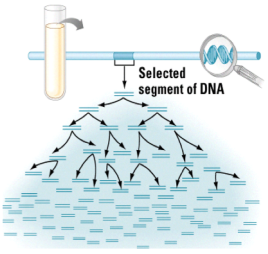
PCR
Predecessors & Variations

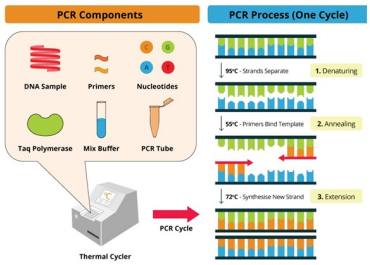
Polymerase Chain Reaction

With PCR, any specific segment—within a DNA sample can be copied many times (amplified) completely *in vitro*.!



- Copies DNA fragments
- Million copies/hr
- Enough for RFLP analysis, probes, sequencing, etc.

Conventional PCR — (Mullins 1983)

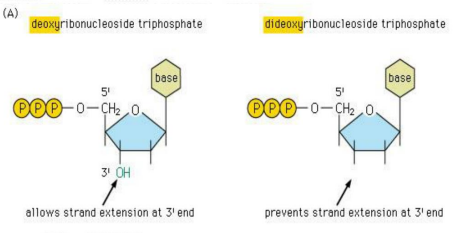


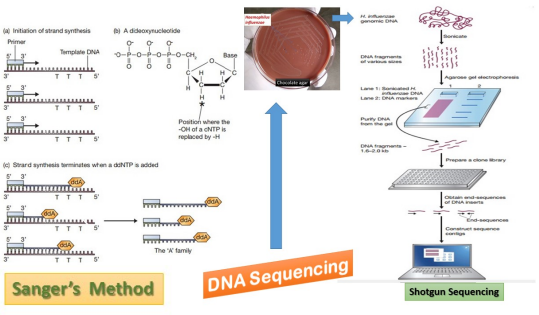
DNA sequencing — the precursor to PCR

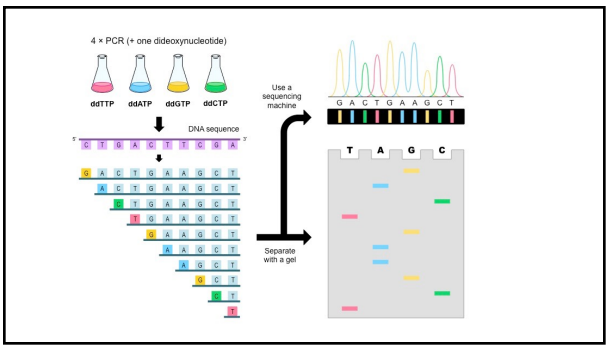
- **Sanger** dideoxy chain termination **method** (1977)

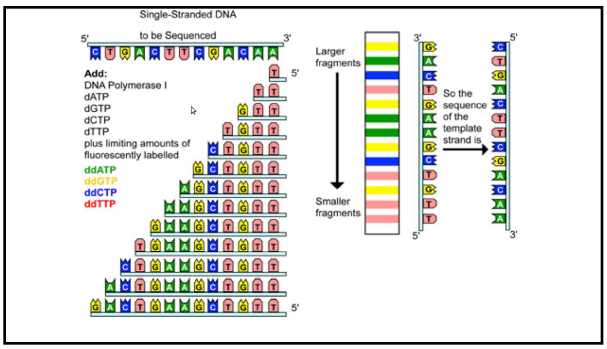
Sanger's Method

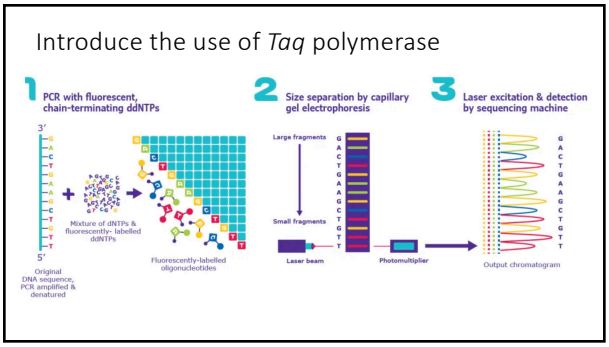
Dideoxy Sequencing of DNA

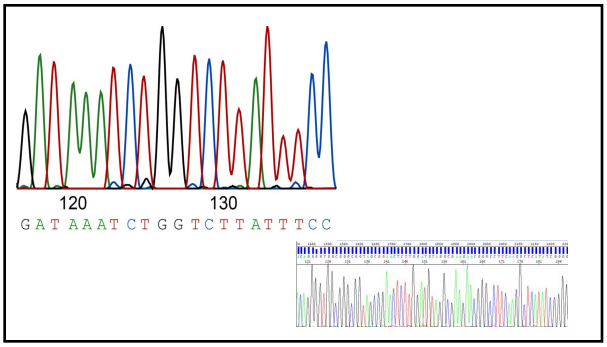


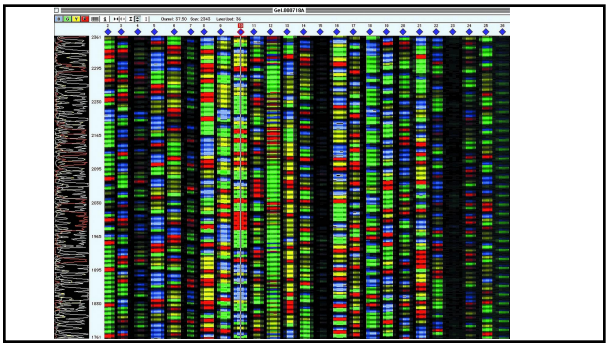


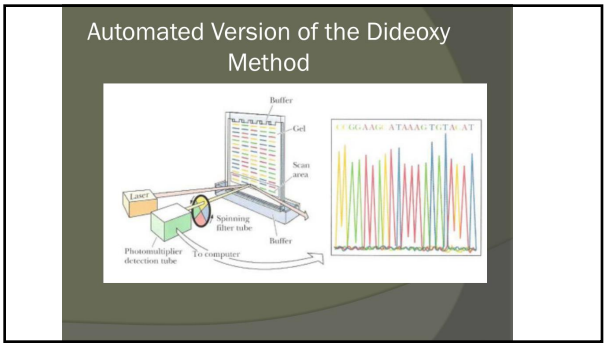

4 × PCR (+ one dideoxynucleotide)
ddTTP
ddATP
ddGTP
ddCTP
DNA sequence
Use a sequencing machine
Separate with a gel
G A C T G A A G C T
T A G C


Single-Stranded DNA
to be Sequenced
Add:
DNA Polymerase I
dATP
dGTP
dCTP
dTTP
plus limiting amounts of fluorescently labelled
ddATP
ddGTP
ddCTP
ddTTP
Larger fragments
Smaller fragments
So the sequence of the template strand is


Introduce the use of *Taq* polymerase
1 PCR with fluorescent, chain-terminating ddNTPs
2 Size separation by capillary gel electrophoresis
3 Laser excitation & detection by sequencing machine
Mixture of dNTPs & fluorescently- labelled ddNTPs
Fluorescently-labelled oligonucleotides
Original DNA sequence, PCR amplified & denatured
Large fragments
Small fragments
Laser beam
Photomultiplier
Output chromatogram


120
130
G A T A A A T C T G G T C T T A T T T C C

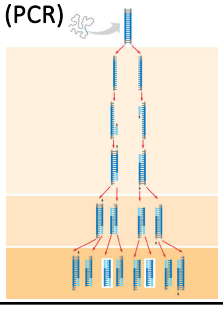



Automated Version of the Dideoxy Method
Buffer
Gel
Scan area
Laser
Spinning filter tube
Buffer
Photomultiplier detection tube
To computer

Polymerase Chain Reaction (PCR)

Mullins' innovation:

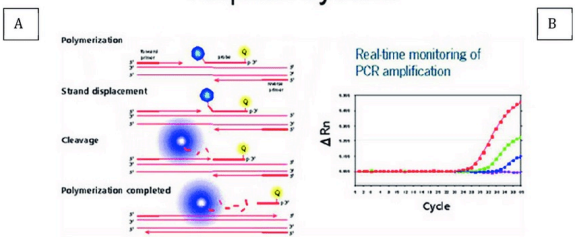
- Start with double stranded DNA
- Use TWO primers, forward and reverse, bracketing the area of interest
- Copy the copies! → "chain reaction" → **amplification**



qPCR

- **Quantitative PCR**
- Use compound that fluoresces when bound to dsDNA
- Measure quantity of fluorescence as measure of quantity of PCR product → proportional to original amount of template DNA

TaqMan system



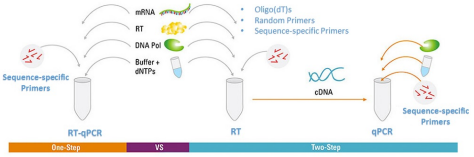
RT-PCR

- **Reverse Transcriptase — PCR**
- Measure gene expression

- Add poly-T primer → bind to poly-A tail of mRNAs
- Use Reverse Transcriptase → cDNA from mRNA template
- Use specific primer + poly-T reverse primer → PCR of cDNA
- Identify specific gene expression in specific cells/tissues

RT-qPCR

- **Reverse Transcriptase Quantitative PCR — "Real-time PCR"**



RT-PCR for COVID-19 Diagnosis