**Developing a super producer of recombinant spider silk protein in *Escherichia coli* BL21 (DE3)**

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Acquiring natural spider silk faces many challenges in terms of logistics and efficiency. That is why we are aiming to produce it in bacteria via inserting protein-encoding genes and modifying them to adapt to the bacterial host system. Recent articles elaborate on hybrid materials with spider silk as their basis to create materials with entirely new properties and added functionality. In this regard, one of our main focuses is to surpass existing projects and enterprises that are researching spider silk applications. With resources such as the spider silk database (spider-silkome.org) and articles that study the correlation between the silk protein genetic code and the resultant fiber features [1], we believe that we can effectively produce new materials that possess different, if not superior, qualities in comparison to the available products.

Spider silk is one of nature’s toughest materials. It is 5 times stronger than steel and has highly elastic properties. However, harvesting natural spider silk is not an efficient process due to the highly cannibalistic nature of spiders which doesn’t allow farming them on a large scale. Moreover, spiders produce minimal amounts of silk that do not suffice on a commercial scale. So, producing the spider silk protein in *Escherichia coli* can be achieved in relatively large quantities and with an economically reasonable cost.

The target protein is Fibroin-4 from *Araneus diadematus* that is coded by the *ADF4* gene. Repetitive region of *ADF4* gene (C-module) was taken from the gene because it is a sufficient part for successful protein production. Design of oligos for assembly and codon optimization for *E. coli* was performed by DNAworks software. C-module was obtained by polymerase chain assemble method. The product of the PCR was cloned to pAL2-T (Evrogen, Russia) and after that transformed into *E. coli* NEB Stable (NEB, USA). Screening of colonies was made by PCR. The target PCR product was sequenced. To multiply C-model a strategy of double digestion approach with compatible restriction enzymes was chosen [2]. Products after each step of multiplication were sequenced. The final product was cloned into pET303 (Invitrogen, USA) and transformed into *E. coli* BL21 (DE3).

*E. coli* strain-producer of Fibroin-4 was made by modification of *E. coli* BL21 (DE3) by pET303 contents 8x C-modules. The current results we have are new genes which are expected to produce tougher and more elastic silks in comparison to the currently available variants. The gene development process is established and the protein production process is under development.

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**References**

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