Using HiFi Assembly to Generate Repetitive Genes in Modular Plasmids to Produce High Molecular Weight Polypeptides

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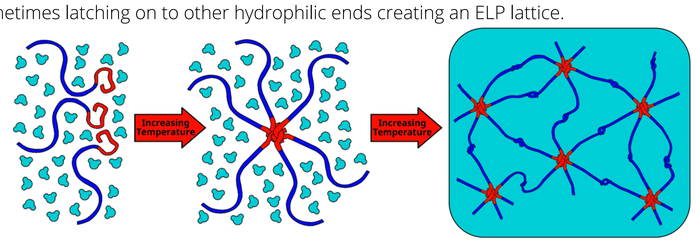
Introduction

Elastin-like polypeptides (ELP) constitute a genetically engineered class of "protein polymers", characterized by having many repeats of GxGyP amino acid monomers. Here, 'x' stands for any amino acid, and 'y' is any amino acid except for proline. ELP's are known for their ability to assemble and disassemble a 3D hydrogel matrix in a reversible process dependent on temperature. This makes them ideal for making bio-inks with embedded cells. It is able to do this based on their hydrophilic and hydrophobic properties. The hydrophobic ends of the ELP (red) will come together naturally, to reduce its surface area exposed to the solution around it. The hydrophilic ends (blue) extend out into solution, sometimes latching on to other hydrophilic ends creating an ELP lattice.



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This transition happens over a narrow temperature range depending on the structure. The transition temperature can be changed on the amino acid substitutes and the length of the polymer, i.e. the number of GxGyP repeats. It is expected that longer hydrophilic blocks will result in lower concentrations of peptides needed to form the gels. This project focused on building a library of ELP's of varying sizes, with the goal of trying to optimize the properties of these gels. The DNA plasmid which contains the gene for the ELP is designed so that the length can be doubled using digestion and HiFi assembly. We started with a DNA plasmid holding a 17xGXGVP repeat, eventually building it to a 513xGXGVP repeat.

Materials & Methods

HiFi Preparation

Before the HiFi assembly is carried out, restriction enzymes are used to cut the circular DNA plasmids into linear DNA fragments. The restriction enzymes used in this process are BamH1 and HindIII, as these can cut the DNA in a place where the remaining overhangs can be ligated back together in the HiFi assembly

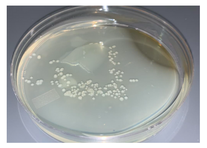
Once the DNA has been cut into linear fragments, the restriction enzymes must be removed so that they do not continue to cut the DNA in future steps. The concentration of DNA is measured to find out how much remained after the cleaning procedure. In this step, a Qubit Fluorometer is used to calculate the concentration of DNA in our samples. If this value is not too low, HiFi assembly can begin.

E. coli Transformation

Many plasmids were made by the HiFi reaction, some of which may be correct, and some may be incorrect. These plasmids currently are all mixed in the same tube of liquid. We want to separate the plasmids from each other, so we only work with the correct plasmid. *E. coli* can only take up 1 plasmid at a time, so by transforming the plasmids into *E. coli* we will separate the plasmids from each other. Because the plasmid contains a Kanamycin resistance gene, only the cells with the correct plasmid will survive on the Kanamycin plate used.

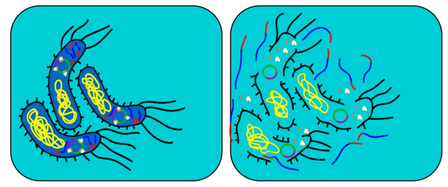
Colony Plate to Culture

Colonies from the plate are selected to verify that the correct plasmid has been acquired from the other plasmids. Once selected, a culture is performed with only that colony. The culture will produce a lot more *E. coli* from this colony, thus producing more plasmid.



Plasmid Preparation

Now that the *E. coli* has grown, it is time to remove the plasmid from the cells, as it cannot be analyzed or quantified if it is still in the *E. coli*. This is done by lysing the cells in solution. After the plasmid has been removed, it is sent to sequencing to verify that the HiFi assembly was successful.



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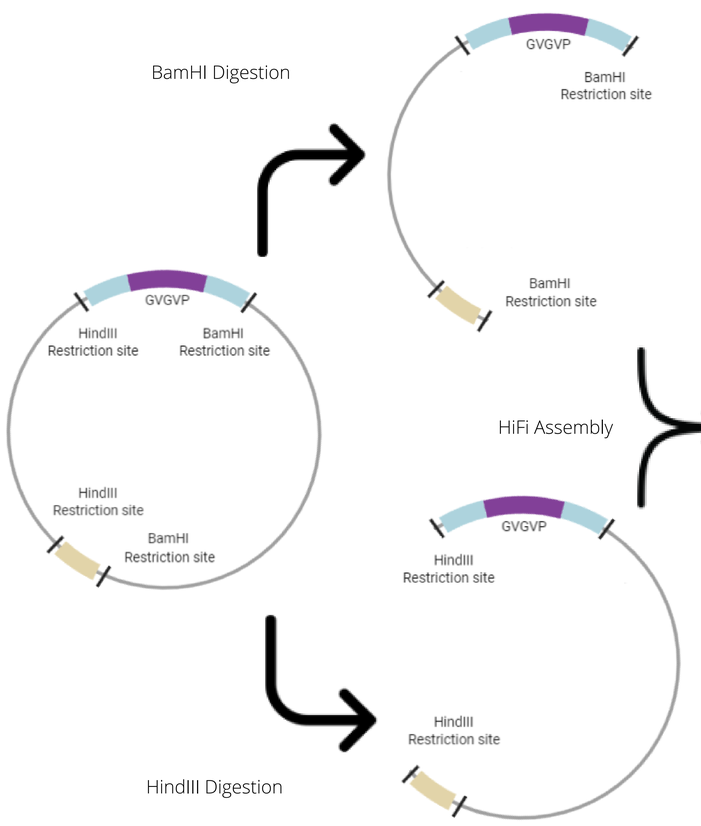
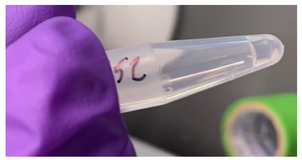
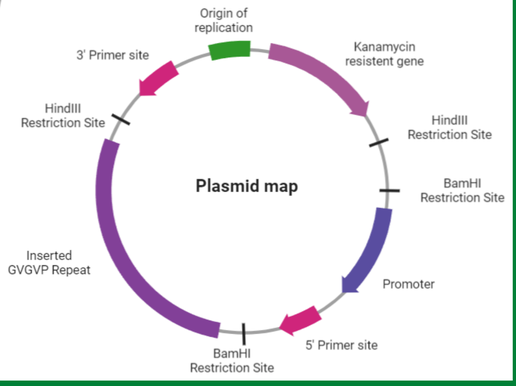
Doubling Gene Length Using HiFi Assembly



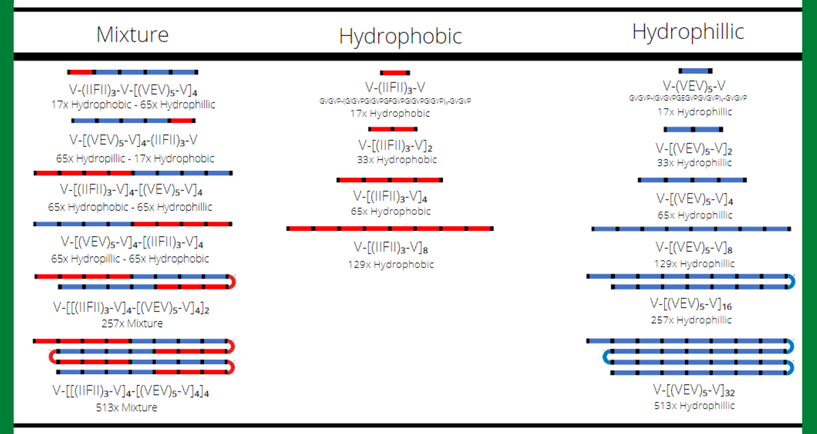
HiFi assembly connects our DNA fragments with overlapping DNA sequences. This allows digested plasmids to be reassembled into whole plasmids again. The result is a new plasmid with double the amount of GXGVP repeats as what was started with.

Our plasmids are designed such that the BamHI and HindIII restriction sites leave complementary overhangs after digestion so that they can be ligated back together efficiently.





Results



In total, a library containing 16 different plasmids was created. Each plasmid encodes for ELP's of different properties due to their number of repeats, as well as hydrophilic and hydrophobic groups. Apart from the initial two, each plasmid was synthesized through HiFi assembly. The next step with these plasmids is to test if they can express the desired ELP's. Then they will be purified and their properties as hydrogels will be tested.

Acknowledgements

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