

**NIH Filariasis Research Reagent Resource Center (FR3)
Experimental Techniques in Filariasis Research**

09 - 13 March 2009

University of Georgia, College of Veterinary Medicine

Class Schedule

NOTE: All lectures and labs will be held in the VetLab (in back of reading room)

Instructors:

Ray Kaplan, DVM, PhD	University of Georgia
Steve Williams, PhD	Smith College
Shelly Michalski, PhD	University of Wisconsin-Oshkosh
Andy Moorhead, DVM, PhD	University of Georgia
Mike Dzimianski, DVM, MS	University of Georgia
Prasit Supakorndej, PhD	University of Georgia
Sue Haynes, MS	Smith College
John McCall, PhD	University of Georgia
Steve Harvey, DVM, MS	University of Georgia
Leanne Alworth, DVM, MS	University of Georgia

Support Personnel:

Bob Storey, MS, RVT and LaDonna Allen

<u>Day</u>	<u>Time</u>	<u>Event</u>
Monday	8:30-9:00	Welcome and Introductions
	9:00-9:15	<u>Lecture</u> (Moorhead): FR3 parasite resources available
	9:15-10:00	<u>Lecture</u> (Williams): FR3 molecular resources available: how to access these; web site, Use of reagents from the FR3 (libraries, clones, etc.).
	10:00-10:45	<u>Lab</u> (Dzimianski/Supakorndej): Use blood from cat with <i>B. malayi</i> microfilariae (mf) to set up membrane-feeders and start infecting mosquitoes.
	10:45-11:00	Break (Refreshments)
	11:00-11:45	<u>Lecture</u> (Williams): The global effort to eliminate lymphatic filariasis
	11:45-12:30	<u>Lecture</u> (Michalski): Overview of nematode biology and introduction to filarioid nematodes

12:30-2:00 Lunch

2:00-3:00 **Lecture** (Williams): Introduction to the isolation of DNA and RNA from filarial parasites. Special considerations compared to isolating DNA from other organisms.

3:00-3:45 **Lab** (Dzimianski/Supakorndej): Collect L3 from mosquitoes, examine L3 under microscope, inject L3 into jirds

3:45-4:00 Break (Refreshments)

4:00-5:30 **Lab** (Williams/Haynes): DNA extraction

Tuesday

8:30-9:30 **Lecture** (Harvey/Alworth): Using gerbils in biomedical research

9:30-10:30 **Lab** (Harvey/Alworth): Using gerbils in biomedical research

10:30-10:45 Break (Refreshments)

10:45-11:30 **Lab** (Williams/Haynes): Finish DNA extraction

11:30-12:30 **Lecture** (McCall): *B. malayi* / *B. pahangi* mosquito vector phase

12:30-2:00 Lunch

2:00-3:00 **Lecture** (McCall): *B. malayi* / *B. pahangi* vertebrate host(s) phase

3:00-4:30 **Lab** (Dzimianski/Supakorndej): Necropsy *B. malayi* infected jirds; Collect adult worms, L4 and microfilariae from peritoneal cavity and examine under microscopy. Identify and sort male and female worms.

4:30-6:00 **Lab** (Williams/Haynes): Isolate RNA from L3 worms.

Wednesday

8:30-9:30 **Lab**: Pick mosquito pupae; put mosquito eggs in water for hatching into larvae.

9:30-10:15 **Lab** (Williams/Haynes): Finish RNA isolation from L3 worms.

10:15-10:30 Break (Refreshments)

10:30-12:00 **Lecture** (Williams): Details of gene expression analysis and RT-PCR, Analysis of EST and other types of expression data, Microarrays

12:00-1:30 Lunch

- 1:30-2:00 **Lecture** (Michalski): Transcriptome analysis I - applications (genome annotation, stage-specific expression, tissue-specific expression, pathogenesis)
- 2:00-2:45 **Lab**: [check RNA on Nano-Drop spec]. Set up RT-PCR reactions.
- 2:45-3:15 **Lecture** (Michalski): Transcriptome analysis II - Methods (sequencing-, hybridization-, and PCR-based, and Verification (RT-PCR, qPCR, in situ hybridization, immunohistochemistry))
- 3:15-3:30 Break (Refreshments)
- 3:30-4:15 **Lecture** (Michalski): Statistical Analysis (the quagmire of statistics) and Reporting Results (who curates databases, who curates genomes, what is reportable?)
- 4:15-6:15 **Lab** (Michalski): Dissect freshly fed (2 hours prior) mosquitoes and quantify midgut penetration of L1. Dissect day 5.5 and day 9.5 mosquitoes – recover and examine L2 and L3 stages
- 7:00- Dinner at East-West Bistro
- Thursday** 8:30-10:00 **Lecture** (McCall): *D. immitis* mosquito vector phase, *D. immitis* vertebrate host(s) phase.
- 10:00-10:15 Break (Refreshments)
- 10:15-11:00 **Lecture** (McCall): Wolbachia
- 11:00-11:30 **Lab** (Dzimianski/Supakorndej): Set up mosquito larval cultures.
- 11:30-12:30 **Lab** (Williams/Haynes): Set up DNA PCR
- 12:30-2:00 Lunch
- 2:00-3:30 **Lab** (Dzimianski/Supakorndej): Necropsy of *Brugia*-infected jirds with lymphatic infection.
- 3:30-4:30 **Lab**: Set up and run gel on RT-PCR and DNA PCR and a second gel for filarial DNA and RNA.
- 4:30-4:45 Break (Refreshments)
- 4:45-5:45 **Lab**: Finish running gel on RT-PCR and DNA PCR. Stain and photograph gel, interpret data.
- 5:45-6:30 **Lecture** (Williams): Interpreting RT-PCR data. Q-RT-PCR.

Friday	8:30-9:15	<u>Lecture</u> (Williams): Finding clones in cDNA and genomic libraries. Bioinformatics.
	9:15-10:15	<u>Lecture</u> (Williams): Bioinformatics of filarial data sets. ESTs and BLAST. Accessing the genomic data at TIGR. Genomics.
	10:15-10:30	Break (Refreshments)
	10:30-12:00	<u>Lecture</u> (Williams): Using online bioinformatics resources. Finishing up unfinished business. END OF COURSE