# Brazilian Spider Silk Protein *Masp2* Production in *E.coli* System with Synthetic Biology

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**Statement of Purpose:** Spider silk fibers have high strength, toughness and elasticity, being stronger than steel and similar to Kevlar. Unfortunately, natural spider dragline silk cannot be obtained because spiders show aggressive behavior and the yield is low. This biomaterial has numerous potential applications in medicine and broader materials-related needs. With synthetic biology it is possible to construct and express genes encoding recombinant spider silk proteins. These proteins are characterized by their highly repetitive specific structural motifs, while dragline Masp2 (major ampullate spidroin) shows glycine, alanine and proline-rich residues and produces an elastic fiber (Hinman, 2000). However, production of high molecular weight spider silk in *Escherichia coli* can be difficult because the DNA may be unstable, resulting in deletions, and the repetitive sequences can lead to transcription and translation errors. Here we show, for the first time, expression of spidroin protein silk (≥100 KDa) from the major ampullate silk *Masp2,* from the Brazilian spider *Parawixia bistriata* in *E. coli*.

**Methods**: A silk monomer gene for Masp2 from *Parawixia bistriata* was designed (DNA2.0) and a 32 mer plasmid constructed using a "head to tail" cloning strategy (Teulé et al, 2009). The construct containing the cloned fragment were confirmed by DNA sequencing. The *E. coli* BL21(DE03) strain, with or without a plasmid with copies of glycyl-tRNA and glycyl-tRNA synthetase and the empty vector pACYC184, for control, (Xiao-xia et al, 2010), were induced with 1 mM IPTG for four hours, at 37 °C, 200 rpm, in LB medium, for spider protein production. Samples were analyzed by SDS-PAGE, stained with Colloidal Blue Staining Kit (Invitrogen) and analyzed by Western blot on PVDF membrane (Invitrogen).

**Results:** All bacteria produced the protein, but the yield from metabolically engineered *E. coli* for glycyl-tRNA was higher on Western blot. In other studies similar protein sizes were produced from *N. clavipes* (Fahnestock & Irwin, 1997), and it is already known that sizes are directly associated with improved fiber properties, but the yields of protein were inversely correlated with size of the synthetic gene (Xia et al, 2010). The amino acid sequence of *Masp2* from *P. bistriata* shows differences in composition when compared to *N. clavipes*. When alternating these motif sequences this suggests a spider silk with different material properties, but this fiber still needs to be characterized.

**Conclusion:** Protein Masp2 with approximately 100 KDa from a Brazilian spider was produced by *E. coli* system, and the metabolically engineered strain showed a larger yield. Different spider silks may result in new types of protein-based biomaterials with broad applications for medicine and industry.

**References: (**Fahnestock SR. Irwin SL Appl. Microbiol. Biotechnol. 1997;47:23-32.) (Hinman MB. Jones JA. Lewis RV. Trends Biotechnol. 2000;18:374-379.) (Teulé F. Cooper AR. Furin WA. Bittencourt D. Rech EL. Brooks A. Lewis RL. Nature Protocols. 2009; 4:341-355.) (Xia X.X. Qian Z.G. Ki C.S. Park YH. Kaplan DL. Lee SY. PNAS. 2010; 107:14059-14063.)

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