Training Agenda

• Real-Time PCR

• GeneXpert Technology
Real Time PCR
Polymerase Chain Reaction (PCR)

Double Stranded DNA, free nucleotides, enzyme, primers, MgCl₂, and buffer

DENATURATION 95°C

ANNEALING 68°C

EXTENSION 72°C
PCR Cycles

TARGET

CYCLE 1

2 COPIES

CYCLE 2

4 COPIES

CYCLE 3

8 COPIES

CYCLE N = $2^N$ COPIES
Real-Time PCR Principles

• **Continuous monitoring of fluorescent signal from polymerase chain reaction throughout the amplification cycles**
  – Target is amplified in the presence of the reporter
  – Instrument excites and detects the reporter and collects fluorescent data

• **Fluorescent signal increases in proportion to the amplified product**

• **Measurement begins when fluorescence rises to a detectable level over background**

• **The number of cycles needed to reach a significant level over background (threshold level) is called the C<sub>t</sub>, or cycles to reach threshold**
Amplification Curve & Ct

- **Baseline**
- **Threshold Line**
- **Plateau**
- **End Point Fluorescence**

Fluorescence levels over cycles:
- **30.0** fluorescence units
- **29.1** cycle point
Taqman® Probe Assay

A. Reporter is quenched when not bound to target sequence

B. 5’ exonuclease degradation of probe separates reporter from quencher
From Cycle to cycle, signal increase with the amount of amplicon
A. Molecular Beacon forms stem loop structure

B. Molecular Beacon hybridizes to a target, the fluorescent reporter and quencher are separated and the fluorescent dye is detectable

C. Temperature increases which causes Molecular Beacon to dissociate
Scorpion

A. Scorpion Primer

B. Scorpions primer extended on target DNA

C. Extended primer is heat-denatured

D. Cooling causes scorpion to rearrange and fluoresce in a target-specific manner

http://www.premierbiosoft.com/tech_notes/Scorpion.html
Reverse Transcription Real-Time PCR

Single Stranded RNA, free nucleotides, enzyme, primer, MgCl₂ and buffer

REVERSE TRANSCRIPTION

cDNA

REAL TIME PCR

AMPLIFICATION AND DETECTION BY REAL-TIME PCR
Nested Real-Time PCR

[Diagram showing the process of Nested Real-Time PCR with labeled primers and probes]
GeneXpert Module

- Syringe Motor
- Motherboard
- Uniframe
- I-CORE
- Ultrasonic Horn
- Module Door
- Valve Drive Motor
GeneXpert Cartridge

PROCESSING CHAMBERS

REACTION TUBE

VALVE BODY
Cepheid Reagent Technology

- **Enzyme Bead**
  - Taq Polymerase
  - General PCR Components

- **TSR beads**
  - Primers and probes for specific target sequences

- **Internal Control**
  - Cepheid Internal Control (CIC)
  - Sample Adequacy Control
  - Sample Processing Control
I-CORE Module
Intelligent Cooling/Heating Optical Reaction Module
## GeneXpert 6 Channels

<table>
<thead>
<tr>
<th>Channel</th>
<th>Excitation Range</th>
<th>Emission Range</th>
<th>Dyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>375-405 nm</td>
<td>420-480 nm</td>
<td>Dye 1</td>
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<tr>
<td>2</td>
<td>450-495 nm</td>
<td>510-535 nm</td>
<td>Dye 2</td>
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<tr>
<td>3</td>
<td>500-550 nm</td>
<td>565-590 nm</td>
<td>Dye 3</td>
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<tr>
<td>4</td>
<td>555-590 nm</td>
<td>606-650 nm</td>
<td>Dye 4</td>
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<td>5</td>
<td>630-650 nm</td>
<td>665-685 nm</td>
<td>Dye 5</td>
</tr>
<tr>
<td>6</td>
<td>630-650 nm</td>
<td>&gt;700 nm</td>
<td>Dye 6</td>
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</tbody>
</table>
GeneXpert Cartridge Video
Waste Disposal

• Biological specimens, specimen collection devices, and used cartridges should be considered capable of transmitting infectious agents and require use of standard precautions.

• Follow your institution’s environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures.

• If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.