# A Biomimetic Manufacturing of Fibers

M98-C5

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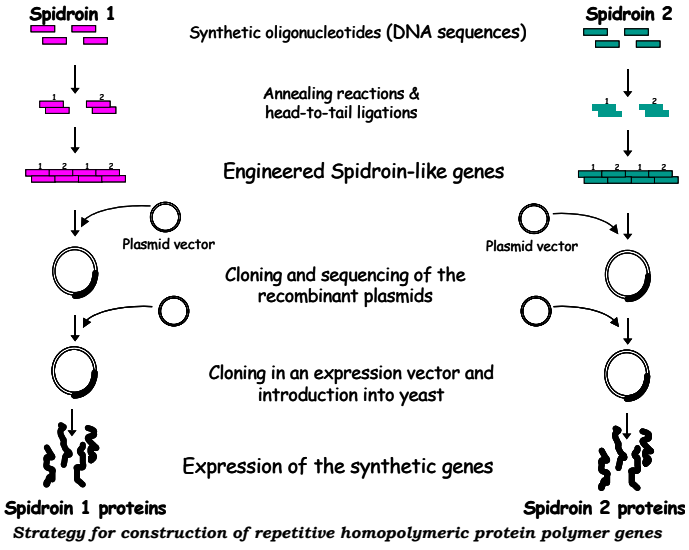
Our ultimate goal is to express synthetic genes in transgenic organisms so we can obtain sufficient quantities of recombinant protein for fiber and film production. We are currently designing spinning technologies based on biological systems and are investigating the role that various protein primary structural components play in fiber production. Our specific objectives are

- to develop the requisite molecular biology for clonal production of fiber-forming protein polymers by genetic expression in yeast and plants.
- to obtain detailed characterization data on native and synthetic silks.
- to use arachnid biology of silk production for the development of biomimetic protein fiber and film manufacturing.

Biotechnology provides the tools to clone and express designed synthetic protein fibers. Spider dragline silk is a strong, elastic, waterproof, stretchable, biodegradable, β-sheet natural protein polymer. The dragline silk of the spider *Nephila clavipes* is the archetype for study of these materials. We have used published sequences for spidroin 1 and spidroin 2 oligonucleotide to design synthetic genes corresponding to repeat units of these two spidroin genes (See Figure), and we now have genes of different sizes for each spidroin. We introduced one of these synthetic spidroin 2 genes into yeast for protein production.




***Strategy for construction of repetitive homopolymeric protein polymer genes***

We are interested in what role the poly alanine repeats (in these spidroin proteins) play in the expression mechanism and in the physical and mechanical properties of the resulting material. To this end, we have made spidroin 1 gene constructs encoding for the normal spidroin 1 protein, spidroin 1 gene constructs encoding for proteins having no alanine runs at all, or less alanine runs, than the native spidroin 1. We have sequenced the genes and have cloned them into yeast for protein production and characterization.

We have also constructed a heteropolymer of spidroin 2 and collagen, using parts of the α-helical (Gly-X-Y) repeat from a nematode (*Meloidogyne incognita*) cuticle collagen and introduced and expressed this synthetic collagen/spidroin gene into yeast. We now have enough of the purified heteropolymer collagen-spidroin protein to test for its ability to form a fiber.

To express this fibrous protein on a more significant scale, we also engineered this same gene in a plant vector for a transgenics experiment. We have performed the first several rounds of tobacco transformation using *Agrobacterium tumefaciens*, a species of bacteria that is capable of incorporating foreign genes into the plant genome, and are currently growing transgenic plants under carefully controlled laboratory conditions. Once these transgenic plants have been harvested, we will examine expression at both the RNA and protein levels in various tissues.

***We are exploiting recombinant DNA and plant transgenic technologies to create and produce novel protein polymers in significant quantities for fiber spinning.***

Ultimately, we believe that plants that produce high protein seeds might provide a good system for high-level expression. To specifically target the synthetic protein production into the transgenic plant seeds, we decided to fuse these synthetic genes to a seed specific promoter (regulatory sequence) that we are currently characterizing in peanuts (peanut omega 9 desaturase gene promoter). We now have clones of this seed-enhanced promoter and are about to test them for promoter activity (GUS assay) in developing peanut seeds.

There is evidence that structural proteins exhibit *in vivo* self-assembly. For this reason, we are examining self-assembly processes in conjunction with the formation of spider silk. Examination of the biology of the *Nephila clavipes* spider and other spider species may discern the molecular events during spider silk spinning. Our studies in the complementary areas of self-assembly and spider biology will be used to further refine the design of our material production system.

We have begun a study of the mechanical properties of silk, including silk from *B. mori*. We are also conducting Raman and X-ray diffraction studies of silk at selected stress levels; these structure and property studies are designed to help us better understand process-structure-property relationships in natural spider silks and biomimetic analogs. These studies will provide the foundation for engineering the genes to produce proteins specific to desired end uses.

Industry interactions: 2

**Project Web Site Address:**
http://hubcap.clemson.edu/~ellisom/biomimeticmaterials

**For Further Information**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