PCR, Microarrays and Sequencing

Introduction to Bioinformatics Course

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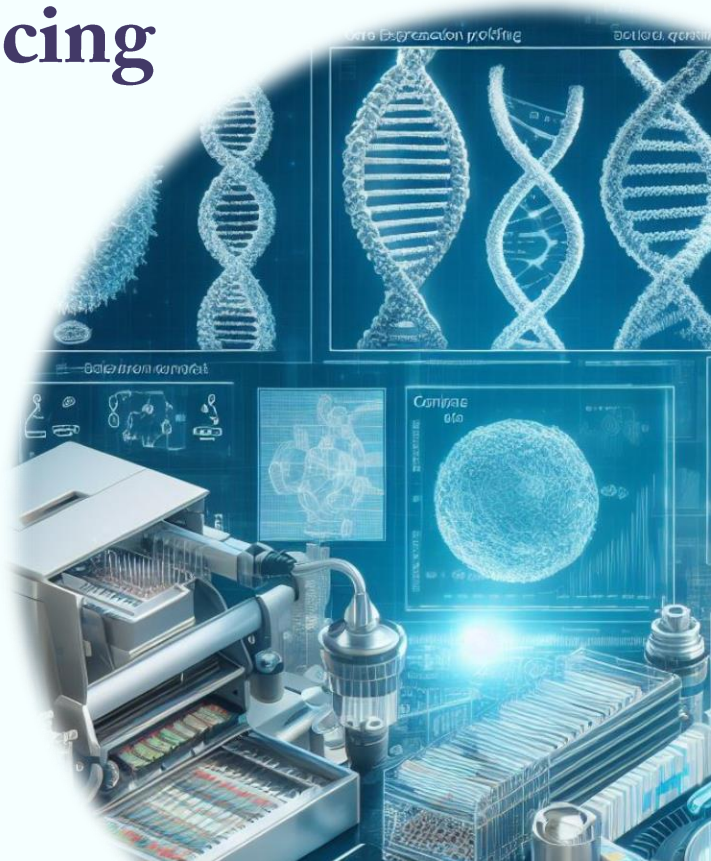
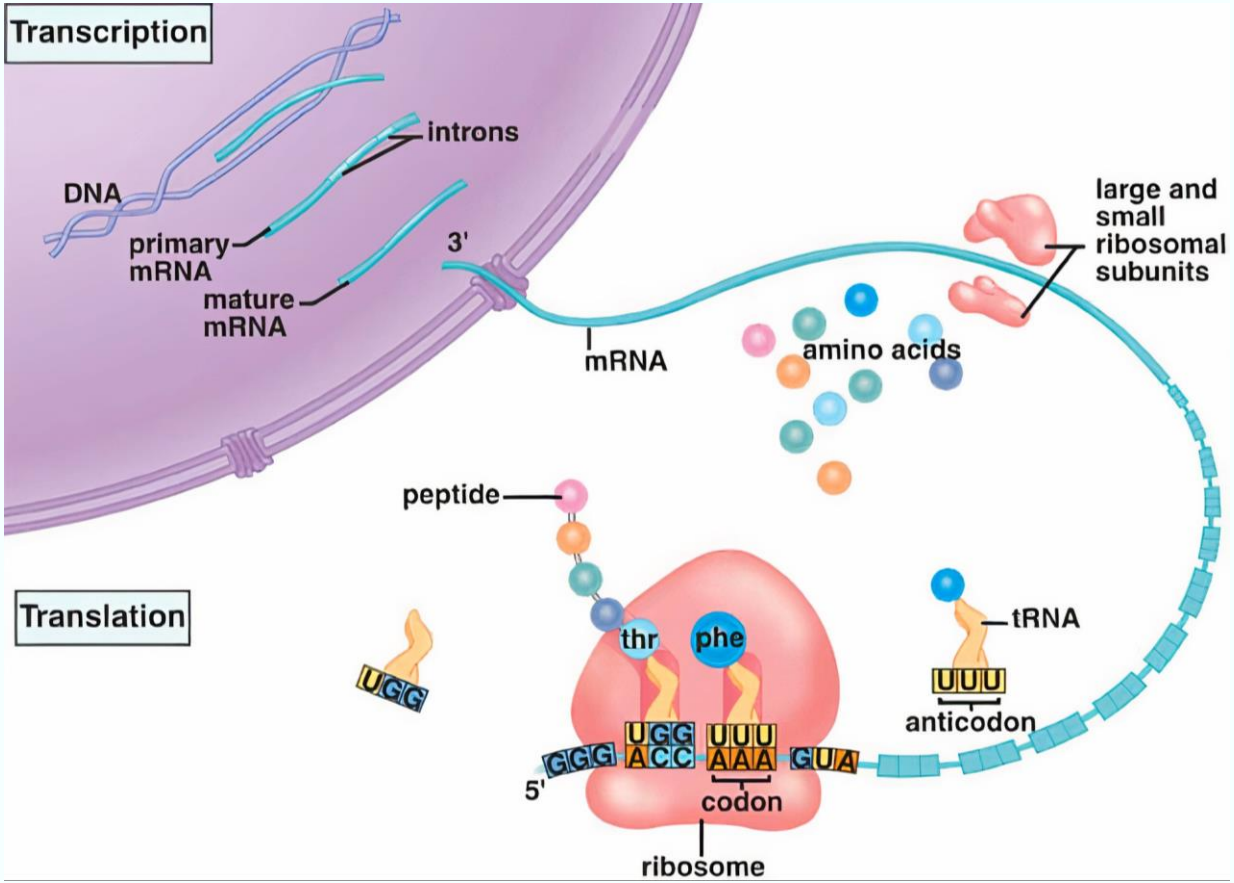


What to Read?


Transcription
DNA
introns
primary mRNA
mature mRNA
3'
mRNA
large and small ribosomal subunits
amino acids
peptide
Translation
thr
phe
tRNA
UUU
anticodon
UCG
UCG
ACC
UUU
AAA
CGG
GUA
5'
codon
ribosome

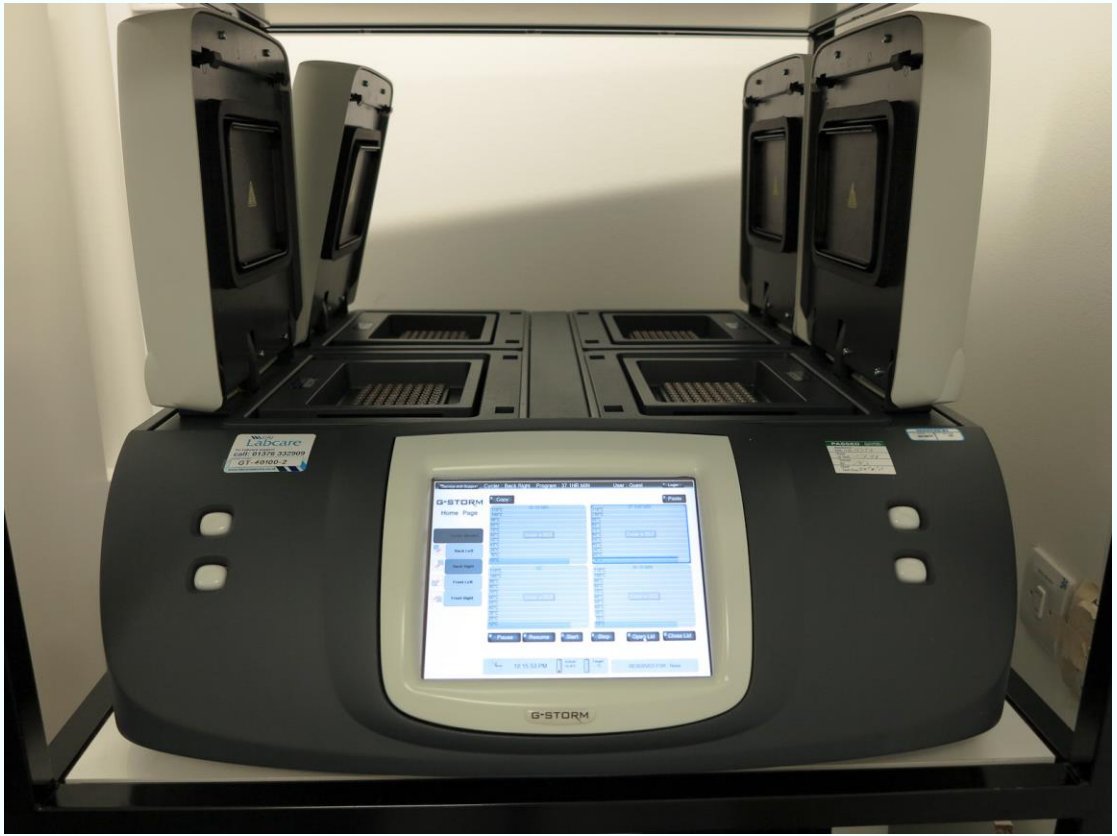
How to Read?

Polymerase Chain Reaction(PCR)

Microarray

Sequencing

Polymerase Chain Reaction (PCR)



Thermal Cycler

PCR Setup: What's in the Mix?

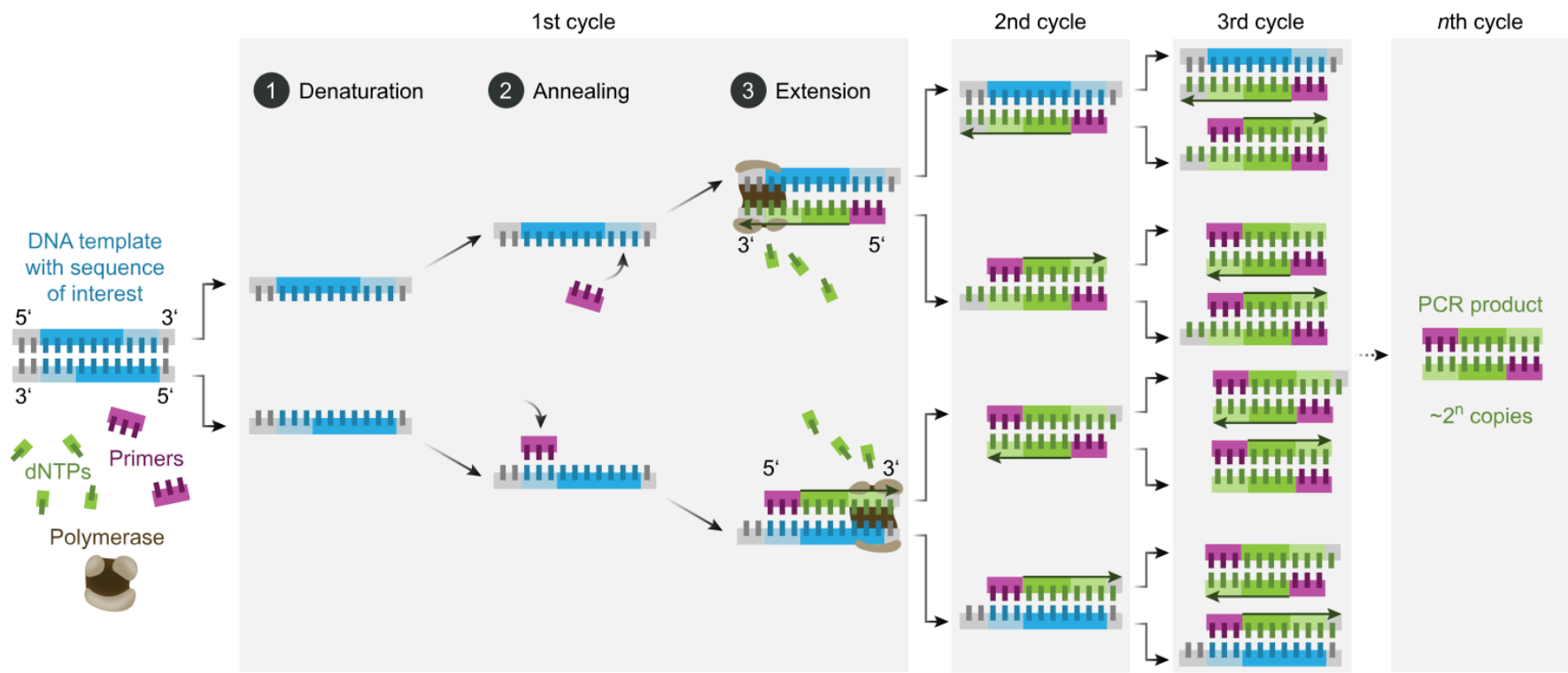
1) A **DNA template** that contains the DNA target region to amplify.

2) **Two DNA primers** that are complementary to the 3' ends of each of the sense and anti-sense strands of the DNA target.

3) **DNA polymerase**; an enzyme that polymerizes new DNA strands; heat-resistant Taq polymerase is especially common

4) **Deoxynucleoside triphosphates**, or dNTPs, the building blocks from which the DNA polymerase synthesizes a new DNA strand.

PCR Procedure



Applications

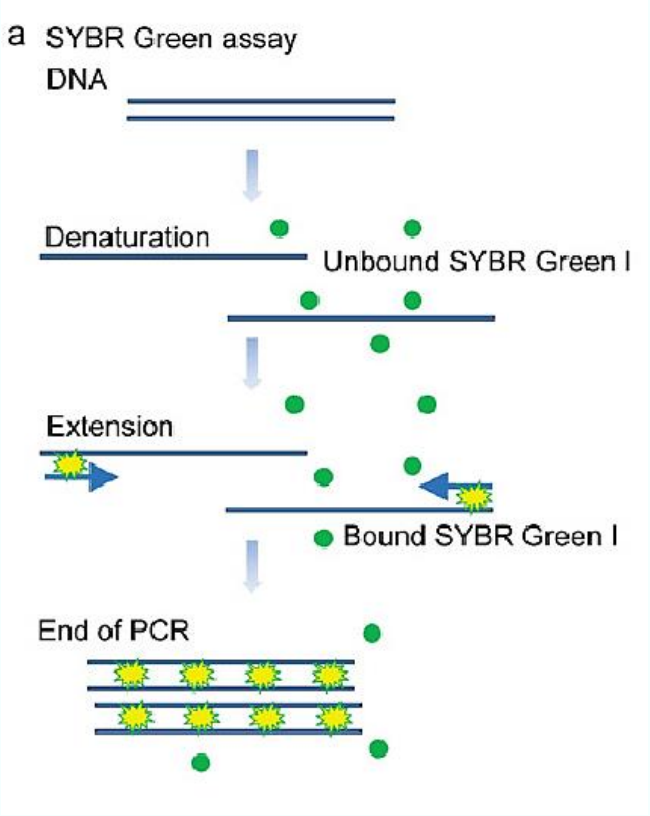
- Medical and Diagnostic
- Forensic Applications
- DNA sequencing can be assisted by PCR
- Detection of Mutations
- And so on …

Quantitative PCR (qPCR)

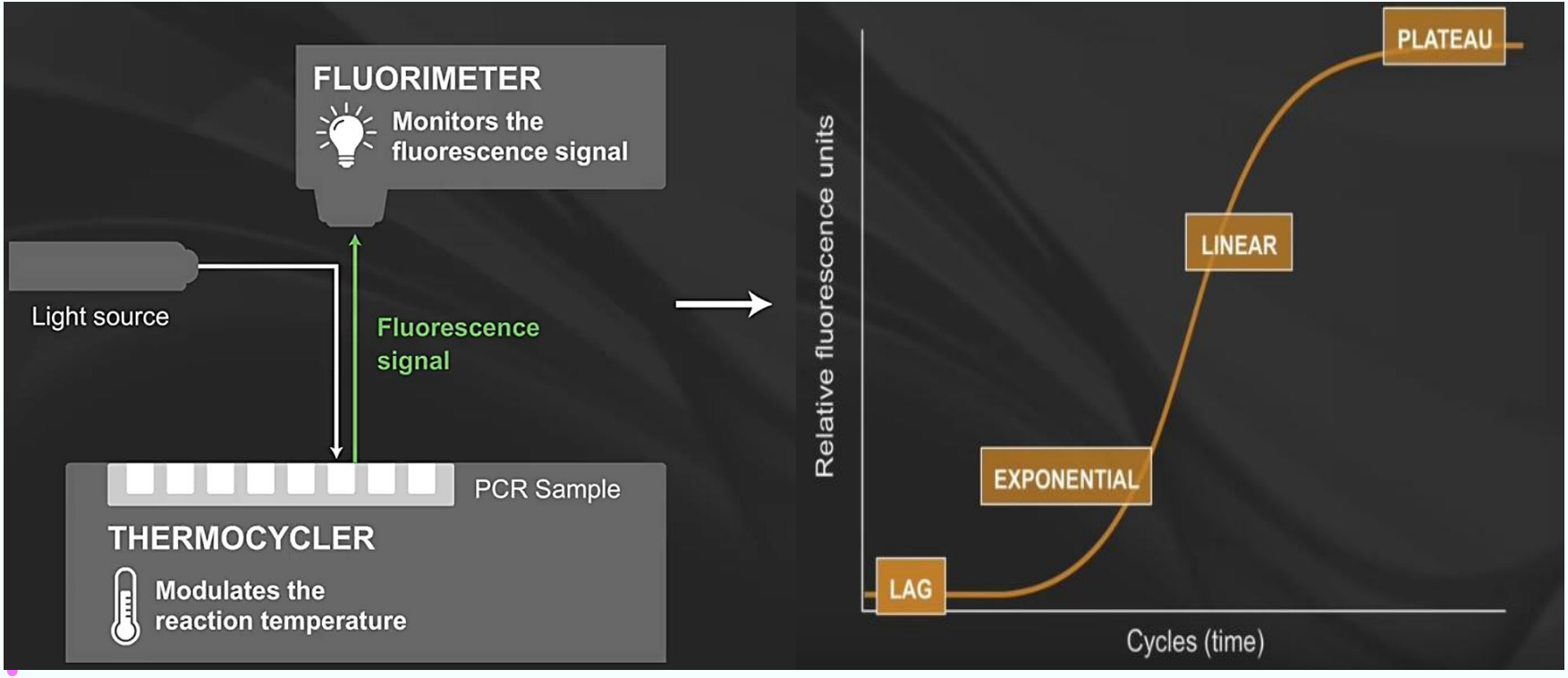
- **qPCR or real-time PCR** is a variation of the PCR technique.
- PCR is a qualitative technique, while qPCR is a quantitative technique.
- qPCR is based on the principle that the amount of fluorescent dye produced during a PCR reaction is proportional to the amount of DNA that is amplified.

SYBR Green is used in qPCR in the following way:

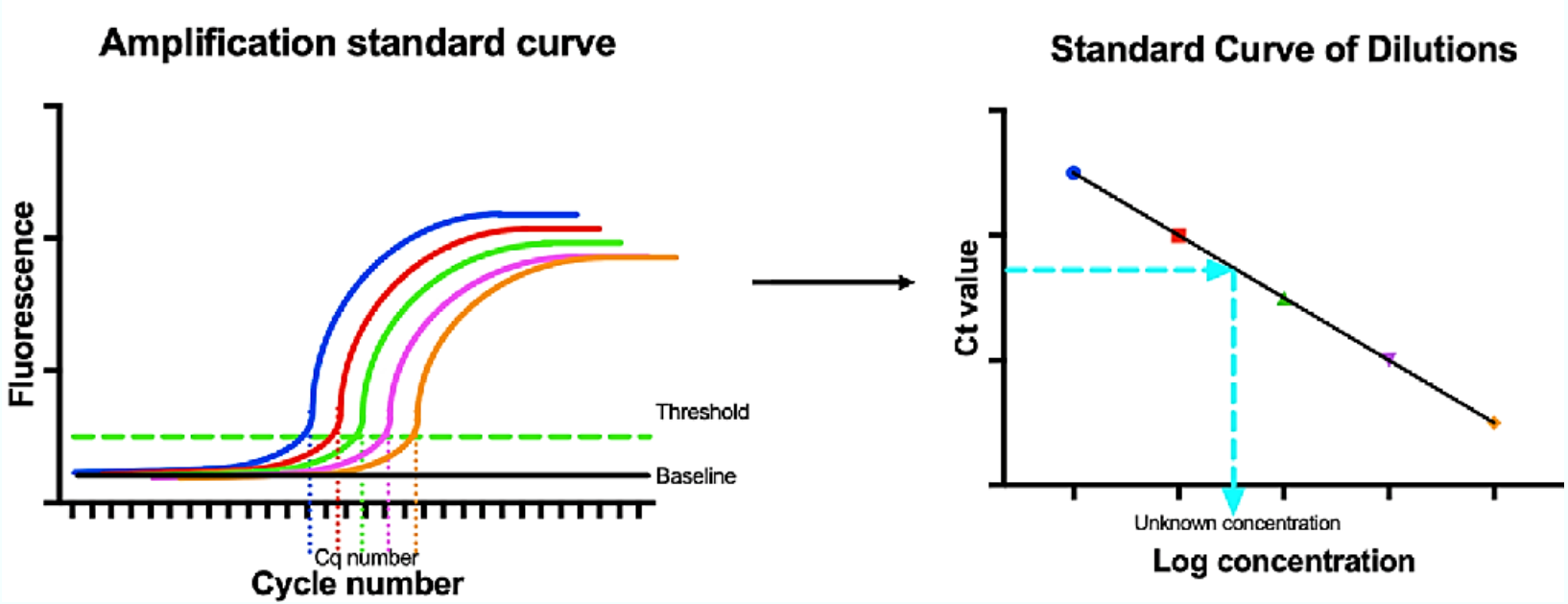
1. The SYBR Green dye is added to the PCR mixture.
2. The PCR reaction is then performed as usual.
3. The fluorescence of the SYBR Green dye is measured at each cycle of the PCR reaction.
4. The amount of DNA that has been amplified is determined by measuring the increase in fluorescence over the course of the PCR reaction.



qPCR Curve

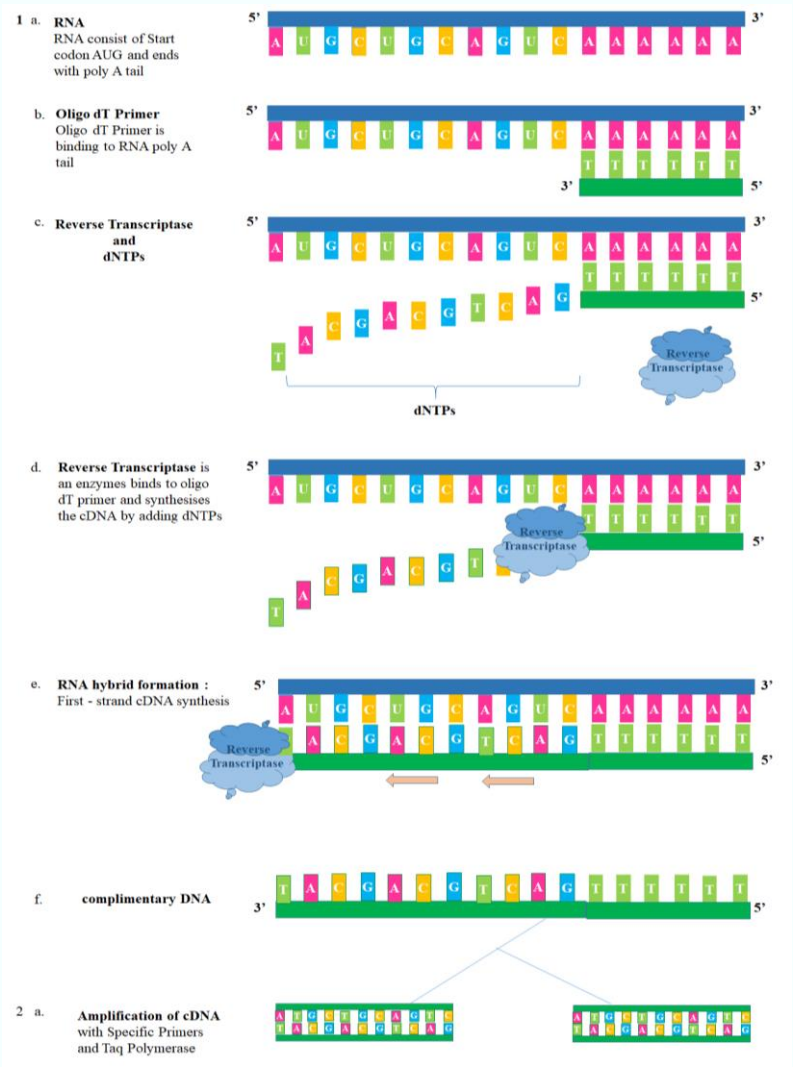

FLUORIMETER
Monitors the fluorescence signal
Light source
Fluorescence signal
PCR Sample
THERMOCYCLER
Modulates the reaction temperature
Relative fluorescence units
PLATEAU
LINEAR
EXPONENTIAL
LAG
Cycles (time)

qPCR Curve


Amplification standard curve
Fluorescence
Threshold
Baseline
Cq number
Cycle number
Standard Curve of Dilutions
Ct value
Unknown concentration
Log concentration

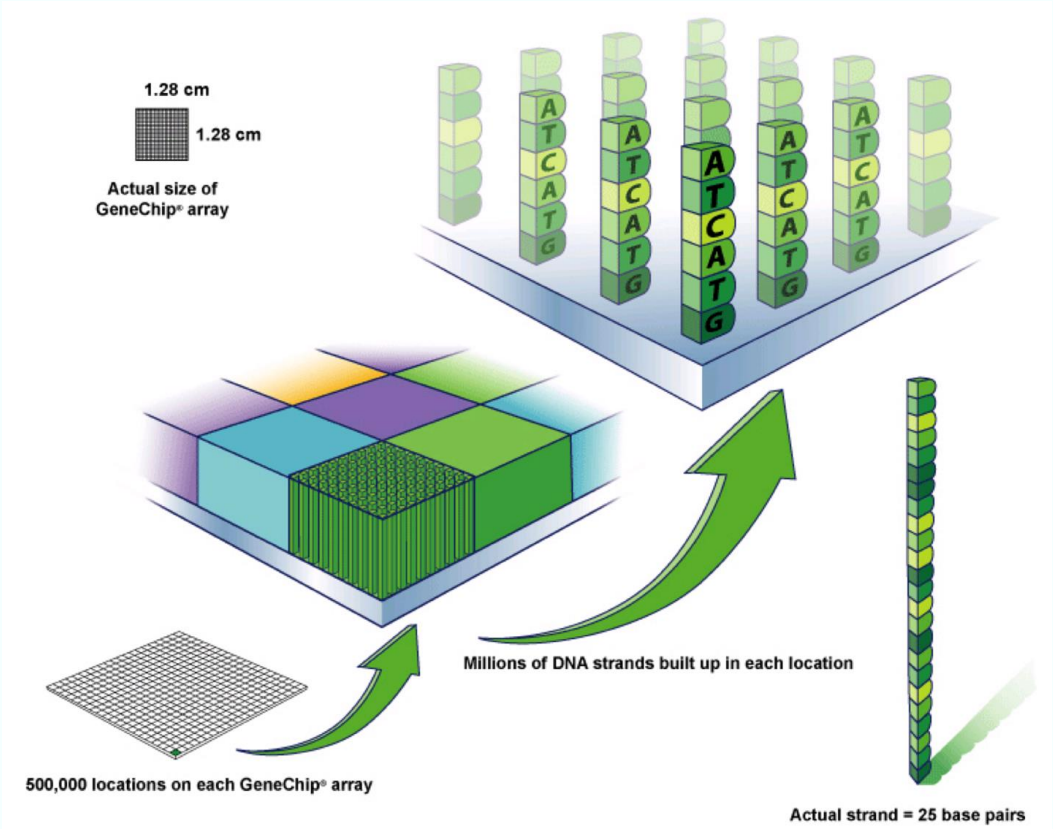
RT-qPCR

- **Reverse transcription qPCR (RT-qPCR)** is a technique that combines reverse transcription (RT) and quantitative PCR (qPCR) to detect and quantify RNA.
- RT is a process by which RNA is converted into complementary DNA (cDNA).
- Common applications of RT-qPCR include gene expression analysis, mRNA quantification, RNA virus detection and so on ..

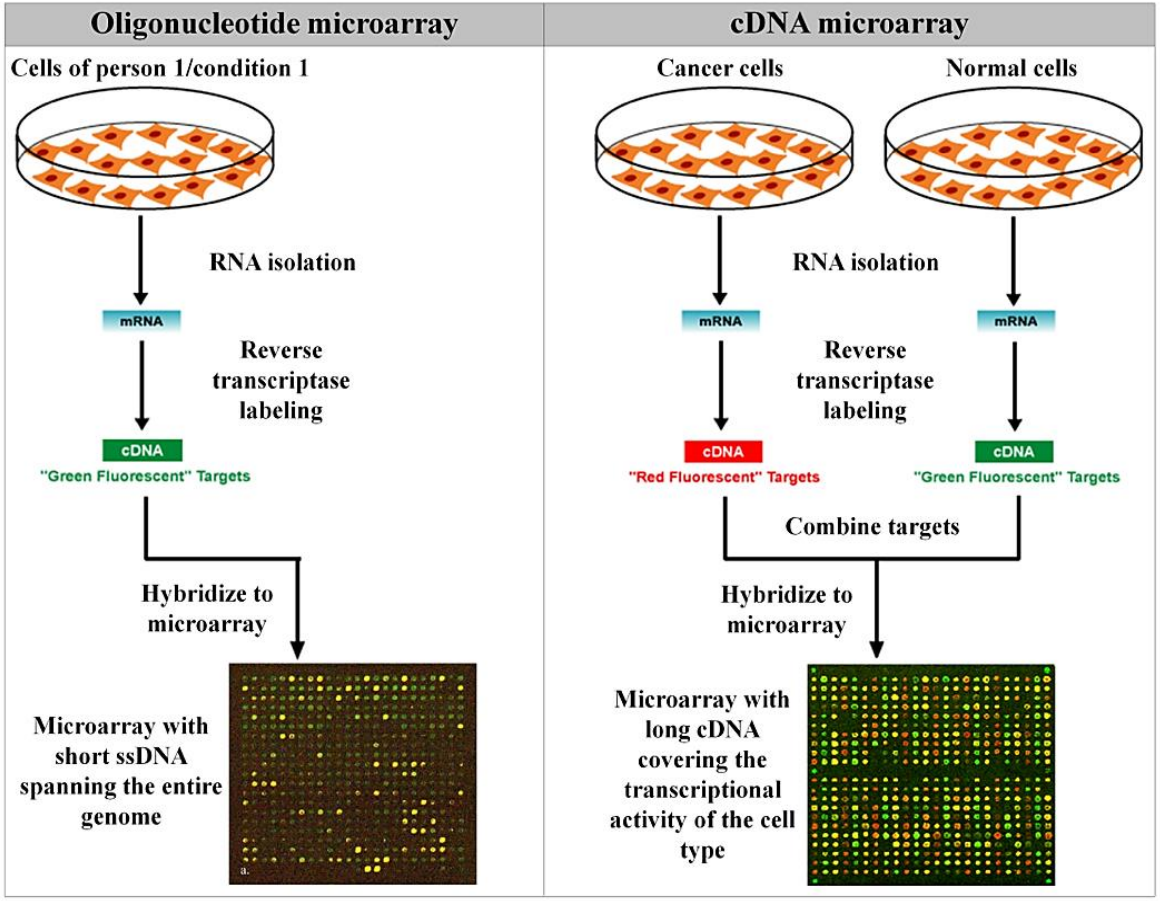


Microarray

- A **DNA Microarray** is a collection of microscopic DNA spots attached to a solid surface.
- DNA microarrays are used to measure the expression levels of large numbers of genes simultaneously.
- Each DNA spot contains a specific DNA sequence, known as probes (or reporters or oligos).
- These can be a short section of a gene or other DNA element that are used to hybridize a cDNA.

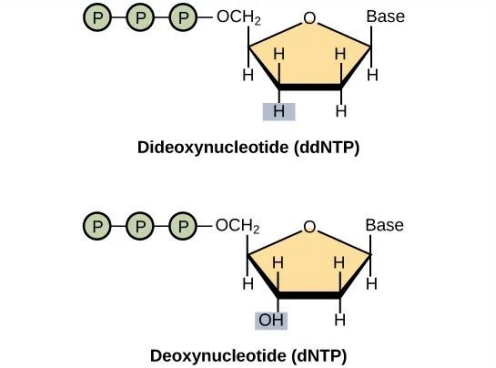


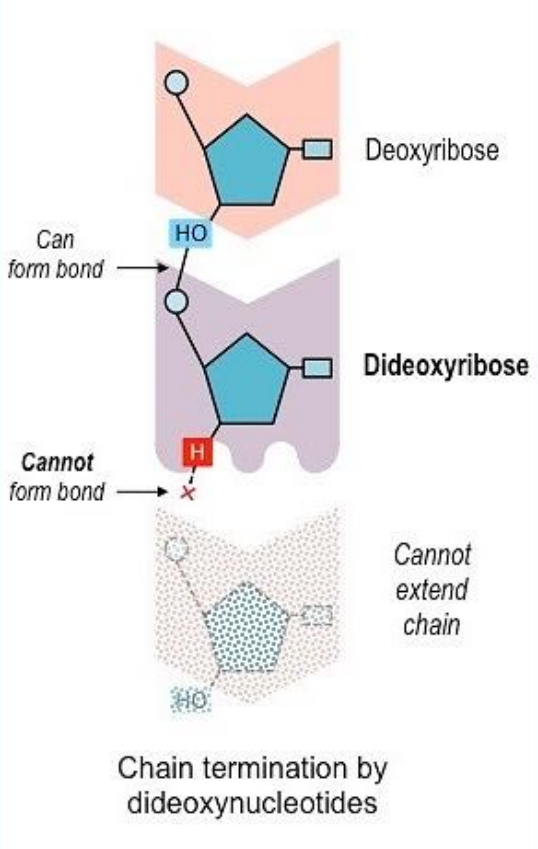
Microarray


Oligonucleotide microarray
Cells of person 1/condition 1
RNA isolation
mRNA
Reverse transcriptase labeling
cDNA
"Green Fluorescent" Targets
Hybridize to microarray
Microarray with short ssDNA spanning the entire genome
cDNA microarray
Cancer cells
Normal cells
RNA isolation
mRNA
mRNA
Reverse transcriptase labeling
cDNA
"Red Fluorescent" Targets
cDNA
"Green Fluorescent" Targets
Combine targets
Hybridize to microarray
Microarray with long cDNA covering the transcriptional activity of the cell type

Sequencing - Sanger

- Sanger sequencing is based on the use of dideoxynucleotides (ddNTPs).
- ddNTPs are similar to normal nucleotides(dNTPs), but they are missing a hydroxyl group on the 3' carbon atom.
- This prevents the DNA polymerase enzyme from adding another nucleotide to the chain, and it therefore terminates the chain.





Sanger Sequencing Steps

1. **Prepare the DNA template.** The DNA template is the DNA sequence that you want to sequence. It can be a single gene, a whole genome, or any other region of DNA.

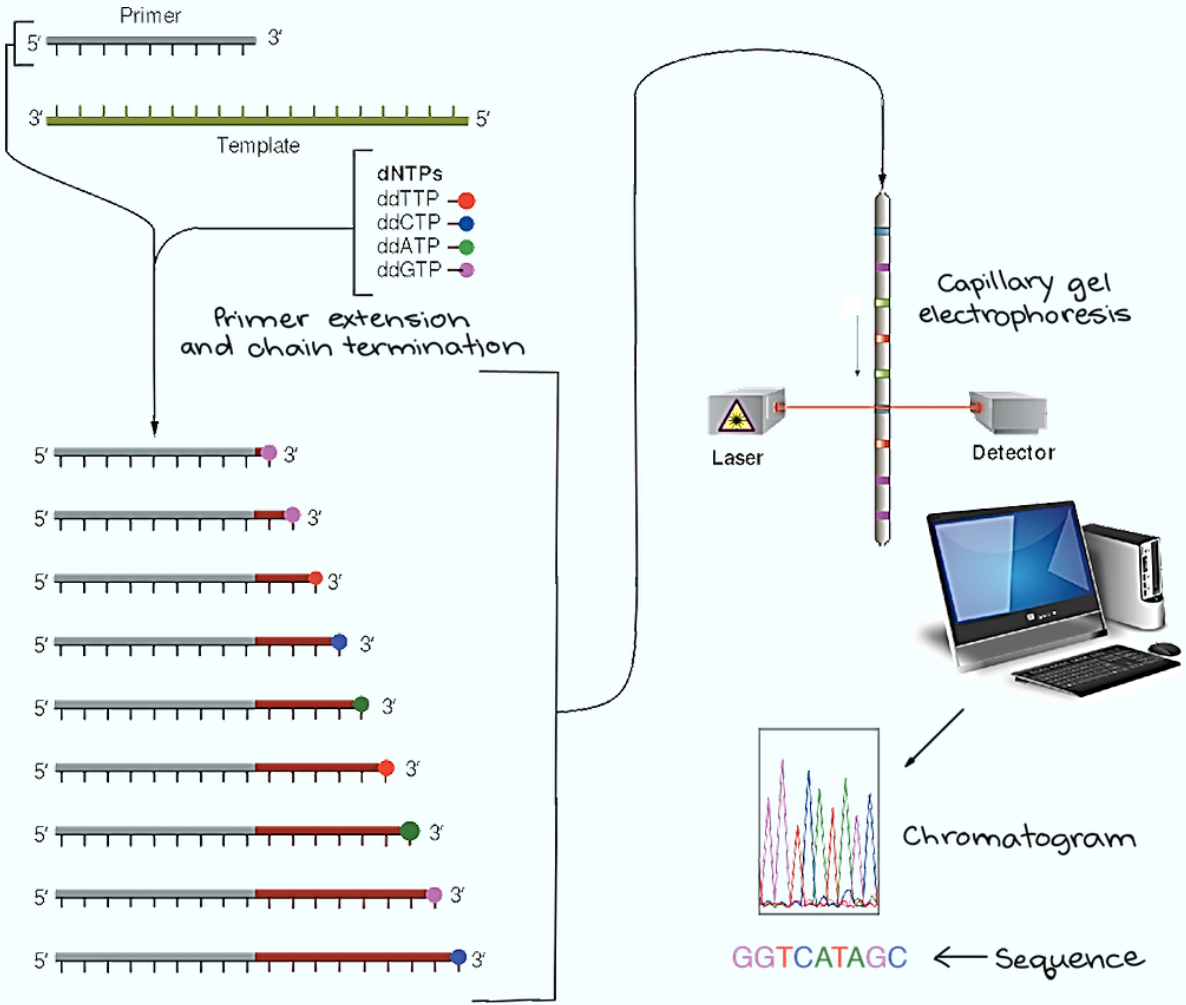
2. **Prime the DNA template.** A primer is a short piece of DNA that binds to the beginning of the DNA template. The primer is used to start the DNA polymerase reaction.

3. **Add the DNA polymerase enzyme, nucleotides, and ddNTPs.** The DNA polymerase enzyme is responsible for adding nucleotides to the DNA chain. The nucleotides are the building blocks of DNA, and the ddNTPs are the chain-terminating nucleotides.

4. **Perform the DNA polymerization reaction.** The DNA polymerase enzyme will add nucleotides to the DNA chain until it encounters a ddNTP. At that point, the chain will terminate.

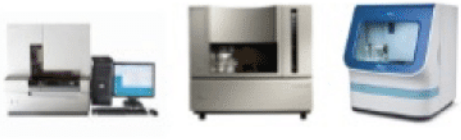
5. **Separate the DNA fragments by size.** The DNA fragments of different sizes are separated using a technique called gel electrophoresis.

6. **Detect the fluorescent labels.** The ddNTPs are labeled with different fluorescent dyes. This allows the DNA fragments to be detected and visualized under ultraviolet light.

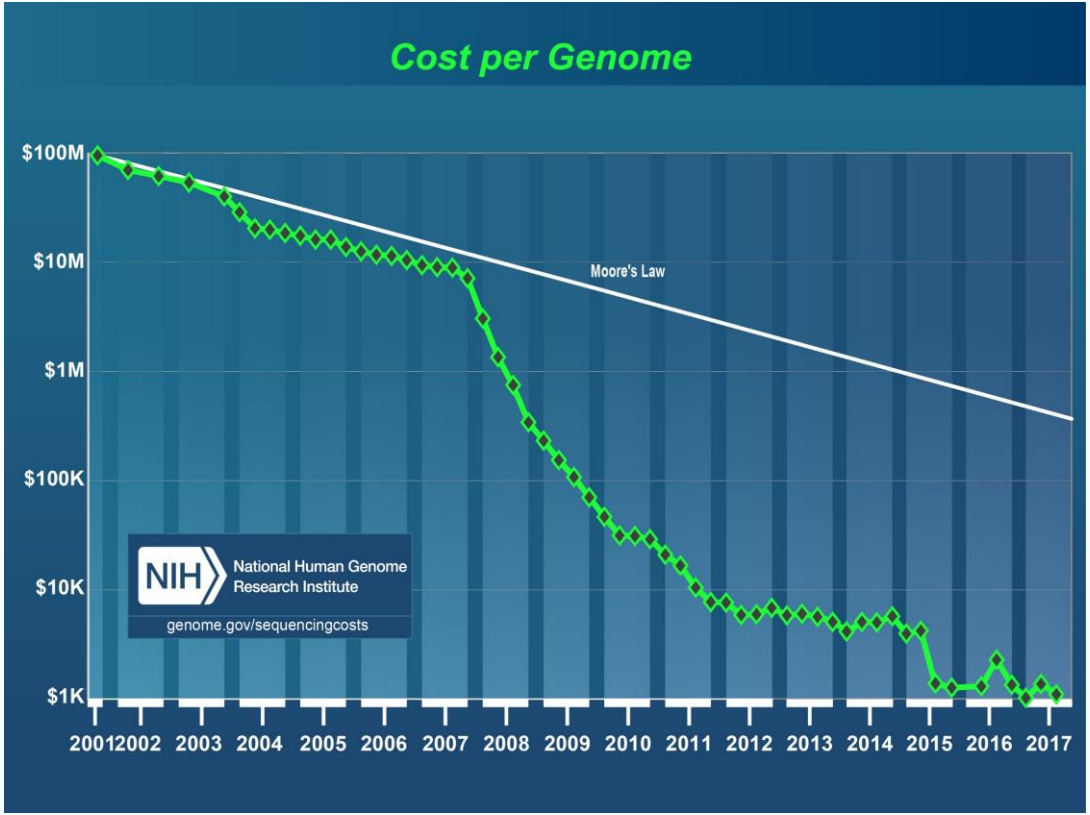

Primer
5′
3′
3′
5′
Template
dNTPs
ddTTP
ddCTP
ddATP
ddGTP
Primer extension
and chain termination
Capillary gel
electrophoresis
Laser
Detector
Chromatogram
GGTCATAGC
Sequence

Sequencing - Sanger

Next Generation Sequencing

First generation	Second generation (next generation sequencing)	Third generation
		
Sanger sequencing Maxam and Gilbert Sanger chain termination	454, Solexa, Ion Torrent, Illumina	PacBio Oxford Nanopore
Infer nucleotide identity using dNTPs, then visualize with electrophoresis	High throughput from the parallelization of sequencing reactions	Sequence native DNA in real time with single-molecule resolution
500–1,000 bp fragments	~50–500 bp fragments	Tens of kb fragments, on average
Short-read sequencing		Long-read sequencing

NGS Cost


Cost per Genome
$100M
$10M
$1M
$100K
$10K
$1K
Moore's Law
NIH
National Human Genome Research Institute
genome.gov/sequencingcosts
2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017