

REAL-TIME PCR USING APPLIED BIOSYSTEMS'S 7500 INSTRUMENT & CHEMISTRIES



GIVEN BY: NORTHEAST REGIONAL FORENSIC INSTITUTE
HOSTED BY: MASSACHUSETTS STATE POLICE CRIME LAB
FUNDED BY: THE NATIONAL INSTITUTE OF JUSTICE

~ funding covers course attendance

JUNE 3 – 5, 2009; SPACES ARE LIMITED!

Instructors: Dr. Bruce McCord, Andy Wist, & Jamie L. Belrose

COURSE DESCRIPTION

This 2.5 day course is designed to provide the theoretical and practical background necessary to perform quantitative PCR and data analysis. Today's advanced technologies have led to an exponential number of cases being submitted to crime labs for DNA testing. For these techniques to be successful, it is imperative that biological evidence be analyzed and interpreted effectively. *Those already using RT-PCR are welcome to attend the one-day advanced lecture by Dr. Bruce McCord.*

ADVANCED LECTURE

WEDNESDAY, JUNE 3, 8:00AM - 4:30PM: MCCORD

- Multicopy methods for real time detection – Sybr Green, Plexor
- The über -male and other interesting issues with Y STRs
- How RT-PCR could be used to evaluate and decide whether to conduct autosomal vs. Y STRs.
- Cautionary notes
- Low copy number and real time PCR.
- Application of realtime PCR for setting laboratory thresholds
- Precision vs. Accuracy, variability with standards, and how labs might incorporate the use of SRMs.
- Validation issues
- Inhibition and mechanisms of how inhibition occurs (causative factors) and how to possibly overcome this dilemma.
- IPC Ct for detecting inhibition
- Efficiency/shape of the amplification plot for detecting inhibition
- Application of DNA melt curves for inhibition detection
- How RT-PCR could be used for screening samples for probative value.
- Methods to detect DNA degradation by real time PCR

INTRODUCTORY LECTURE

THURSDAY, JUNE 4, 8:00AM - 4:30 PM: NERFI

- Why Quantitate DNA??? FBI QAS Standard 9.3
- Several Methods of Quantitation (Yield Gels, Spectrophotometry, UV-Vis, & Sybr Green)
- How the amount of input DNA can/will impact PCR - adding too much or too little...
- Theory of Real-Time PCR
- Advantages of Quantitative PCR
- Fluorescent Detection (Stokes Shift)
- Kits from Applied Biosystems: Quantifiler™ Human, Quantifiler™ Y Human Male & Quantifiler® Duo
- Taqman® Probes & 5' Nuclease Activity
- Fluorescence Resonant Energy Transfer or FRET
- Instrument Optics (Tungsten Lamp, Virtual Filters & Charged-Coupled Device Camera)
- Definitions: Threshold, Cycle Threshold, Internal PCR Control, Slope, Y-intercept, & Correlation Coefficient
- Hands-on demonstration

FRIDAY, JUNE 5, 8:00AM – 12:00PM: NERFI

- Calibrations: Background, Region of Interest, and Pure Dye
- Sequence Detection Software (SDS):
- Absolute Quantification (Standard Curve)
- Troubleshooting outlier standards & changes in slope and Y-intercept

The course will conclude with a multiple-choice exam and issuance of completion certificates.