

Workshop Instructors:

Martin Wiedmann (Cornell University)
Kendra Nightingale (Colorado State University)
Haley Oliver (Cornell University)
Mark Carter (Silliker, Inc.)
Sarita Raengpradub-Wheeler (Silliker, Inc.)

Advisory Board:

Tim Freier, Director, Global Food Safety Technologies, Cargill
Paul Hall, Food Safety Consultant
Scott Hood, Senior Manager, Microbiology, General Mills
Yuewei Hu, Microbiologist, General Mills
Randy Huffman, Chief Food Safety Officer, Maple Leaf Foods
Keith Lampel, U.S. Food and Drug Administration
Jennifer Lee, Quaker Oats, PepsiCo
Mark Moorman, Senior Director, Food Safety and Chemistry, Kellogg Company
Theo Morille-Hinds, Associate Program Director of Global Manufacturing and Food Safety, Kraft Foods
Rob Reinhard, Director Food Safety, Sara Lee Food and Beverage
Les Smoot, Director, Nestle Quality Assurance Center
Kathryn Boor, Cornell University
Yuhuan Chen, Grocery Manufacturers Association

FOR MORE DETAILS AND WORKSHOP REGISTRATION PLEASE VISIT OUR WEBSITE (<http://ansci.colostate.edu/content/view/808/112/>)

Contact:

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Goal of the Workshop:

The overall goal of the “Molecular Methods in Food Microbiology Symposium and Workshop Series” is to address and fill-in knowledge gaps regarding molecular detection and subtyping of foodborne pathogens and spoilage organisms for industry professionals and graduate students being trained to work in the food industry along with current and future food safety professionals in regulatory agencies and academia. In order to realize this goal, we have assembled an advisory committee to address the challenges the industry faces today with respect to molecular methods.

Colorado
State
University

DEPARTMENT OF ANIMAL SCIENCES

Third Annual Symposium and Workshop Series on Molecular Methods in Food Microbiology

June 28 - July 2, 2010

Colorado State University
Fort Collins, CO



Colorado
State
University

DEPARTMENT OF ANIMAL SCIENCES

Lecture Series and Workshop Schedule*



Colorado State University

Monday, June 28

Lecture Series Day 1
Grey Rock Room in the Lory Student Center

Morning - Arrival in Fort Collins

1:30 p.m. - Welcome and Introduction

1:45 p.m. - Review of fundamentals: Nucleic Acids, DNA replication, transcription and translation and their application to assay development (Kendra Nightingale)

2:15 p.m. - Review of concepts in microbial taxonomy and diversity and implications for molecular detection, including new challenges (e.g., STEC) (Martin Wiedmann)

3:00 p.m. - Coffee Break and Vendor Exhibits

3:15 p.m. - PCR: Fundamentals, primers, amplification conditions (Kendra Nightingale and Sarita Raengpradub-Wheeler)

4:00 p.m. - Primer design, including hands-on activity (Kendra Nightingale assisted by all instructors and TAs)

4:45 p.m. - PCR Variations (multiplex PCR; real-time PCR; reverse transcriptase PCR) (Sarita Raengpradub-Wheeler)

5:30 p.m. - Break for the day

6:00 p.m. - Opening reception (from 6:00 to 6:30 p.m.); Keynote Address speaker - TBA (from 6:30 to 7:15 p.m.); and dinner (beginning at 7:15 p.m.) at the Hilton Hotel (425 W. Prospect Road)

Tuesday, June 29

Lecture Series Day 2
Grey Rock Room in the Lory Student Center

8:30 a.m. - PCR Controls, GLPs, QC&QA, set-up of a PCR lab

9:00 a.m. - Use and misuse of the sensitivity and specificity with examples (Kendra Nightingale)

9:30 a.m. - Steps before PCR: sampling and enrichment, concentration procedures, compositing vs. pooling, true real-time PCR without enrichment (Mark Carter and Sarita Raengpradub-Wheeler)

10:15 a.m. - Ins and outs of commercially available detection assays: what questions should you ask to identify the assay that best suits your needs

10:30 a.m. - Coffee break and vendor exhibits

10:45 a.m. - Presentations from invited companies that market commercially available molecular detection systems (companies and speakers TBA)

12:00 p.m. - Working lunch/question and answer session with company representatives and workshop instructors on selection of the molecular detection assay that fits your needs

1:30 p.m. - Case studies on interpretation of results from molecular detection assays (i.e., questions such as "What does a positive PCR test mean?" and "How to deal with a PCR positive culture negative result?") / Breakout into groups and troubleshoot with examples of "gray area" results.

2:30 p.m. - 16s rDNA based detection (Martin Wiedmann and Kendra Nightingale)

3:00 p.m. - New trends on molecular detection (2008 to 2010) and outlook into the future (Martin Wiedmann)

3:30 p.m. - Discussion and wrap-up question and answer session

4:00 p.m. - Mixer with happy hour and vendor exhibits (Grey Rock Room, Lory Student Center)

5:00 p.m. - Break for day

5:30 p.m. - Depart for an evening in Estes Park (optional)

Wednesday, June 30

Hands-on Workshop Day 1
Room A201 Microbiology Building

8:30 a.m. - Introduction to DNA sequence databases, uploading/downloading sequence data, primer BLAST (Kendra Nightingale)

9:00 a.m. - Design custom PCR assay for target organism of interest (e.g., *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, *Listeria* spp., *Pseudomonas fluorescence*, *Paenibacillus* spp., *Alicyclobacillus*) and Order Primers. (Participants may send DNA as target to develop custom PCR assay to Kendra Nightingale prior to the workshop)

11:30 a.m. - Prepare lysates for 16s rDNA PCR

12:00 p.m. - Lunch on your own

1:30 p.m. - Set-up 16s rDNA PCR reactions and begin thermal cycling

2:00 p.m. - Prepare lysates for Bax → 8 hour *Listeria* Detection PCR.

3:00 p.m. - Set-up multiplex PCR for detection of STEC

4:00 p.m. - Determine reaction master mix and thermal cycling conditions for custom PCR

5:00 p.m. - Break for day; dinner on your own

Thursday, July 1

Hands-on Workshop Day 2
Room A201 Microbiology Building

8:30 a.m. - Prepare 16s rDNA PCR products for sequencing and submit to core facility

10:00 a.m. - Set-up Bax → 8 hour *Listeria* Detection PCR and begin thermal cycling

11:00 a.m. - Set-up custom PCR reactions and begin thermal Cycling

12:00 p.m. - Lunch on your own

1:30 p.m. - Load and run gel from custom PCR

2:00 p.m. - Lecture and discussion "DNA Sequencing" (Kendra Nightingale)

3:00 p.m. - Troubleshooting custom PCR results

4:00 p.m. - Optimization of custom PCR or purification of custom PCR products and send for sequencing (optional)

5:00 p.m. - Break for day

6:00 p.m. - Workshop closing dinner at The Moot House (2626 S. College at Drake); mixer from 6:00 p.m. to 6:30 p.m. followed by dinner at 6:30 p.m. Vans will pick up participants from the Hilton Hotel at 5:45 p.m.

Friday, July 2

Hands-on Workshop Day 3
Room A201 Microbiology Building

8:30 a.m. - Realtime PCR to Detect *Salmonella* (MicroSeq *Salmonella* kit; Applied Biosystems); run gels from optimization of custom PCR.

10:00 a.m. - Lecture/Demonstration on TaqMan primers and probes (Sarita Raengpradub-Wheeler)

11:00 a.m. - Demonstration of DNA sequence assembly, proofreading, and multiple sequence alignment software (Kendra Nightingale)

12:00 p.m. - Lunch on your own

1:30 p.m. - Assemble and proofread 16S rDNA sequences and perform BLAST and RDP searches for identification

3:00 p.m. - Optional wrap-up discussion session and individual discussions with instructors

*All times Mountain Standard Time.