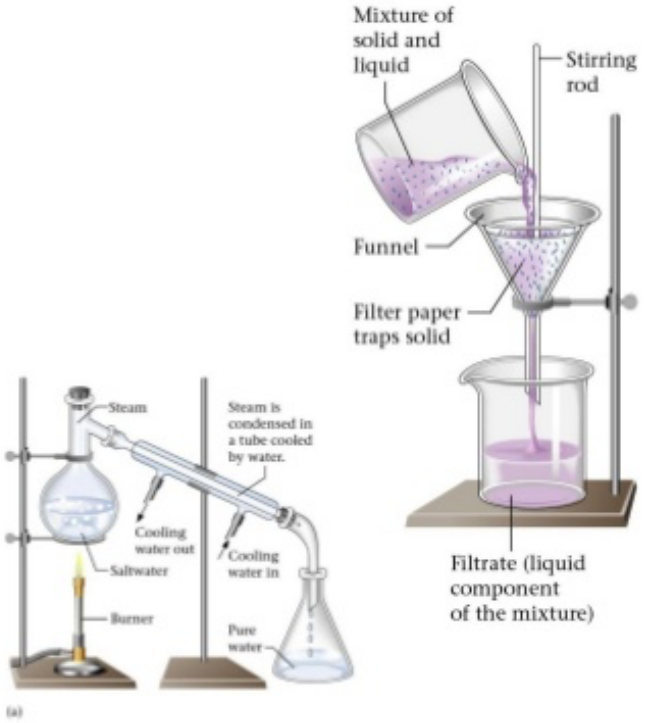
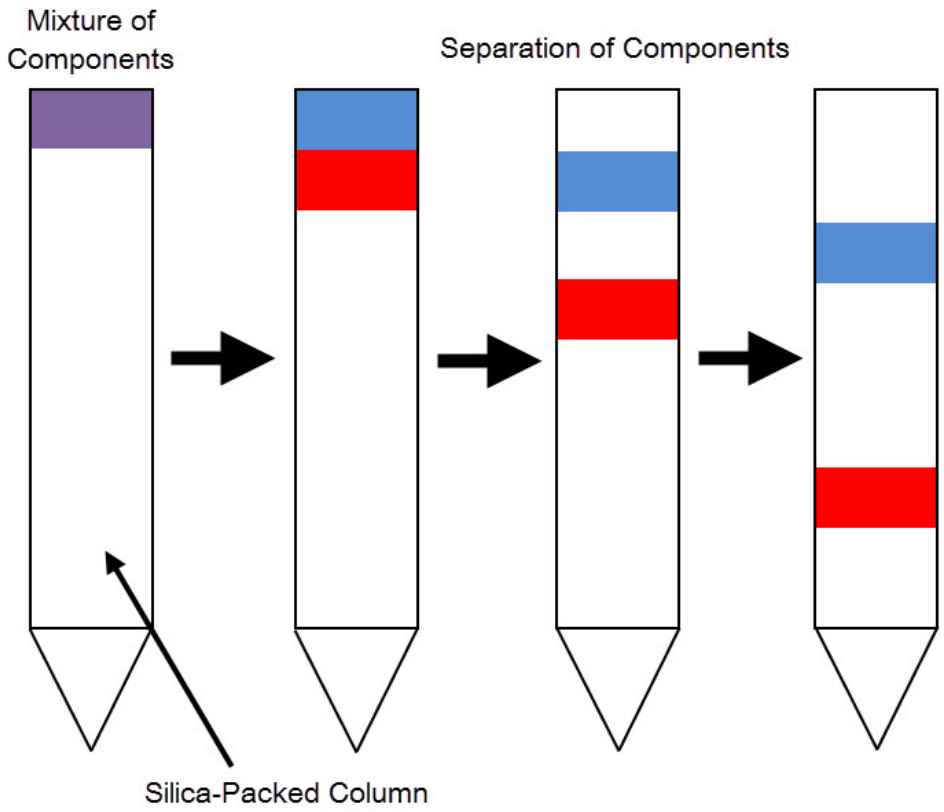
Methods of Separating Mixtures

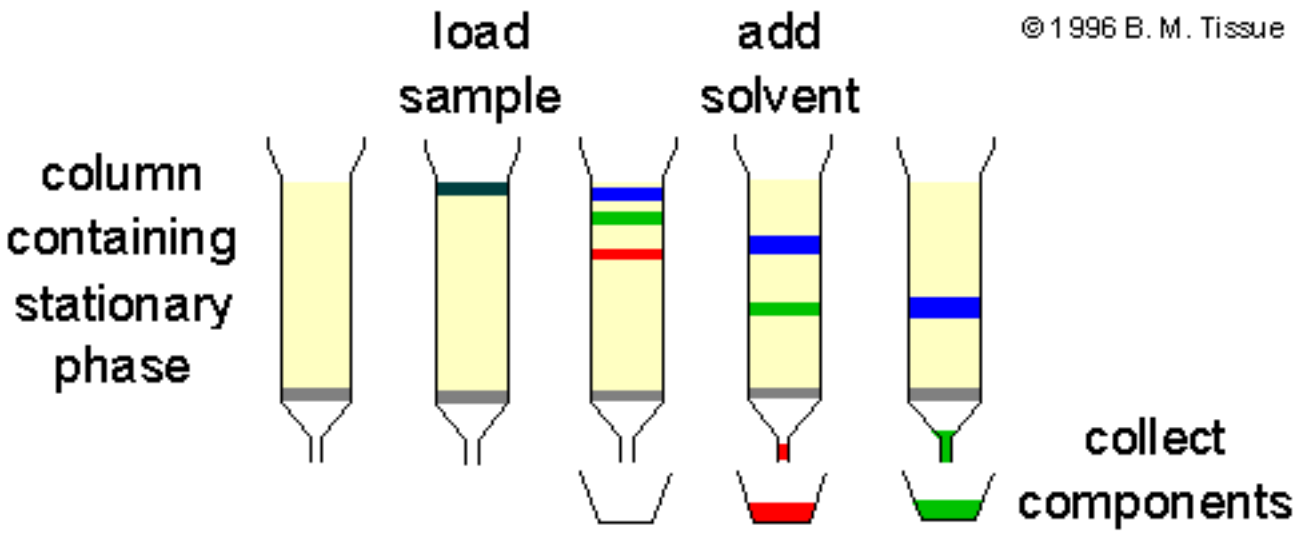
- Magnet
- Filter
- Decant
- Evaporation
- Centrifuge
- Chromatography
- Distillation



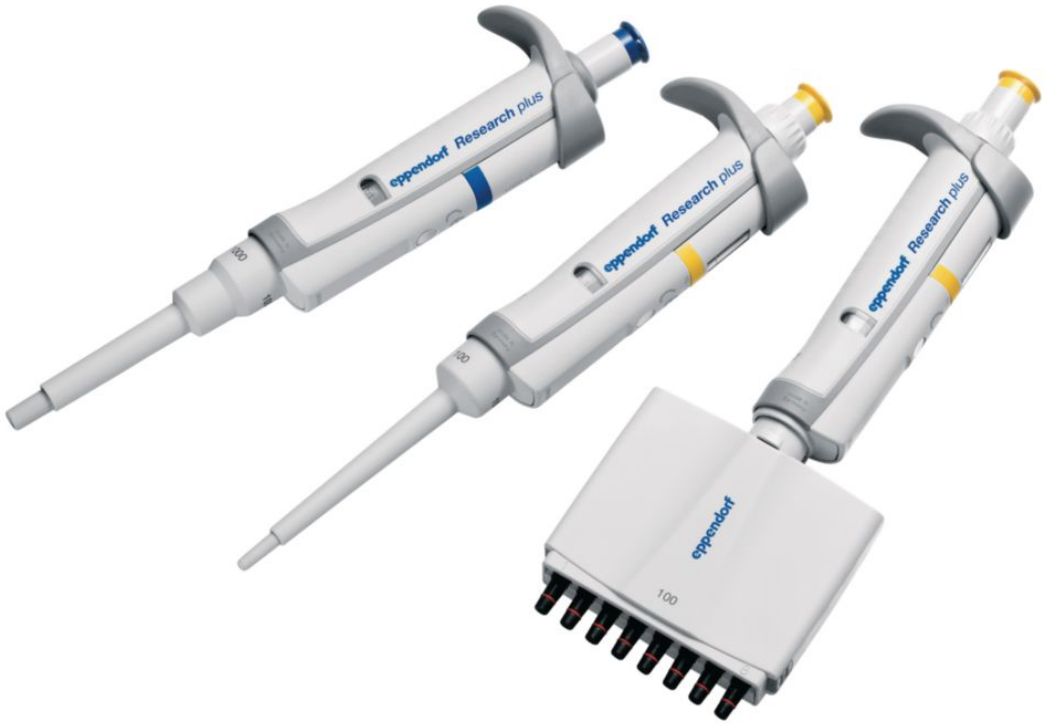
Swing-out centrifuge

Microfuge



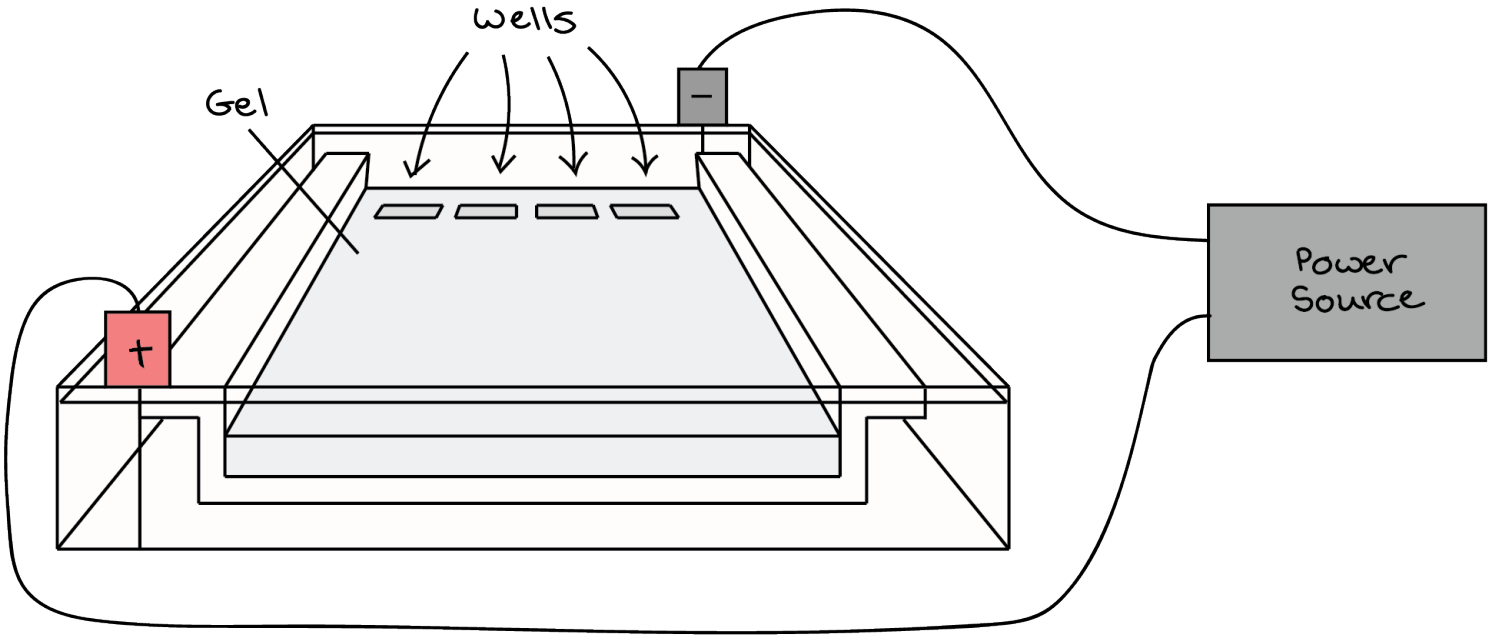


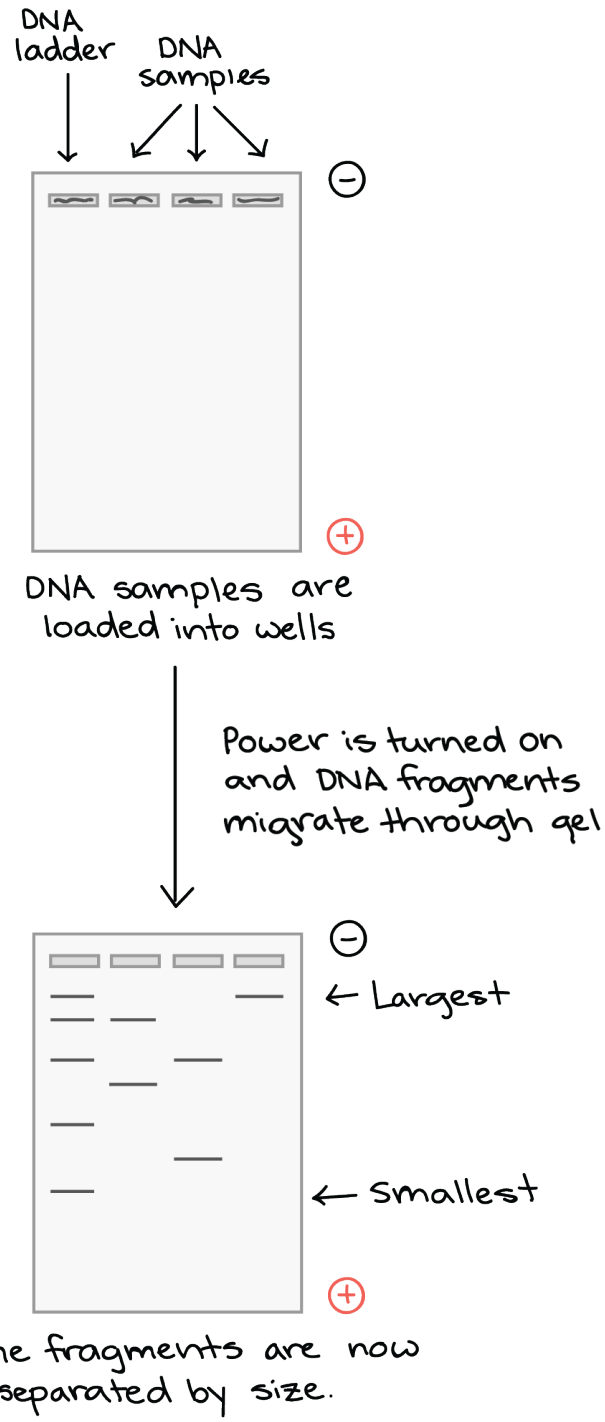
Liquid transfer with micropipets



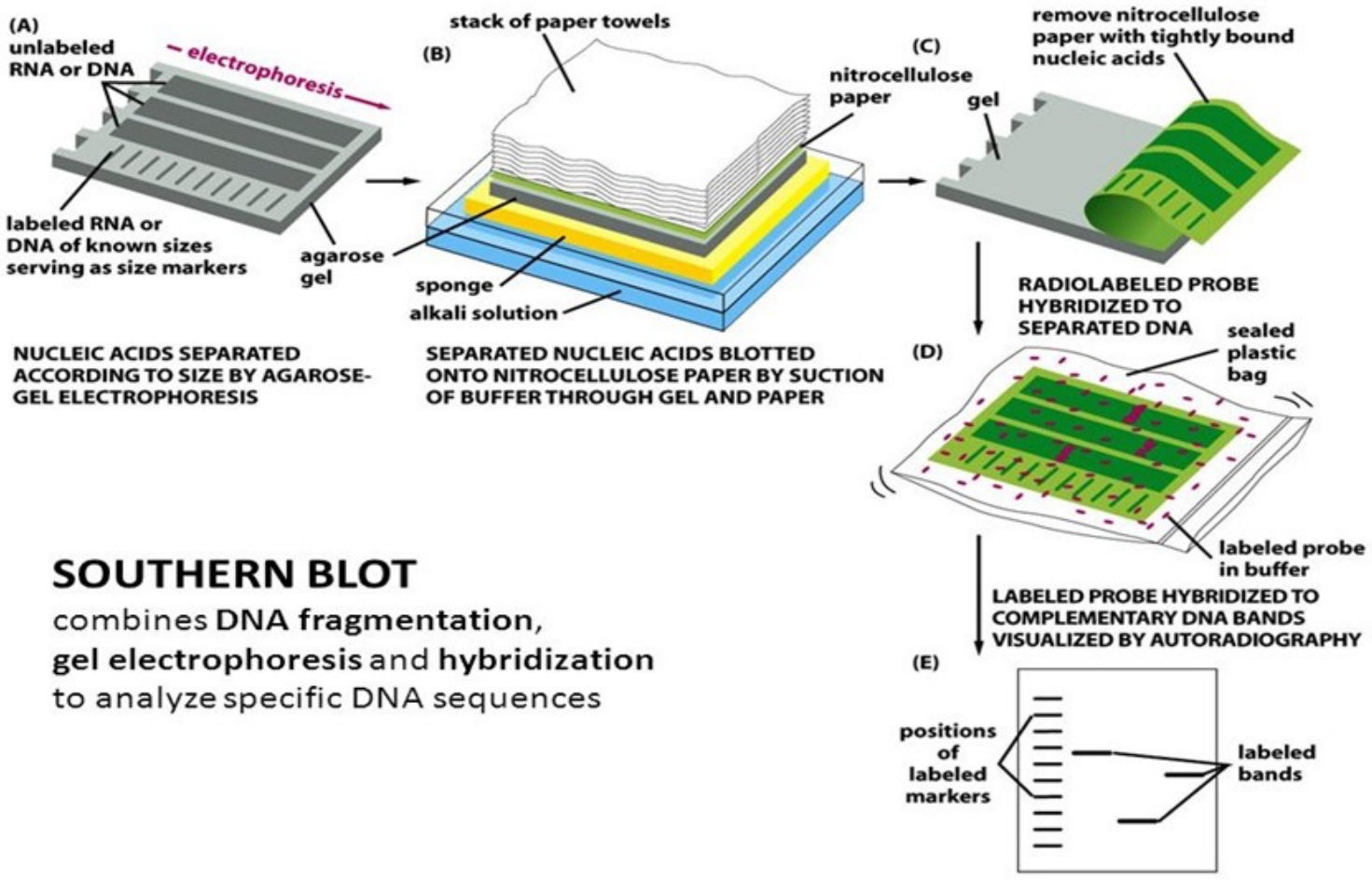
Micropipets multi vs single channel

Micropipetting techniques


Wells
Gel
−
+
Power Source


DNA ladder
DNA samples
DNA samples are loaded into wells
Power is turned on and DNA fragments migrate through gel
Largest
Smallest
The fragments are now separated by size.

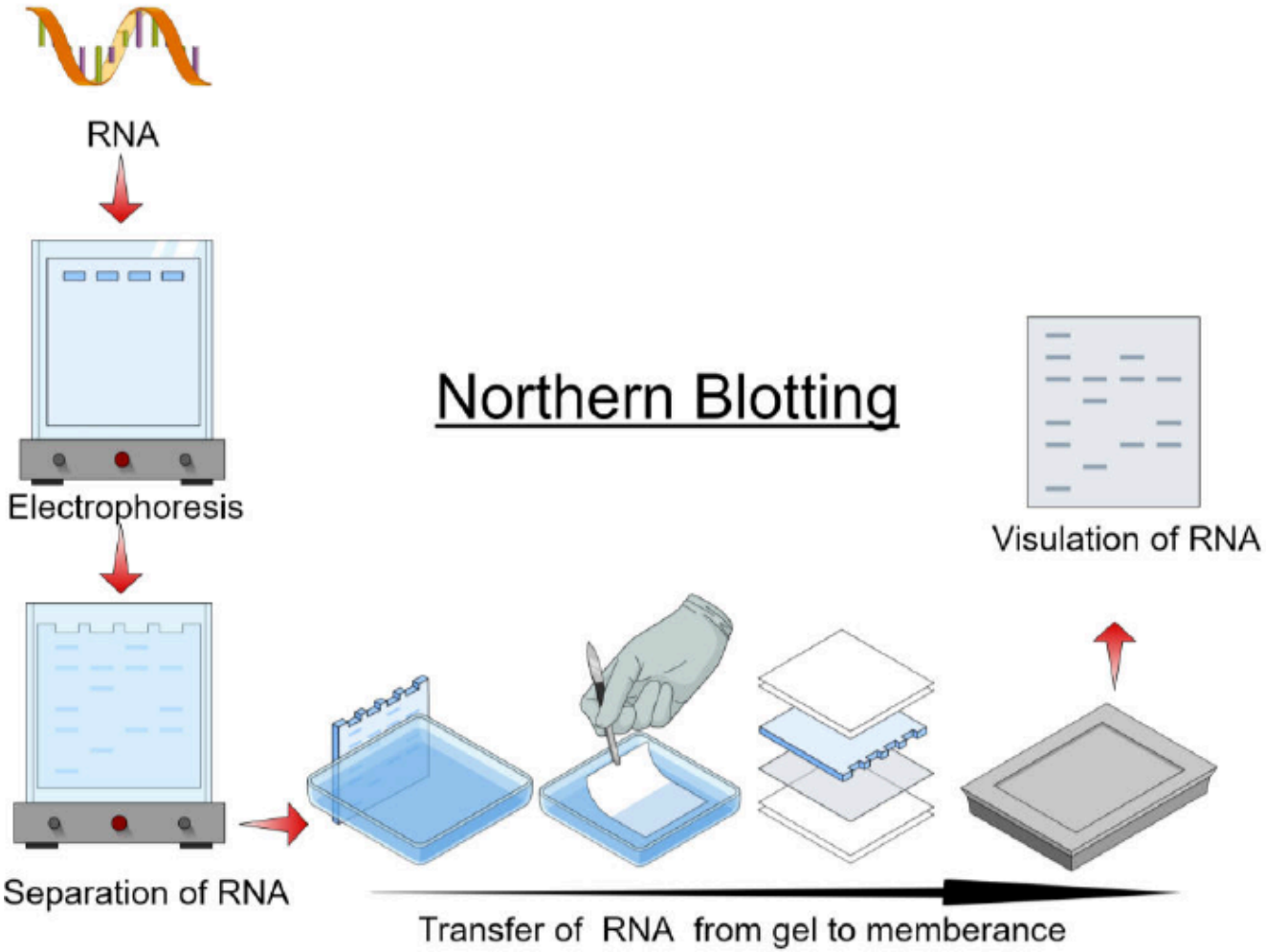
Southern Blotting Technique

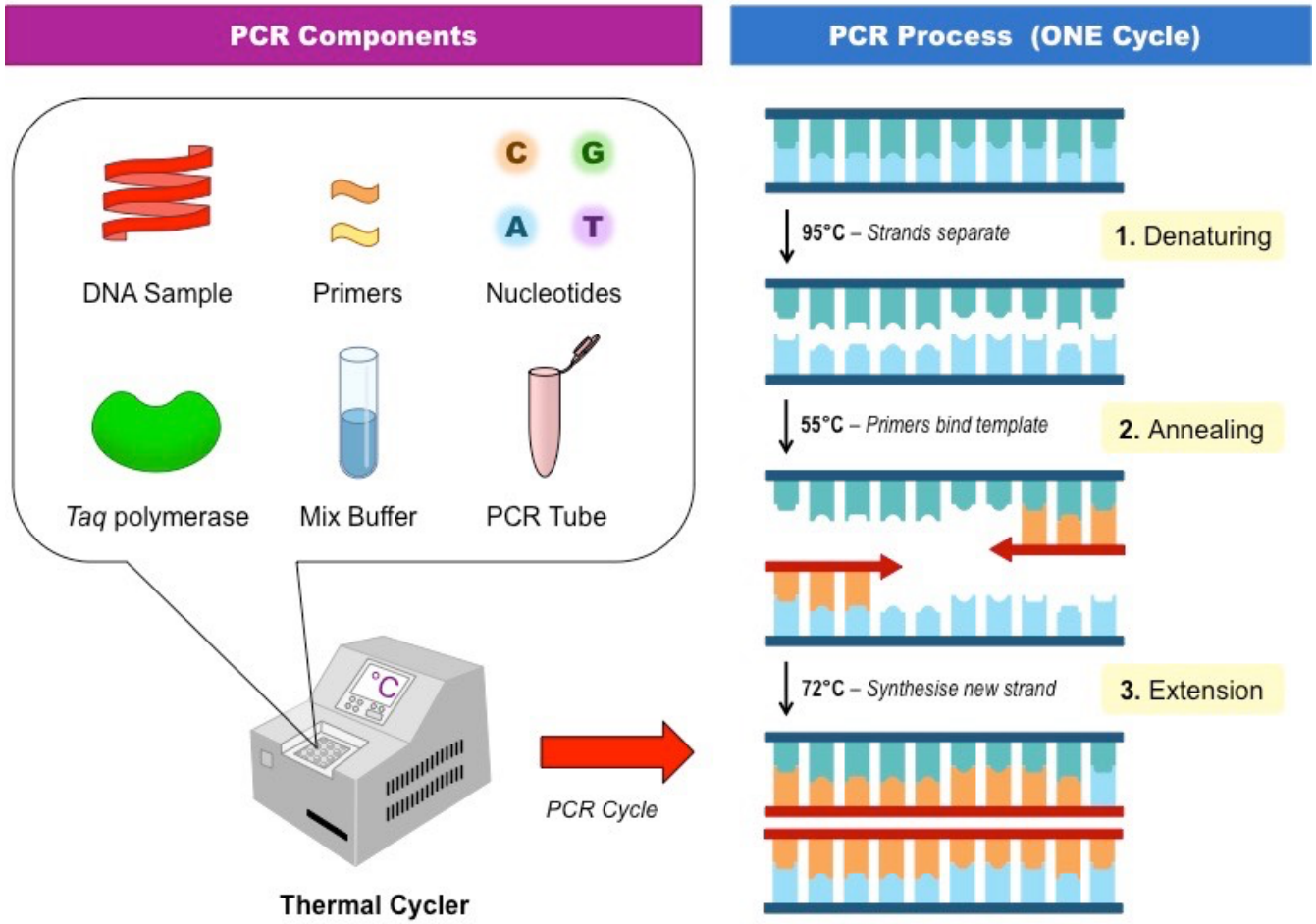


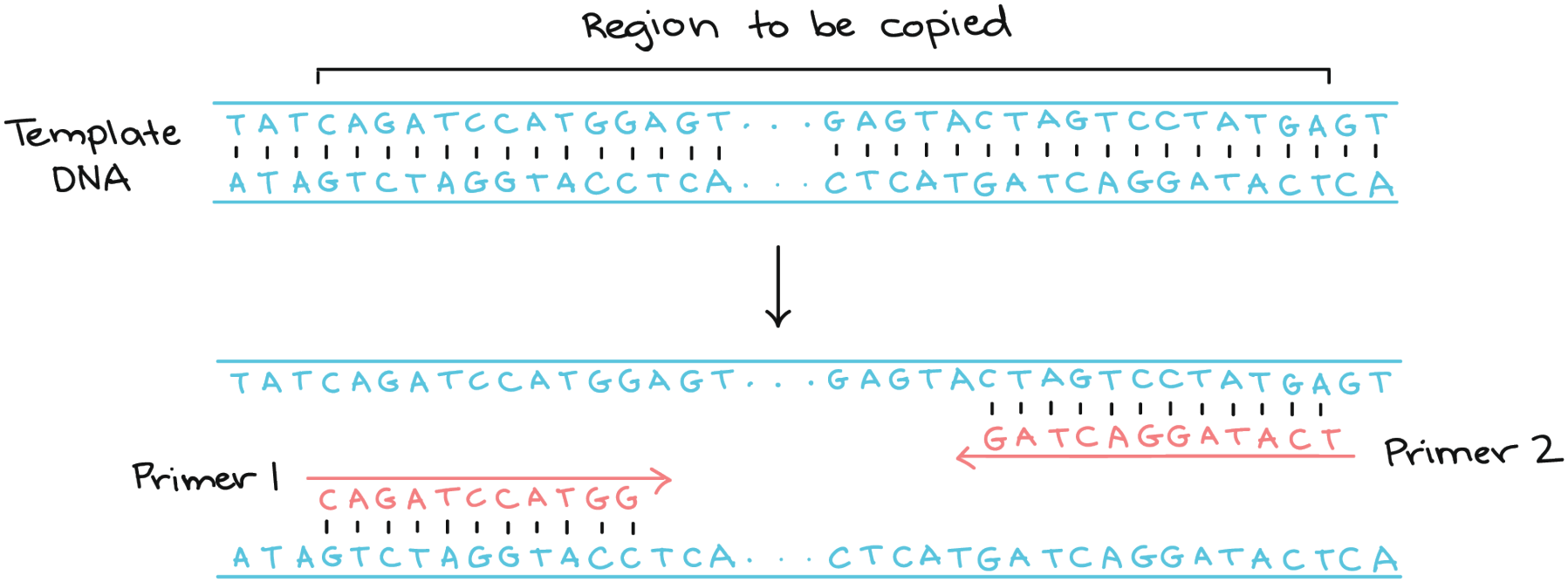
SOUTHERN BLOT

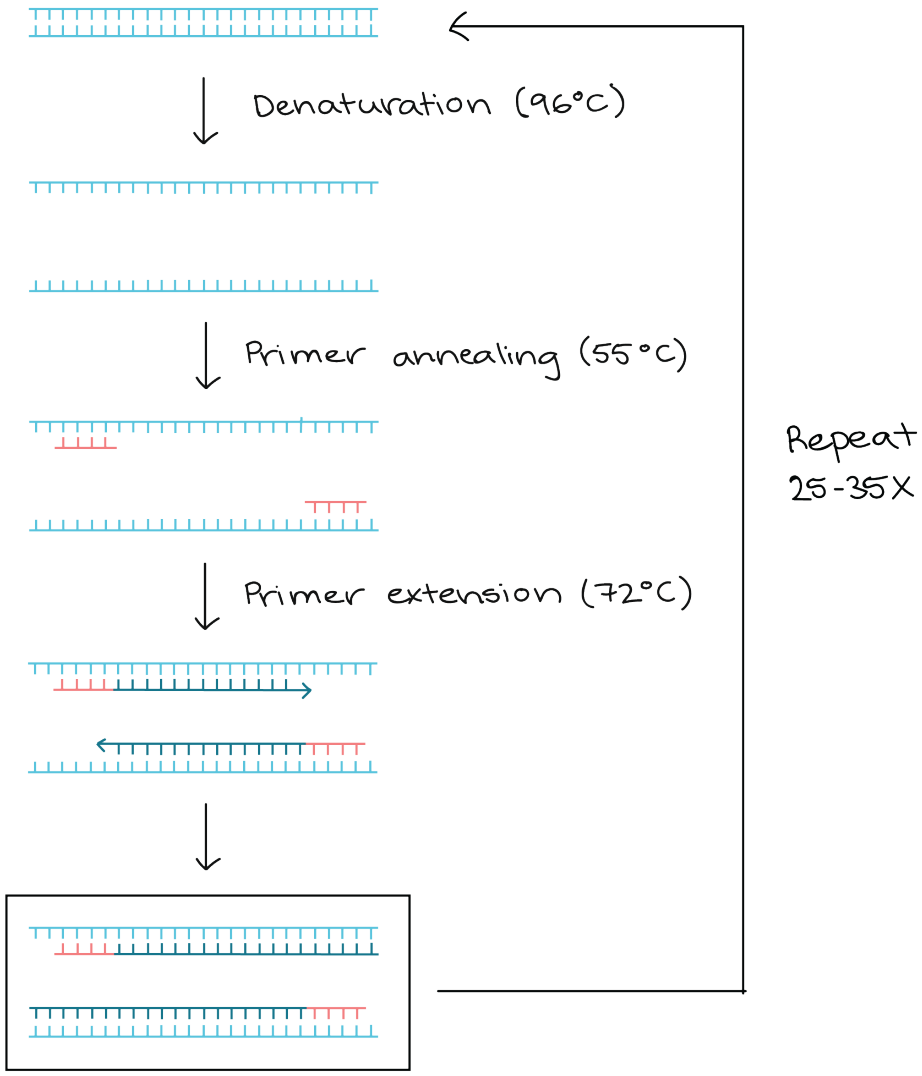
combines **DNA fragmentation**, **gel electrophoresis** and **hybridization** to analyze specific DNA sequences

Northern Blotting

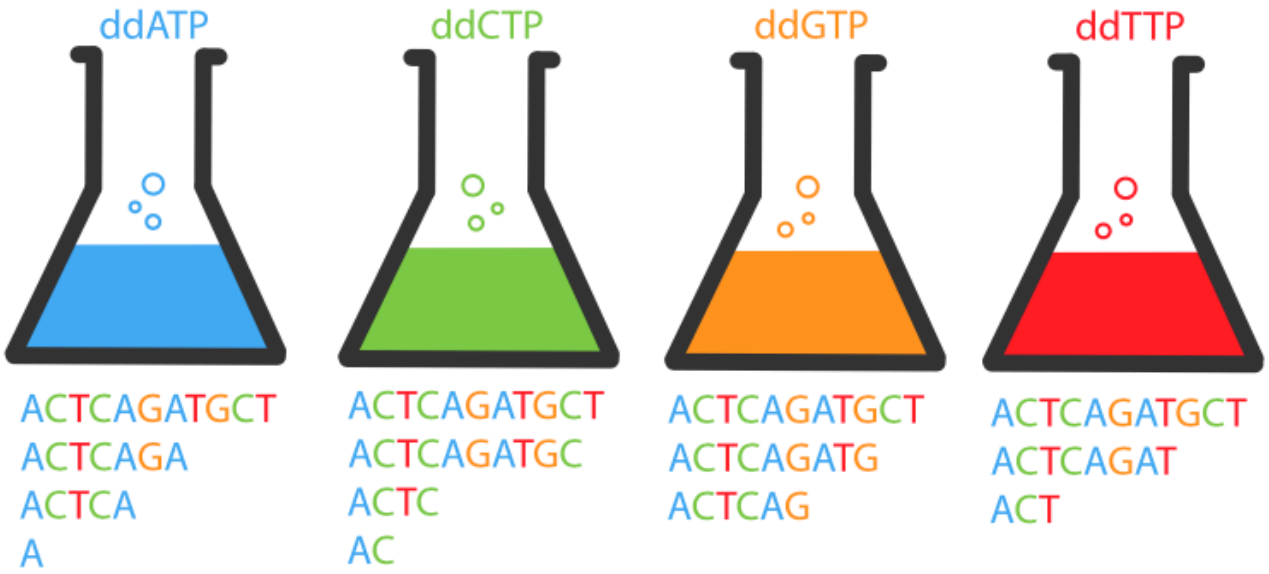



PCR Components
DNA Sample
Primers
C
G
A
T
Nucleotides
Taq polymerase
Mix Buffer
PCR Tube
°C
Thermal Cycler
PCR Cycle
PCR Process (ONE Cycle)
95°C – Strands separate
1. Denaturing
55°C – Primers bind template
2. Annealing
72°C – Synthesise new strand
3. Extension

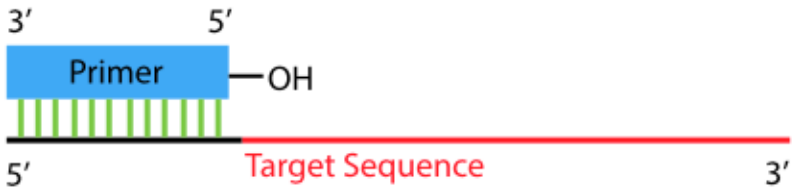

Region to be copied
Template DNA
TATCAGATCCATGGAGT. . .GAGTACTAGTCCTATGAGT
ATAGTCTAGGTACCTCA. . .CTCATGATCAGGATACTCA
TATCAGATCCATGGAGT. . .GAGTACTAGTCCTATGAGT
GATCAGGATACT
Primer 2
Primer 1
CAGATCCATGG
ATAGTCTAGGTACCTCA. . .CTCATGATCAGGATACTCA


Denaturation (96°C)
Primer annealing (55°C)
Primer extension (72°C)
Repeat
25-35X
Result after 1 cycle:
# of DNA molecules
doubled

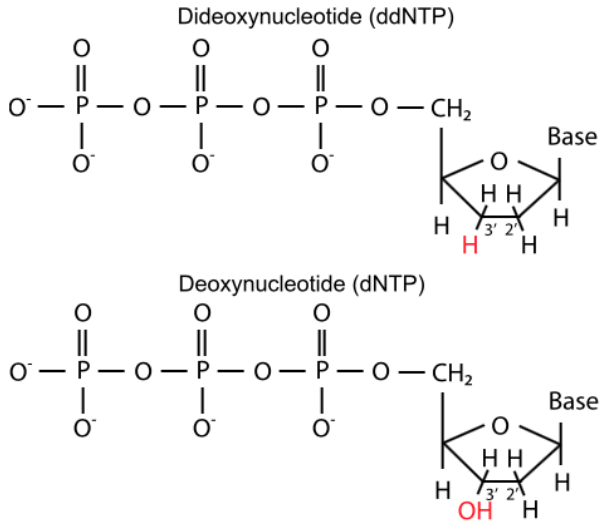
dATP + dCTP + dGTP + dTTP
DNA Polymerase
Template DNA
Primer



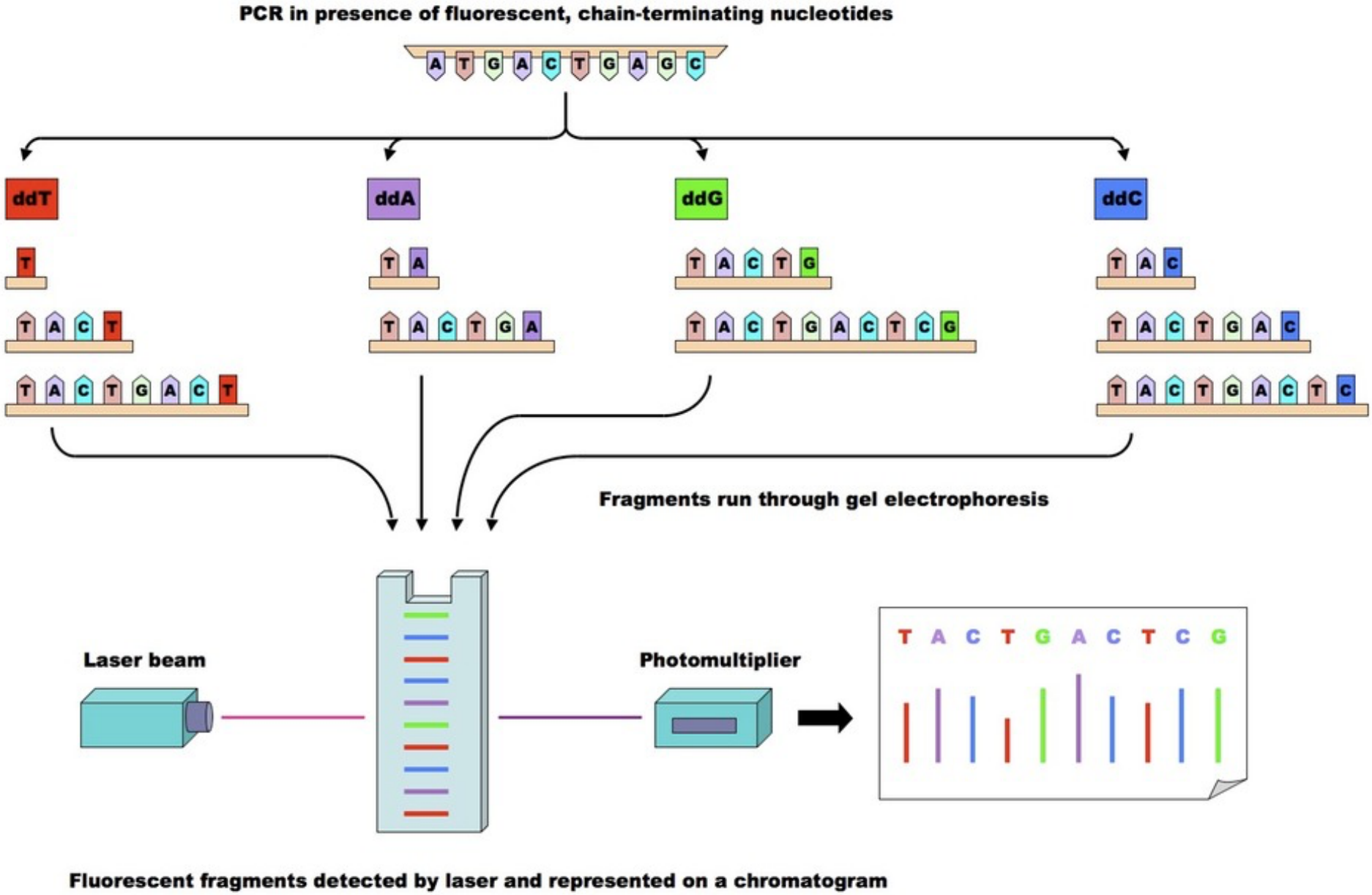
Since ddNTP is added, some of the strands cannot be elongated any further. Note that these colors are for illustrative purposes - they do not mean that each dNTP is fluorescently labeled.

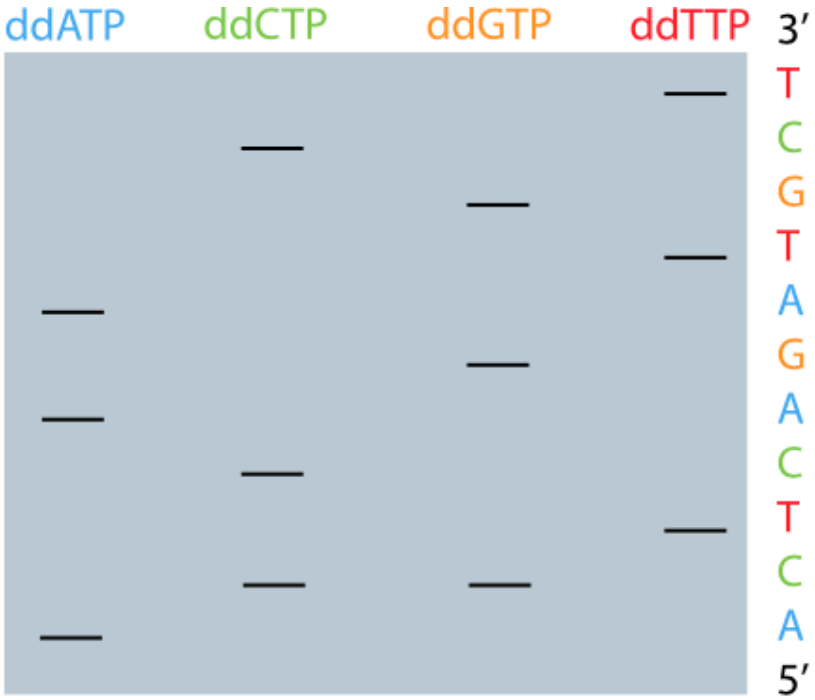


An oligonucleotide is annealed, and a primer is attached. The 5'-OH group allows for DNA elongation.



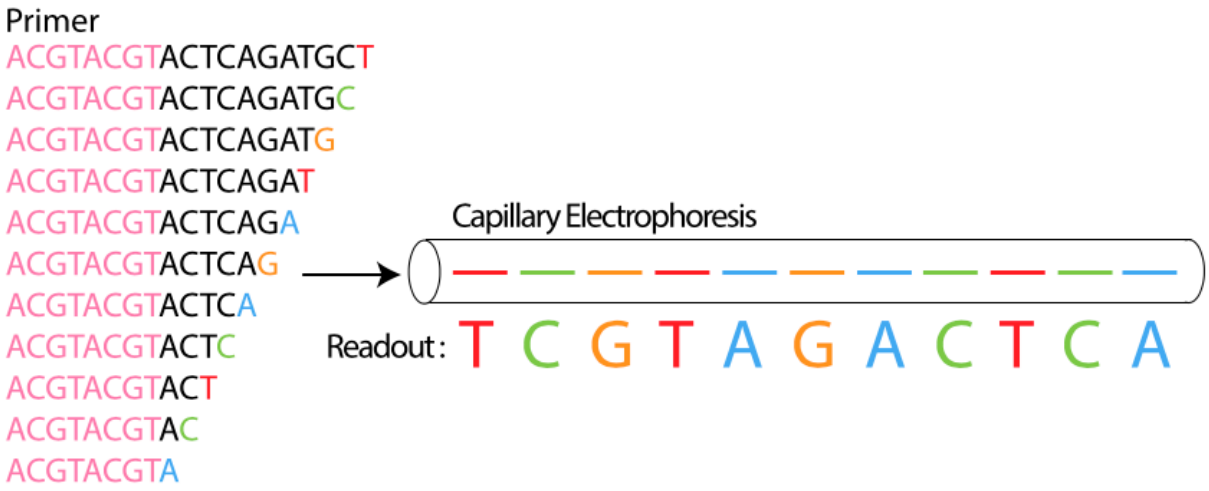
A comparison of the ddNTP and dNTP molecules.


PCR in presence of fluorescent, chain-terminating nucleotides
A T G A C T G A G C
ddT
ddA
ddG
ddC
T
T A
T A C
T A C T
T A C T G
T A C
T A C T G A
T A C T G A C T C G
T A C T G A C
T A C T G A C T
T A C T G A C T C
Fragments run through gel electrophoresis
Laser beam
Photomultiplier
T A C T G A C T C G
Fluorescent fragments detected by laser and represented on a chromatogram



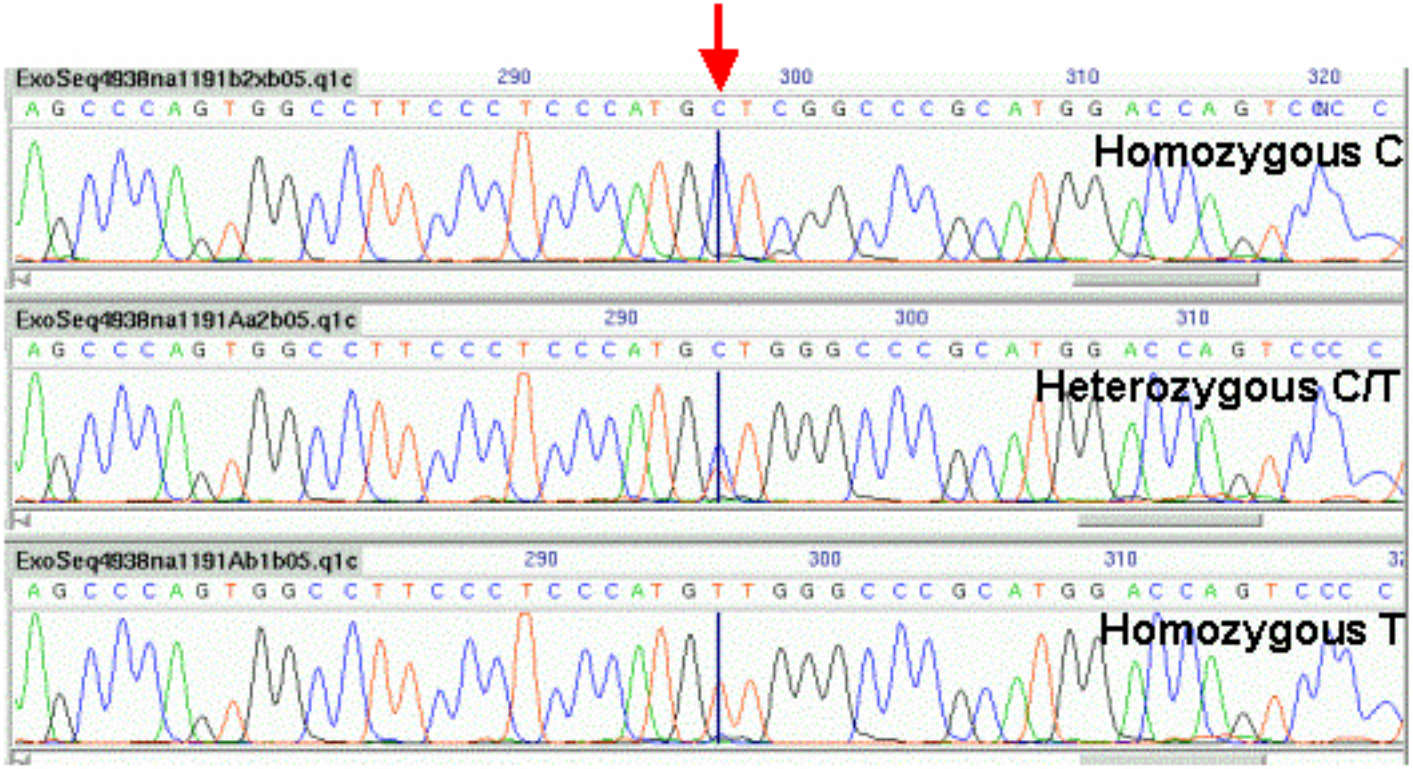
Gel Electrophoresis

Smaller strands migrate to the bottom, while larger strands stay up top. We can read each molecule in order to find the DNA sequence.



The fluorescently-labeled DNA sequences are run through capillary electrophoresis and their order is resolved by color.

Capillary Electrophoresis Machines


ExoSeq4938na1191b2xb05.q1c
290
300
310
320
Homozygous C
ExoSeq4938na1191Aa2b05.q1c
290
300
310
Heterozygous C/T
ExoSeq4938na1191Ab1b05.q1c
290
300
310
Homozygous T

Sanger di deoxy sequencing
Polyacrylamide Gel Electrophoresis