Appendix 2
Sample Weekly Schedule from Collaborative Learning Model

General Topic: Crayfish Molecular Biology

Monday:

Goals: To begin to use molecular biological techniques to explore the crayfish nervous system. Practice designing primers, including degenerate primers. Review how to use NCBI and other on-line databases.

Reading Assignment: Ch. 4 (Required Reading: The Molecular Basis of Heredity), Ch. 6 (Getting the Message Out: Transcription of Genes to Produce Messenger RNA), and Ch. 9 (Messing About with DNA) all from *Molecular Biology Made Simple and Fun.*

Handouts: What is a PCR Primer? and "The Big Picture"

Activity: Prepare for nucleic acid extraction by dissecting out the brain and nerve cords from 2 or 3 crayfish (either the complete nerve cord and brain, or 3 separate segments, brain, thorax, and abdomen). Dissect using RNase free techniques and immediately freeze tissue on dry ice for storage. Explore NCBI database at http://www.ncbi.nlm.nih.gov/blast/.

Questions for Experimentation: What primers are you going to use and why? What does degeneracy mean, with regards to primer design? Explain what the Tm is and how it is determined.

Primer suggestions: A few possible choices include the nicotinic-acetylcholine receptor, NMDA, one of the serotonin (5-HT) receptors, innexin/connexin (gap junctions), CHH (crustacean hyperglycemic hormone), MIH (molt inhibiting hormone), or GIH (growth inhibiting hormone).

Tuesday:

Goal: Extract DNA and RNA from the crayfish nerve cord.

Procedure: Using TRIzol reagent extract total RNA and DNA from the crayfish nerve cord. The total RNA will be used in cDNA synthesis and the DNA will be used in a Southern Blot (for DNA fingerprinting). Determine the concentration of your total RNA using the Biophotometer.

Reading Assignment: Section 1-1 SS-Phenol/Chloroform Extraction and Ethanol Precipitation (Davis et al, 1994).

Handouts: Protocol for nucleic acid (RNA, DNA) extraction using TRIzol, and Purification of Total RNA from Cells and Tissues.

Questions for Experimentation: Why are the nucleic acids found in the aqueous layer? Why are amino acids found in the organic layer? What are some sources of RNA degradation and how do you avoid them?

Wednesday:

Goal: Continue with nucleic acid extraction, treat the total RNA with DNase enzyme using the Gene Clean Kit. Check total RNA quality using gel electrophoresis.

Procedure: Make a mini agarose gel and run the total RNA (follow directions on handout).

Handouts: Gene Clean protocol; Gel Electrophoresis and Making Agarose Gels

Questions: Why is it important to remove all DNA from the total RNA before cDNA synthesis and PCR? What is the difference between genomic DNA and cDNA? What size bands should you see for total RNA?

Thursday: Off campus reading in am; Weekly group seminar in pm.

Friday:

Goal: Use reverse transcriptase to generate cDNA from total RNA

Procedure: Follow the cDNA synthesis protocol (Invitrogen Cloned AMV First-Strand cDNA Synthesis Kit).

Reading: 13-1 Generation of cDNA insert from Eukaryotic mRNA (from Davis et al, 1994), and Protocol 1: The Basic Polymerase Chain Reaction and Protocol 2: Purification of PCR Products in Preparation of Cloning (from Sambrook and Russell, Vol. 2, 2001).

Handout: What is PCR handout?; Invitrogen instructions for cDNA kit.
Questions: What primers did you use for the cDNA synthesis? What is the difference between random hexamers and oligo d(T) primers?

Guidelines:
1. Do not eat or drink at your bench.
2. Always wear gloves to both protect yourself and the RNA you extract and use.
3. Please DO NOT wear open-toed shoes or sandals when doing the RNA extraction procedure.
4. RNases are everywhere! Use and follow RNase-free lab procedures. RNases are enzymes that destroy RNA.
5. Keep all enzymes and reagents on ice at all times.
6. Please help keep the lab clean. Molecular biology can be messy. You are responsible for cleaning up your lab bench, putting away all reagents and chemicals, washing your dishes, and cleaning any messes.

References

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Davis, L., Kuehl, M. & Batty, J. 1994. Basic Methods in Molecular Biology, 2nd ed., Appleton & Lange, Norwalk, CT, 777 pp.
Hockfield, S., Carlson, S., Evans, C., Levitt, P., Pintar, J. & Silberstein, L. 1993. Selected Methods for Antibody and Nucleic Acid Probes, Vol. 1. CSHL Press, Cold Spring Harbor New York. 679 pp.
Mount, D.W. 2001. Bioinformatics: Sequence and Genome Analysis CSHL Press, Cold Spring Harbor New York. 564 pp.
Sambrook, J. & Russell, D.W. 2001. Molecular Cloning: A Laboratory Manual, Vols. 1-3. CSHL Press, Cold Spring Harbor New York. Various pp. These lab manuals are the “Bibles” of molecular biology. These were originally written by T. Maniatis, J. Sambrook, and E. F. Fritsch; the series is often referred to as "Maniatis".