Molecular Cloning

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Abstract

Molecular cloning is a process that manipulates the spider silk sequence to select for a proper sequence size and specific vector into which it can be expressed. This process is achieved using techniques such as digests, transformations, purifications, and ligations. This semester, our work has centered around the mPRI(alfalfa), pOET2(insect), and pmk(ecoli) vectors, into which we have, or will, insert 3 time, 6 time, and 9 time sequences of the spider silk amino acid chain. With each repetition of the silk sequence, its properties improve and become more like natural silk. Vector specification allows these sequences to be produced in different hosts, with each providing beneficial and detrimental circumstances to the silks eventual development and production. These processes are ongoing but we hope to produce usable protein in both the mPRI and pOET2 vectors in the coming months, allowing us to refine our production process until the best possible product is achieved.

The Cloning Process

1: Digest

Restriction enzymes KpnI, BamHI, and NdeI (see below) separate the silk sequence from the vector, preparing it for manipulation. These enzymes create distinct amino acid ends that allow for selectivity when ligating.

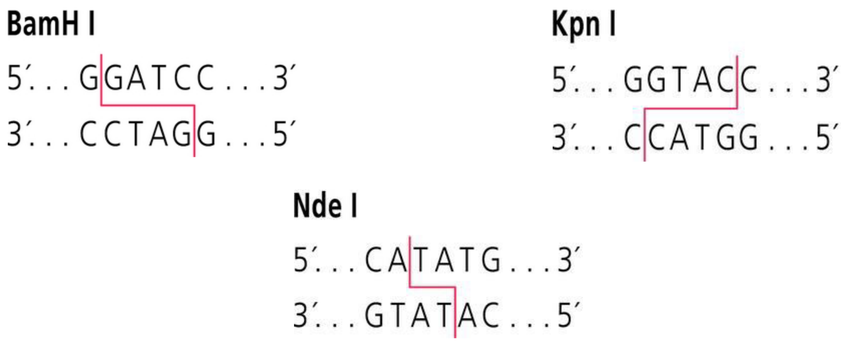

Figure 1: amino acid sequence for BamHI, KpnI, and NdeI

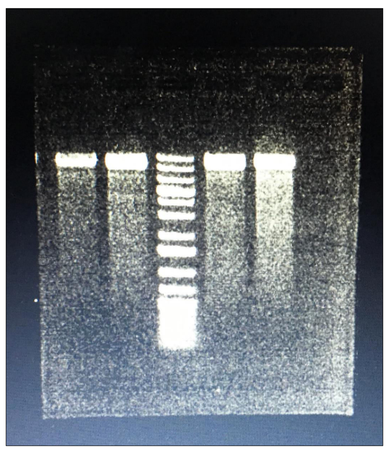

Figure 2: separation of silk sequence (bottom band) from vector (top band) on a protein gel

2: Extract/ Purify

After separating bands by amino acid pairs in a DNA gel, the silk sequence is extracted and purified. Extraction physically removes the sequence, after which chemical differences in pH purify.

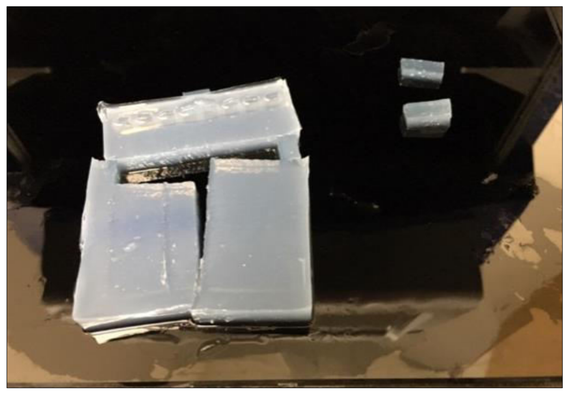

Figure 3: extracts of silk sequence from protein gel

3: Ligate

Once purified the band is then ligated or attached to another purified vector or silk sequence by using matching ends formed by restriction enzymes at digestion.

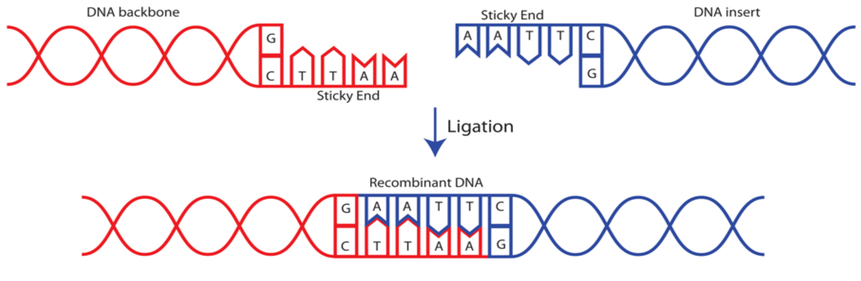

Figure 3: representation of the ligation process

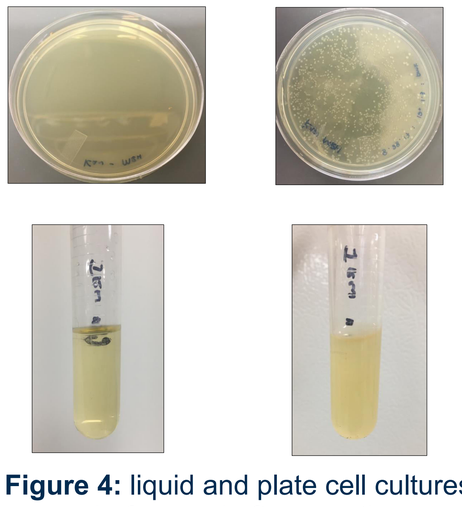

Figure 4: liquid and plate cell cultures before and after growth

4: Transform

The complete vector formed by ligation is then cultured and transformed into a cell line which replicates and produces thousands of copies of the desired inserted silk sequence.

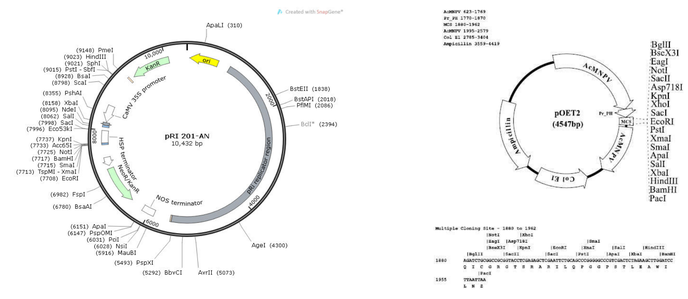

Figure 5: vector maps from left to right: pri201an(mPRI), pOET2

Future

In the near future we hope to finalize the transformation of the silk sequences into expression vectors, and then transfect those completed vectors into their hosts. Alfalfa(mPRI) is one of the few hosts capable of producing a protein similar in size to the natural silk and insect cell lines(pOET2) glycosylate naturally, allowing the silk to form a natural glue. These are exciting opportunities that we hope to take advantage of in coming months and continue to refine in order to create a usable silk product.

Setbacks

Contamination and mutation are the two biggest stumbling blocks when working with molecular cloning. Because both cells are sensitive and particular about their ideal growing conditions, they must be given large amounts of nutrients in order to grow. This high nutrient level also increases the growth of other cells and bacteria, making the difficult process of cell isolation critical. If contamination can be avoided, the cell still has the ability to mutate, or incorrectly digest, ligate, or transform. This makes repetition of ligations and digestions key in patiently waiting for the cells to accept the desired form and build a complete and replicating vector.

Acknowledgements

I must acknowledge the support and encouragement of Doctors Randy Lewis and Justin Jones, as it has allowed me the opportunity to work and progress in the lab. I would also like to thank my wonderful graduate student Micheala Hugie for her hard work and patience with me as I have worked to learn and understand the methods and techniques used in our research. These individuals, along with other graduate and post doctorate researchers, have influenced and shaped my incredible research experience.

Figure 6: From left to right: Doctor Justin Jones and Doctor Randy Lewis