Finding some cryptic plasmids from rat guts and an approach to synthetic biology by redesigning a small plasmid

Farzana Shamim

Synthetic biology is a relatively new and emerging field of science that combines the knowledge from many disciplines such as biology, engineering, mathematics and physics. It deals with synthesis and redesigning of biological devices and systems with novel functions, which are not exactly present in nature. Attempting to create biological part, which is a collection of genetic parts that are used in the assembly of systems and devices in synthetic biology, is not something new. The journey of synthetic biology started approximately 50 years back with the attempt of creating a biological part with circuit-like connectivity. But in the last decade many successful attempts have been made. For instance in 2003 Smith and co-workers synthesized a bacteriophage from oligonucleotides. After the success of making this synthetic bacteriophage, in 2008 Gibson and co-workers successfully synthesized a whole organism, *Mycoplasma genitalium*, genome from oligoneucleotides - the basic building blocks of life.

One of the aims of this project was to isolate and purify small plasmids from the microorganisms isolated from the gut of the 8 different rats. Different methods were applied which showed that some methods give far better results than others. This was done because after characterization and sequencing of these plasmids, they could be use as potential natural cloning vectors, which is a DNA molecule used as a vehicle to artificially carry foreign material into another cells and eventually can be replicated and/or expressed. In addition to that, it also gives a possibility to modify and recreate those plasmids synthetically to make them more efficient, which was the purpose of the other experiment in this project. In that case we targeted to redesign a small gene (cloning vector) of around 3 kb isolated from wastewater with several bioinformatics tools and recreate it synthetically from the oligonucleotides. As this plasmid was isolated from waste-water, which is a very good reservoir of various kinds of microorganisms, especially those belonging to the Enterobacteriaceae family, it was supposed to be a broad host-range vector. That means this synthetic vector was supposed to adapt and replicate inside multiple hosts, especially those from the Enterobacteriaceae family. Modifications of the vector were successfully done and a protocol was developed to recreate it synthetically. Unfortunately the experiment could not be completed, as we did not get the expected results needed to carry out the next steps of the experiment, and also there was a time limit. Although, the procedures were not completed, it is assumed that they could be successful with some more optimization reactions, as the same procedures were successfully worked out before in order to synthesize an entire plasmid from oligonucleotides.

Degree project in Biology, Master of science (2 years), 2012
Examensarbete i biologi 45 hp till masterexamen, 2012
Biology Education Centre, Uppsala University, and Department of Biology, University of Copenhagen
Supervisors: Leise Riber and Lars H. Hansen