



REAL TIME  
PCR

## What is Real-Time PCR?

PCR, or the Polymerase Chain Reaction, is a process for the **amplification** of **specific** fragments of DNA.

Real-Time PCR a specialized technique that allows a PCR reaction to be visualized “in real time” as the reaction progresses.

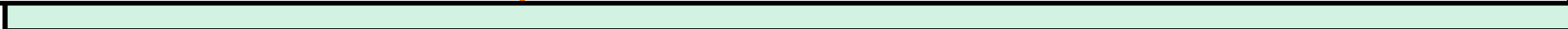
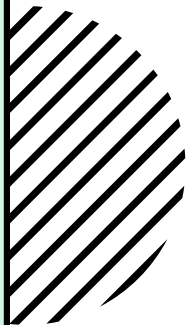
As we will see, Real-Time PCR allows us to measure minute amounts of DNA sequences in a sample!



**What is  
Real-Time  
PCR?**

**Conventional PCR**  
tells us “what”.

**Real-Time PCR**  
tells us “how much”.



# What is Real-Time PCR used for?

Real-Time PCR has become a cornerstone of molecular biology. Just some of the uses include:

- **Gene expression analysis**
  - Cancer and Drug research
- **Disease diagnosis and management**
  - Viral quantification
- **Food testing**
  - Percent GMO food
- **Animal and plant breeding**
  - Gene copy number
- **Forensics**

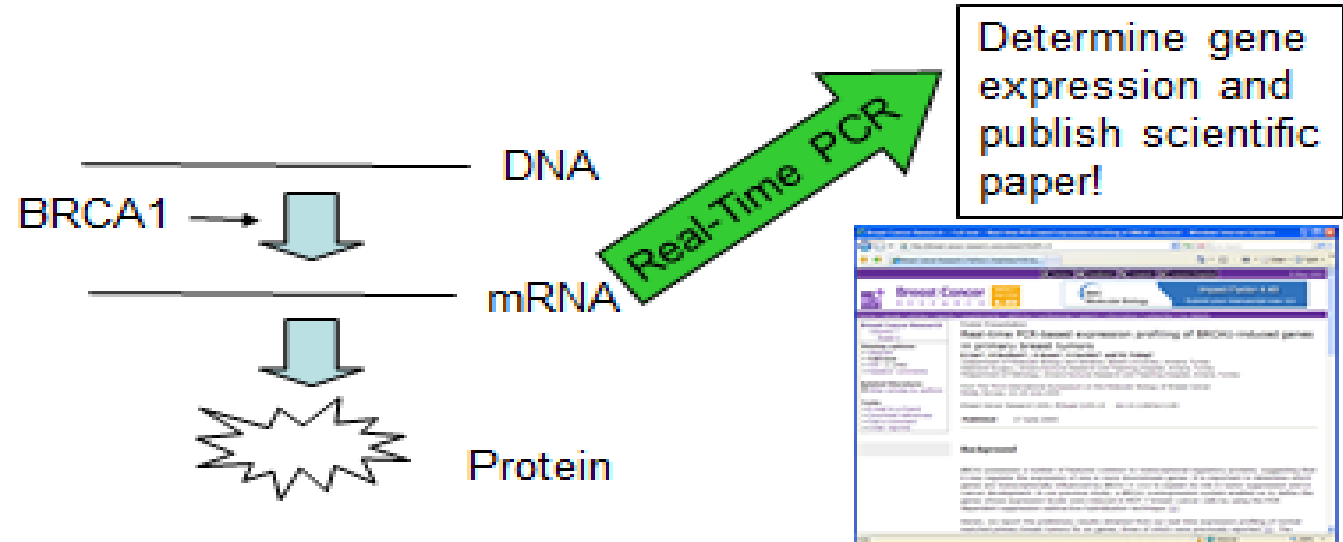
Sample identification and quantification

# Real-Time PCR in Gene Expression Analysis



## Example: BRCA1 Expression Profiling

BRCA1 is a gene involved in tumor suppression. BRCA1 controls the expression of other genes. In order to monitor level of expression of BRCA1, real-time PCR is used.



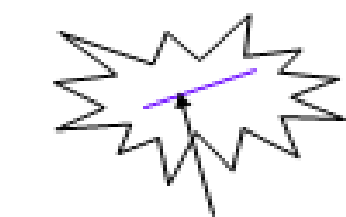
# Real-Time PCR in Disease Management



## Example: HIV Treatment

Drug treatment for HIV infection often depends on monitoring the “viral load”.

Real-Time PCR allows for direct measurement of the amount of the virus RNA in the patient.

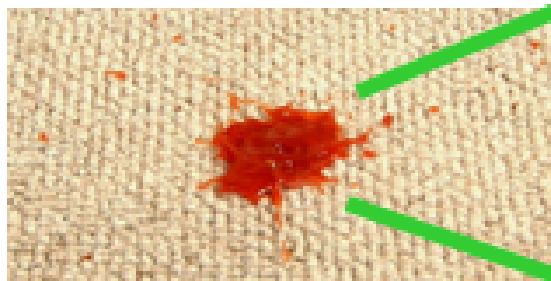


Viral RNA



Measure amount of virus, adjust prescriptions.

# Real-Time PCR in Forensics



What is it ??

Enough DNA to ID ??

## Example: Real-Time PCR in Forensic Analysis!

### STAIN IDENTIFICATION

The Use of Real-Time PCR for Forensic Stain Identification

By Tasha L. Hernandez-Cook, Ph.D., and Greg Best, Ph.D.,  
Wisconsin Forensic Laboratory, Department of Public Safety

### Stain Identification:

New Real-Time methods can be directly used to identify the composition of unknown stains, with much better accuracy than traditional “color-change” tests.



### DNA Quantification:

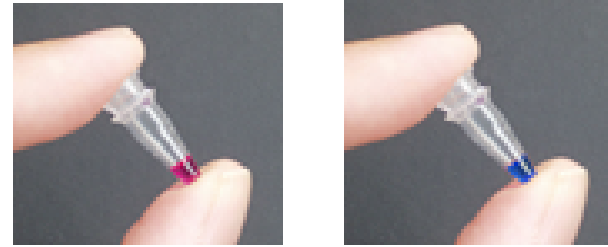
Since standard forensic STR Genotyping requires defined amounts of DNA, Real-Time PCR can be used to accurately quantify the amount of DNA in an unknown sample!

# Imagining Real-Time PCR

## Measuring Quantities

What if YOU started with EIGHT times LESS DNA template than I did?

Cycle 25 →

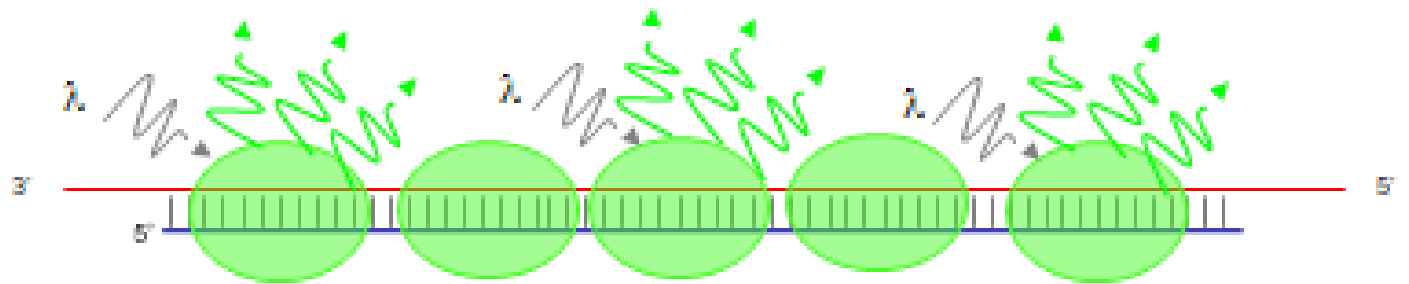


Cycle	Me	You
25	1,000,000	125,000
26	2,000,000	250,000
27	4,000,000	500,000
28	8,000,000	1,000,000



## How do We Measure DNA in a PCR Reaction?

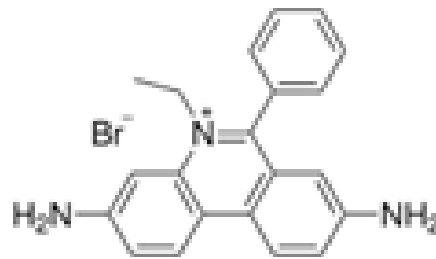
We use reagents that fluoresce in the presence of amplified DNA!



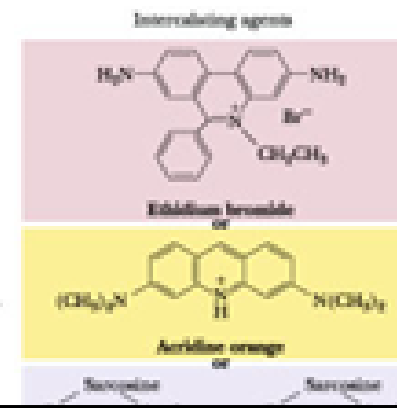
# Measuring DNA: Ethidium Bromide

## Ethidium Bromide

- + = common and well known
- = toxic, not very bright



B-DNA before intercalation



B-DNA after intercalation



# Trouble-Shooting

- A successful real-time PCR experiment will have the following characteristics:

