



# GeneXpert Technology Overview

# Training Agenda

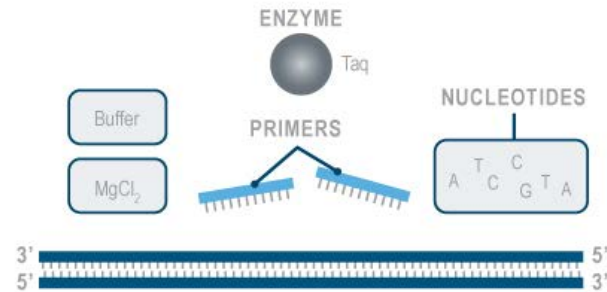
- **Real-Time PCR**
- **GeneXpert Technology**

# Real Time PCR

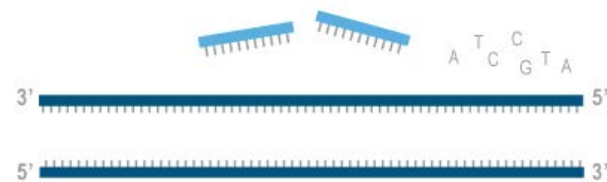


# Polymerase Chain Reaction (PCR)

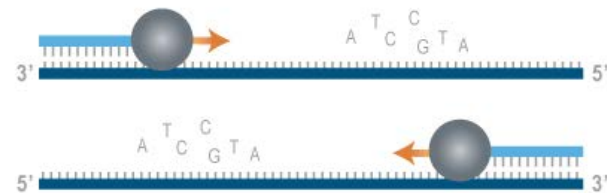
Double Stranded DNA,  
free nucleotides, enzyme,  
primers,  $MgCl_2$  and buffer



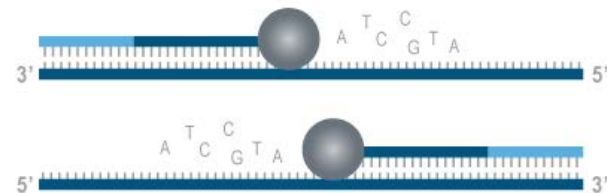
DENATURATION  
95°C



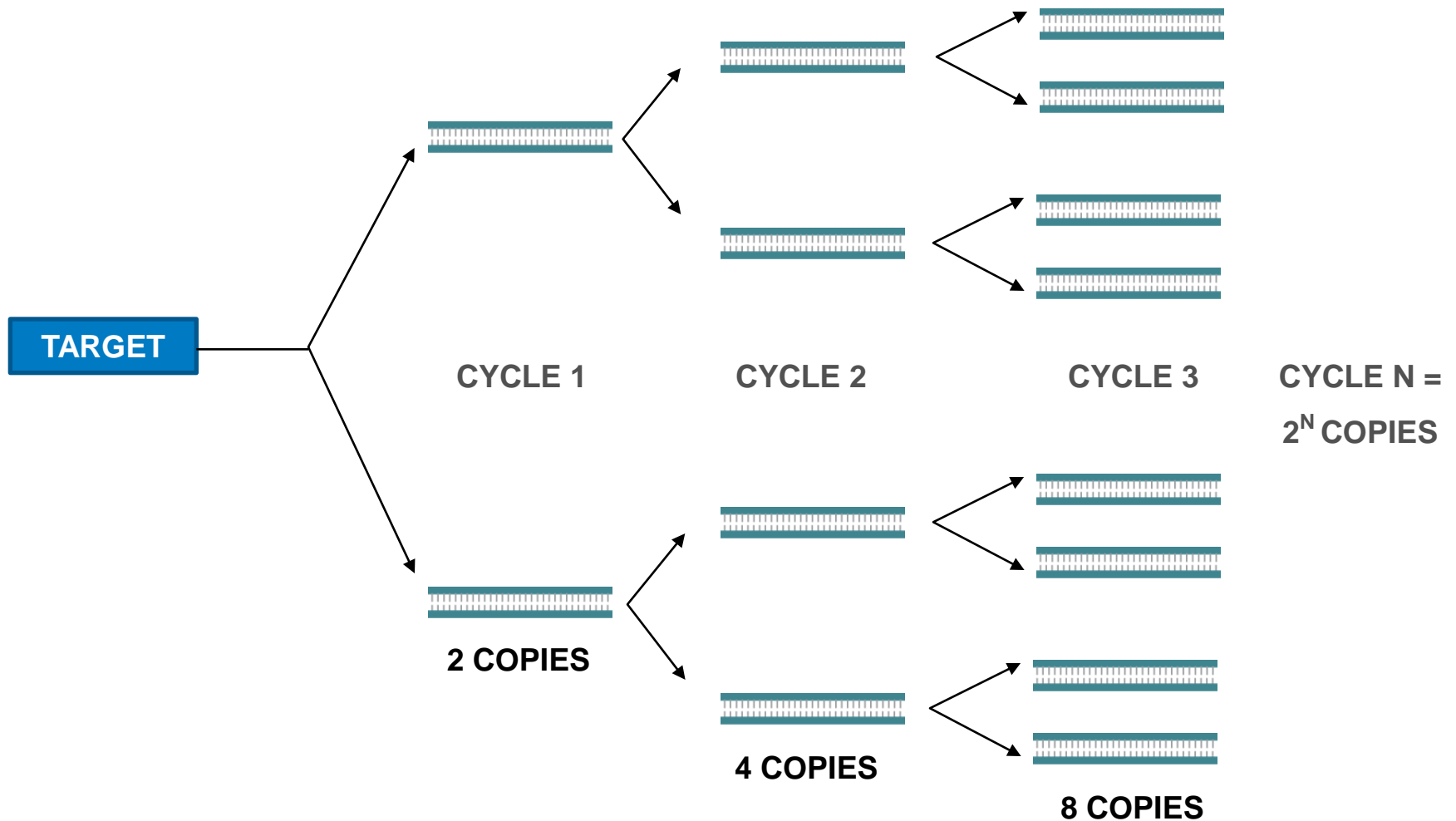
ANNEALING  
68°C



EXTENSION  
72°C



# PCR Cycles

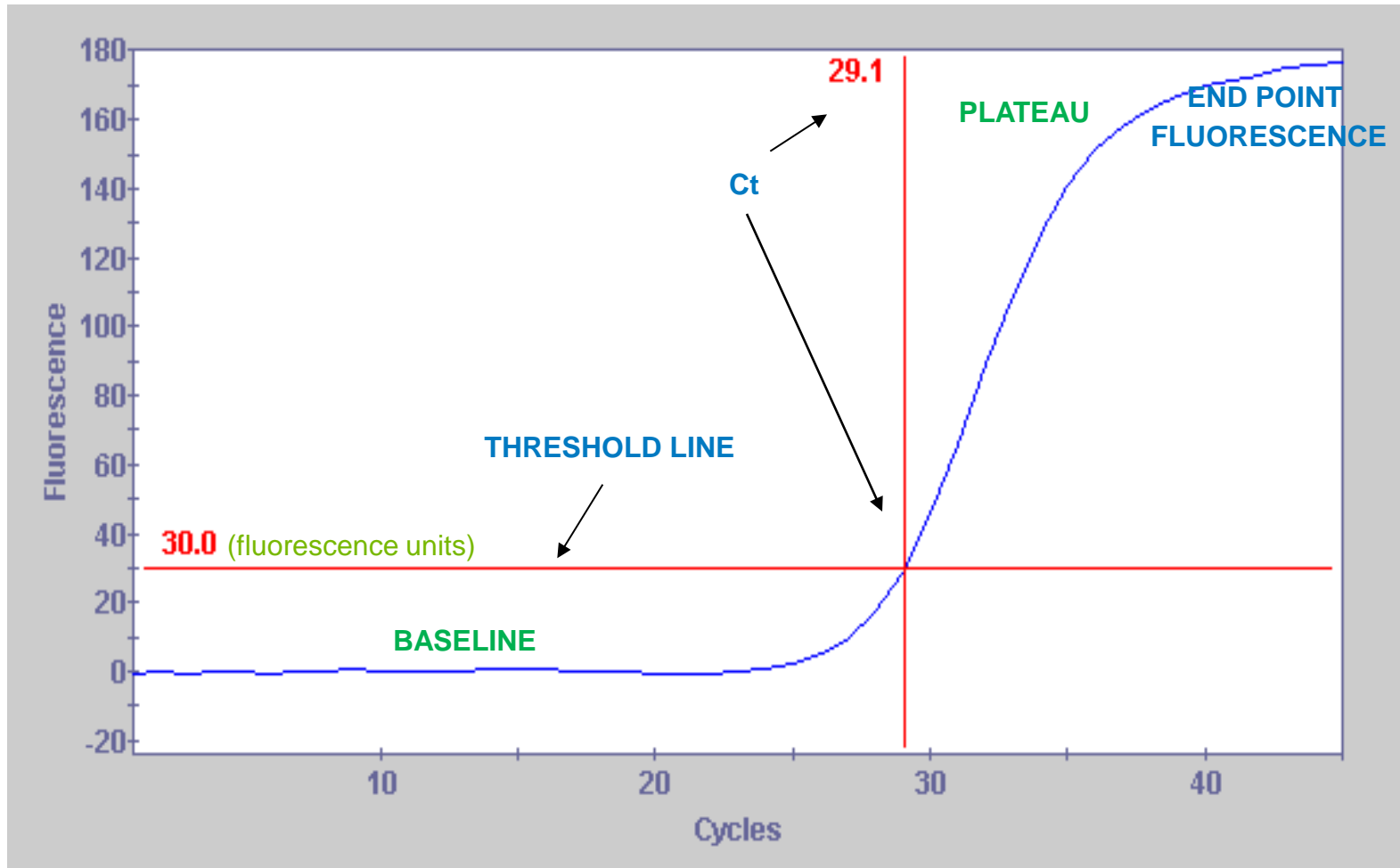


# Real-Time PCR Principles

- **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  $C_t$ , or cycles to reach threshold**

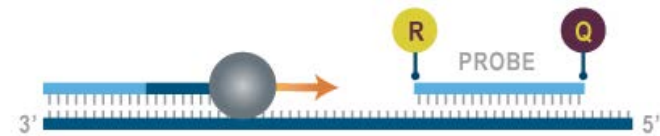
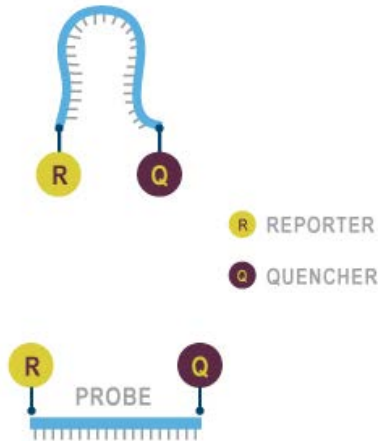


# Amplification Curve & Ct



# Taqman<sup>®</sup> Probe Assay

## A. Reporter is quenched when not bound to target sequence



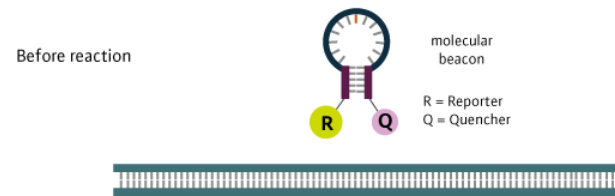
## B. 5' exonuclease degradation of probe separates reporter from quencher From Cycle to cycle, signal increase with the amount of amplicon



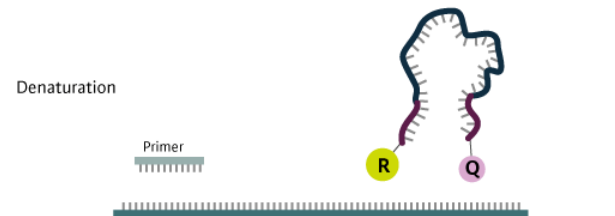


# Molecular Beacon Probe

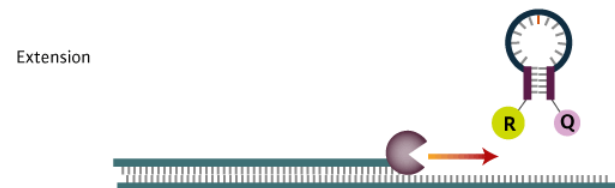
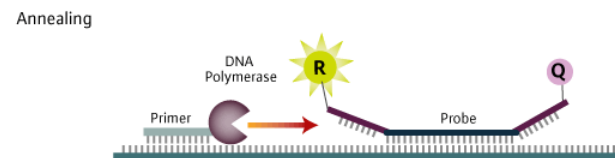
A. Molecular Beacon forms stem loop structure



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

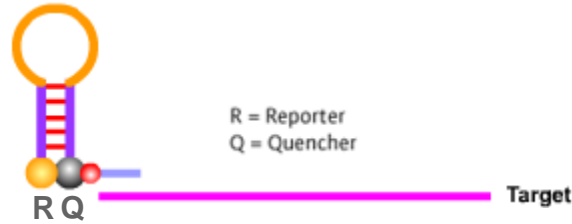


C. Temperature increases which causes Molecular Beacon to dissociate

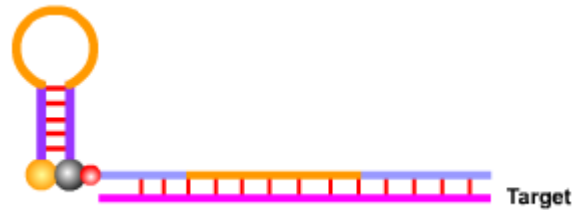


# Scorpion

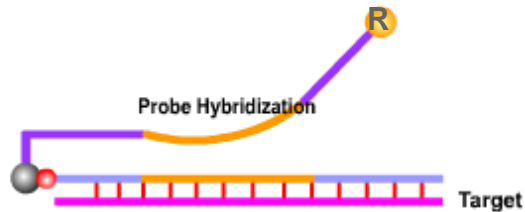
A. Scorpion Primer



B. Scorpions primer extended on target DNA



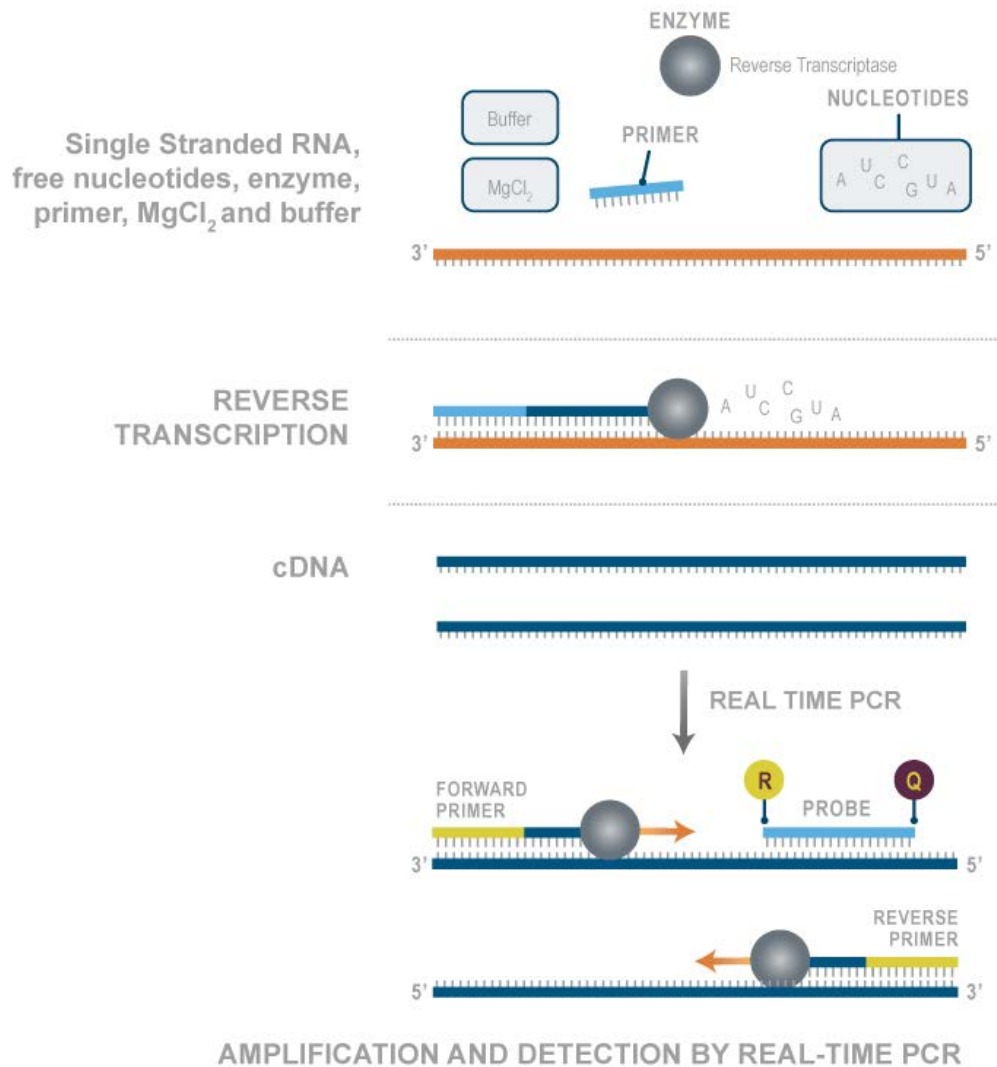
C. Extended primer is heat-denatured



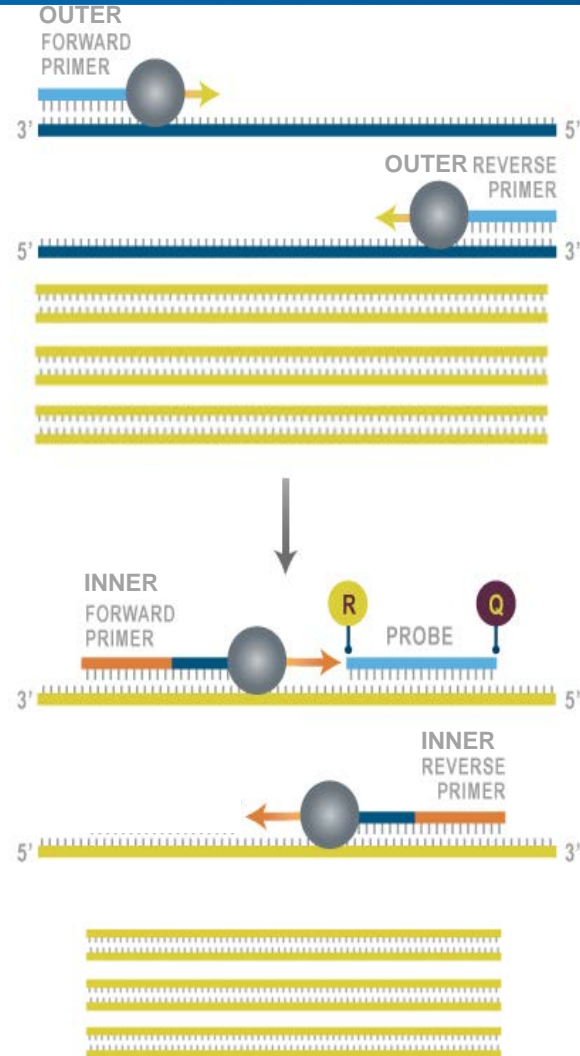
D. Cooling causes scorpion to rearrange and fluoresce in a target-specific manner



# Reverse Transcription Real-Time PCR



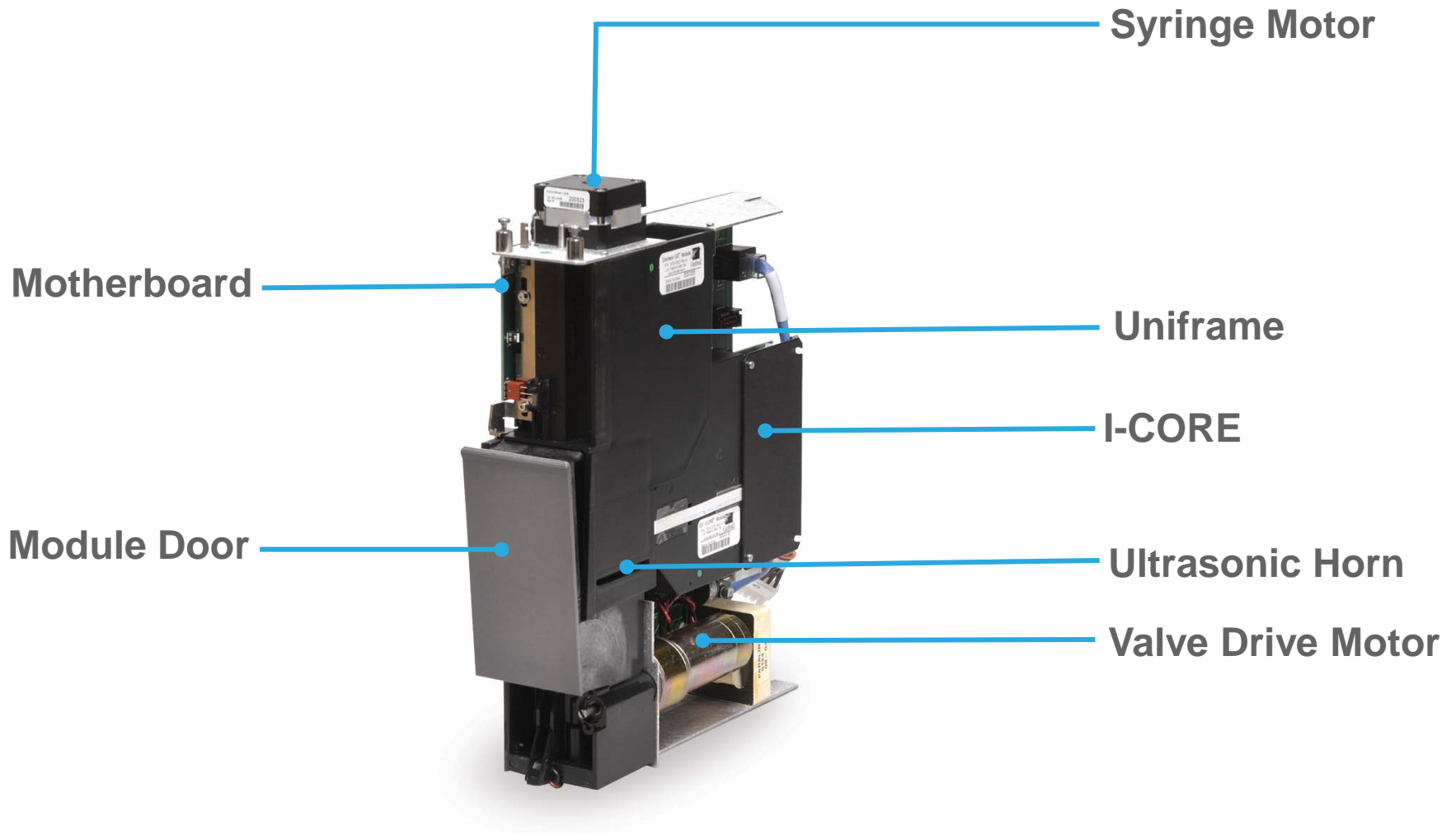
# Nested Real-Time PCR



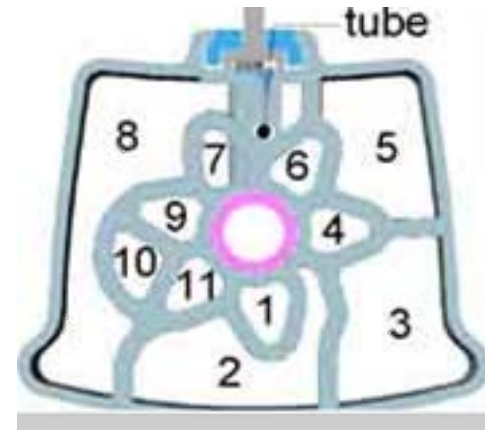
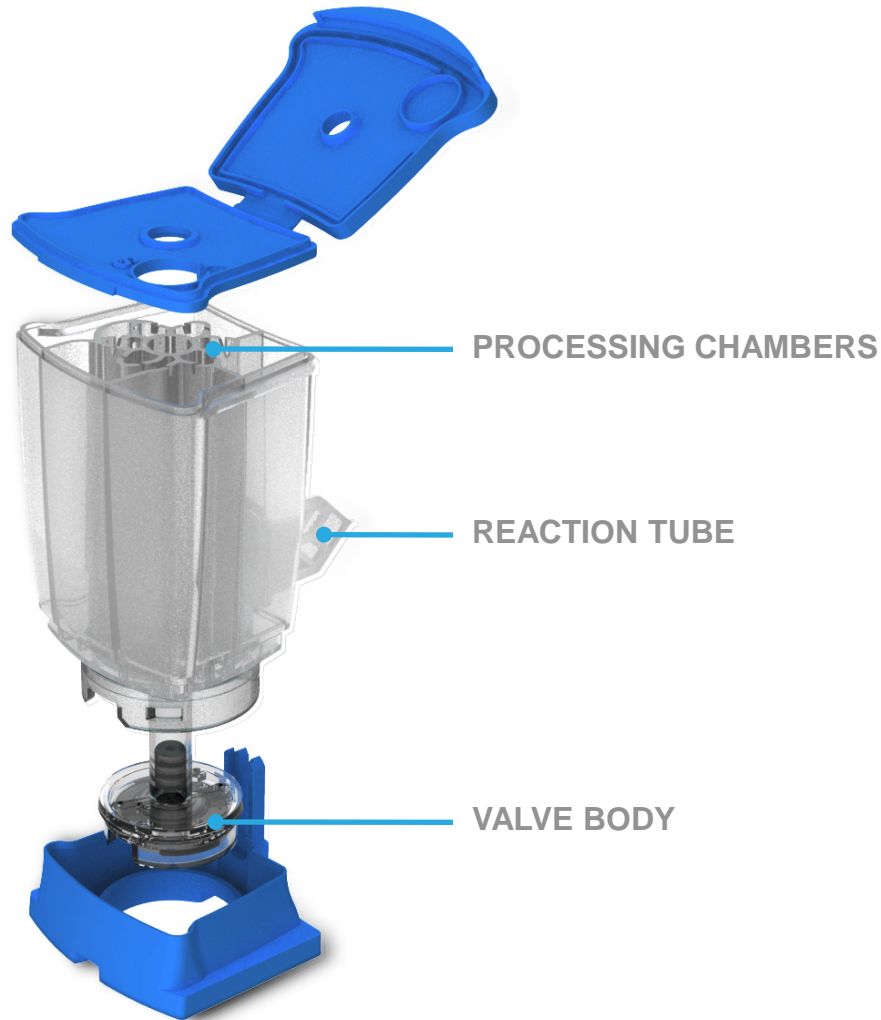
# Cepheid Technology



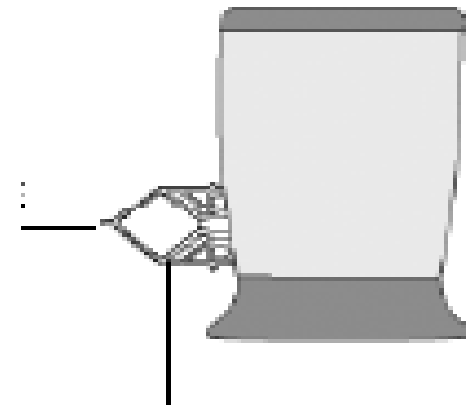
# GeneXpert Module



# GeneXpert Cartridge

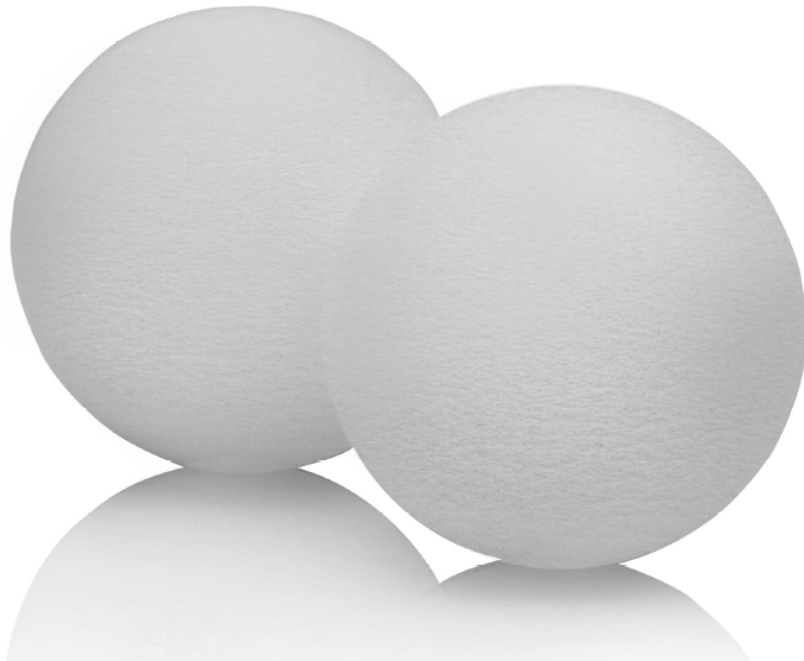


Cartridge





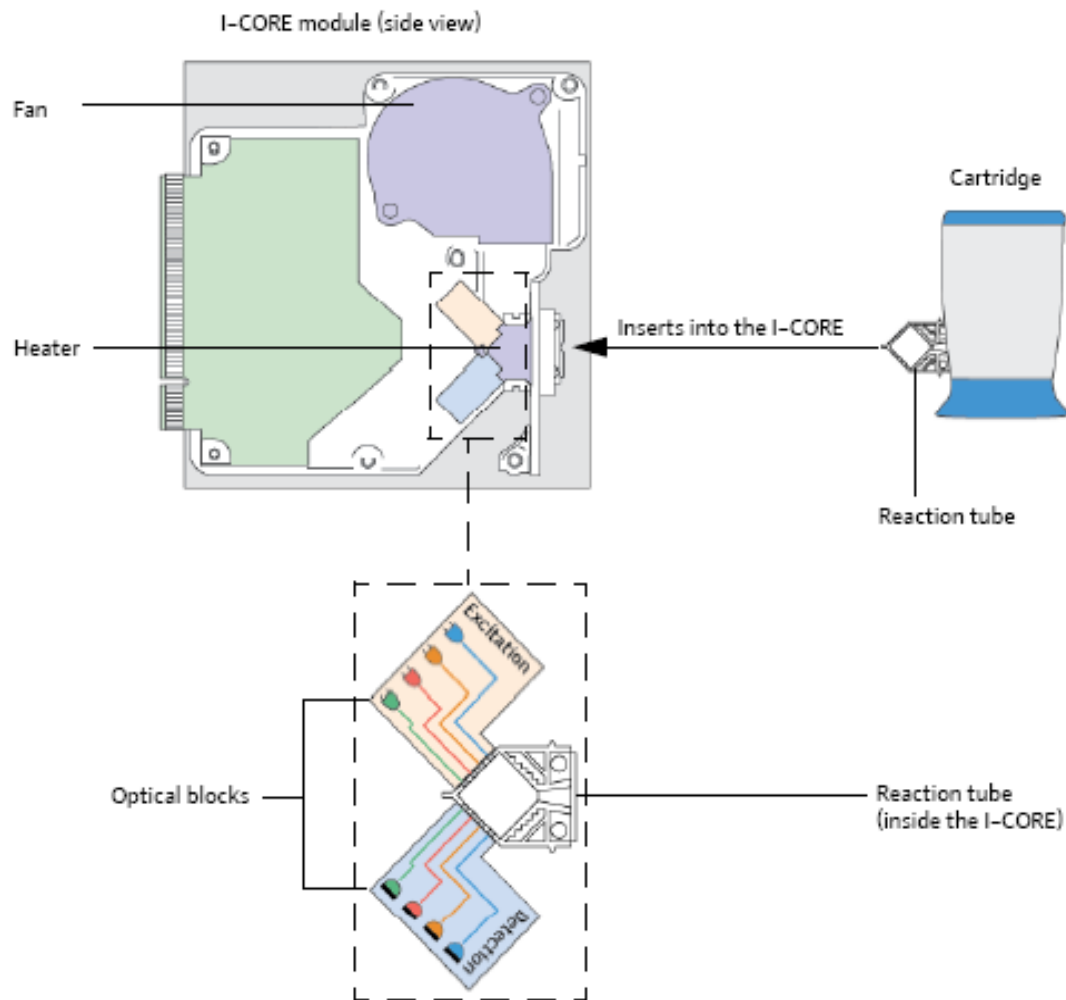
# Cepheid Reagent Technology



- **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

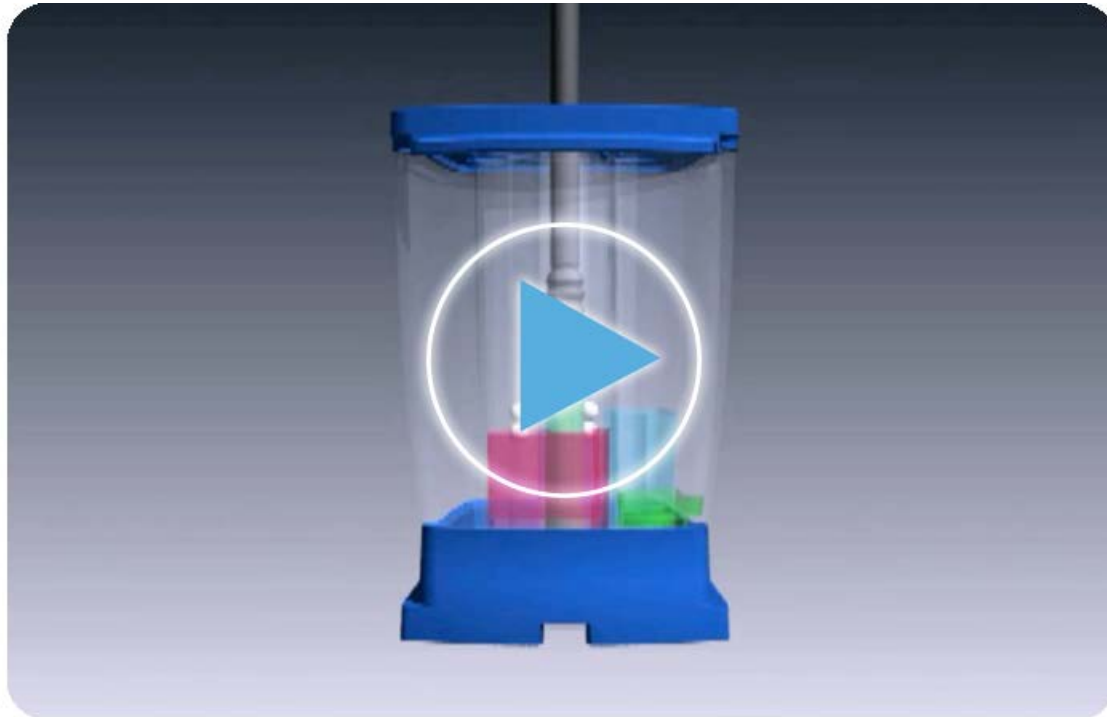
## Intelligent Cooling/Heating Optical Reaction Module



# GeneXpert 6 Channels

Channel	Excitation Range	Emission Range	Dyes
1	375-405 nm	420-480 nm	Dye 1
2	450-495 nm	510-535 nm	Dye 2
3	500-550 nm	565-590 nm	Dye 3
4	555-590 nm	606-650 nm	Dye 4
5	630-650 nm	665-685 nm	Dye 5
6	630-650 nm	>700 nm	Dye 6

# 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.**



Thank You.

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