

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

05 - 09 May 2008

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
John McCall, PhD	University of Georgia
Pat Lammie, PhD	Centers for Disease Control
Shelly Michalski, PhD	University of Wisconsin-Oshkosh
Mike Dzimianski, DVM, MS	University of Georgia
Prasit Supakorndej, PhD	University of Georgia
Sue Haynes, BS	Smith College
Tiffany Weinkopff, MS	Centers for Disease Control

Support Personnel:

Bob Storey, MS, RVT, and Pam Murray

<u>Day</u>	<u>Time</u>	<u>Event</u>
Monday	8:30-8:45	Welcome and Introductions
	8:45-9:30	<u>Lecture</u> (Kaplan): Nematode biology and introduction to Filarioid nematodes and FR3 parasite resources
	9:30-10:15	<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.
	10:15-10:30	Break (Refreshments)
	10:30-11:15	<u>Lecture:</u> (Lammie): Pathogenesis and treatment of filarial infections
	11:15-12:00	<u>Lecture</u> (Lammie): The global effort to eliminate lymphatic filariasis
	12:00-12:30	Questions – Discussion
	12:30-2:00	Lunch
	2:00-3:00	<u>Lecture</u> (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	<u>Lab</u> (Dzimianski/Supakorndej): Collect L3 from mosquitoes, examine L3 under microscope, inject L3 into jirds

	3:45-4:00	Break (Refreshments)
	4:00-5:15	<u>Lab</u> (Williams/Haynes): DNA extraction
Tuesday	8:30-9:30	<u>Lecture</u> (Williams): FR3 molecular reagents available: how to access these; web site.
	9:30-10:30	<u>Lecture</u> (Williams): Use of reagents from the FRRRC (libraries, clones, etc.). Methods for studying gene expression (Northern, RT-PCR, microarrays).
	10:30-10:45	Break (Refreshments)
	10:45-12:15	<u>Lab</u> (Dzimianski/Supakorndej): Necropsy <i>B. malayi</i> infected jirds; Collect adult worms, L4 and microfilariae from peritoneal cavity and examine under microscopy. Identify and sort male and female worms.
	12:15-1:45	Lunch
	1:45-3:30	<u>Lab</u> (Williams/Haynes): Isolate RNA from adult worms.
	3:30-4:30	<u>Lecture</u> (McCall): <i>B. malayi</i> / <i>B. pahangi</i> mosquito vector phase.
	4:30-5:30	<u>Lab</u> (Williams/Haynes): Finish RNA isolation from adult worms.
Wednesday	8:30-9:15	<u>Lab</u> : Pick mosquito pupae; put mosquito eggs in water for hatching into larvae.
	9:15-10:15	<u>Lecture</u> (McCall): <i>B. malayi</i> / <i>B. pahangi</i> vertebrate host(s) phase.
	10:15-10:30	Break (Refreshments)
	10:30-11:15	<u>Lecture</u> (Williams): Microarrays.
	11:15-12:30	<u>Lecture</u> (Williams): Details of gene expression analysis and RT-PCR and microarrays. Analysis of EST and other types of expression data.
	12:30-2:00	Lunch
	2:00-3:00	<u>Lab</u> : [check RNA on Nano-Drop spec]. Set up RT-PCR reactions. Run a DNA gel.
	3:00-3:45	<u>Lecture</u> (Michalski): Transcriptome analysis I - applications (genome annotation, stage-specific expression, tissue-specific expression, pathogenesis)
	3:45-4:30	<u>Lecture</u> (Michalski): Transcriptome analysis II - Methods (sequencing-, hybridization-, and PCR-based, and Verification (RT-PCR, qPCR, in situ hybridization, immunohistochemistry)

	4:30-5:00	<u>Lab</u> : Stain gels and examine results.
	5:00-7:00	Dinner
	7:00-9:00	<u>Lab (Michalski)</u> : 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
Thursday	8:30-9:30	<u>Lecture</u> (McCall): <i>D. immitis</i> mosquito vector phase, <i>D. immitis</i> vertebrate host(s) phase.
	9:30-10:30	<u>Lecture</u> (Michalski): Statistical Analysis (the quagmire of statistics) and Reporting Results (who curates databases, who curates genomes, what is reportable?)
	10:30-10:45	Break (Refreshments)
	10:30-11:00	<u>Lab</u> (Dzimianski/Supakorndej): Set up mosquito larval cultures.
	11:00-12:00	<u>Lab</u> (Williams/Haynes): Set up DNA PCR
	12:00-1:30	Lunch
	1:30-2:45	<u>Lab</u> : Run gel on RT-PCR. Stain and interpret data.
	2:45-3:45	<u>Lecture</u> (Williams): Interpreting RT-PCR data. Q-RT-PCR.
	3:45-4:00	Break (Refreshments)
	4:00-5:00	<u>Lecture</u> (Williams): Finding clones in cDNA and genomic libraries. Bioinformatics.
Friday	8:30-8:45	<u>Lab</u> (Williams/Haynes): Run DNA PCR gel in fast buffer.
	8:45-10:30	<u>Lab</u> (Dzimianski/Supakorndej): Necropsy of <i>Brugia</i> -infected jirds with lymphatic infection.
	10:30-12:30	<u>Lecture</u> (Williams): Bioinformatics of filarial data sets. ESTs and BLAST. Accessing the genomic data at TIGR. Genomics.
	12:30-2:00	Lunch
	2:00-3:30	<u>Lecture</u> (Williams): Using online bioinformatics resources. Finishing up unfinished business. END OF COURSE