Lab Report

# Artificial Silk

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The artificial silk has the potential to revolutionize the biomaterials industry. Artificial silk could be applied in protective clothing, human tissue repair, such as tendon repair and ligament repair. The silk proteins allow to programme and change the strength and tension properties of biomaterials.. The gene coding for honey bee silk protein was used because it has fewer DNA sequence repeats and the sequence is shorter than more well known producers such as spiders and silkworms. Producing artificial honey bee silk would be advantageous because it holds potential to downstream applications in the biomaterials industry. The artificial silk gene was cloned into pUC19 vector, followed by transformation and expression in *Escherichia coli.*

**Keywords:** Biomaterial, honey bee silk, artificial silk

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## Background

Artificial silk has a long list of potential uses, ranging from stronger fishing lines, to repairing tendons within the body. Some of the other endless possibilities of artificial silk could include bulletproof clothing, musical instrument strings, repairs in human body tissues, and protective helmets. Since artificial silk is a non-toxic biomaterial, synthesised by a living organism, it is eco-friendly and safe for humans. Generally the body does not recognize biomaterials as toxic so there is no reaction (ASM International Team, 2003). Artificial silk is composed of crystalline beta sheets responsible for its non-toxic property (Vepari, Kaplan, 2007). They have been used in *in vitro* and *in vivo* studies have proved to be compatible (Vepari, Kaplan, 2007). Artificial silk can not help with the amount of violence people face, but it could help with protection from bullets and debris. It could be used for protective and bulletproof clothing.. Spider silk proteins on their own have one sixth the strength of steel. However, if the silk is layered and wrapped together, it has potential to match up in durability, while weighing less (Bhanoo, 2014). This means that if the artificial silk were processed into clothing, it would not be overly heavy and it would be light weight like normal silk, yet with far greater strength.

Honey bees (*Apis mellifera*) create silk from the process of making an insoluble filament from a protein solution. As this insect turns its head in different directions, the labial gland starts spinning silk (Hepburn, 2013). The labium–hypopharynx and the spinneret is the spot where the silk exits from the bee's insect body. The silk ends up being 3 μm in diameter. The silk is made up of α-helical proteins assembled into ordered coiled-coil structures (Hepburn, 2013). Honey bee silk is made up to be extended β-sheets (Hepburn, 2013).The hydration in the silk loosens the interaction between neighboring crystalline regions thereby reducing stiffness. Potentially, by looking at how hydrated a cell is, the strength could be inferred.

Since artificial silk is a biomaterial it could be used in human medical applications. This could help with tendon and ligament repair, because the strength could be adjusted. Artificial silk could also be used for stitches, which would make taking stitches out less painful, and patients would be less susceptible to infections entering the wound.

The goal of this project is to produce artificial silk using the silk gene from honey bees. A plasmid containing the silk coding gene will be inserted into *Escherichia coli* through transformation, consequently expressed and artificial silk could be mass produced and used for all the advantages it has to offer.

## Materials and Methods

The goal of this work is to insert the gene responsible for producing honey bee protein silk into a plasmid (pUC19) then to have it transformed in *E.coli* and express the silk protein. It starts off with preparing the Luria-Bertani (LB) culture broth supplemented with ampicillin to grow *E. coli transformed cells*.. The pUC19 is then inserted into *E. coli* and then *E. coli* cells are incubated in LB broth plus ampicillin. After this, the plasmid is extracted from transformed cells and goes through digestion to be loaded and isolated into the gel bed. The liquid for the gel has to be made and set in the mold. Once the gel bed is ready for electrophoresis, the deoxyribonucleic acid (DNA) ladder is loaded, as well as the suspended plasmid after has been mixed with loading dye. After the gel bed is run, it will show where the extracted pUC19 is by comparing with the running profile of the DNA ladder fragments. The plasmid will then be cut out of the gel bed using a DNA clean up kit for later use. The gel of the bed must be purified in order to extract plasmid from the gel bed. Once sole DNA is obtained it will be prepped to 1000 ng and the pUC19 cloned with the silk gene through Gibson Assembly. After that, the plasmid is transformed in *E.coli cells*, which should start to express the silk gene present in the plasmid. Since bacteria multiply fast, it should create many copies of the plasmid that now has the honey bee silk gene and express that DNA. The DNA must be checked to make sure the gene of interest is being exposed, and then it will be run through another gel bed. Once the protein is produced and expressed, the silk protein will be extracted and purified through dialysis tubing. When the silk reaches a certain concentration, the strength can be tested. The Monarch Plasmid Miniprep Kit (NEB, USA), Bio-Rad EZ Load 170-8356, Purple Loading Dye (NEB), Carolina BioClave,Corning Benchtop Shaking Incubator, and NEBC2987 bacteria were used in our experiments.

## Results

### Plasmid Digestion Results

To ensure that the plasmid digestion step went correctly our group made up a gel bed to show base pair matching of the pUC19 DNA sequence. The first gel bed did not show proper results, in fact noDNA dying was detected. The DNA ladder did not show up and neither did the pUC19. There was another group sharing our lab that used a 2% gel bed, so we thought we would try it as well since we were not having the best luck with yielding results. Although the agarose gel chart said a 2% gel bed would work best for sequences between 50-2,000, the ladder showed up for us, along with bands from our DNA sample (Figure 1A). We compared the bands from our digested plasmid with the ladder and thought we had the right amount. Since the ladder used only indicated up to 1,000 base pairs, we had to create a graph and an equation to find the length of the DNA sample used (Figure 1B). We used Google Sheets to generate a log transformed equation to work with the easier equation of $y = mx + b$. We looked at the distance travelled from the ladder, since we already knew the base pair matches. We found that we had digested our plasmid correctly because the base pair matching was around 2800 bp.

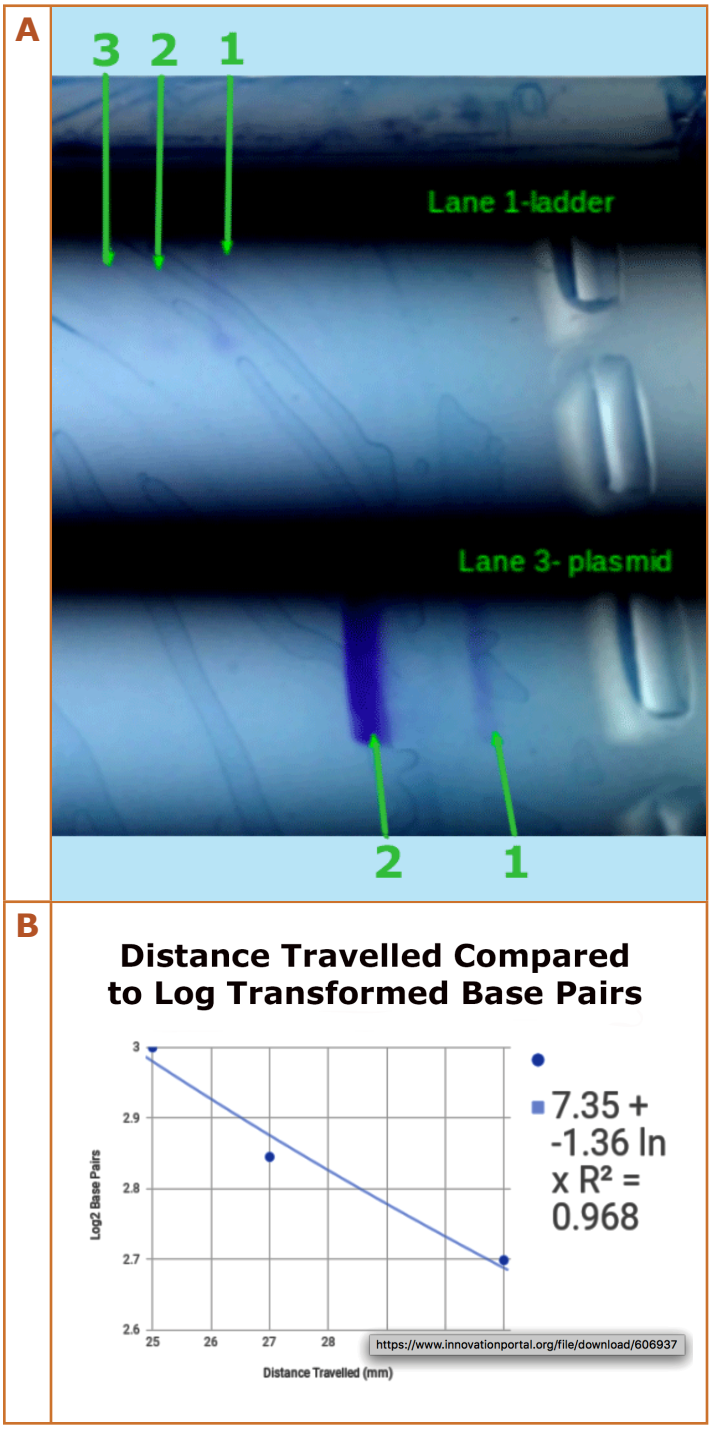


**Figure 1.** *A) Gel bed of the digested pUC19 plasmid. B) Shows the length of the bands in the gel bed log transformed to find base pair matches.*

### Gibson Assembly Results

To see results of Gibson Assembly we had to make LB broth and ampicillin plates. We made two plates, one for the control and one for transformed plasmid from Gibson Assembly. Then we transform the plasmid created from

Gibson Assembly into *E. coli* and put it into the incubator overnight. We then transferred the *E. coli* cells onto the LB broth and ampicillin plates. We put unmodified pUC19 on a different plate to use as a control, since it did not contain the honey bee silk gene (Figure 2). Our team discovered that when we were designing the plasmid, the BsaI cut site was in the ampicillin resistant coding sequence of the plasmid. Since this happened the protein was not assembled correctly, so the cells could not go through transformation.

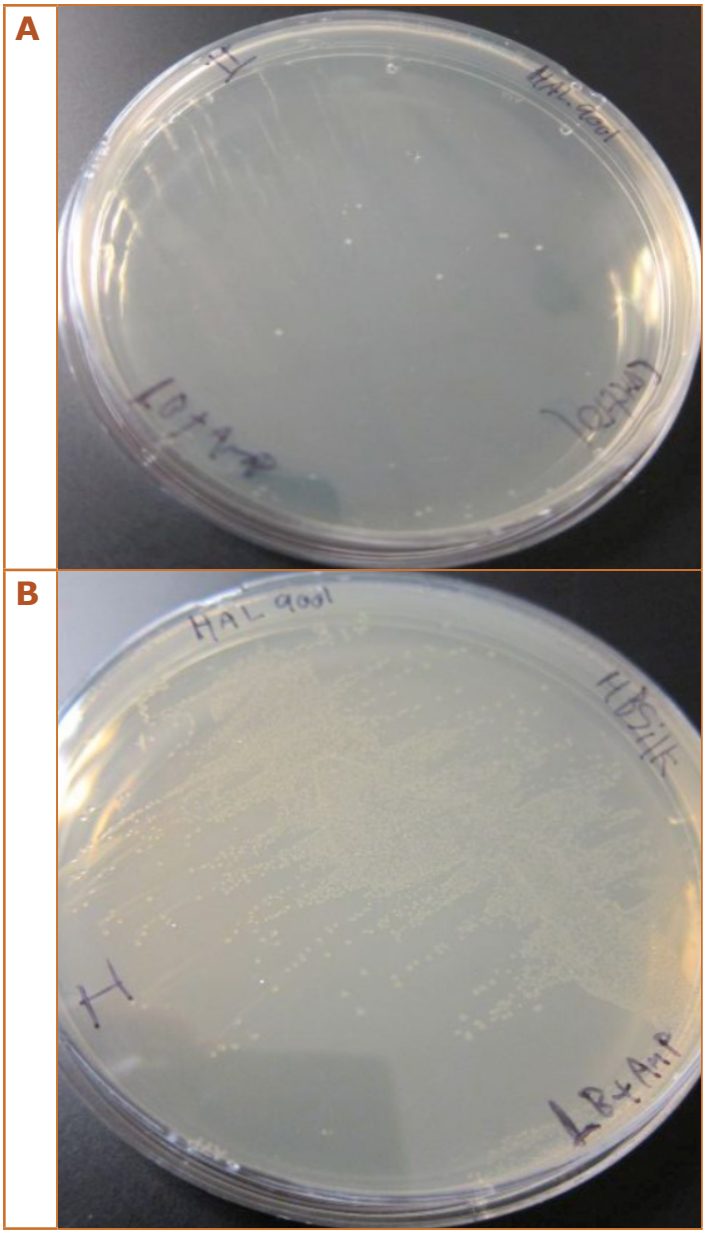


**Figure 2.** *A) Control plate of just pUC19 with no gene insertion. As you can see, there is no growth. B) Colonies of transformed E. coli cells with pUC19 cloned with the honey bee silk gene.*

## Discussions

We failed to meet our objective, not because of any of the difficulties we had foreseen as possible problems, but because of gel bed deficiencies at first, which slowed us down greatly. Then, we based our cut site on a version of the pUC19 plasmid that was different from the one we had to use, which resulted in the cutting of the antibiotic resistance gene. When this lab is performed in the future, *BsaI* must be placed somewhere on the plasmid where there is non-coding DNA so important parts of the plasmid, like the ampicillin resistance gene in this case, is not cut.

This is not all, or really at all, a bad thing. We've learned more, gained more experience with every failure. We better understand how to use materials and equipment that once confused us. As an opportunity to learn and grow, this has been a complete success.

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