

Project name

Date

What are you doing?

Opportunity



Why are you doing it?

Scientific considerations



Why are you doing it?

Ethical considerations



How else could you do it?

Alternatives



What will this lead to?

Outcomes



Take action!

So what?



What are you doing?

Opportunity

I am trying to construct a plasmid from six DNA fragments. I now tried to do this by enzymatic restriction and ligation, but it did not work. [problem]



Why are you doing it?

Scientific considerations

I want to express several proteins inside a bacterial cell, with regulatory elements in front, since I want to find out how regulation works.



Why are you doing it?

Ethical considerations

The bigger aim of this research is to discover new biological functions to contribute to the scientific field. In this way, other researchers can use our findings to solve urgent questions, like curing cancer.



How else could you do it?

Alternatives

Construct the plasmid by using homologous recombination in yeast or in *E. coli*, Gibson Assembly, LCR or EO-PCR. Express the proteins on separate (similar) plasmids instead of on one plasmid.



What will this lead to?

Outcomes

For homologous recombination in yeast, a new ori and marker and homologous flanks are necessary. For Gibson Assembly, LCR, and EO-PCR homologous flanks are needed. GA is the most viable approach. Expressing proteins on separate plasmids can be done, but only as proof of principle.



Take action!

So what?

I will try to use Gibson Assembly for plasmid construction, as this is the most viable approach. In parallel, I will express proteins on separate plasmids as proof of principle. If this does not work, the design needs to be changed.



Project name

Date

Example creating minimal cell

08/02/2018

What are you doing?

Opportunity

We want to create a minimal cell using the bottom up approach.



Why are you doing it?

Scientific considerations

We want to understand and discover new biological functions of the cell. This can probably result in new insights in diseases.



Why are you doing it?

Ethical considerations

This can result in finding new medicine to cure people. This can save a lot of money for health care. Besides, fascinates me.



How else could you do it?

Alternatives

New biological functions can also be discovered by only looking at separate processes, by DNA/protein analysis, following metabolites inside the cell using fluorescence etc. Getting more insights in diseases and medicine can also be done more directly, targeting one specific disease.



What will this lead to?

Outcomes

Different ways of finding new biological functions of the cell can be done, probably leading to faster results on insights in diseases and medicine, but neglecting the bigger picture of creating a minimal cell, which is less fascinating.



Take action!

So what?

Creating a minimal cell is one of the approaches to understand and discover new biological functions. Using the whole picture instead of separate processes, will give extra insights. Also, I found out that fascination and curiosity are a major incentives for me to do this work. No further action will be taken.

