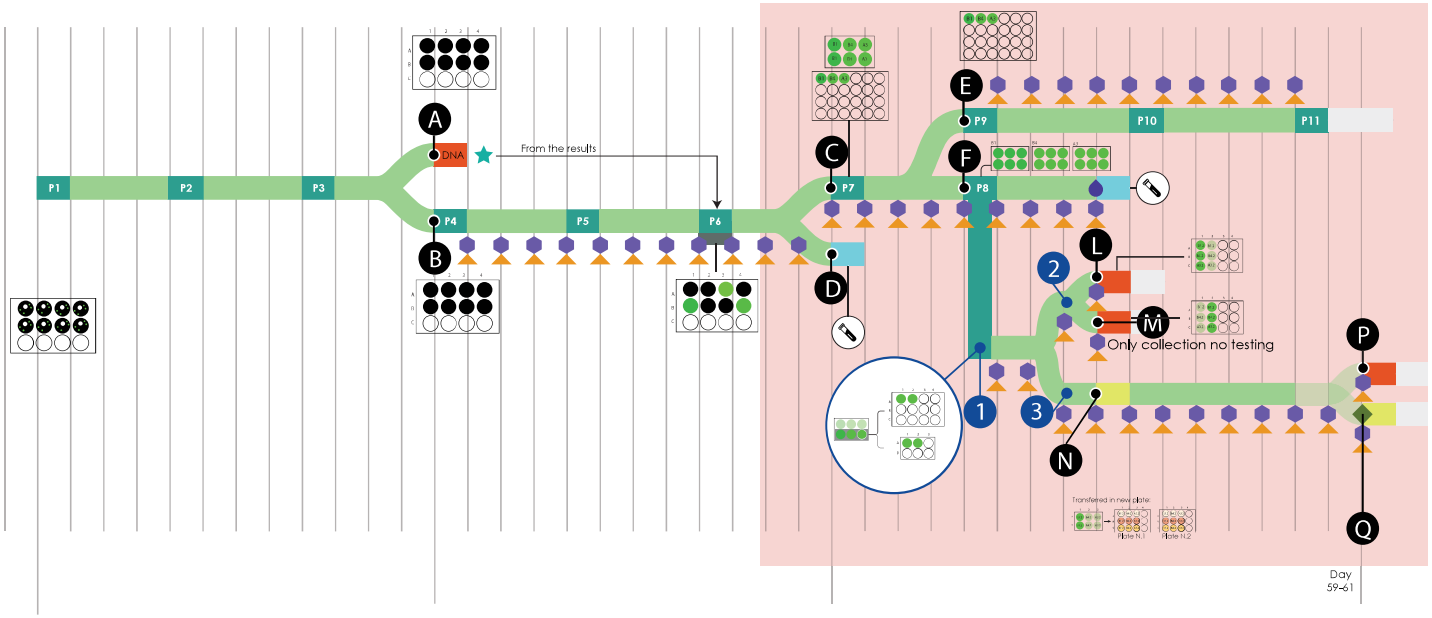
Function						
Transport well plates through the deck						
Open an incubator and transfer wells to a pipetting robot						
Insert a well into a pipetting robot						
Move a well plate in 4 different directions on a plane						
How to center a well over a microscopic lense	Measure the distance between Center A1, A2, A3,A4 and move the microscope ...ctly in that direction with the measured distance		magnet alignment (electromagnets)	Press fitting	Automated AI	
How to clean the surfaces of a cabinet during down... times	Vaporized Hydrogen Peroxide	UV light	positive air pressure	heat sterilization	Sound (vibration) or Steam	Alcohol wipe
How to selectively pipette well plates	Automated pipetting supported by directional data (... of where is which plate in the space)	Separate pipettes	Cover well plate openings Optical tracking	Do not load pipeting tips Gate recording		
How to take track of one well in one well plate through the process	barcode position tracking	NFC Tracking				
Transportation of material to the machine wothout contamination	Glovebox interaction	Biological isolator	Biological safety cabinet			
Liquid handling in plates process management	All wells at the same time	Two different channel pipettes at the same time	One plate at a time			

Appendix VI - Process Analysis

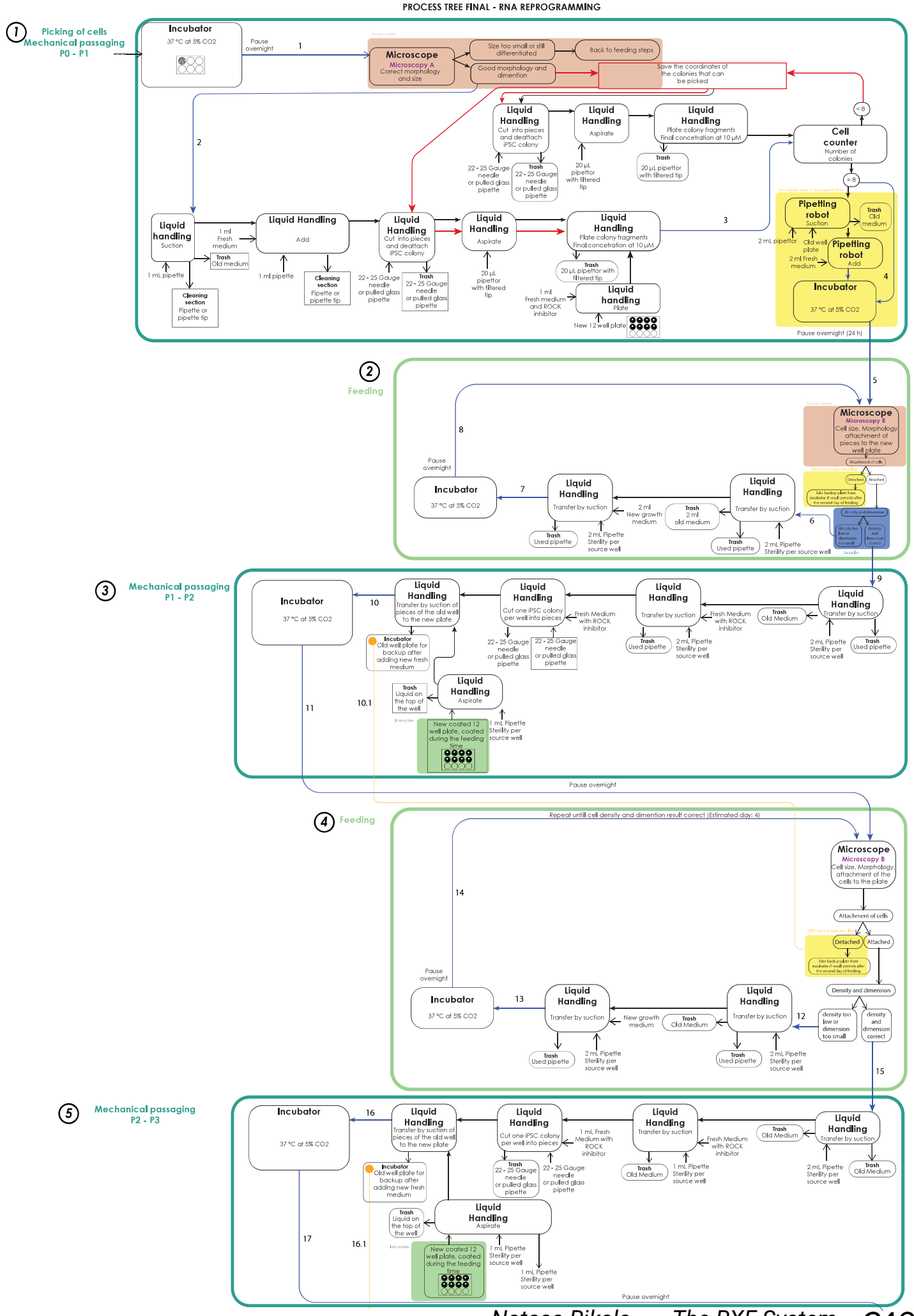
PROCESS OVERVIEW - RNA Reprogramming

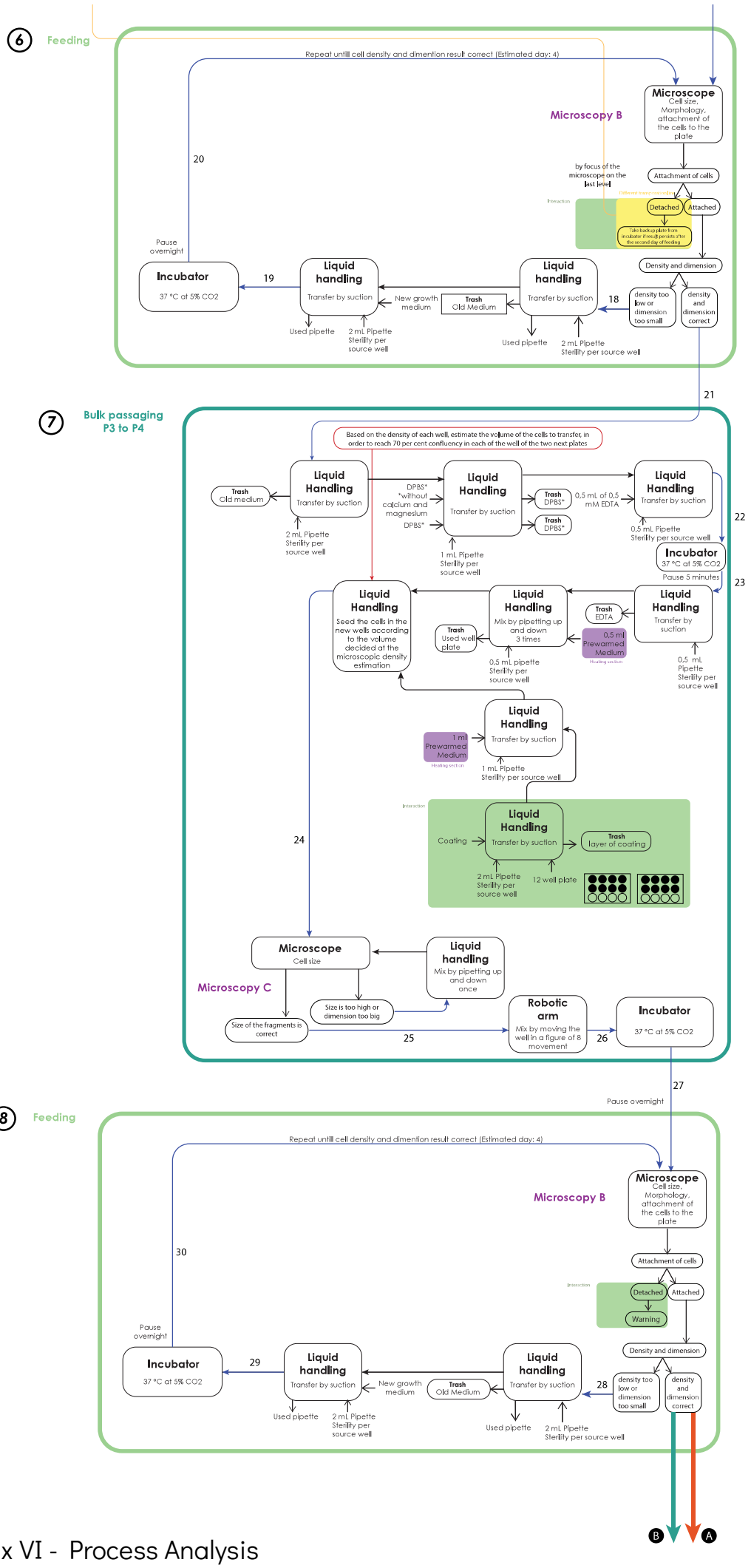


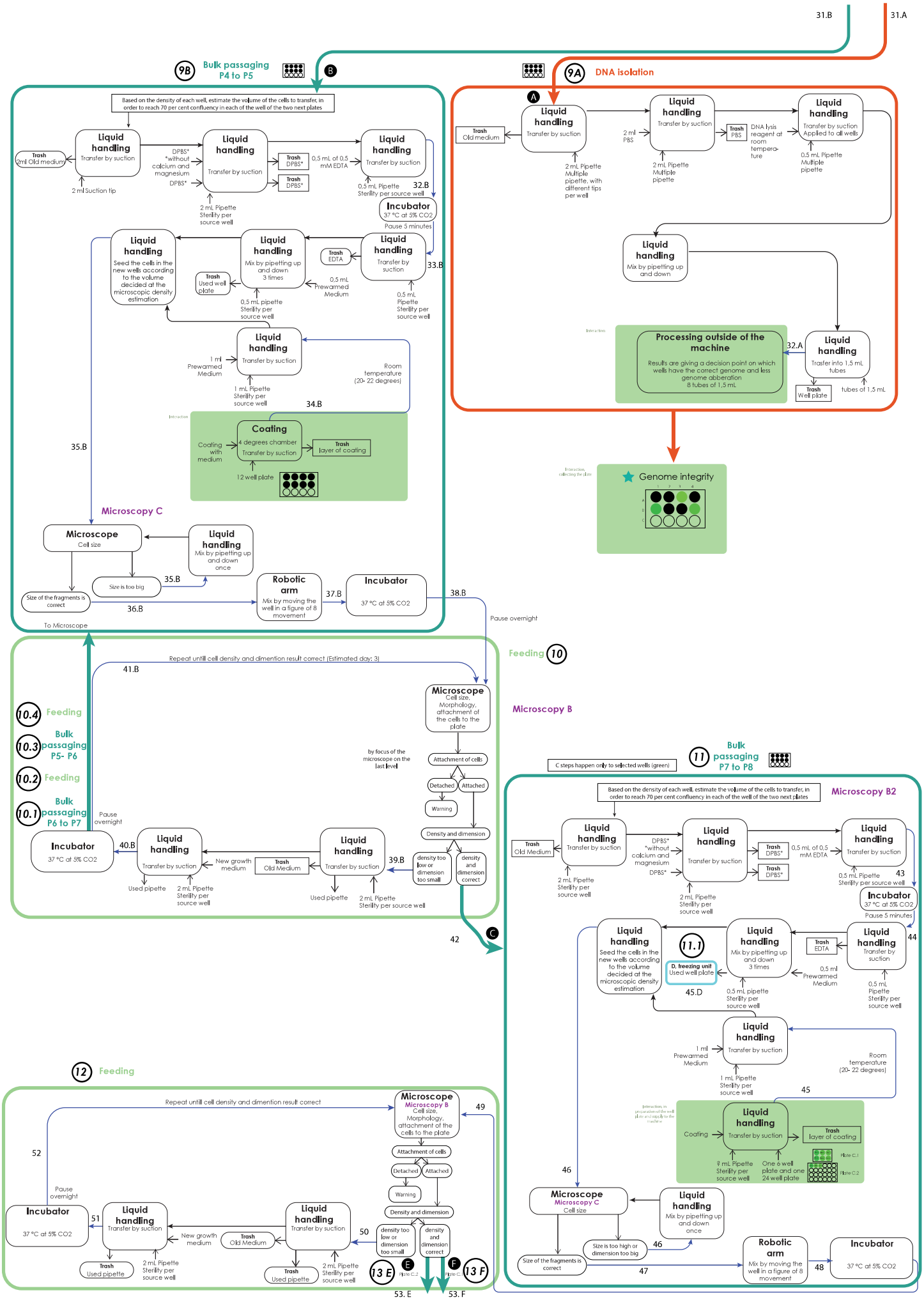
CHECKPOINTS in the selected area of the process



Check points	What does it have to take in account?
Quality control, bacteria and fungi	Turbidity of the medium
Quality control, microplasma	DNA, done outside of the machine
Genome integrity	DNA/ RNA, done outside of the machine
Validation of the identity of the cell	It is done through staining
Correct functioning of the machine For having a record and train the machine learning process	Cell morphology, cell density and cell size
Observation that the cells are ready for splitting	


PROCESS TREE FINAL - RNA REPROGRAMMING
1 Picking of cells Mechanical passaging P0 - P1
Incubator
37 °C at 5% CO2
Pause overnight
1
Microscope
Microscopy A
Correct morphology and size
Size too small or still differentiated
Back to feeding steps
Good morphology and dimention
Save the coordinates of the colonies that can be picked
2
Liquid Handling
Cut into pieces and deattach iPSC colony
22 - 25 Gauge needle or pulled glass pipette
Trash
22 - 25 Gauge needle or pulled glass pipette
Liquid Handling
Aspirate
20 µL pipettor with filtered tip
Liquid Handling
Plate colony fragments Final concetration at 10 µM
Trash
20 µL pipettor with filtered tip
< 8
Cell counter
Number of colonies
= 8
Liquid handling
Suction
1 ml Fresh medium
Trash
Old medium
1 ml pipette
Cleaning section
Pipette or pipette tip
Liquid Handling
Add
1 ml pipette
Cleaning section
Pipette or pipette tip
Liquid Handling
Cut into pieces and deattach iPSC colony
22 - 25 Gauge needle or pulled glass pipette
Trash
22 - 25 Gauge needle or pulled glass pipette
Liquid Handling
Aspirate
20 µL pipettor with filtered tip
Liquid Handling
Plate colony fragments Final concetration at 10 µM
Trash
20 µL pipettor with filtered tip
3
1 ml Fresh medium and ROCK inhibitor
Liquid handling
Plate
New 12 well plate
Pipetting robot
Suction
Trash
Old medium
2 mL pipettor
Old well plate
2 ml Fresh medium
Pipetting robot
Add
4
Incubator
37 °C at 5% CO2
Pause overnight (24 h)
2 Feeding
5
Microscope
Microscopy B
Cell size, Morphology attachment of pieces to the new well plate
Attachment of cells
Detached
Attached
Take backup plate from incubator if result persists after the second day of feeding
Density and dimension
density too low or dimension too small
density and dimension correct
6
Liquid Handling
Transfer by suction
Trash
Used pipette
2 mL Pipette Sterility per source well
Liquid Handling
Transfer by suction
2 ml New growth medium
Trash
2 ml old medium
Trash
Used pipette
2 mL Pipette Sterility per source well
7
Liquid Handling
Transfer by suction
Trash
Used pipette
2 mL Pipette Sterility per source well
Pause overnight
8
Incubator
37 °C at 5% CO2
3 Mechanical passaging P1 - P2
9
Liquid Handling
Transfer by suction
Trash
Old Medium
Trash
Used pipette
2 mL Pipette Sterility per source well
Liquid Handling
Transfer by suction
Fresh Medium with ROCK inhibitor
Trash
Used pipette
2 mL Pipette Sterility per source well
Liquid Handling
Cut one iPSC colony per well into pieces
Fresh Medium with ROCK inhibitor
22 - 25 Gauge needle or pulled glass pipette
22 - 25 Gauge needle or pulled glass pipette
10
Liquid Handling
Transfer by suction of pieces of the old well to the new plate
Incubator
Old well plate for backup after adding new fresh medium
Trash
Liquid on the top of the well
10.1
Liquid Handling
Aspirate
New coated 12 well plate, coated during the feeding time
1 mL Pipette Sterility per source well
11
Incubator
37 °C at 5% CO2
Pause overnight
4 Feeding
Repeat untill cell density and dimention result correct (Estimated day: 4)
Microscope
Microscopy B
Cell size, Morphology, attachment of the cells to the plate
Attachment of cells
Detached
Attached
Take backup plate from incubator if result persists after the second day of feeding
Density and dimension
density too low or dimension too small
density and dimension correct
12
Liquid Handling
Transfer by suction
Trash
Used pipette
2 mL Pipette Sterility per source well
Liquid Handling
Transfer by suction
New growth medium
Trash
Old Medium
Trash
Used pipette
2 mL Pipette Sterility per source well
13
Liquid Handling
Transfer by suction
Trash
Used pipette
2 mL Pipette Sterility per source well
Pause overnight
14
Incubator
37 °C at 5% CO2
15
5 Mechanical passaging P2 - P3
Liquid Handling
Transfer by suction
Trash
Old Medium
2 mL Pipette Sterility per source well
Trash
Old Medium
Liquid Handling
Transfer by suction
Fresh Medium with ROCK inhibitor
Trash
Old Medium
1 mL Pipette Sterility per source well
Liquid Handling
Cut one iPSC colony per well into pieces
1 mL Fresh Medium with ROCK inhibitor
Trash
22 - 25 Gauge needle or pulled glass pipette
22 - 25 Gauge needle or pulled glass pipette
16
Liquid Handling
Transfer by suction of pieces of the old well to the new plate
Incubator
Old well plate for backup after adding new fresh medium
Trash
Liquid on the top of the well
16.1
Liquid Handling
Aspirate
New coated 12 well plate, coated during the feeding time
1 mL Pipette Sterility per source well
1 mL Pipette Sterility per source well
17
Incubator
37 °C at 5% CO2
Pause overnight

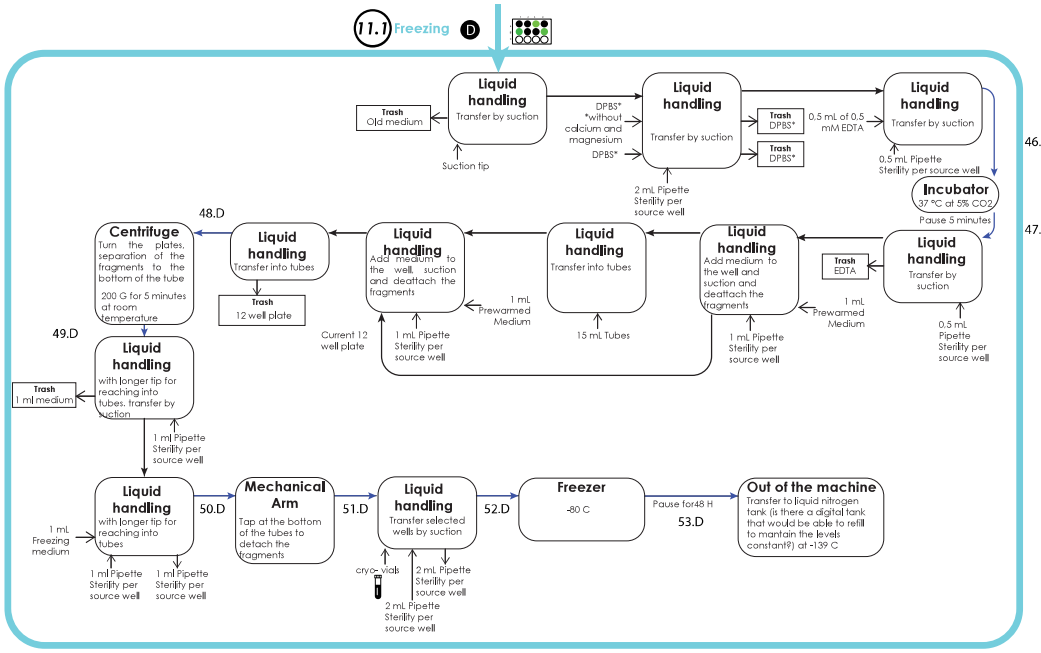

6 Feeding
Repeat untill cell density and dimention result correct (Estimated day: 4)
Microscope
Cell size, Morphology, attachment of the cells to the plate
Microscopy B
20
by focus of the microscope on the last level
Attachment of cells
Detached
Attached
Take backup plate from incubator if no_ji persists after the second day of feeding
Density and dimension
density too low or dimension too small
density and dimension correct
18
Liquid handling
Transfer by suction
Trash
Old Medium
New growth medium
Liquid handling
Transfer by suction
Used pipette
2 mL Pipette
Sterility per source well
19
Incubator
37 °C at 5% CO2
Pause overnight
21
7 Bulk passaging P3 to P4
Based on the density of each well, estimate the volume of the cells to transfer, in order to reach 70 per cent confluency in each of the well of the two next plates
Liquid Handling
Transfer by suction
Trash
Old medium
2 mL Pipette
Sterility per source well
DPBS*
*without calcium and magnesium
DPBS*
Liquid Handling
Transfer by suction
1 mL Pipette
Sterility per source well
Trash
DPBS*
Trash
DPBS*
0,5 mL of 0,5 mM EDTA
Liquid Handling
Transfer by suction
0,5 mL Pipette
Sterility per source well
22
Incubator
37 °C at 5% CO2
Pause 5 minutes
23
Liquid Handling
Transfer by suction
Trash
EDTA
0,5 ml Prewarmed Medium
0,5 mL Pipette
Sterility per source well
Liquid Handling
Mix by pipetting up and down 3 times
Trash
Used well plate
0,5 mL pipette
Sterility per source well
Liquid Handling
Seed the cells in the new wells according to the volume decided at the microscopic density estimation
1 ml Prewarmed Medium
Liquid Handling
Transfer by suction
1 mL Pipette
Sterility per source well
24
Coating
Liquid Handling
Transfer by suction
Trash
layer of coating
2 mL Pipette
Sterility per source well
12 well plate
Microscopy C
Microscope
Cell size
Liquid handling
Mix by pipetting up and down once
Size is too high or dimension too big
Size of the fragments is correct
25
Robotic arm
Mix by moving the well in a figure of 8 movement
26
Incubator
37 °C at 5% CO2
27
Pause overnight
8 Feeding
Repeat untill cell density and dimention result correct (Estimated day: 4)
Microscope
Cell size, Morphology, attachment of the cells to the plate
Microscopy B
30
Attachment of cells
Detached
Attached
Warning
Density and dimension
density too low or dimension too small
density and dimension correct
28
Liquid handling
Transfer by suction
Trash
Old Medium
New growth medium
Liquid handling
Transfer by suction
Used pipette
2 mL Pipette
Sterility per source well
29
Incubator
37 °C at 5% CO2
Pause overnight
B
A

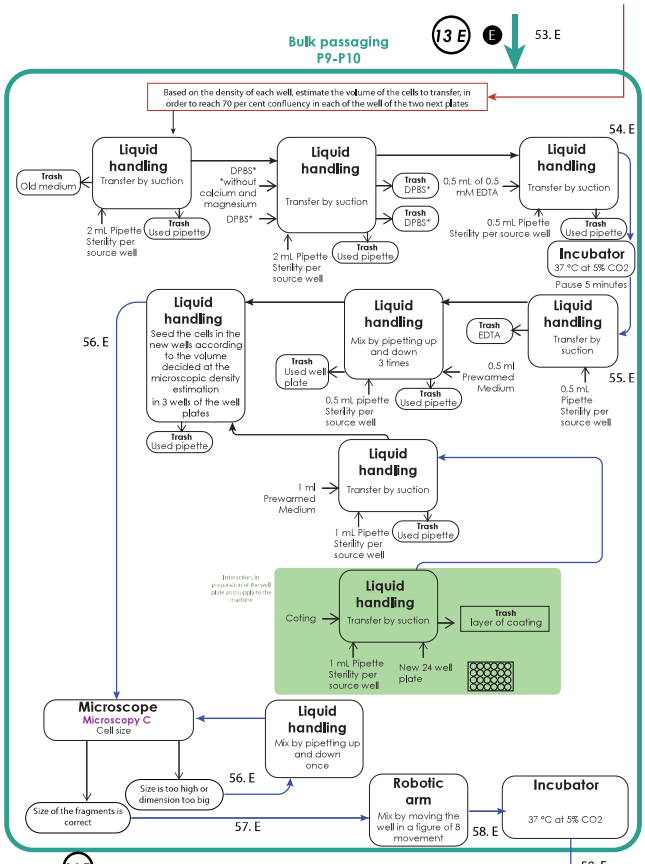

31.B
31.A
9B Bulk passaging P4 to P5
B
9A DNA isolation
A
Based on the density of each well, estimate the volume of the cells to transfer, in order to reach 70 per cent confluency in each of the well of the two next plates
Liquid handling
Transfer by suction
Trash 2ml Old medium
2 ml Suction tip
DPBS* *without calcium and magnesium
DPBS*
2 mL Pipette Sterility per source well
Trash DPBS*
0,5 mL of 0,5 mM EDTA
0,5 mL Pipette Sterility per source well
32.B
Incubator
37 °C at 5% CO2
Pause 5 minutes
Liquid handling
Seed the cells in the new wells according to the volume decided at the microscopic density estimation
Mix by pipetting up and down 3 times
Trash Used well plate
0,5 mL pipette Sterility per source well
Trash EDTA
0,5 mL Prewarmed Medium
0,5 mL Pipette Sterility per source well
33.B
1 ml Prewarmed Medium
1 mL Pipette Sterility per source well
Room temperature (20- 22 degrees)
34.B
Interaction
Coating
4 degrees chamber
Transfer by suction
Coating with medium
Trash layer of coating
12 well plate
35.B
Microscopy C
Microscope
Cell size
Mix by pipetting up and down once
Size is too big
Size of the fragments is correct
36.B
Robotic arm
Mix by moving the well in a figure of 8 movement
37.B
38.B
To Microscope
Trash Old medium
2 ml PBS
2 mL Pipette Multiple pipette, with different tips per well
2 mL Pipette Multiple pipette
Trash PBS
DNA lysis reagent at room tempera- ture
0,5 mL Pipette Multiple pipette
Mix by pipetting up and down
Interaction
Processing outside of the machine
Results are giving a decision point on which wells have the correct genome and less genome abberation
8 tubes of 1,5 mL
32.A
Trasfer into 1,5 mL tubes
Trash Well plate
tubes of 1,5 mL
Interaction, collecting the plate
Genome integrity
Pause overnight
Feeding 10
Repeat untill cell density and dimention result correct (Estimated day: 3)
41.B
Microscope
Cell size, Morphology, attachment of the cells to the plate
Microscopy B
by focus of the microscope on the last level
Attachment of cells
Detached
Attached
Warning
Density and dimension
density too low or dimension too small
density and dimension correct
39.B
Liquid handling
Transfer by suction
New growth medium
Trash Old Medium
Used pipette
2 mL Pipette Sterility per source well
40.B
Incubator
37 °C at 5% CO2
Pause overnight
10.4 Feeding
10.3 Bulk passaging P5- P6
10.2 Feeding
10.1 Bulk passaging P6 to P7
42
C
11 Bulk passaging P7 to P8
C steps happen only to selected wells (green)
Microscopy B2
Based on the density of each well, estimate the volume of the cells to transfer, in order to reach 70 per cent confluency in each of the well of the two next plates
Trash Old Medium
DPBS* *without calcium and magnesium
DPBS*
2 mL Pipette Sterility per source well
Trash DPBS*
0,5 mL of 0,5 mM EDTA
0,5 mL Pipette Sterility per source well
43
Incubator
37 °C at 5% CO2
Pause 5 minutes
44
Liquid handling
Seed the cells in the new wells according to the volume decided at the microscopic density estimation
11.1
D. freezing unit
Used well plate
45.D
Mix by pipetting up and down 3 times
Trash EDTA
0.5 ml Prewarmed Medium
0,5 mL Pipette Sterility per source well
0,5 mL pipette Sterility per source well
1 ml Prewarmed Medium
1 mL Pipette Sterility per source well
Room temperature (20- 22 degrees)
45
Interaction, in preparation of the well plate and supply to the machine
Coating
Trash layer of coating
9 mL Pipette Sterility per source well
One o well plate and one 24 well plate
Plate C.1
Plate C.2
46
Microscopy C
Cell size
Mix by pipetting up and down once
Size is too high or dimension too big
Size of the fragments is correct
47
Robotic arm
Mix by moving the well in a figure of 8 movement
48
Incubator
37 °C at 5% CO2
12 Feeding
Repeat untill cell density and dimention result correct
49
Microscope
Microscopy B
Cell size, Morphology, attachment of the cells to the plate
Attachment of cells
Detached
Attached
Warning
Density and dimension
density too low or dimension too small
density and dimension correct
50
Liquid handling
Transfer by suction
New growth medium
Trash Old Medium
Used pipette
2 mL Pipette Sterility per source well
51
Incubator
37 °C at 5% CO2
Pause overnight
52
13 E
E
Plate C.2
13 F
F
Plate C.1
53. E
53. F

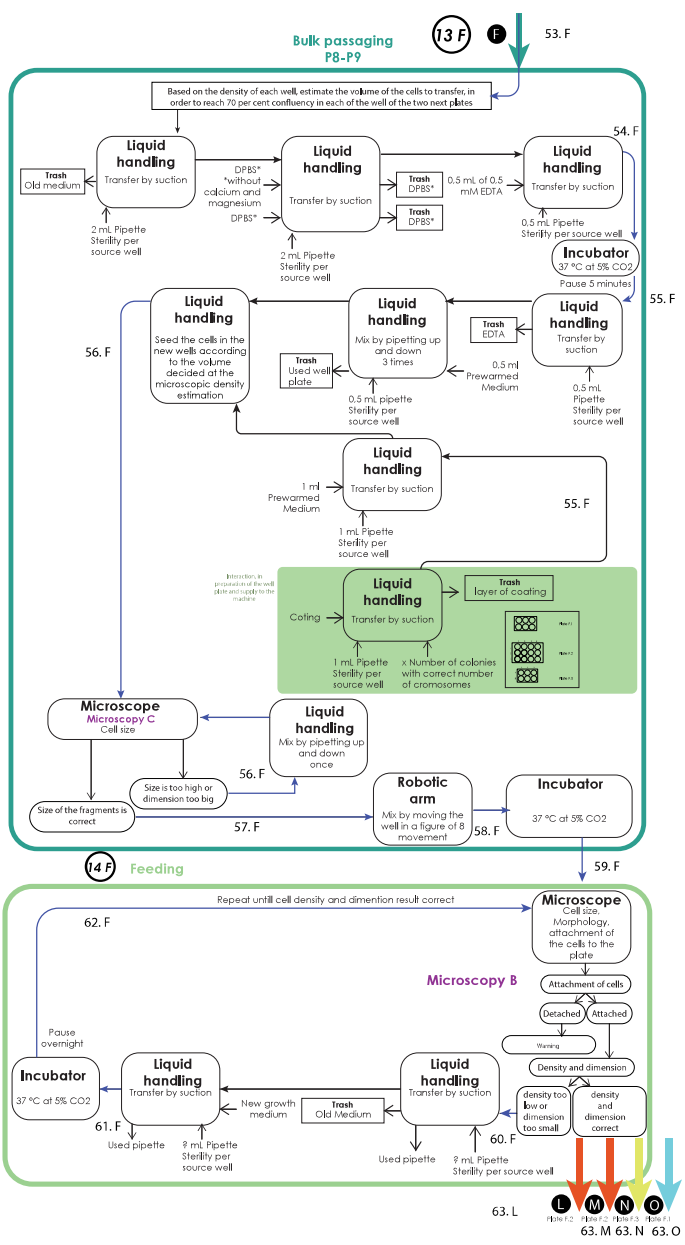
Genome integrity

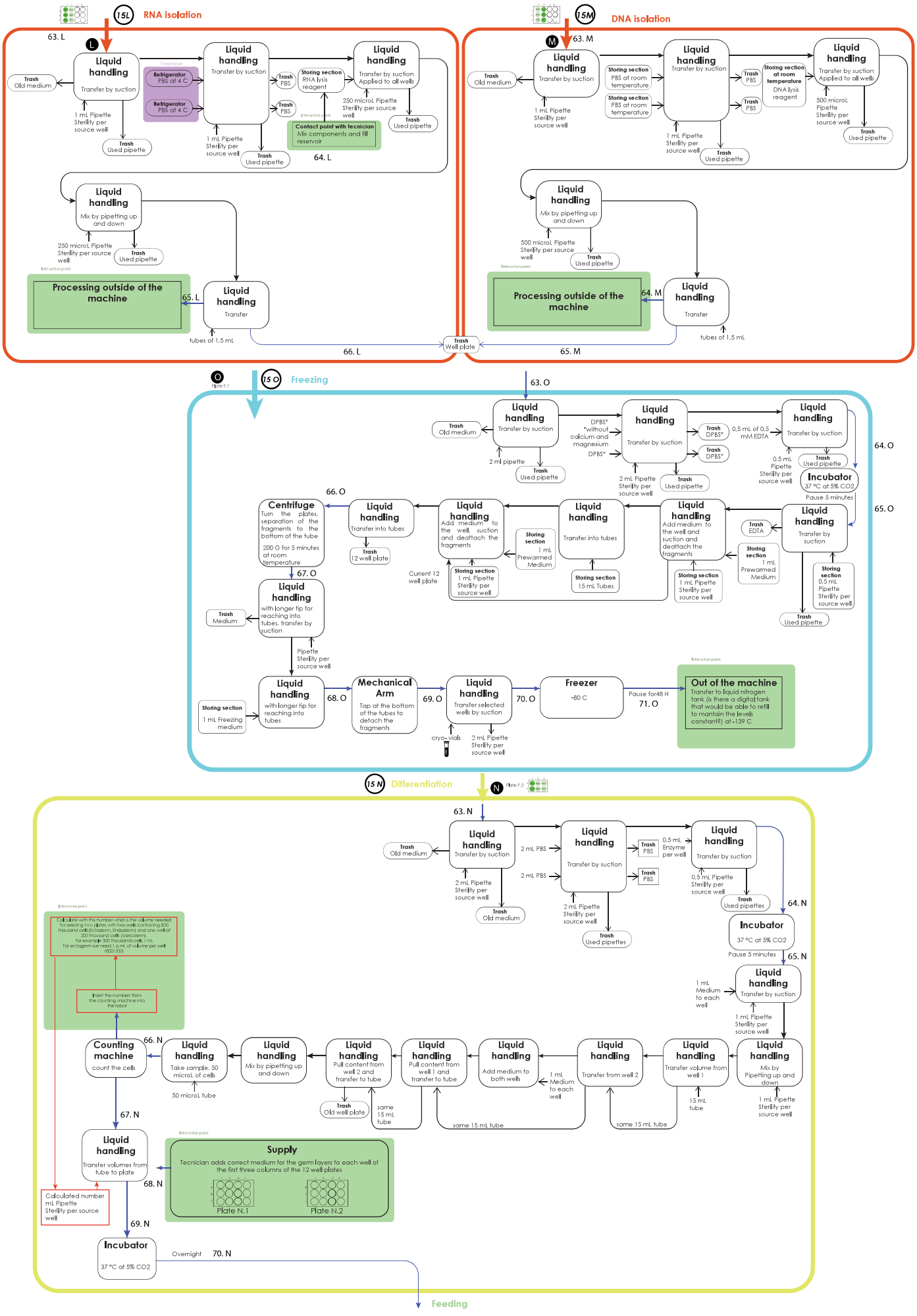
Contact point with the researcher that has to give imput on which wells are selected for D and for F.

Usually only 3 colons continue to F while the rest goes to D.
All the colons that continue to F have correct genome.

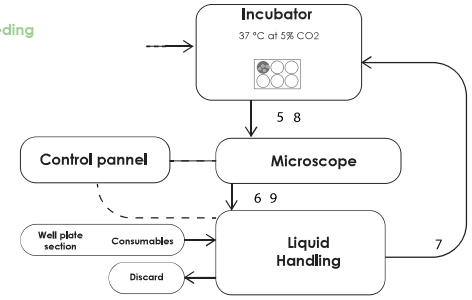




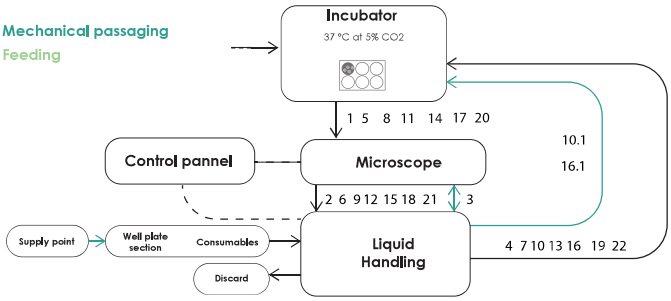



15L RNA isolation
63. L
Liquid handling
Transfer by suction
Trash Old medium
Refrigerator PBS at 4 C
Refrigerator PBS at 4 C
Trash PBS
Storing section RNA lysis reagent
Trash PBS
Liquid handling
Transfer by suction
Liquid handling
Transfer by suction Applied to all wells
1 mL Pipette Sterility per source well
Trash Used pipette
1 mL Pipette Sterility per source well
Trash Used pipette
250 microL Pipette Sterility per source well
Trash Used pipette
Contact point with tecnician
Mix components and fill reservoir
64. L
Liquid handling
Mix by pipetting up and down
250 microL Pipette Sterility per source well
Trash Used pipette
Processing outside of the machine
65. L
Liquid handling
Transfer
tubes of 1,5 mL
66. L
15M DNA isolation
63. M
Liquid handling
Transfer by suction
Trash Old medium
Storing section PBS at room temperature
Storing section PBS at room temperature
Liquid handling
Transfer by suction
Trash PBS
Storing section at room temperature DNA lysis reagent
Trash PBS
Liquid handling
Transfer by suction Applied to all wells
1 mL Pipette Sterility per source well
1 mL Pipette Sterility per source well
Trash Used pipette
500 microL Pipette Sterility per source well
Trash Used pipette
Liquid handling
Mix by pipetting up and down
500 microL Pipette Sterility per source well
Trash Used pipette
Processing outside of the machine
64. M
Liquid handling
Transfer
tubes of 1,5 mL
65. M
Trash Well plate
O Plate F.1
15 O Freezing
63. O
Liquid handling
Transfer by suction
Trash Old medium
2 ml pipette
Trash Used pipette
DPBS* *without calcium and magnesium
DPBS*
Liquid handling
Transfer by suction
Trash DPBS*
Trash DPBS*
2 mL Pipette Sterility per source well
Trash Used pipette
Liquid handling
Transfer by suction
0,5 mL of 0,5 mM EDTA
0,5 mL Pipette Sterility per source well
Trash Used pipette
Incubator 37 °C at 5% CO2
Pause 5 minutes
64. O
65. O
Liquid handling
Transfer by suction
Trash EDTA
Storing section 1 mL Prewarmed Medium
Storing section 0,5 mL Pipette Sterility per source well
Trash Used pipette
Liquid handling
Add medium to the well and suction and deattach the fragments
Storing section 1 mL Pipette Sterility per source well
Liquid handling
Transfer into tubes
Storing section 15 mL Tubes
Liquid handling
Add medium to the well, suction and deattach the fragments
Storing section 1 mL Prewarmed Medium
Storing section 1 mL Pipette Sterility per source well
Current 12 well plate
Liquid handling
Transfer into tubes
Trash 12 well plate
66. O
Centrifuge
Turn the plates, separation of the fragments to the bottom of the tube
200 G for 5 minutes at room temperature
67. O
Liquid handling
with longer tip for reaching into tubes, transfer by suction
Trash Medium
Pipette Sterility per source well
Liquid handling
with longer tip for reaching into tubes
Storing section 1 mL Freezing medium
68. O
Mechanical Arm
Tap at the bottom of the tubes to detach the fragments
69. O
Liquid handling
Transfer selected wells by suction
cryo-vials
2 mL Pipette Sterility per source well
70. O
Freezer
-80 C
Pause for48 H
71. O
Out of the machine
Transfer to liquid nitrogen tank (is there a digital tank that would be able to refill to mantain the levels constant?) at -139 C
15 N Differentiation
N Plate F.3
63. N
Liquid handling
Transfer by suction
Trash Old medium
2 mL Pipette Sterility per source well
Trash Old medium
2 mL PBS
2 mL PBS
Liquid handling
Transfer by suction
Trash PBS
Trash PBS
2 mL Pipette Sterility per source well
Trash Used pipettes
Liquid handling
Transfer by suction
0,5 mL Enzyme per well
0,5 mL Pipette Sterility per source well
Trash Used pipettes
64. N
Incubator 37 °C at 5% CO2
Pause 5 minutes
65. N
Liquid handling
Transfer by suction
1 mL Medium to each well
1 mL Pipette Sterility per source well
Liquid handling
Mix by Pipetting up and down
1 mL Pipette Sterility per source well
Liquid handling
Transfer volume from well 1
15 mL tube
Liquid handling
Transfer from well 2
same 15 mL tube
Liquid handling
Add medium to both wells
1 mL Medium to each well
same 15 mL tube
Liquid handling
Pull content from well 1 and transfer to tube
Liquid handling
Pull content from well 2 and transfer to tube
Trash Old well plate
same 15 mL tube
Liquid handling
Mix by pipetting up and down
Liquid handling
Take sample, 50 microL of cells
50 microL tube
66. N
Counting machine
count the cells
Insert the numbers from the counting machine into the robot
Calculate with this number what is the volume needed for seeding two plates with two wells containing 800 thousand cells (Ectoderm, Endoderm) and one well of 200 thousand cells (Mesoderm). For example 300 thousands cells / mL For ectoderm we need 1,6 mL of volume per well (900/300)
67. N
Liquid handling
Transfer volumes from tube to plate
68. N
Calculated number mL Pipette Sterility per source well
69. N
Supply
Tecnician adds correct medium for the germ layers to each well of the first three columns of the 12 well plates
Plate N.1
Plate N.2
Incubator 37 °C at 5% CO2
Overnight
70. N
Feeding

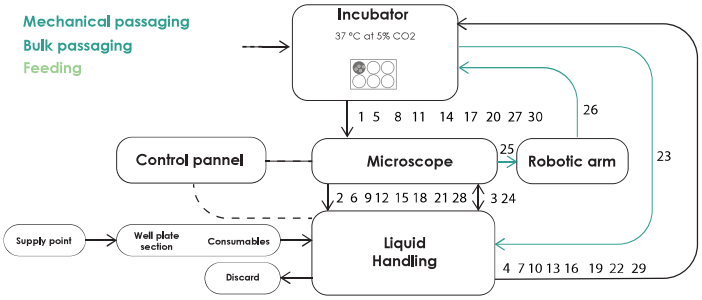
Feeding



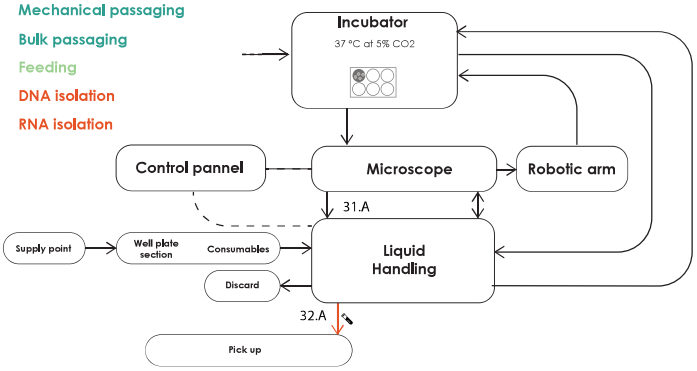
Mechanical passaging
Feeding



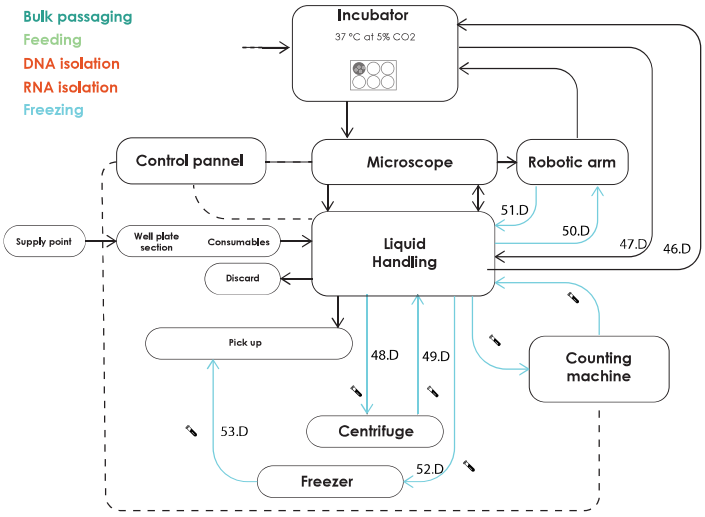
Mechanical passaging
Bulk passaging
Feeding



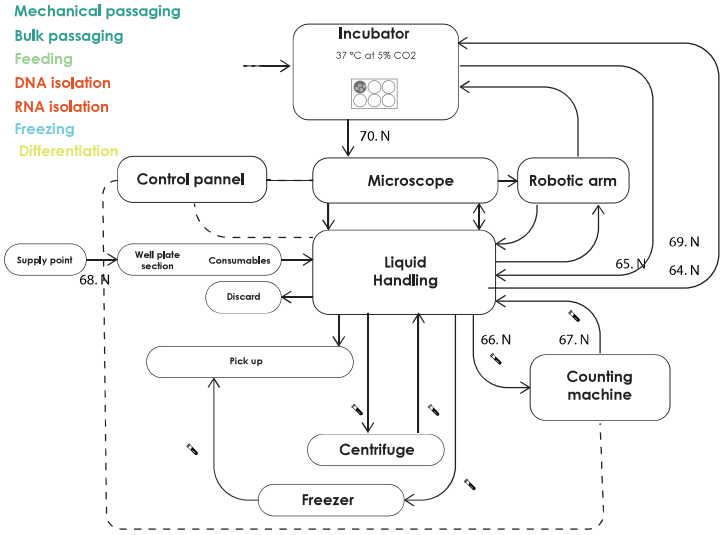
Mechanical passaging
Bulk passaging
Feeding
DNA isolation
RNA isolation



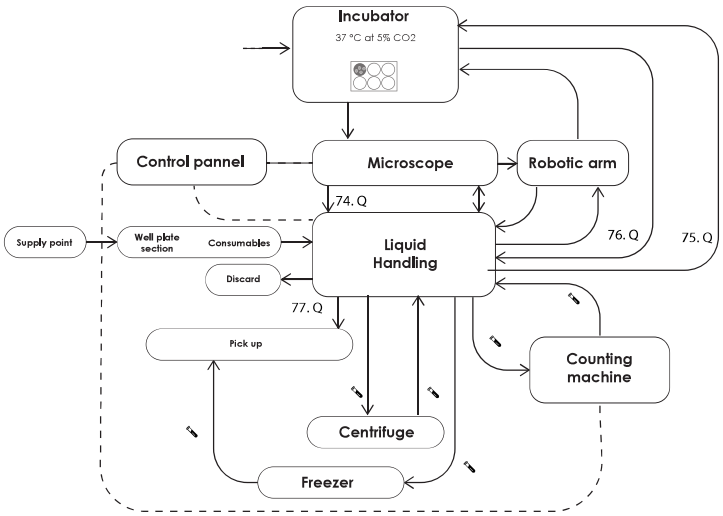
Mechanical passaging
Bulk passaging
Feeding
DNA isolation
RNA isolation
Freezing

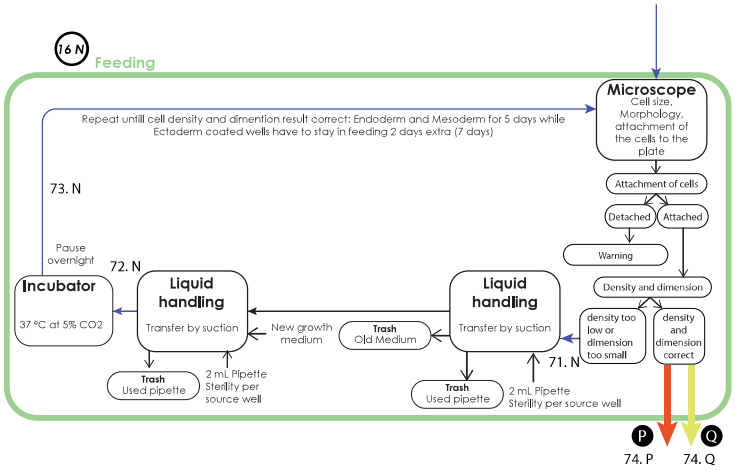


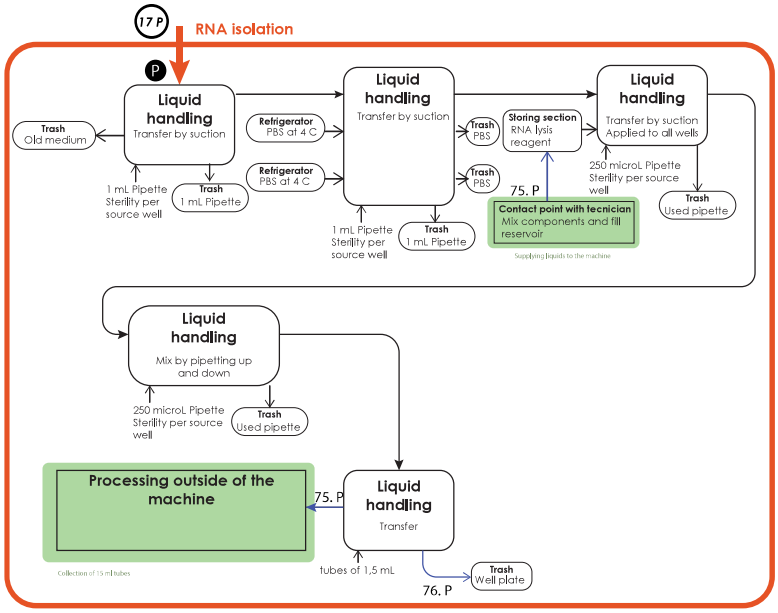
Mechanical passaging
Bulk passaging
Feeding
DNA isolation
RNA isolation
Freezing
Differentiation

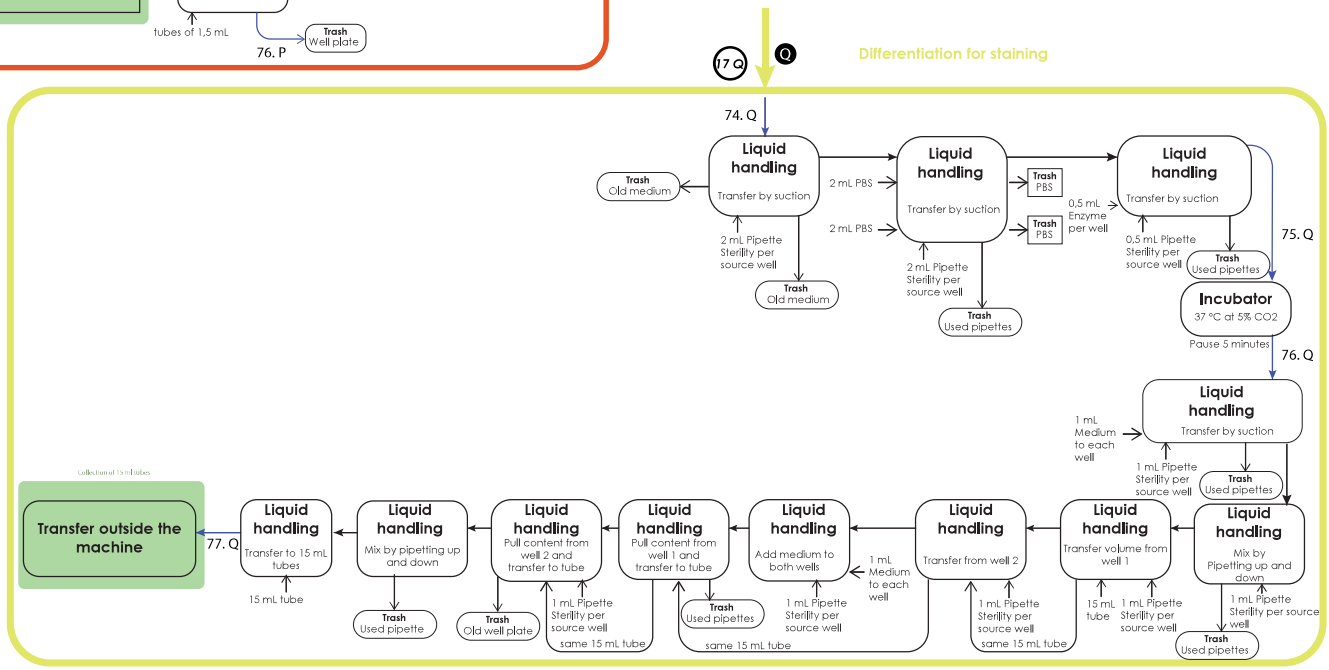


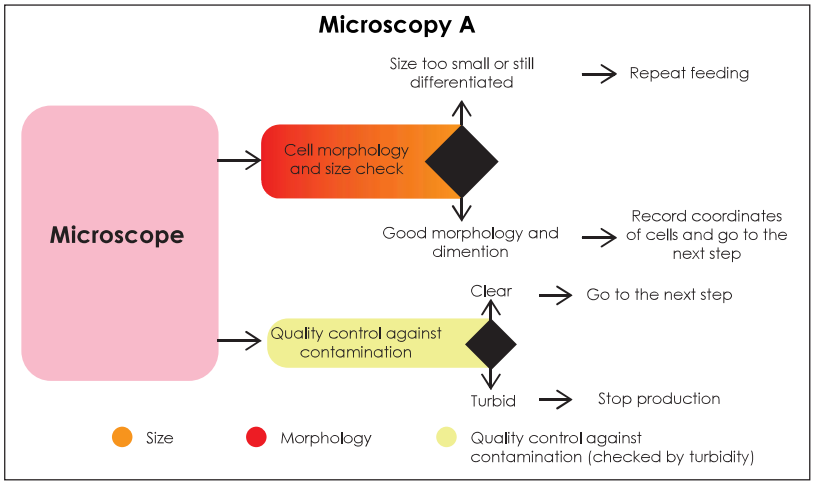
Mechanical passaging
Bulk passaging
Feeding
DNA isolation
RNA isolation
Freezing
Differentiation for staining




16 N
Feeding
Microscope
Cell size, Morphology, attachment of the cells to the plate
Repeat untill cell density and dimention result correct: Endoderm and Mesoderm for 5 days while Ectoderm coated wells have to stay in feeding 2 days extra (7 days)
73. N
Attachment of cells
Detached
Attached
Microscopy B
Warning
Density and dimension
density too low or dimension too small
density and dimension correct
Pause overnight
72. N
Incubator
37 °C at 5% CO2
Liquid handling
Transfer by suction
New growth medium
Trash
Old Medium
Trash
Used pipette
2 mL Pipette
Sterility per source well
71. N
Trash
Used pipette
2 mL Pipette
Sterility per source well
P
Q
74. P
74. Q


17 P
RNA isolation
P
Liquid handling
Transfer by suction
Trash
Old medium
1 mL Pipette
Sterility per source well
Trash
1 mL Pipette
Retrigerator
PBS at 4 C
Retrigerator
PBS at 4 C
Liquid handling
Transfer by suction
1 mL Pipette
Sterility per source well
Trash
1 mL Pipette
Trash
PBS
Trash
PBS
Storing section
RNA lysis reagent
75. P
Contact point with tecnician
Mix components and fill reservoir
Liquid handling
Transfer by suction
Applied to all wells
250 microL Pipette
Sterility per source well
Trash
Used pipette
Liquid handling
Mix by pipetting up and down
250 microL Pipette
Sterility per source well
Trash
Used pipette
Processing outside of the machine
75. P
Liquid handling
Transfer
tubes of 1,5 mL
Trash
Well plate
76. P


17 Q
Q
Differentiation for staining
74. Q
Liquid handling
Transfer by suction
Trash
Old medium
2 mL Pipette
Sterility per source well
Trash
Old medium
2 mL PBS
2 mL PBS
Liquid handling
Transfer by suction
2 mL Pipette
Sterility per source well
Trash
Used pipettes
Trash
PBS
Trash
PBS
0,5 mL Enzyme per well
Liquid handling
Transfer by suction
0,5 mL Pipette
Sterility per source well
Trash
Used pipettes
75. Q
Incubator
37 °C at 5% CO2
Pause 5 minutes
76. Q
Liquid handling
Transfer by suction
1 mL Medium to each well
1 mL Pipette
Sterility per source well
Trash
Used pipettes
Liquid handling
Mix by Pipetting up and down
1 mL Pipette
Sterility per source well
Trash
Used pipettes
Liquid handling
Transfer volume from well 1
15 mL tube
1 mL Pipette
Sterility per source well
Liquid handling
Transfer from well 2
1 mL Pipette
Sterility per source well
same 15 mL tube
Liquid handling
Add medium to both wells
1 mL Medium to each well
1 mL Pipette
Sterility per source well
Liquid handling
Pull content from well 1 and transfer to tube
Trash
Used pipettes
same 15 mL tube
Liquid handling
Pull content from well 2 and transfer to tube
1 mL Pipette
Sterility per source well
same 15 mL tube
Trash
Old well plate
Liquid handling
Mix by pipetting up and down
Trash
Used pipette
Liquid handling
Transfer to 15 mL tubes
15 mL tube
77. Q
Transfer outside the machine



Functions:

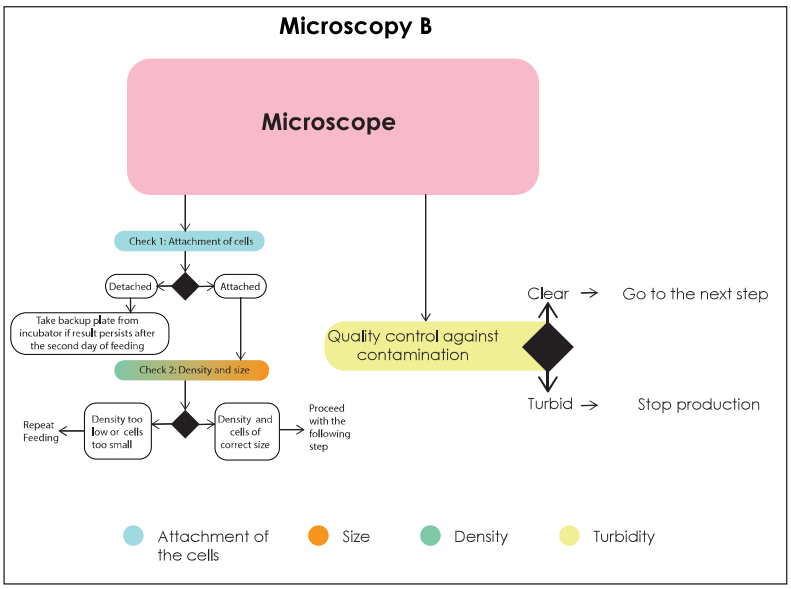
This step of microsocopy should collect informations regarding the cell size, the cell shape and the brightness of cell edges.
It should also check against fungi contamination

A picture of the well plate should be recorded and mapped in order to gain information on where are the colonies of cells that can be picked.
This information also controls the pippetting robot.

Interactions

Send report and immage of the well to the researcher, wait for the approval on continuing to the next step, which would control the transition to the next step through the robotic arm.
One immage of the well is stored in database.

Me



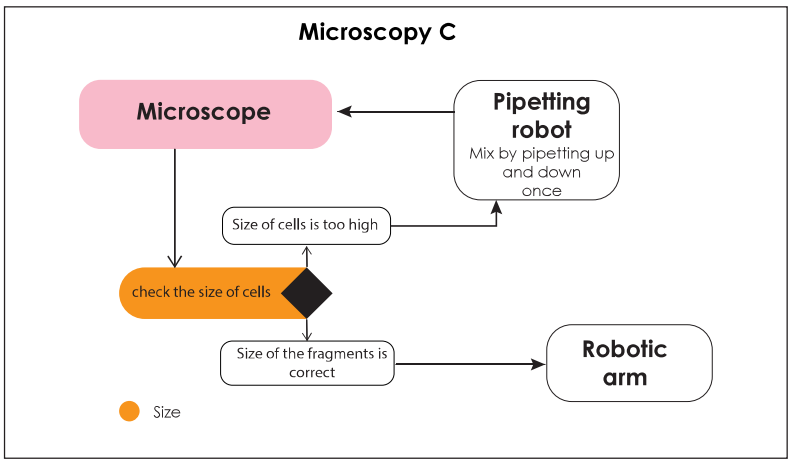
Functions:

This step of microsocopy should collect subsequently two informations:
Check 1 regarding the attachment of the cells to the bottom of the plate, if the cells are not attached at the second day of feeding the process should be repeated with an other plate that is better attached.
Check 2: should give informations on the cell density and their size and although leading in both ways to the pipetting robot it leads to either the repetition of the previous feeding day or the next Bulk passaging step, DNA isolation, RNA isolation, freezing or preparation for staining.

Interactions

Send immage ony at check point 2, regarding the dimention of the cells.

If the cells result not attached to the bottom of the well the tecnician has to reprogram the machine to start the program again with the remaining old well plate.



Functions:

Determines if the pipetting up and down should be repeated or not to reduce the dimension of the cells

Interactions

Send immage regarding the dimention of the cells and wait for approval by tecnician

At what stages is it needed	Properties it checks
chanical passagings	Cell size: it should go from X to Y Cell morphology: What are the characteristics that describe the morphology?

At what stages is it needed	Properties it checks
End and start of the feeding	Which is the layer of the well in which the cells are suspended. Density of the cells and their dimension

At what stages is it needed	Properties it checks
Bulk passaging	Size of the cells.

Appendix VII - Risk evaluation

#	Risk	Probability of occurrence	Impact of consequences
	Pre use		
1	Budget not present or unclear, difficulty in starting production	Low	Average
2	Missing ethical approval	Very low	Very large
3	Availability of cells is not as initially envisioned, there are less cells then planned	Very low	Average
4	Software or hardware malfunction at the set up of the mechine	Low	Very large
	Use		
	Reprogramming unit		
5	Contamination of cells during seeding and possible cell death due to less variability of the cells	High	Large
6	Contamination of well from external factors	Very high	Large
7	Cross contamination of well	Considerable	Large
8	Picking a clone that is not completely reprogrammed	Considerable	Large
9	Not successful mechanical passaging, there are no new clones to be picked	Very low	Small
10	Plate falls from the hands of the operator during transportation	Low	Average
	Expansion unit		
11	The system has problems in recognizing the well plate	Considerable	Large
12	Failed RNA isolation and need to redo the control step, problematic dependant from an other facility	Low	Average
13	External contamination	Very high	Large
14	The technician is hit by the robotic arms while loading the liquid handler	High	Very large
15	Wrong functioning of the AI system in prediction of confluency	Considerable	Large
	Freezing unit		
16	The plates fall from the hands of the operator during transport	Low	Small
17	Contamination	High	Large
18	The technician is hit by the robotic arms while loading the liquid handler	High	Very large
19	The cells are inserted inside the freezer abruptly, without a slow decrease of temperature	Considerable	Very large
20	Delivery of wrong tubes	Low	Large
	Post use		
21	At the moment of refurbishment, the new machine that is substituting the old one doesn't have the same performance as the initial device because of changes of models	Considerable	Large
22	At the moment of mantainance, broken parts remain inside the machine and obstruct propper functioning.	Low	Very large

Figure 1 : Ranking

Figure 1 indicates the ranking of the identified risks through the interaction overview. The criteria of evaluation used for this ranking are described in Figure 2.

Impact

As impact it has been considered the incidence of the risk factor on the future activities done at the IPS Facility.

Probability

For the evaluation on probability two units have been taken into account: the number of times the event can happen within the extension of time of one cell line production, and the number of times it can happen through the entire yearly production of the facility.

In the next pages the Bow-Ties for each High risk

Impact	
Very large	Blocks completely the production
Large	It can lead to wrong results or death of cells
Average	Provides loss in time and money
small	A back up plan can be organized
Very small	Affects one well plate and not cell line

Probability	
Extremely high	On more occasions for each cell line
High	At each cell line
Considerable	Every 20 lines
Low	Once every 500 cell lines
Very low	Once a year

Figure 2: values used for the ranking of risks.

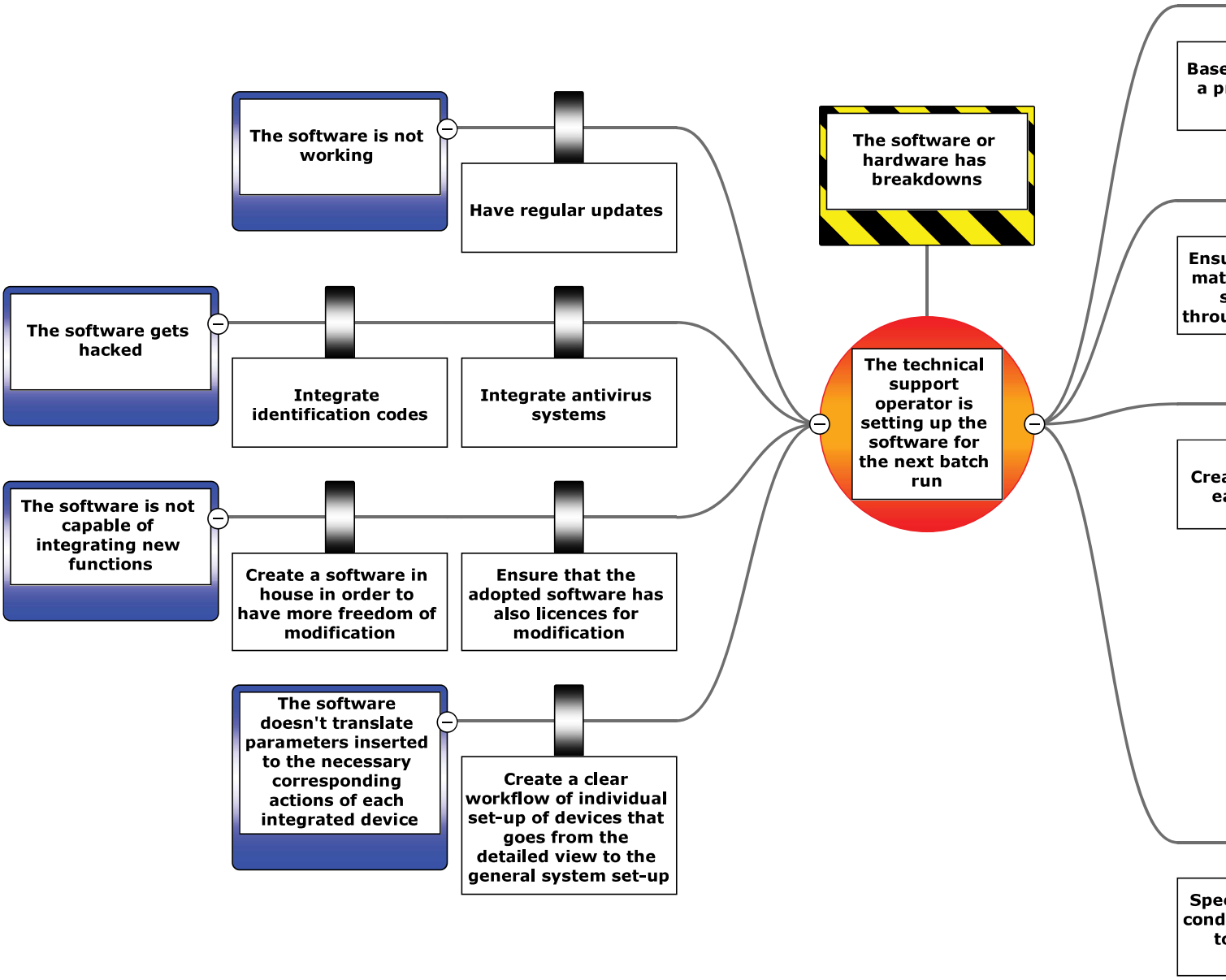
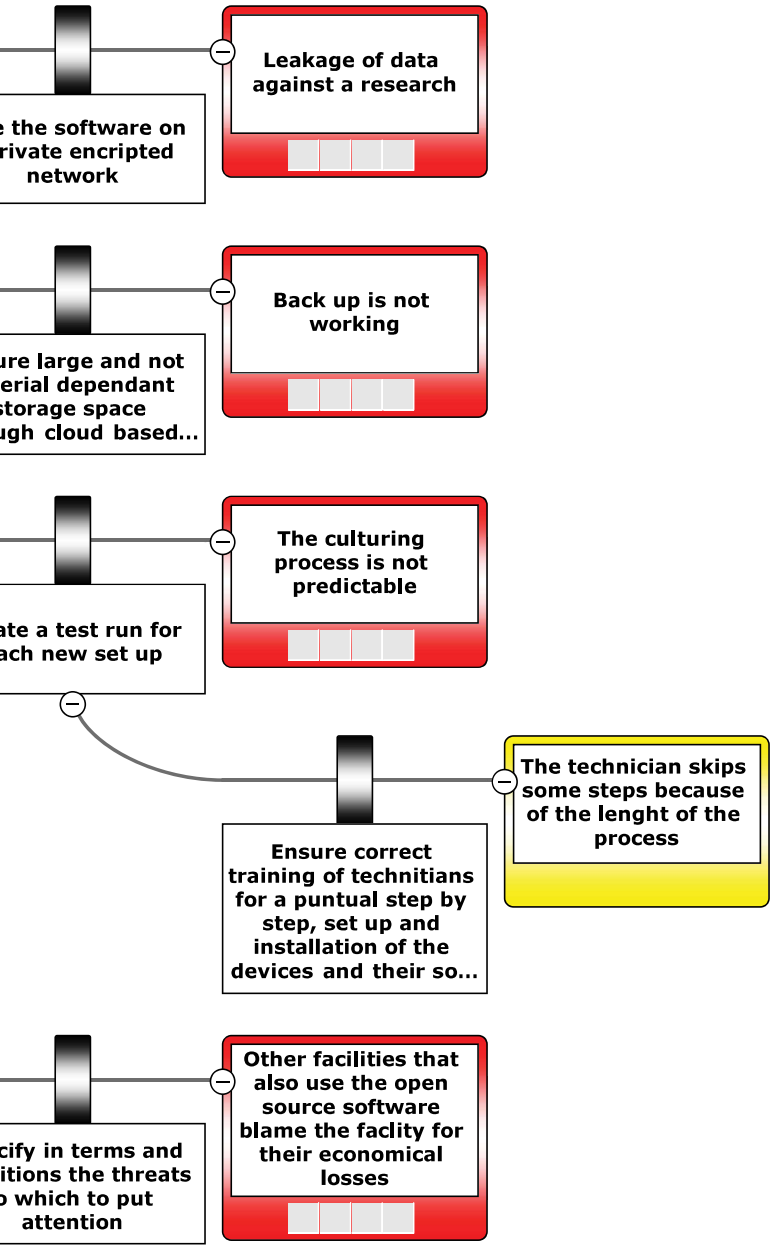


Figure A: Bow tie Risk number 4


Leakage of data against a research
e the software on rivate encripted network
Back up is not working
ure large and not erial dependant storage space ugh cloud based...
The culturing process is not predictable
ate a test run for ach new set up
The technician skips some steps because of the lenght of the process
Ensure correct training of technitians for a puntual step by step, set up and installation of the devices and their so...
Other facilities that also use the open source software blame the faclity for their economical losses
cify in terms and itions the threats o which to put attention

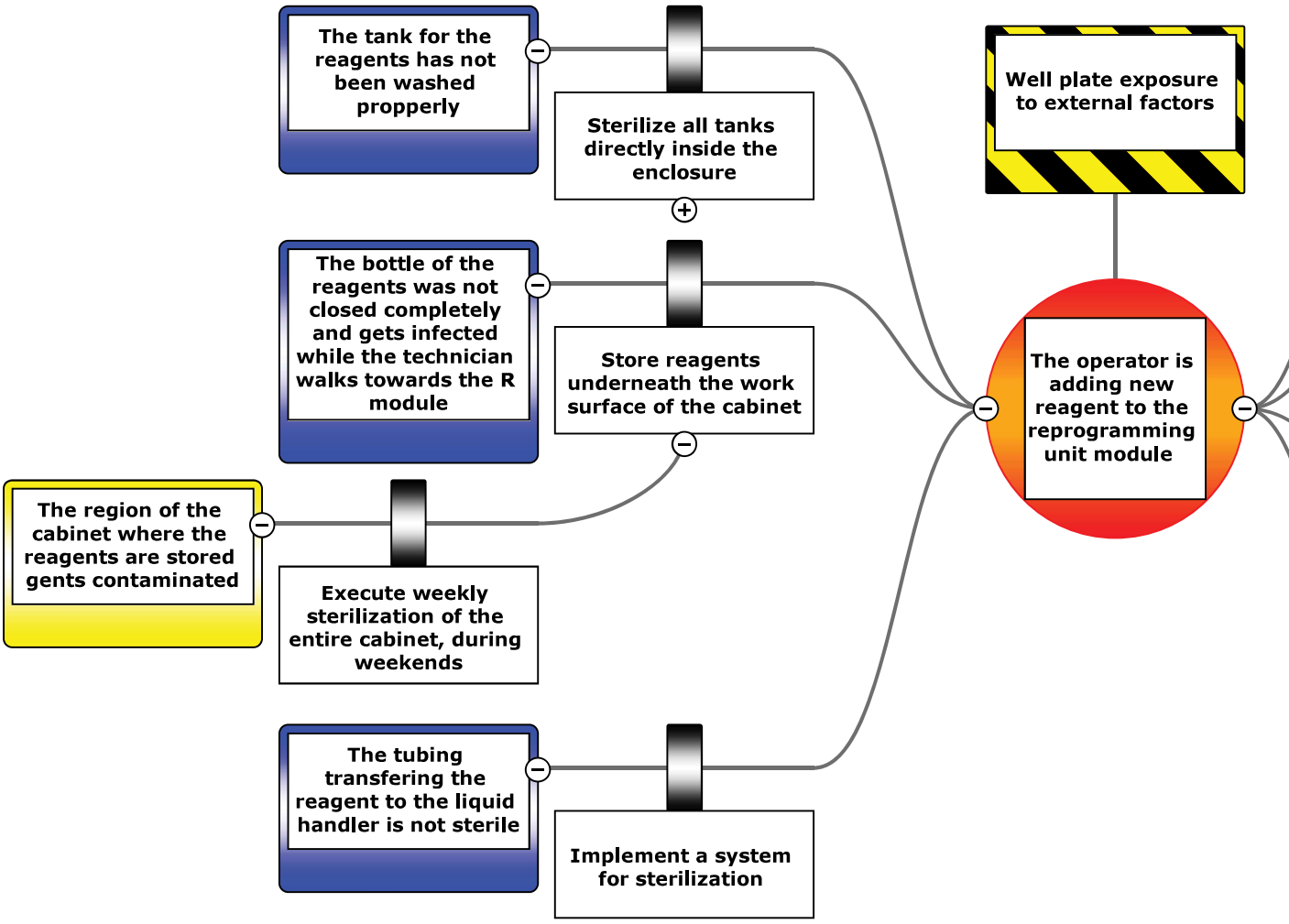
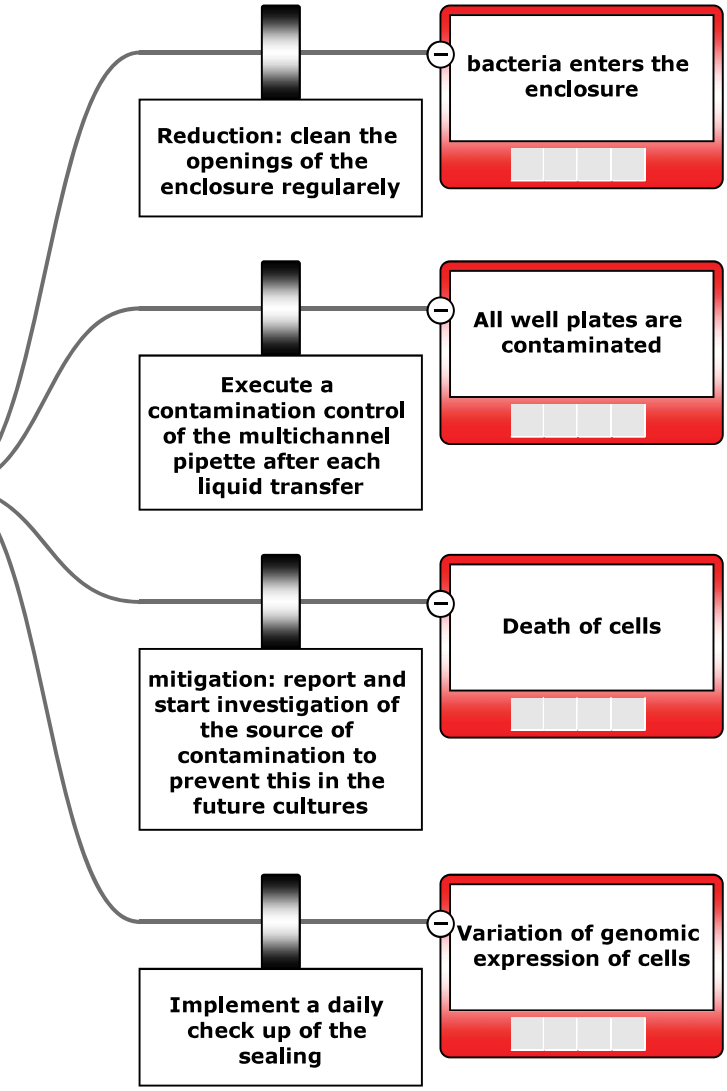
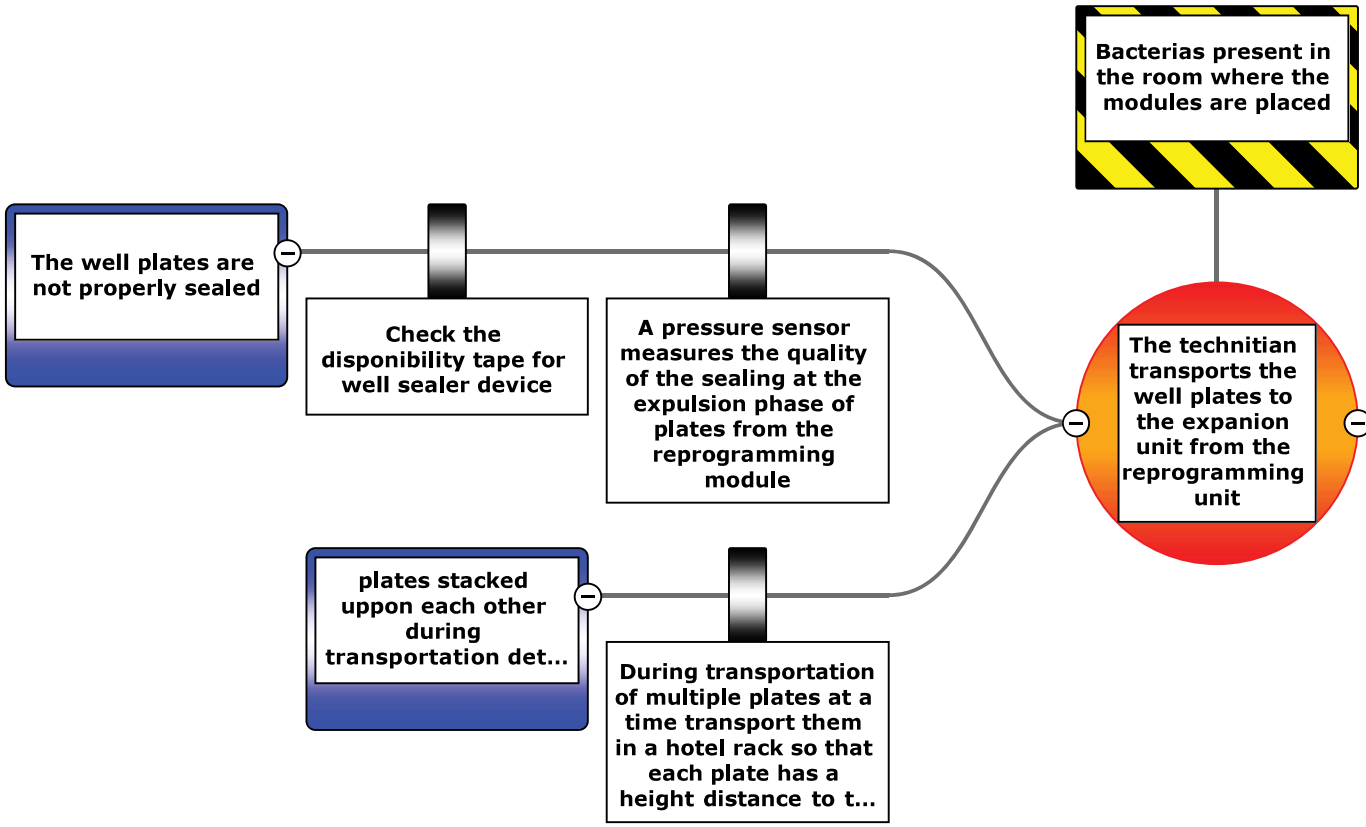
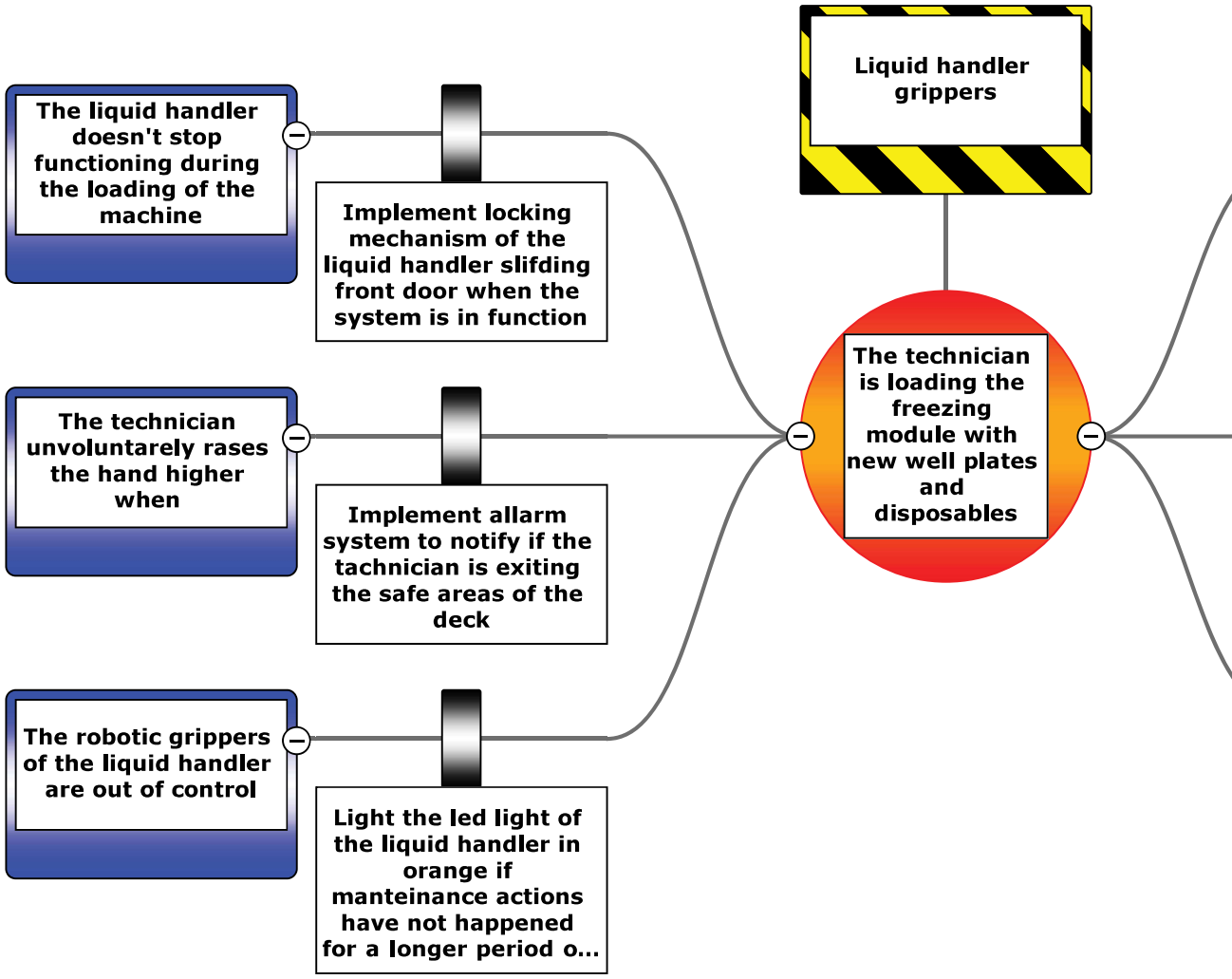


Figure A: Bow tie Risk number 6


bacteria enters the enclosure
Reduction: clean the openings of the enclosure regularely
All well plates are contaminated
Execute a contamination control of the multichannel pipette after each liquid transfer
Death of cells
mitigation: report and start investigation of the source of contamination to prevent this in the future cultures
Variation of genomic expression of cells
Implement a daily check up of the sealing


Bacterias present in the room where the modules are placed
The well plates are not properly sealed
Check the disponibility tape for well sealer device
A pressure sensor measures the quality of the sealing at the expulsion phase of plates from the reprogramming module
The technitian transports the well plates to the expanion unit from the reprogramming unit
plates stacked uppon each other during transportation det...
During transportation of multiple plates at a time transport them in a hotel rack so that each plate has a height distance to t...


Liquid handler grippers
The liquid handler doesn't stop functioning during the loading of the machine
Implement locking mechanism of the liquid handler slifding front door when the system is in function
The technician is loading the freezing module with new well plates and disposables
The technician unvoluntarely rases the hand higher when
Implement allarm system to notify if the tachnician is exiting the safe areas of the deck
The robotic grippers of the liquid handler are out of control
Light the led light of the liquid handler in orange if manteinance actions have not happened for a longer period o...

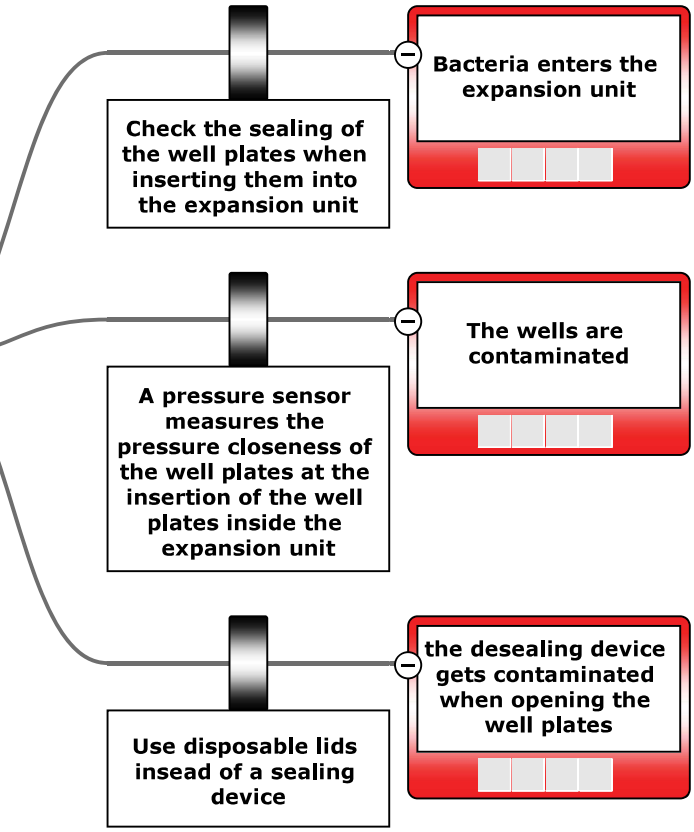


Figure : Bow tie Risk number 13

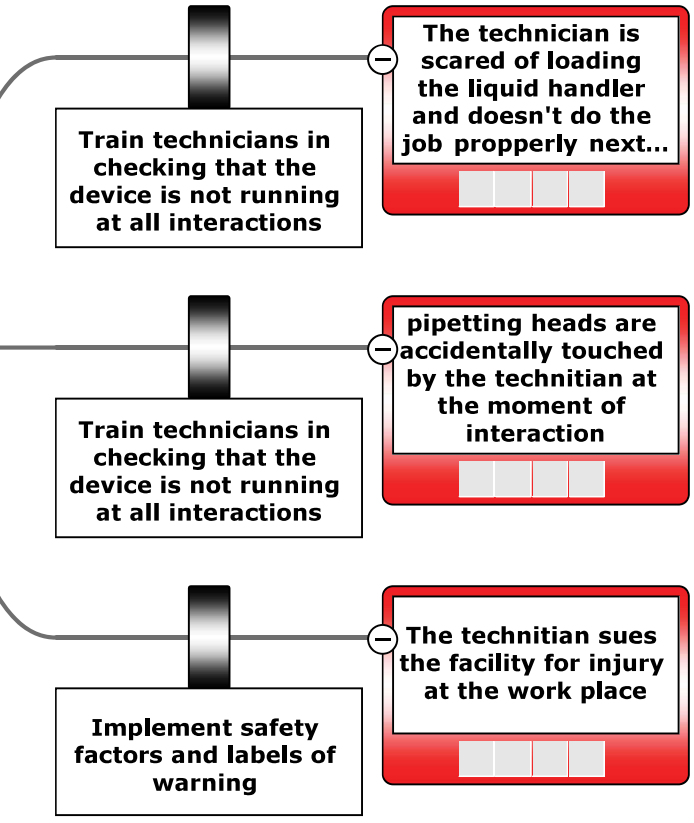


Figure : Bow tie Risk number 14, 18

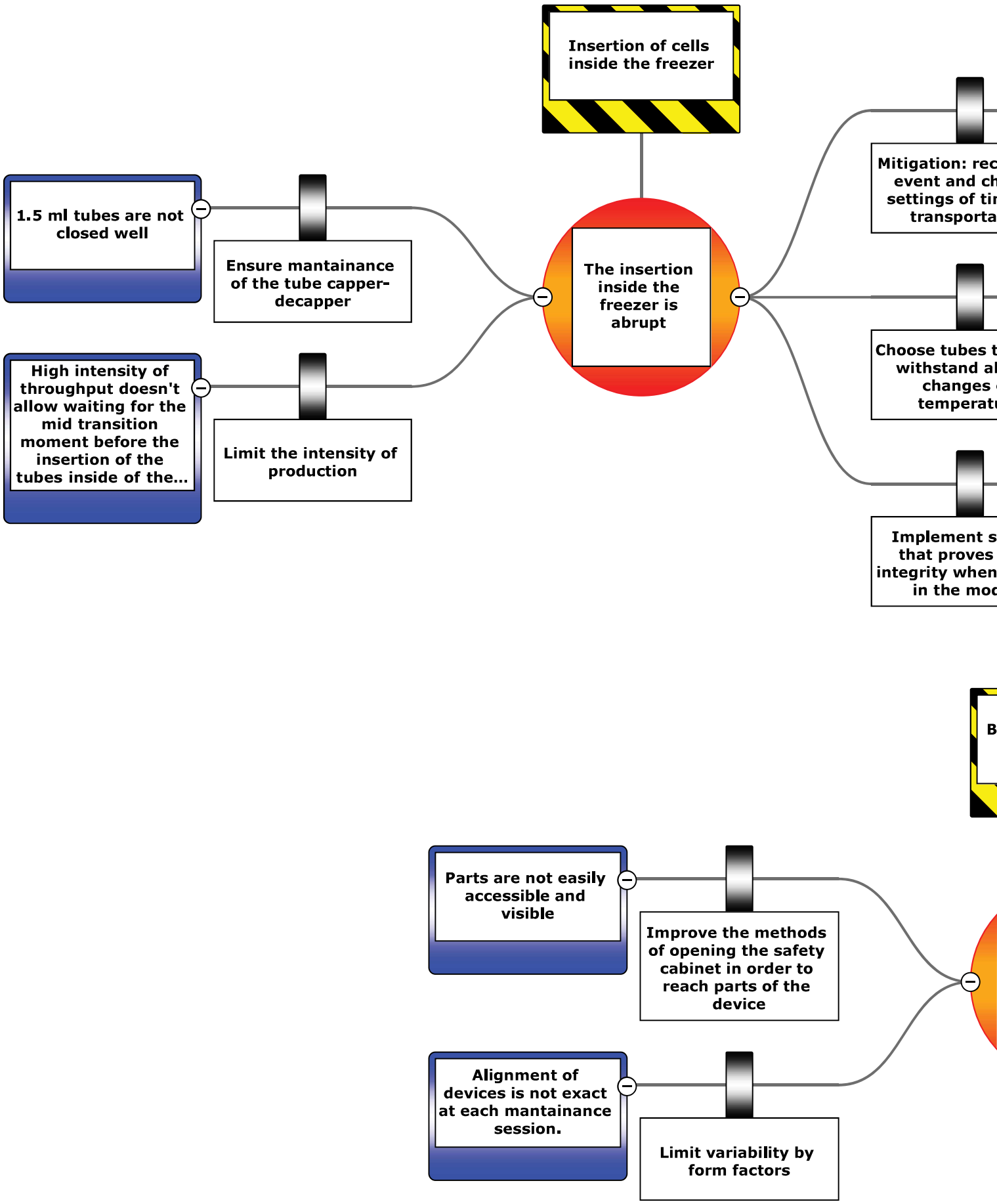


Figure A: Bow tie Risk number 22

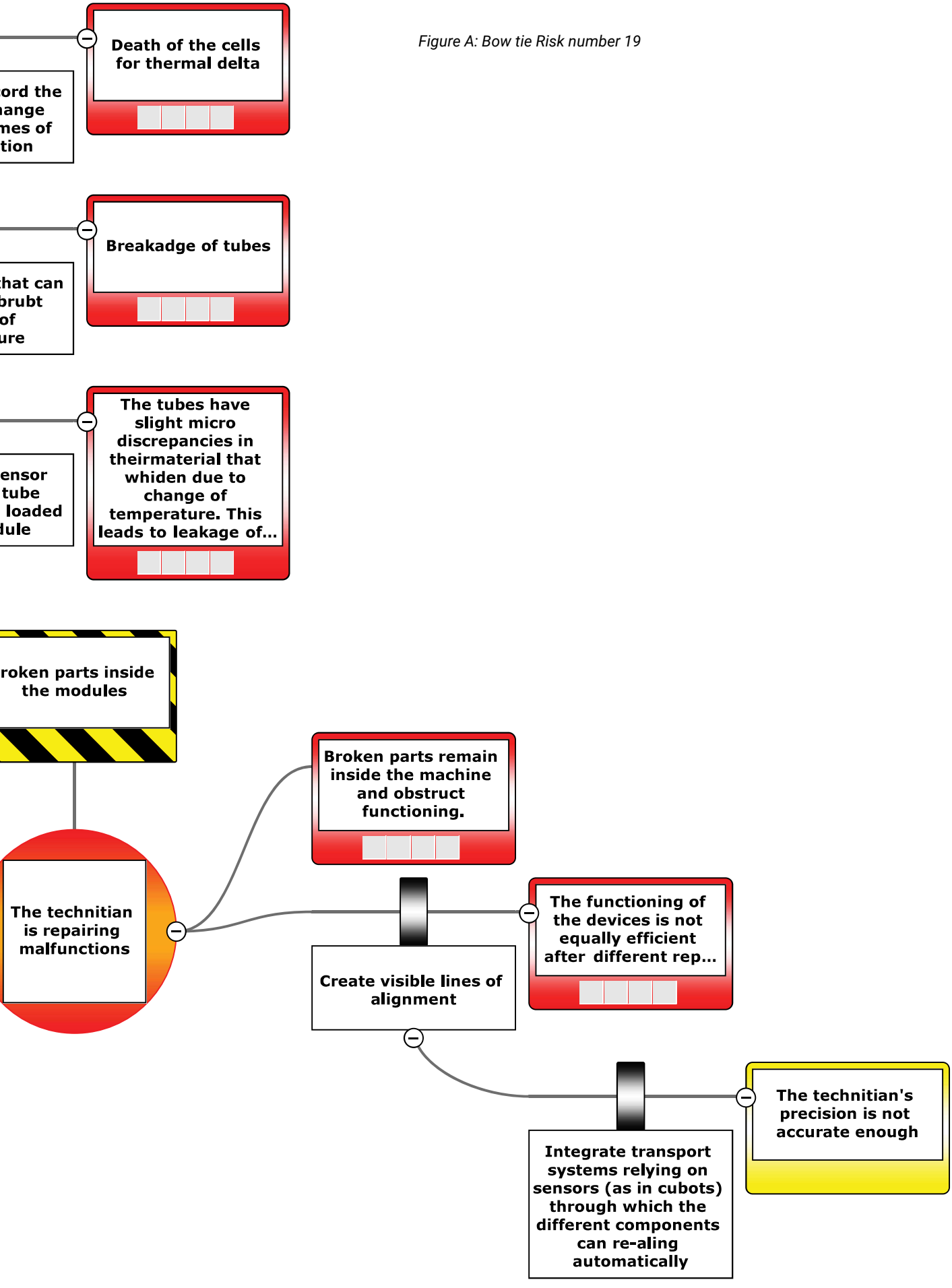


Figure A: Bow tie Risk number 19

INTERNAL COMPONENTS OF THE MODULES				
Component	Qty	Price (€) x 1	Source	Total Price
Reprogramming Unit				
Computer with desktop set-up	1	€ 800,00	Estimation of general price of Desktop computers	€ 800,00
Display for pick up interaction	1	€ 80,00	Estimation based on Alibaba.com	€ 80,00
Liquid handler - **Biomek i7 dual Multichannel without enclosure**	1	€ 168.800,00	Beckman Coulter Price File attachment found at eandi.org (199,900.00 USD)	€ 168.800,00
Etichette printer for well plates - **Sci-Print PM 2 or PlateCrane EX Robotic Arm Microplate Handler**	1	€ 5.000,00	*Estimation, no source*	€ 5.000,00
Well plate Peeler - **X-Peel**	1	€ 5.000,00	Estimation based on other similar devices on bio-rad.com	€ 5.000,00
*Well seeler - **ALPS 3000***	1	€ 34.000,00	Estimation based on other similar devices on thomassci.com	€ 34.000,00
Column robotic arm - **Precise PF70 Microplate handling robot**	2	€ 844,00	Estimation, from globalspec.com a robotic arm cost can go from $25,000 to $400,000, but Robots for schools, universities and other non-industrial applications can be found for $1,000 or less	€ 1.688,00
Small robotic arm - As model **Meca500**	1	€ 400,00	Estimation, from globalspec.com a robotic arm cost can go from $25,000 to $400,000, but Robots for schools, universities and other non-industrial applications can be found for $1,000 or less, this is smaller then the other one.	€ 400,00
Conveyor belts - **Faulhaber conveyor bellts with angle rotation**	3	€ 400,00	*Estimation, no source*	€ 1.200,00
Spectrometer, turbidity reader - SPECTROStar Nano	1	€ 11.000,00	Requested quote from producer	€ 11.000,00
Cytometer, cell counting - **Celigo imaging Cytometer**	1	€ 100.000,00	Requested quote from producer, the list prices given are in a range of 110,000-175,000 Euros	€ 100.000,00
Cell picking device - **ASL Cell celector**	1	€ 210.110,00	Estimation based on the reselling price of a used ASL CellCelector on dotmed.com and further confirmation from the managing director (range from 220k and 320k Euro)	€ 210.110,00
Incubator - **Incubator Cytomat C10**	1	€ 6.000,00	Estimation based on the reselling prices of CO2 incubators at troutunderground.com/24/Incubators/	€ 6.000,00
Tilting deck stations	?	?		
*Waste reagents discard - **Liquid disposable ASL***	?	?		
Subtotal	1			€ 544.078,00
Expansion Unit				
Liquid handler - **Biomek i7 Hybrid (MC + Span-8) without enclosure**	1	€ 190.501,00	Beckman Coulter Price File attachment found at eandi.org (225,600.00 USD)	€ 190.501,00
Incubator - **Incubator Cytomat C10**	1	€ 6.000,00		€ 6.000,00
Shaking ALP Single Position	3	€ 5.066,00	Beckman Coulter Price File attachment found ateandi.orgg (6,000.00 USD)	€ 15.198,00
Heating/ Cooling position ALP Single position	2	€ 3.597,00	Beckman Coulter Price File attachment found at eandi.org (4,260.00 USD)	€ 7.194,00
Etichette printer for well plates - **Sci-Print PM 2**	1	€ 5.000,00	*Estimation, no source*	€ 5.000,00
Automated high speed inverted microscope - **Ti2-E microscope**	1	€ 80.000,00	Requested quote from producer, they gave a range of 60-100k euros for this microscope s	€ 80.000,00
Conveyor belts - **Faulhaber conveyor bellts with angle rotation**	1	€ 400,00	*Estimation, no source*	€ 400,00
Small robotic arm - As model **Meca500**	1	€ 400,00	Estimation, from globalspec.com a robotic arm cost can go from $25,000 to $400,000, but Robots for schools, universities and other non-industrial applications can be found for $1,000 or less, this is smaller then the other one.	€ 400,00
*Well seeler - **ALPS 3000***	1	€ 34.000,00	Estimation based on other similar devices on thomassci.com	€ 34.000,00
*Waste reagents discard - **Liquid disposable ASL***	?	?		
Tilting deck stations	?	?		
Subtotal	1			€ 338.693,00
Freezing Unit				
Liquid handler - **Biomek i5 Span-8 without enclosure**	1	€ 101.753,00	Beckman Coulter Price File attachment found at eandi.org (120,500.00 USD)	€ 101.753,00
Well plate Peeler - **X-Peel**	1	€ 5.000,00	Estimation based on other similar devices on bio-rad.com	€ 5.000,00
Cytometer, cell counting - **Celigo imaging Cytometer**	1	€ 100.000,00	Requested quote from producer	€ 100.000,00
Column robotic arm - **Precise PF70 Microplate handling robot**	1	€ 844,00	Estimation, from globalspec.com a robotic arm cost can go from $25,000 to $400,000, but Robots for schools, universities and other non-industrial applications can be found for $1,000 or less	€ 844,00
Centrifuge - **Sigma 4-5KRL** (91309) with rotor 91318, buckets 91319 with custom adapter	1	€ 35.310,00	Requested quote from producer (33000 excl. BTW)	€ 35.310,00
*(Subtotal B) Automated freezer (-80 °C) - **Hamilton SAM HD***	1	€ 200.000,00	Requested quote from producer (this system can hosy 10000 samples)	€ 200.000,00
*(Subtotal A) **Redimentioned Automated freezer***	1	€ 70.000,00	Probably a freezer 50 times this capacity is what is really needed, as an estimation a third of the price of the Hamilton SAM HD is taken into account	€ 70.000,00
*Waste reagents discard - **Liquid disposable ASL***	?	?		
Tilting deck stations	?	?		
Shaking ALP Single Position	1	€ 5.066,00	Beckman Coulter Price File attachment found ateandi.orgg (6,000.00 USD)	€ 5.066,00
Subtotal A	1			€ 317.973,00
Subtotal B	1			€ 517.973,00
SAFETY CABINETS				
Reprogramming Unit				
Safety cabinet class II enclosure	1	100 k - 200 k	Consultation with enclosure builder, it comprehends, engineering, shipping and transporta	€ 200.000,00
Expansion Unit				
Safety cabinet class II enclosure	1	100 k - 200 k	Consultation with enclosure builder, it comprehends, engineering, shipping and transporta	€ 200.000,00
Freezing Unit				
Safety cabinet class II enclosure	1	30 - 50 k	Consultation with enclosure builder, standard big sized safety cabinet	€ 50.000,00
Subtotal only for the frames				€ 400.000,00
EMBODIMENT				
Engineering	?		25 weeks of work(estimation of the necessary time for the enclosure buiders work, 8 h a day)	
Transportation of devices to the facility	?			
Installation, labour for assembling	?	20 €/h		
Device Software engineering	?			
Scheduling software purchase	?			
Shipping of devices	?			
Subtotal	?			

Appendix VIII - Cost price evaluation

In this table prices of the devices embedded inside the RXF System are listed.

As it can be seen by the table, the costs for which it is possible to derive more detailed information are relative to the devices integrated inside each module.

Although stakeholders have been contacted through this project, as it can be seen by the interviews conducted with Beckman Coulter, Hamilton Company and Clean Air, it is very difficult to estimate, at this moment, elements relative to the price of manufacturing, transportation, engineering or installation.

Moreover, the device, in use, would have overhead costs, that would also need more time and details in order to be calculated.

For the overall price of the Freezing unit two subtotal values are shown, one with the automated freezed finally adopted and one with an estimated freezer that could be of the dimentions really necessary for the throughputs of the IPS Facility.
Because of this also the final total price for the overall RXF System presents two values.

As described in the Cost Evaluation Chapter, the calculated cost should be taken as a starting base cost, to which more elements, may need to be added with more future iterations of the project.

Total prices of System without production				
Reprogramming unit (R module)	1	(Internal devices + Frame)	€ 744.078,00	
Expansion Unit (X module)	1	(Internal devices + Frame)	€ 538.693,00	
Freezing unit (F module)	1	(Internal devices + Frame)	€ 367.973,00 –	€ 567.973,00
RXF System	1	All internal devices + Frames	€ 1.650.744,00	€ 1.850.744,00

Appendix IX - Evaluation of concept by safety cabinet builder

Main topics discussed

1. It is important to understand if there should be only product safety or also personal safety. Other automated systems used for the production of hIPSC (such as the Luxemburg University system) mention the use of biosafety cabinets of class II. These cabinets provide also person safety by the inflow of air and re-circulation of 70 % of the air from the cabinet, through filtration.
2. In the case in which protection should be provided only to the products, the procedure is easier because there is only a need for a clean environment and no need for an upstream flow of air.
3. Adopting only product-based protection should be discussed further since, even if the reagents used for the process of RNA reprogramming are not hazardous for the technicians it should be sure that no human-endangering virus or bacteria could develop within the process.
4. For a class II cabinet, extra air is needed for the inflow which will provide person protection. When using only a downflow you do not need to recirculate the air in the cabinet. You can just blow it out underneath the system. When using a class II cabinet solution you must provide a big return duct for all air.
5. In case of need to give also protection to people in the environment where the safety cabinet is placed, suiting such a system could be more complex. In fact, there would be the need of creating also a stream for the flow, a return duct, to circulate in the air in the upper direction. Due to the big dimensions of the units, the airflow would need to be of very big amounts (more or less 10.000.000 liters/ hour of air supply: Calculation: 4 meters x 2 meters downflow area x 0,36m/s airspeed x 3600 sec = 10.368 m3/h. This is only for the downflow with a downflow air velocity of 0,36 m/s).
6. This column of air would also need to be extended over the full area of one of the sides of the cabinet, not allowing openings from this side.
7. Integration of devices could cause problems to the flow of air, this is the case for full objects such as the incubator.
8. The integration of a freezer inside of the cabinet would create smaller problems to the cabinet because of the lower density of cooler air in respect to warm air. One aspect to take into account, in this case, is the need of having access to the opening of the freezer in order to manage to clean the opening from possible water condensation
9. The bigger the cabinet gets the more difficult it will be to reach the center of the cabinet, so the positioning of the elements to reach should be very close to the openings, which should not be higher than 20 cm. The reason behind it is that the air that is sucked in has a smaller impact than the air blowing from the top so this could cause the downflow to have overpressure and therefore lose protection for the person.
10. A solution could be still worked out for example by lowering the working deck so that the technician can lean better with the arms.

11. Also with safety cabinets of these dimensions, the opening can be left open. This should be avoided in the case in which elements close to the opening are moving. In this case, the cabinet is usually made so that it can be closed. The reason for this is to avoid technicians getting hurt by a moving robotic arm or other devices, but if it can be of help another solution can also be to implement safety nets.
12. The safety cabinet can be designed to be opened in the phase of installation. This means that all the devices and surfaces are also cleaned after the cabinet is closed.
13. In order to do this the usual way is to use vaporized hydrogen peroxide (VHP) sterilization. The devices that are placed on the deck, therefore, should be resistant to this typology of gasses. If it is possible to clean the work area manually there is no need to use VHP for cleaning the return duct. The VHP is only necessary when the class II cabinet has to be opened in the contained part. For instance for exchanging the HEPA filters.
14. The fact that devices inserted in the cabinet are not “open”, i.e. they are an obstruction to the airflow, makes it necessary to find other solutions and facilitate airflow. One way could be by lifting the incubator or other devices slightly up.
15. Moving devices and parts could facilitate cross-contamination between wells because they cause other movements of air.
16. The models presented have a very high budget due mainly to the needs of engineering costs and on transportation costs (also standard safety cabinets of big dimensions of 90 cm x 180 cm have to be dismantled from the production place and be installed in the facility because they can not pass through doors). At this time it is almost impossible to give a good estimation of costs but as an indication factor, the enclosure builder gave the information that for the big modules, the budget could be of 100 k - 200 k euros each. If the devices could be stacked together even more tightly for example in the freezing module (dimensions closer to the standard safety cabinet), the budget could be of 30 - 50 k euros.

Result

There are elements to be improved in future stages of development of the project, but solutions can be found. There are no real ways of containing the budget because of need for investment in engineering and transportation, except for the fact that the freezing module could reduce the costs by being readapted to a standard safety cabinet.

The models showed are a good starting point because they give an indication of all the elements necessary to go further. In fact, in order to continue with the project, it is necessary to have clear what are:

- The devices to be integrated into the system and their dimensions

- The areas that are necessary to reach

- What are the elements important for the maintenance

- A flow showing the sequence of use.

Future steps

Timings: the process of development could take, in an estimation, 22 to 25 weeks (4-6 weeks for engineering decisions after having a final concept, 10 weeks for the supply of material, 2-3 weeks for building, an additional variable).

It is a very complex case with several challenges to tackle and more experts should be gathered around the project if the disponibility of budgets is given.

Appendix X Spread of introduction for ideation sessions

iPS Core Facility

The Erasmus MC IPS core facility within Erasmus MC is a department that supports research on X-linked diseases, cancer, infertility and aging by providing high quality induced pluripotent stem (iPS) cell lines and embryonic stem (ES) cell lines to researchers within and outside the Erasmus MC.

Who are the tecnicians

Currently 6 technicians and one facility manager work in the laboratory.

Technicians have a Bachelor degree background preparation in Biology and build their knowledge on the procedure steps over 2 to 3 months of training.

Each technician takes care of any phase of the process, all of them work on all the cell lines in production and by getting feedback from more experienced peers they build up on each other's knowledge.

The facility manager keeps an eye on the procedure, instructs new technicians and has the final word if problems or doubts come up.

In the future scenario of automation the facility manager will be the interacting with the automized system while all the other technicians will be responsible for manual simple supply of material to the system and procedures regarding the interpretation of the results.

hIPSC - human indu

What are human induced plu
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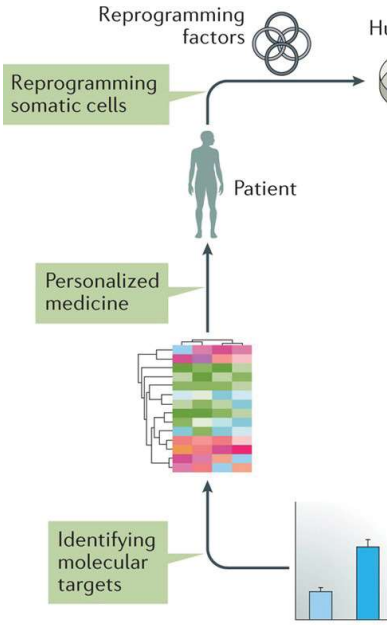

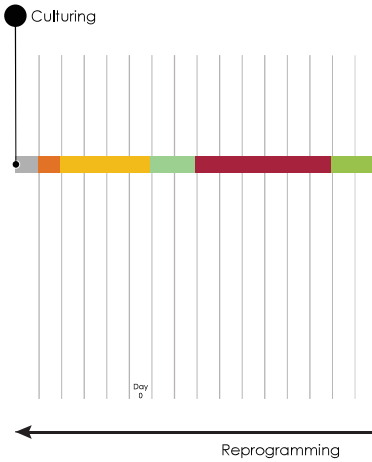

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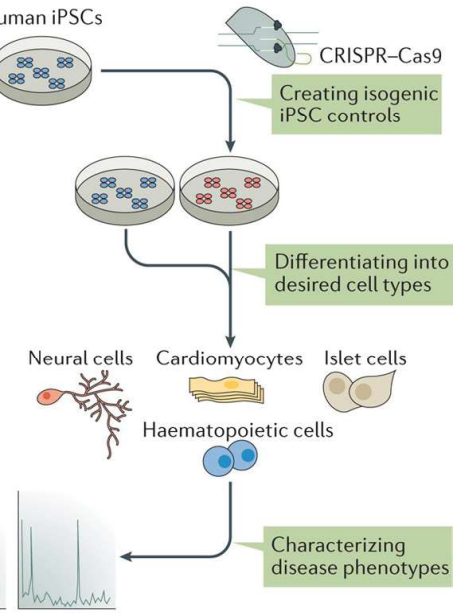


iPS Cell expansion

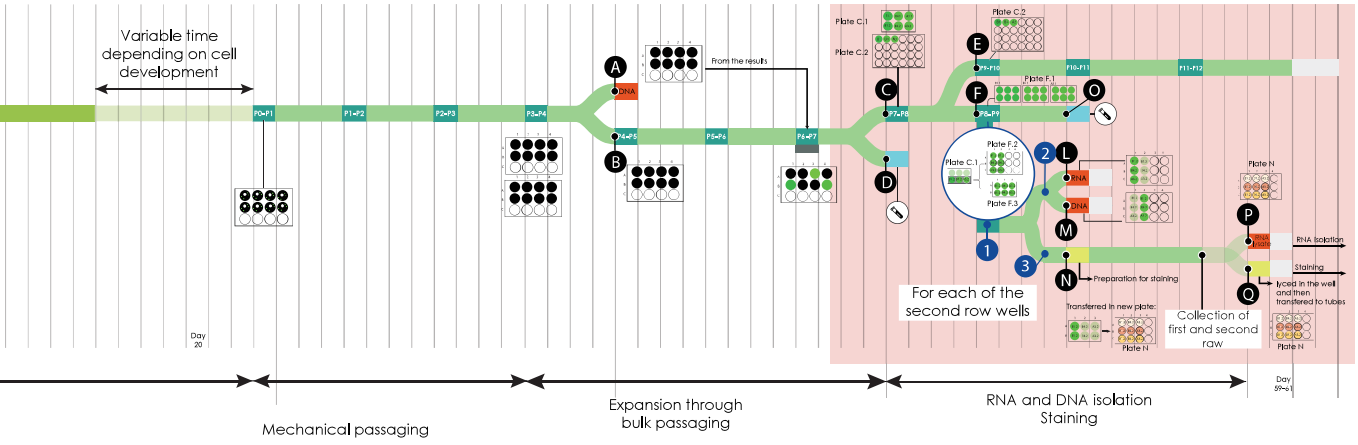
There are many different methods used for the production, expansion and storing of the biological materials, the procedure followed by Erasmus MC is directed towards a medium-high throughput.
The current thoughput (production quantity) of the iPS Core Facility is of 50 lines per year while they aim to increase to a throughput of 1000 lines per year. The only way of doing so, due to the very high expenses that go into technicians salaries and due to the labouriousness of the method is to transition to automation.

Automation is stable in the production of products as well as in pharmaceutical processes, while it includes several variables when it concerns biological material such as human cells.

Automation uses machine learning in order to substitute the decisions over the process made by technicians thanks to their experience in evaluating the quality of the culture.

This is mainly why there are control steps to which the technician will have to give feedback to the system or through which he/she will provide the system with new material.

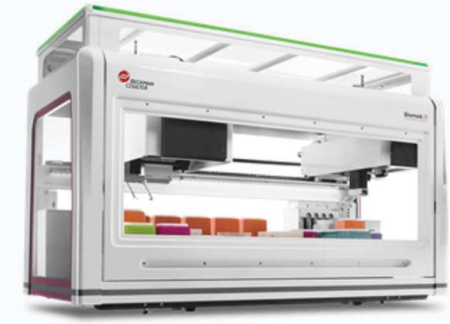
The manual process for the production of each cell line has been documented in the past phases of the research and a final estimation of the devices to be used during the different steps has been identified.
In the following picture you can see a general picture of the process ramifications.



What are the components of the system

These are the devices that are taken into account for the initial concept of the system, all to be
cabinet. We are going to take them only as boxes that do a function, this is just for you to get fa

Screening liquid handler
BioMek FX



Laser microscope for iPSC cleaning
Zeiss Palm

Tube c

Liquid hanc
BioMek NX

Cell viability checking device
ViCell

Sealin
Alps 30

Acustic dispencer
Labcyte Echo550

Peeling foil on plate
X-Peel

Centrif
Sigma

Confocal high content microscope
Opera

Robotic arm
SCARA

Incubat
Cytomat

enclosed in a biosafety
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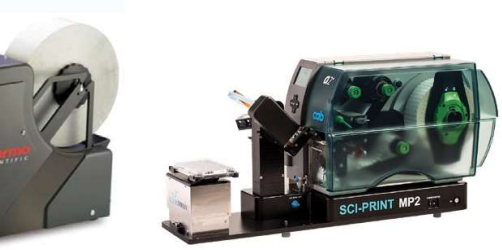

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Ideation session aim

The aim of this ideation session is within the **diverging phase** of the project.
A process complex as the one described earlier has a lot of very strict requirements and it can be approached it in an engineering inside-the-box manner.

In order to disumpt from this analytical approach I would like to explore with you three topics that still leave some design space to explore

Brainstorming - Functional alternatives:

Aside from the technical functions done by the devices you can see in the pictures in this page, the device is going to be composed by additional elements, starting from their functions we will make a brainstorming session for each function as part of a morphological chart.

SCAMPER - Configurations:

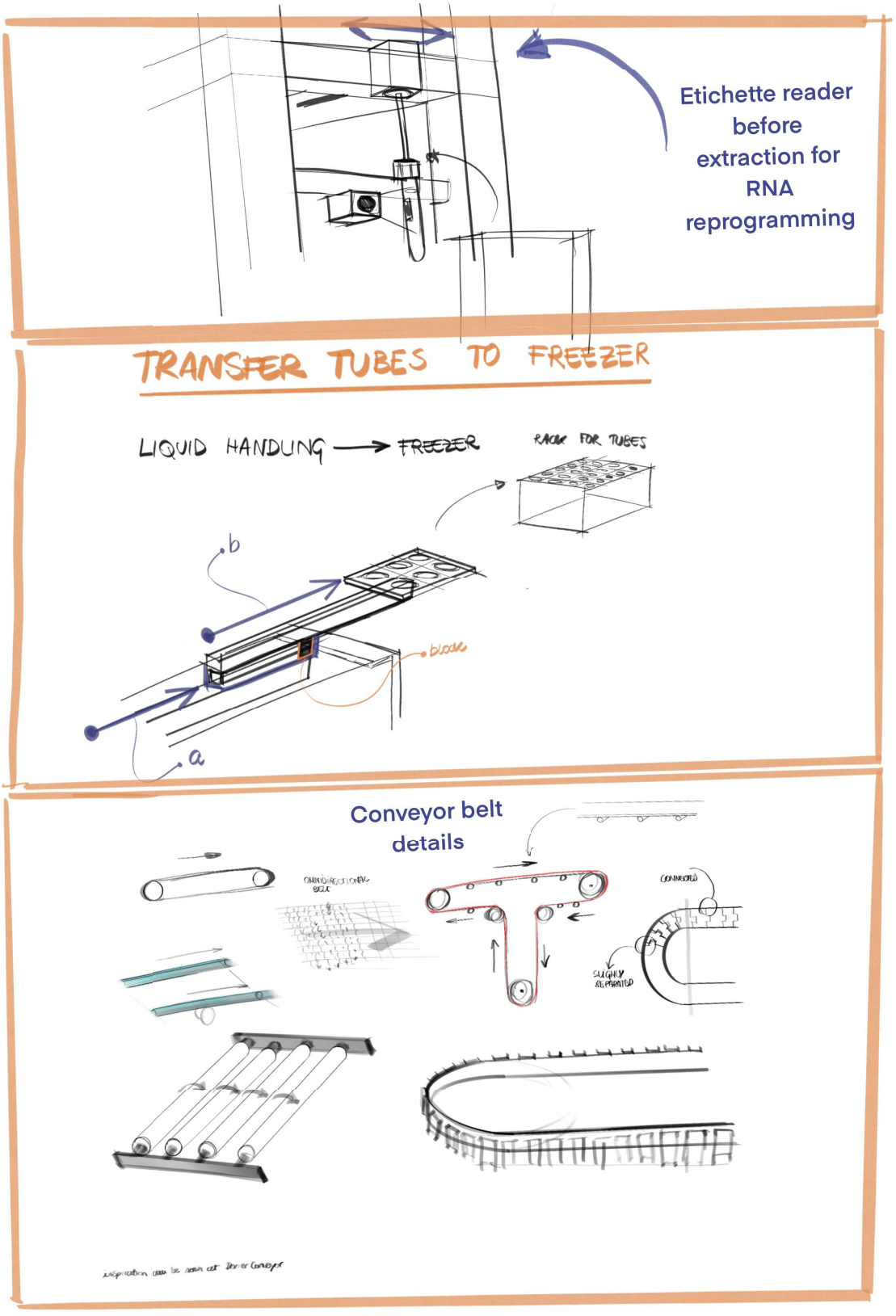
We will explore different ways of configuration of the devices between each other within a given room and within an illimited area.

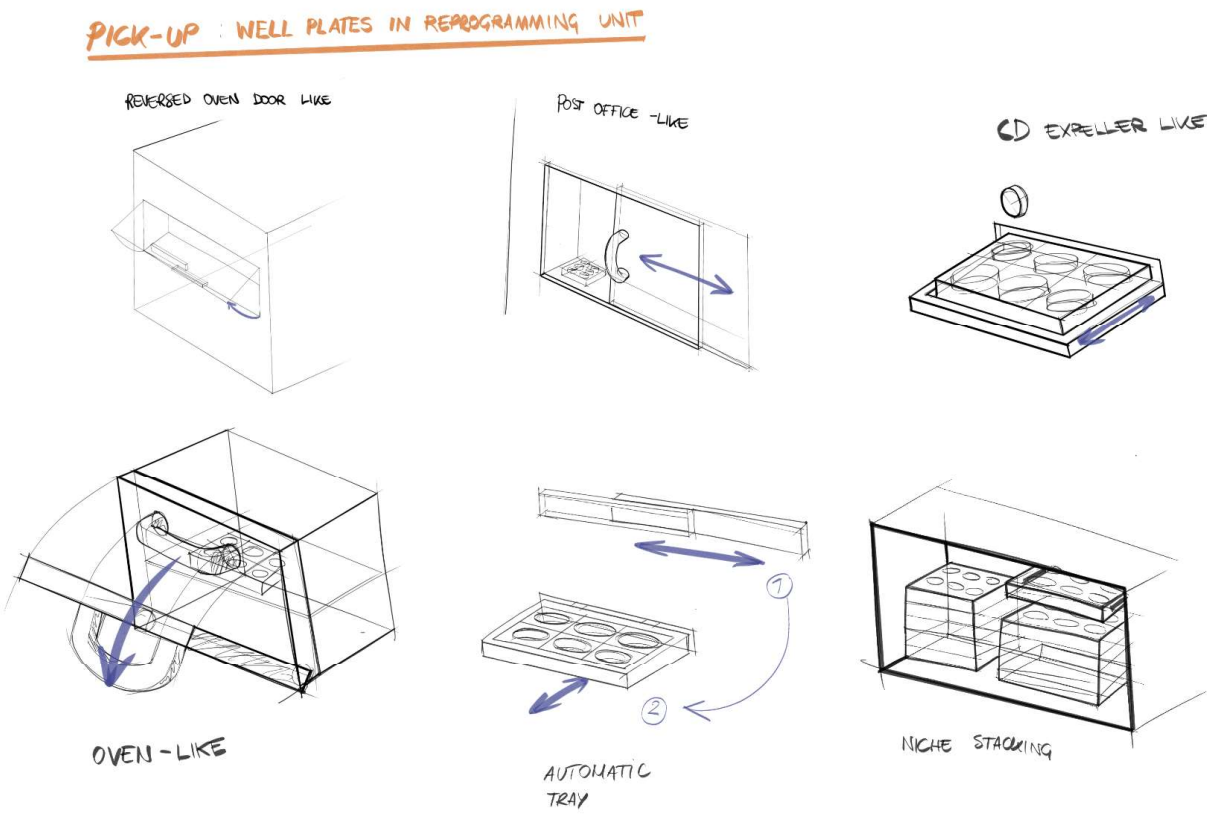
Interaction with a robot

This last section will be focused on ways in which we could envision the interaction between the technician and the robot during specific edge scenarios.

Thank you for your collaboration,
Looking foreward,

Natasa R.


Etichette reader before extraction for RNA reprogramming
TRANSFER TUBES TO FREEZER
LIQUID HANDLING → FREEZER
RACK FOR TUBES
b
a
Conveyor belt details



Here are reported only some of the sketches done during ideations, which were mainly conducted digitally

Appendix XIII- Minutes from interview with Hamilton Germany

Main Research Questions:

Does the Software have different modules that can be adjusted when the application steps change? How independently can we be in the adjustment of the software?

Is it possible to change the parameters of the programming and of the modules integrated into the system?

Conversation Stefan Baumler, Furst Rainer, Dupon Mieke. Working inside the Hamilton international support team.

combination of devices and orchestration of their functionalities.

Short description of our process:

Methodologies used are Lentivirus reprogramming, Sendai Reprogramming, and RNA reprogramming.

After initial reprogramming 8 different clones are picked and left to grow until passage 6, here their genome integrity is checked, the most promising 4 clones are selected and go further into validation including the check on the STEM cells markers and pluripotency (differentiation ability).

In total 6 master vials are frozen: 3 master vials are delivered to the researchers to expand the cells and start doing their research, while 3 master vials are banked for future needs of the researchers and are not given/used without their permission.

Ideas on modularity

The use of the robot should be made most efficient by using different platforms: Similar functions are represented in different stages of the process and can be used by the same module (for example for refreshment and differentiation).

Typology of labware

Typology of labware used goes from 12 well plates, used for picking, and 6 well plates, used to collect RNA or for the freezing of the cells. With automatization, clones can be picked in smaller wells (24 well plates) because of easier handling.

Nowadays the culturing of the cells starts in a T25 flask but can be done also in a 6 well plate.

Lines at the same time:

We don't have an exact estimation yet on how many lines to have in parallel at the same time. At the moment, with the manual process, every two weeks 3-4 lines in parallel start the reprogramming process (that lasts approximately 3 months). The aim of automation is to have 1000 lines produced per year.

Hamilton Germany

Software:

Interface:

The operator doesn't use codes but the GUI identifies different lines with different color coding. Steps are scheduled on different days and the due date is visible.

The steps are sorted by the due date and the arrangement of steps can be modified per priority.

An overview of the system needs for loading of resources is available A prediction is given on the safety of the walk-away times in relation to the availability of resources

Possibilities of modification:

New projects can be created.

A new experiment or line can be defined.

There is a list of steps that can be performed and the steps can be changed in their details.

The step types can be selected from an already created library.

Parameters within a step can be changed each and every time.

The number of batches can be changed for each step.

Management of disruptions:

If a batch is not running as intended the plate is secured inside the incubator

and it can be selected to be delivered to the technician with an automated

pick-up. Also, the delivery back to the system is automated.

The functioning of the software:

Steps are clearly identified and individual application modules are created from their specifics.

Hamilton's software engineers create strong flexibility for accessible parameters that have to be changed from day to day.

These modules are delivered as a toolbox and can be used freely to create individual workflows in the Editor.

Templates can be saved and imported into new experiments without the need for setting them up again.

All steps come as a part of the Hamilton Star line equipment in the ***Venus software***, the user interface calls the steps for the Editor.

The code is open and the steps can be defined and changed for variants.

In case of a change of devices, new drivers will need to be installed, but the Venus software will still be able to integrate the newly installed devices in the automation.

Some Hamilton customers are able to program and switch the devices or functions themselves, but the most common approach is to collaborate again with Hamilton for the preparation of test methods for new integrations.

Training for the manipulation of the software is needed, after 3 days of training the trained technicians will be able to change the basic settings.

Appendix XIV- Minutes from interview with Beckman Coulter

Main Research Questions:

Does the Software have different modules that can be adjusted when the application steps change? How independently can we be in the adjustment of the software?

Is it possible to change the parameters of the programming and of the modules integrated into the system?

Conversation Stefan Baumler, Furst Rainer, Dupon Mieke. Working inside the Hamilton international support team.

combination of devices and orchestration of their functionalities.

Short description of our process:

Methodologies used are Lentivirus reprogramming, Sendai Reprogramming, and RNA reprogramming.

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In total 6 master vials are frozen: 3 master vials are delivered to the researchers to expand the cells and start doing their research, while 3 master vials are banked for future needs of the researchers and are not given/used without their permission.

Ideas on modularity

The use of the robot should be made most efficient by using different platforms: Similar functions are represented in different stages of the process and can be used by the same module (for example for refreshment and differentiation).

Typology of labware

Typology of labware used goes from 12 well plates, used for picking, and 6 well plates, used to collect RNA or for the freezing of the cells. With automatization, clones can be picked in smaller wells (24 well plates) because of easier handling.

Nowadays the culturing of the cells starts in a T25 flask but can be done also in a 6 well plate.

Lines at the same time:

We don't have an exact estimation yet on how many lines to have in parallel at the same time. At the moment, with the manual process, every two weeks 3-4 lines in parallel start the reprogramming process (that lasts approximately 3 months). The aim of automation is to have 1000 lines produced per year.

Beckman Coulter

Software:

Interface:
The interface is easy to read and communicative in an icon-based way

The overview of the progress is presented on a daily basis by showing the currently running processes

Warnings at the bottom of the page give information on the conflicts and needs of interaction

Users have to either manually give the instruction for a process to start or set it to start automatically for a later time.

Possibilities of modification:

The software has 4 layers, the technician is

going to interact is the Sami level in order to implement changes. if the changes are relative to a separate system the interaction to apply the changes is also needed in those systems and therefore also in the process manager.

- Volumes and the possibilities of liquid volumes can be changed very easily
- Extra codes can be integrated as well on different layers
- Small and fast pipetting

The main changes can be done at the Sami software (scheduling that regulating the optimization of the process) but also in the scheduling software (connected to the process management), this can be done independently by the workers at Erasmus MC.

The tracking and documentation system used at the laboratory can be integrated within the DART system but documentation is also going to run within DART

There is no limit to the steps to be changed

There is a possibility of using several units that are regulated by the same DART system and therefore function as one unified system.

It is possible to incorporate parallel methodologies for different typologies of cells through different models of the same system.

Management of disruptions:
The tracking works on a well level so it can avoid the treatment of the different wells.

It allows to track if some steps will happen outside office hours, the method of solutions could be done by changing times, give further instructions on automation or postpone the step for working time.

The calendar shows also where and when there could be overlappings of the process and offers a prompt message for decision making on how to resolve the conflicts

The functioning of the software:

The software has 4 layers, data is recorded from previous runs from the DART method.

The calendar can be re-organized by the technicians

Training is given for the technicians to change the Sami software, no previous programming knowledge is needed

Appendix XV- Interview Access Orchestrate

The following notes are deriving from an interview with Rod Schregardus on the 17 July 2020. It was conducted in order to research what are the possibilities of planning software to be adopted for the system activity planning

Access Orchestrate is used by many companies both in many industries, some examples are Siemens, Alexion, Catalent, Merck, Surepharma, Shire, Seqirus, Pepsico, land rover, Novo Nordisk, Gilead, monopoly, Jaguar or also academic institutions such as the Duke University.

It is usually used for management by replacing usual spreadsheets that omit certain elements of complex systems such as dependencies between elements labor, tooling, and maintenance.

The needs it is able to satisfy are relative to day to day scheduling, capacity planning, and “what if” analysis. All of these elements should be included in a clear, simple way, easy to implement, and with dedicated support.

The Access Orchestrate is a cloud-based software that embeds in one platform several aspects of project management: warehouse management, construction, production planning, scheduling, and manufacturing.

The connection between the company enterprise resource planning (ERP) and the staging database is not offered by Access, since their area of focus is relative to the connection of the staging database to the orchestrate database.

Different typologies of tables can be used in order to manage their data through Orchestrate: for example, customer data, sales orders data, sales orders items data, work orders data, purchase orders data, work or sales orders specific pegging data, suppliers data, items data, item rates data, item types data, Bill of Materials data, stock data and resources data.

The benefits of using Orchestrate are relative to higher efficiency and easiness in the planning process, ability to optimize the planning and have improved communication between departments through the usage of one central production schedule, being able to reduce the downtime between shifts for batch handover, and for planners to plan batches. The use of such software enables, furthermore, to reduce the overall batch footprint, as tasks can be accurately planned forward and downtime can be tracked more effectively with the schedule, resulting in focused continuous improvement projects.

The software has two main views, one used for organizing the data imported and plan the production and a second section more userfriendly for the operators that is designed for concentrating on the specific tasks.

During the interview, a demo of a pharmaceutical application was done. In the configuration presented, realized for a pharmaceutical application, the use of the software was presented as follows:

The process is defined by inserting details of the typology of the task, its description, its relation to the operation step, and process duration.

Each operation is manually related to other dependencies, resources, costs, and outcomes.

Opening the plan tab its possible to have an overview of the timed forecast of the steps

At the moment of scheduling, new activity boundaries of time can be set for starting a determinate task and links to activities that

are taking place in neighboring devices are highlighted

Reports on the process can be visualized relative to costs and outcomes, in different time frames or production specifications. In this way, for example, it is possible to visualize the periodic utilization of reagents in tanks across a period of time

Report data can also be used for comparing the current plan with a hypothetical scenario in order to test optimization.

Once the planning is completed it is possible to have an overview of the activities that take place in each device and to which operation they are connected to.

Visualization can also be relative to the activities that take place through the day in an agenda-like view

Back in the main view of the process, the Operator can now view both in a daily, weekly or monthly manner the activities to do

Once operations are completed they can be visualized in a new chart that highlights the time of start and finish of all the processes. Here the technician or planner can change properties of all activities such as quantity, timing, or status of the competition.

Conclusion and evaluation

Access Orchestrate is a very useful software for planning and foreseeing needs of the system, reducing times and costs of production and management. However, because of its possibility of receiving input data only from a company ERP and not from devices used it is not suitable for applications such as automated system management. Nonetheless, its organized way or handling disruptions can be taken as an example since the management and planning activities would find its functions beneficial.

In terms of adoption of the software Access Orchestrate, it was confirmed that if there are additional needs to the model to be added or taken into account changes could be possible and that the software would be able to retrieve data from other software and could be embedded, therefore, with other software that input data to the system's devices.

The price of the software depends on the number of users it should be given access to, which for a university faculty it could be of an incentivized license fee of 30 thousand euros with a two-year support plan.

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