

Homework 1 - Fruit Flies and Malaria Meals

Due August 28, 2000

Research Objective

The trypsin gene encodes a protease that is required for digestion of a mosquito's blood meal. If a female mosquito cannot obtain nutrients from a blood meal due to inability to digest proteins, then it would effectively prevent egg laying. A graduate student reasons that isolation and characterization of the *Aedes aegypti* trypsin gene could lead to biochemical assays designed to develop mosquito trypsin inhibitors that might make useful pesticides. The *Drosophila* trypsin gene has been cloned and is available. Based on the likely homology between the Fly and Mosquito trypsin genes, the graduate student wants to use the *Drosophila* DNA as a molecular probe to isolate the Mosquito trypsin coding sequence from a Mosquito cDNA library.

Available Information and Reagents

Complementary DNA (cDNA) is synthesized from mRNA using the enzyme reverse transcriptase and an oligo dT primer that anneals to the poly A⁺ tail of mRNA.

1. A full-length *Drosophila* trypsin cDNA was obtained by this method and was found to contain 2 kb of coding sequence (ORF; open reading frame) and 4.5 kb of 3' untranslated region (UTR). UTR sequences often contain repetitive sequence elements that are imbedded in this "non-functional" region.
2. Purified mRNA from *Drosophila melanogaster* and *Aedes aegypti* is available for preliminary studies and a Mosquito cDNA library with 1 million recombinant phage has been purchased from commercial sources. The student predicts that there is 80% identity between the coding sequence of the Fly and Mosquito trypsin genes, but only a 20% identity between the 3' UTRs.

1. (3 pts.) **What would be the best preliminary experiment to perform in order to determine if the cloned *Drosophila* cDNA would in fact be a useful reagent to screen the large Mosquito cDNA library? Name the technique and describe (the best you can) what the actual experiment would be and what the expected outcome would be.**

One experiment would be to use the *Drosophila* trypsin cDNA as a probe on a Northern Blot using the available Mosquito RNA to determine if the mosquito trypsin gene is similar enough to be detected. A control would be to load the gel with Mosquito RNA in one lane and *Drosophila* RNA in another lane to be sure the hybridization worked. You would expect [hope] to see a transcript band show up in the lane with Mosquito RNA that hybridized uniquely to the probe; it may or may not be the same MW as the *Drosophila* trypsin mRNA. A possible drawback to this strategy would be insufficient levels of trypsin RNA in the Mosquito prep due to low levels of gene expression (see the answer to question 4 below regarding RNA isolated from female mosquitoes after feeding). This could be overcome by probing a Southern blot using Mosquito genomic DNA to see if the Mosquito genome encodes a complementary sequence that may have not been detectable in the Northern blot.

2. (3 pts.) How might the method need to be optimized if after the first attempt there is no clear indication that the *Drosophila* cDNA will be a useful probe to screen the Mosquito library?

The two most critical conditions to change are; 1) temperature of hybridization and membrane washing - lower the temperature to permit annealing of non-identical sequences, and 2) change the salt conditions - increase the salt concentration to lower the stringency of hybridization reaction. Other conditions to experiment with to increase sensitivity would be a longer time of hybridization to push the Cot value as far as possible due to sequence complexity, and to include dextran sulfate in the hybridization reaction to increase the "effective concentration" of the cDNA probe.

3. (2 pts.) Assuming that the preliminary experiment eventually gave convincing results, describe which of these *Drosophila* cDNA probes would be the most suitable for screening the Mosquito cDNA library; a) the entire 6.5 kb cDNA, b) the 4.5 kb UTR, or c) the 2 kb ORF? Justify your answer.

The best probe would be the 2 kb ORF because it is most likely that evolutionary conservation between the *Drosophila* and Mosquito genomes would be in the trypsin coding sequence rather than the UTR (which could contain repetitive sequence elements). The best hybridization conditions to use (hybridization and wash temperatures, salt concentration/formamide concentration and time of hybridization) would be those that were found to be optimal in the Northern/Southern blotting experiments.

4. (2 pts.) Biochemical studies had previously shown that the enzymatic activity of Mosquito trypsin increases rapidly in the gut of females soon after a blood meal. What experiment could be done using the newly isolated Mosquito trypsin cDNA, and sample material obtained from a laboratory Mosquito colony, to determine if the observed increase in trypsin enzyme activity was due to an increase in trypsin gene expression?

To determine if the increase in trypsin enzyme activity following a blood meal were the result of activation of trypsin gene expression, you would want to do a Northern blot using RNA isolated from female mosquitoes at various times after feeding. The Northern blot would be probed with the Mosquito cDNA sequence that had been isolated.