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Recombinant Expression of Human Semenogelin Proteins and Creation of Novel Antibodies for the Detection of Human Semen

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Introduction

The semenogelin proteins (SEMG1 and SEMG2) make up nearly 50% of the total protein in semen, and protect spermatozoa by undergoing non-covalent crosslinking to form the coagulum after ejaculation. Semenogelin can also be used to detect the presence of seminal fluid in suspected cases of sexual assault. By optimizing methods for creating peptide fragments of the semenogelin proteins, we can create novel antibodies for improved detection of human semen.

Methodology

- Amplification
- Purification
- Cloning into Expression Vector (pScript)
- Transfection into 293T Cells
- Sequence Confirmation
- Protein Expression
- Antibody Creation

Preliminary Results

Four total fragments were chosen to be amplified and tested. Each segment was amplified from the start codon through the end of a specific segment of the gene. Chosen amplicons consisted of the start codon through the la, ila, and ila segments, as well as the full length semenogelin 1 gene.

- With fragments confirmed in a transfer vector, a double digest using EcoR1 and HindIII will allow for the movement into an expression vector
- Lower bands are the confirmed isolated fragments and can be purified via gel purification
- Segments again transferred to a mammalian expression vector to allow to the production of semenogelin protein
- Fragments cultured in HEK-293T cells and expression monitored using and SDS-Page gel and western blot assays

Discussion & Conclusions

So far we have been able to successfully:
- Amplify human semenogelin 1 fragments la, ila, ila
- Purify and digest amplified fragments
- Optimize amplification and ensure complete digest
- Move amplicons into transfer vector and confirm via sequencing

The work done so far has created an optimized method for the consistent amplification of the semenogelin 1 fragments and a method to ensure complete digestion of the fragments before transformation. The transfer vector has also been very successful in accepting the fragments for large scale growth and further transformations.

- Methods are equally useful for semenogelin 2
- Transfected segments into human 293T cells to test expression with no observable protein production
- Protocols and antibodies can be altered to try and optimize the process

Future Directions

- Repeat processes with Human Semenogelin 2
- Double check all sequences for error
- Optimize transfection process to get proper expression of protein (using full length SEMG1 gene)
- Test commercially available antibodies for their ability to detect the semenogelin fragments and whole SEMG1c ene
- Determine an appropriate method for the optimized creation of antibodies and test their ability of detection

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