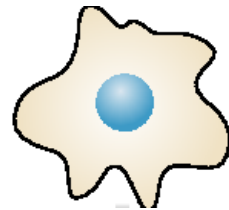
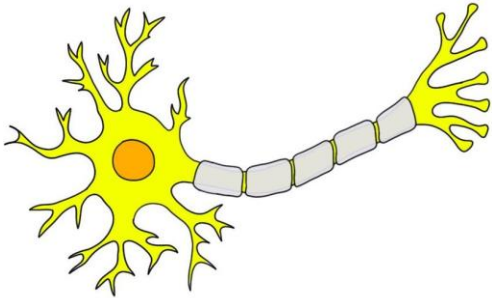


Molecular Biology Techniques

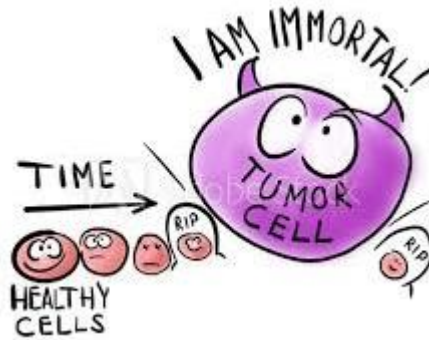
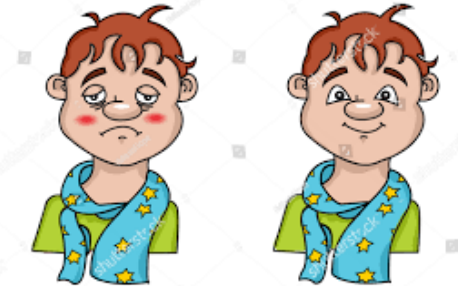
Majid Lotfinia
Pharmaceutical biotechnology, PhD
majid.lotfinia@gmail.com

Differences & Changes in Biology

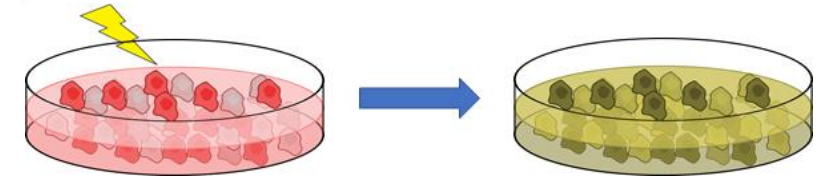
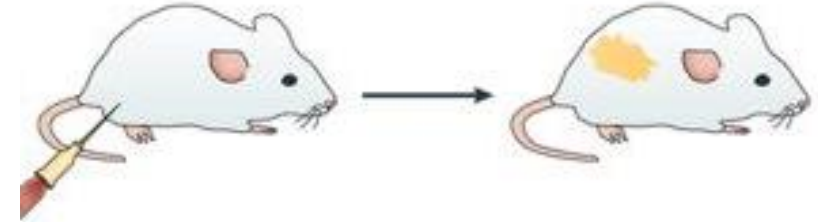
Cell types Brain vs Liver



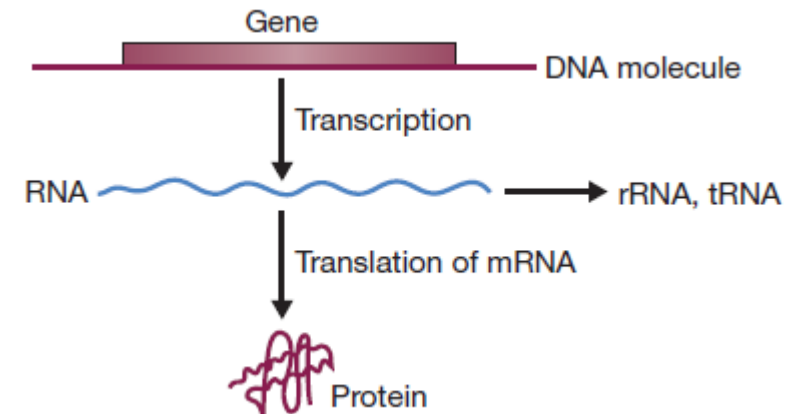
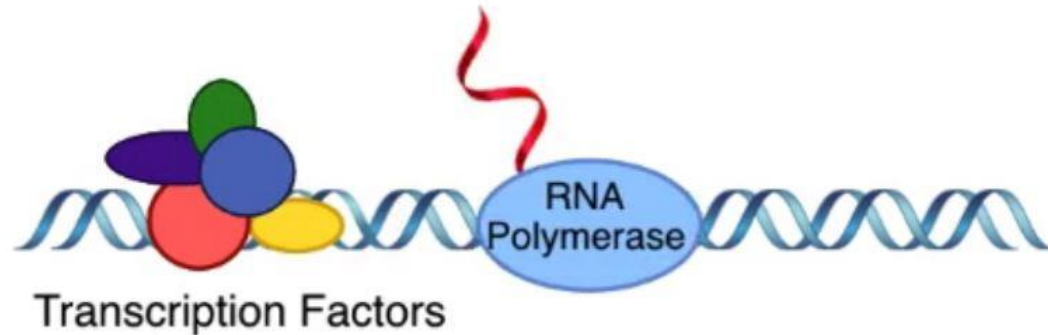
Disease vs Normal



Treatments Before vs After



Gene regulation



Molecular Biology Methods

Central dogma

Techniques

PCR-sequencing

PCR-RFLP

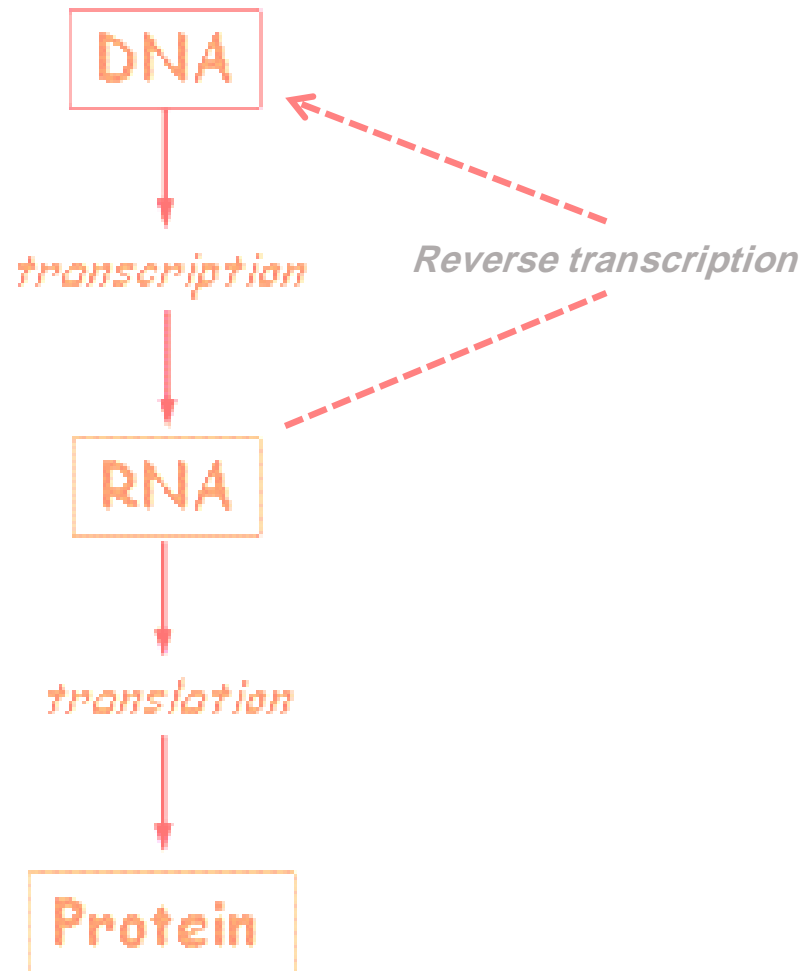
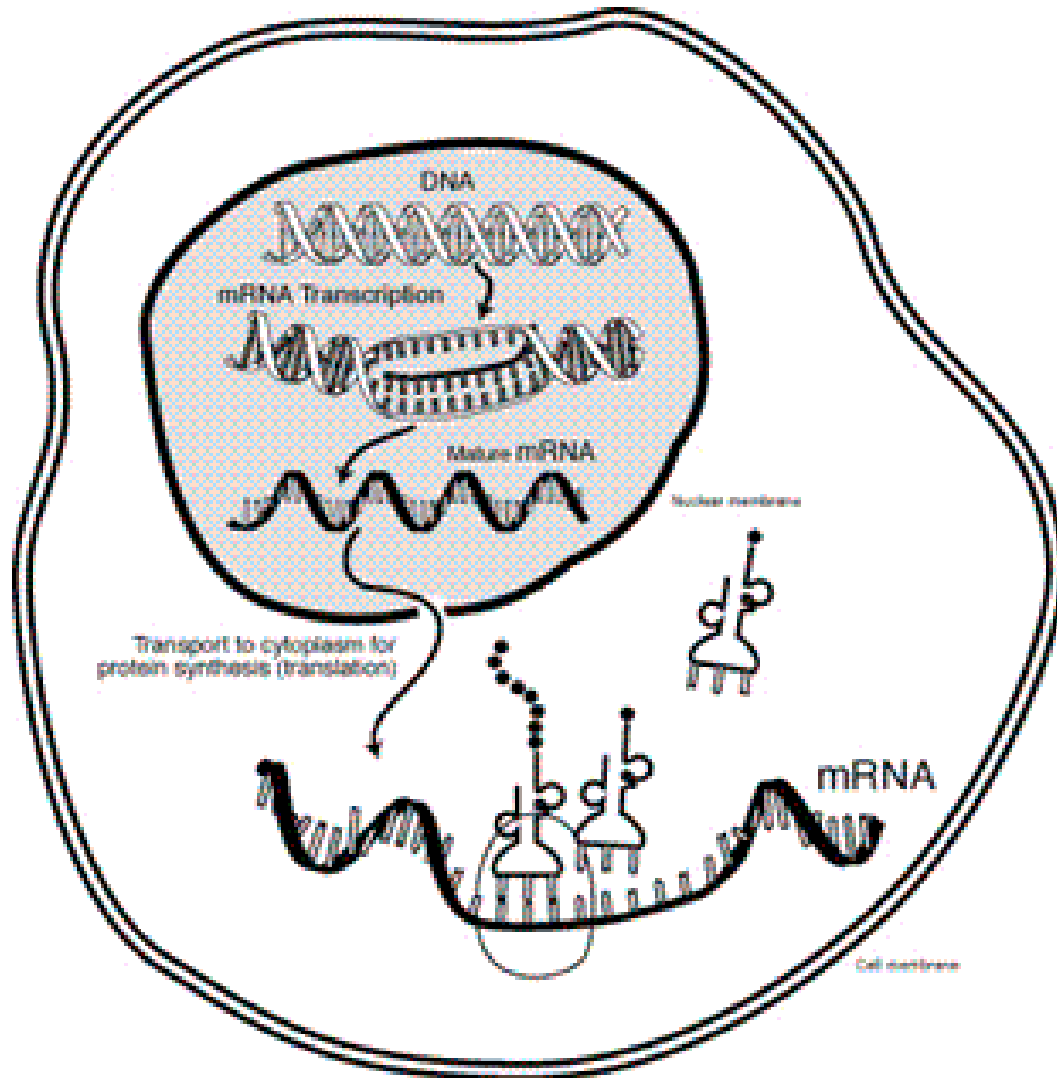
ARMS-PCR

PCR-HRM

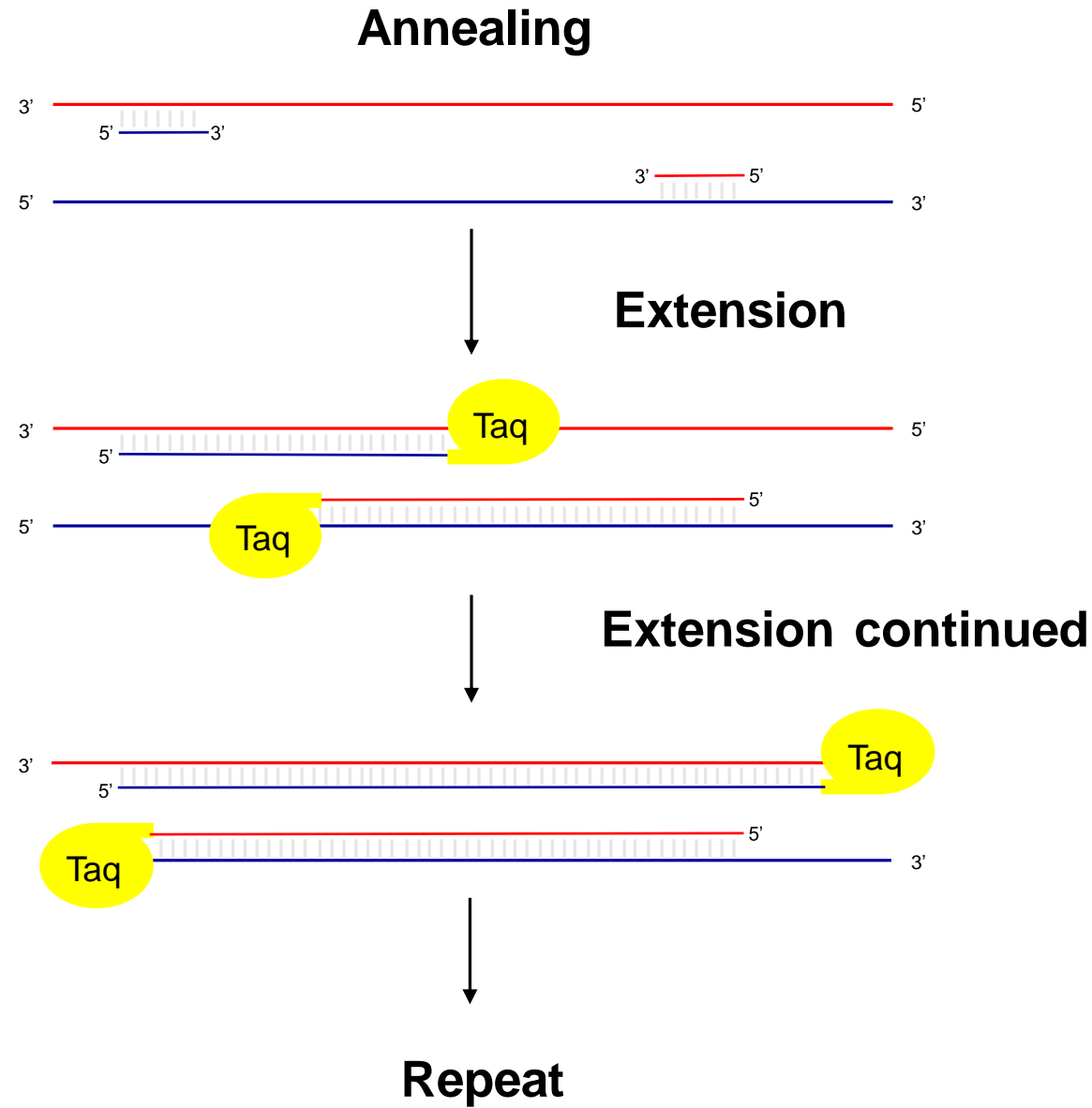
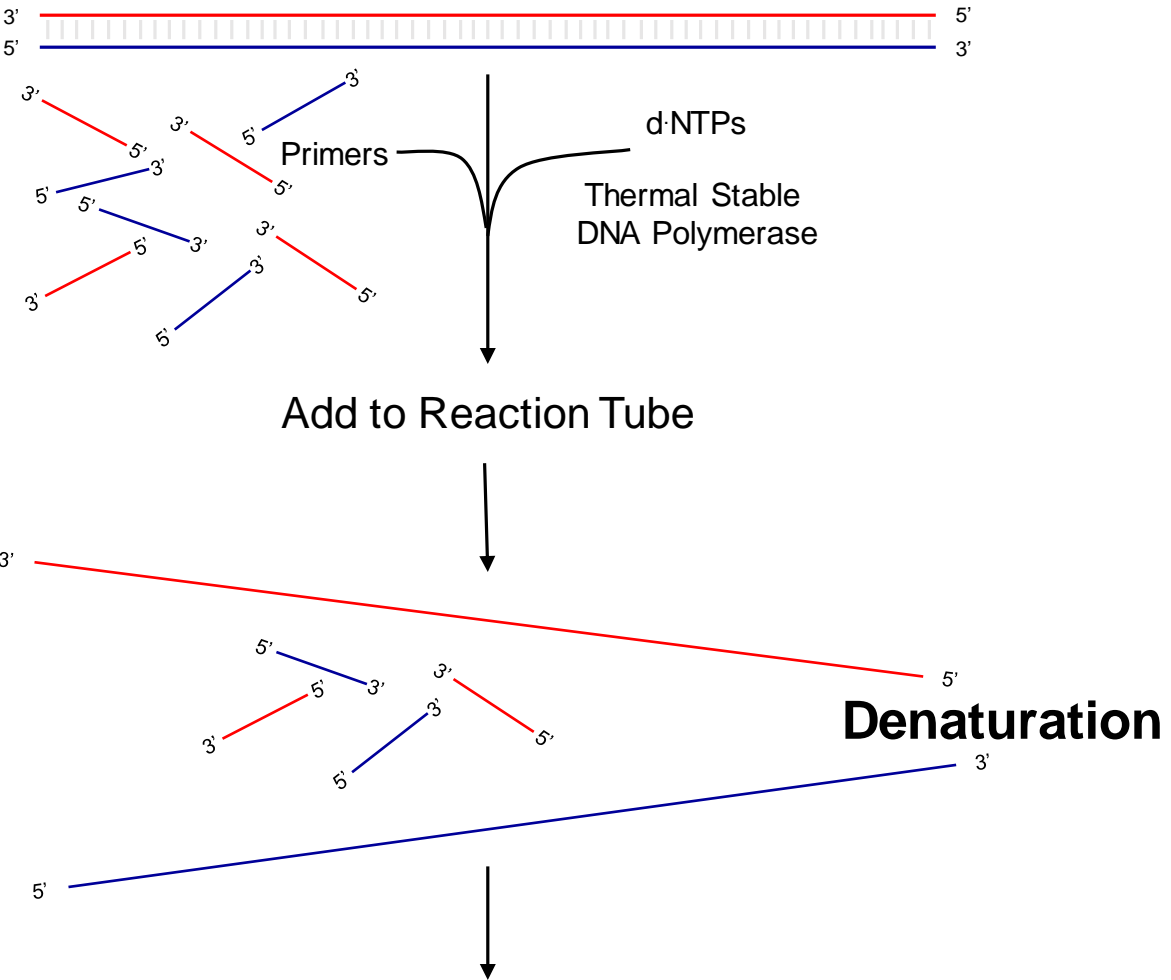
TaqMan

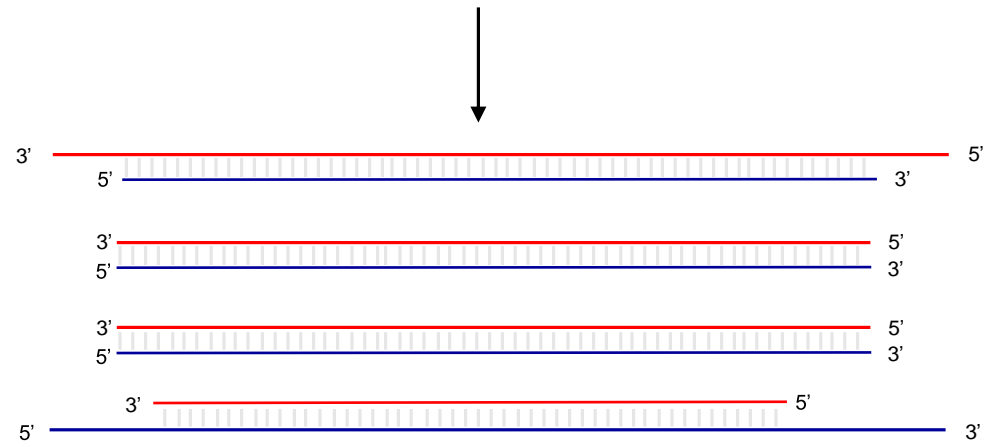
Real-time qPCR

Western blot



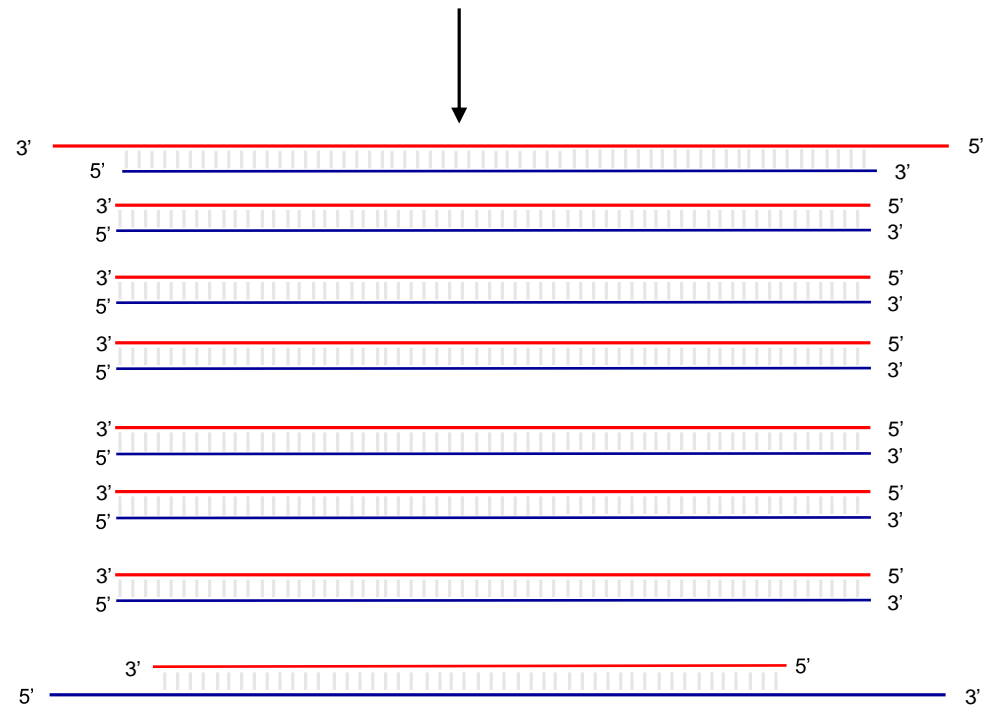
Polymerase Chain Reaction (PCR)





Cycle 2

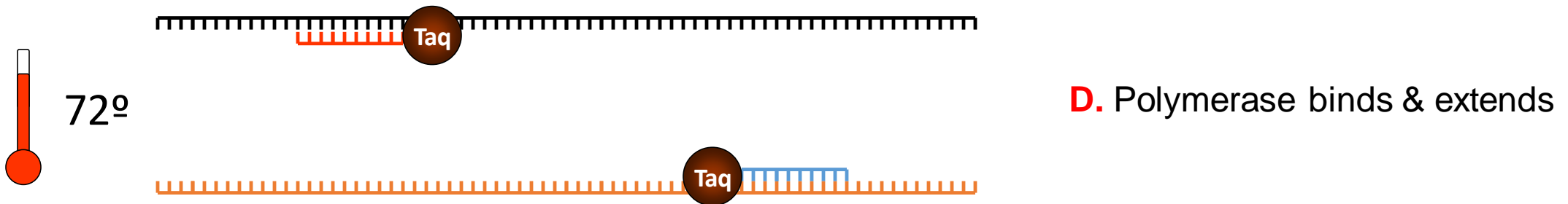
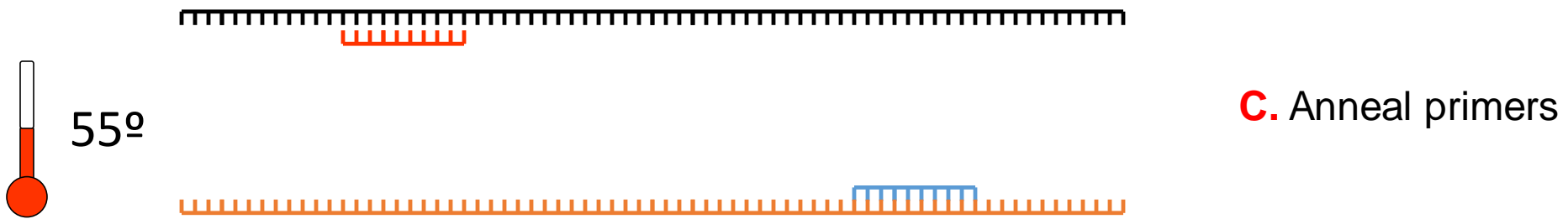
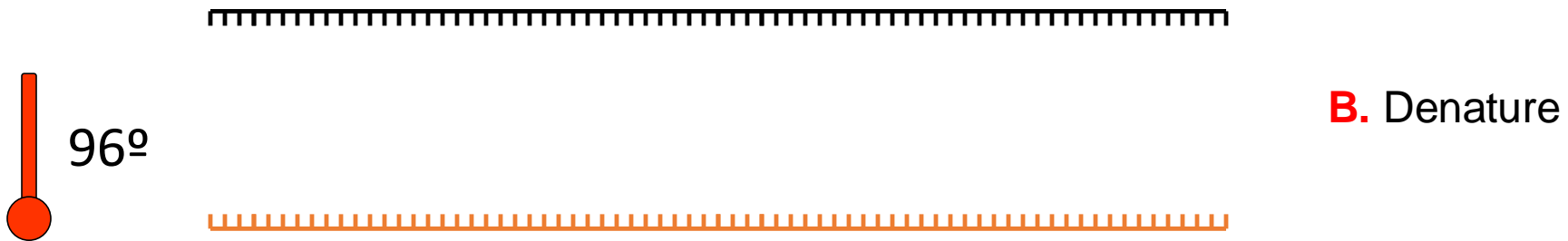
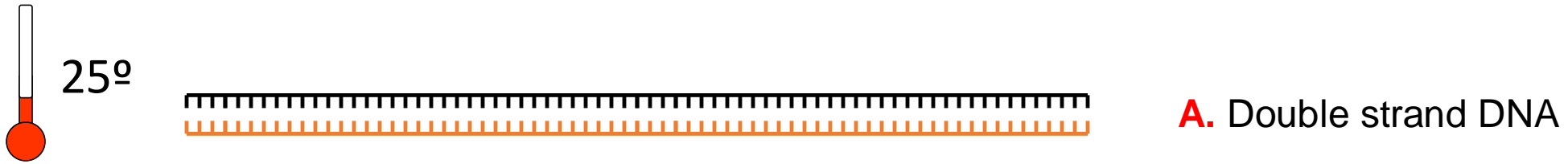
4 Copies



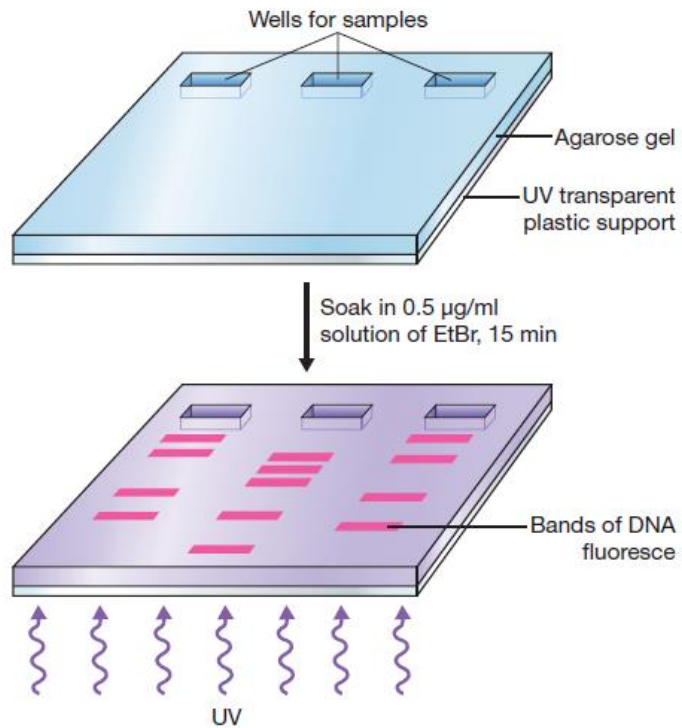
Cycle 3

8 Copies

Thermal Cycles

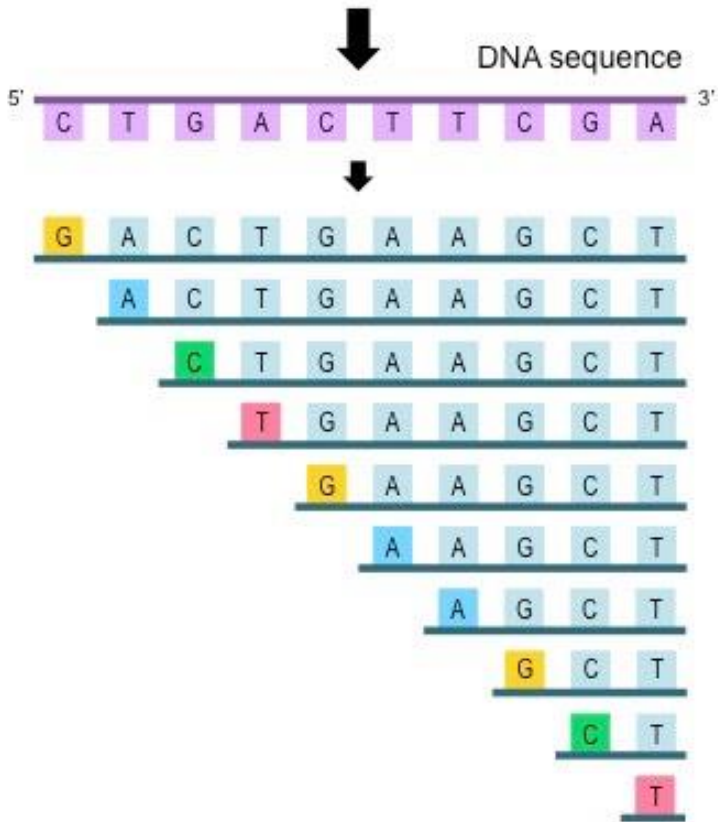
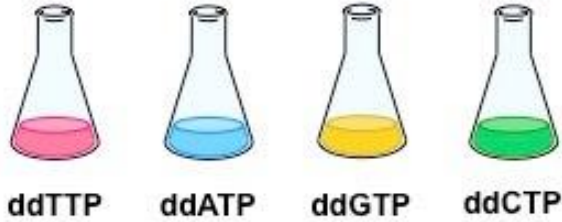


PCR Product Visualization

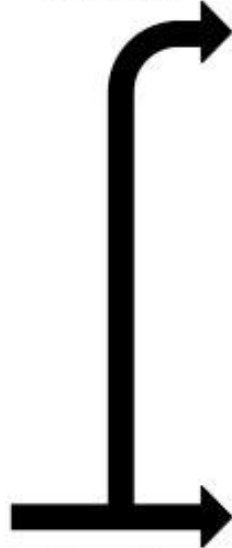
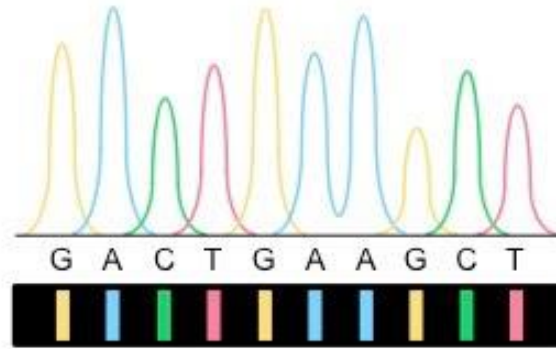


DNA Sequencing

4 × PCR (+ one dideoxynucleotide)



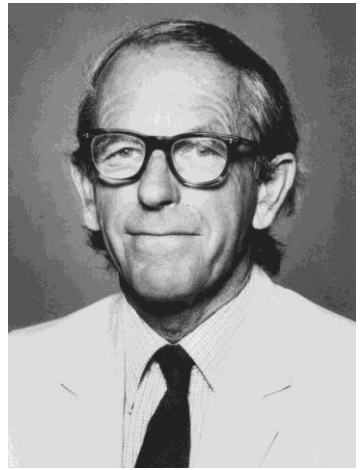
Use a sequencing machine



Separate with a gel

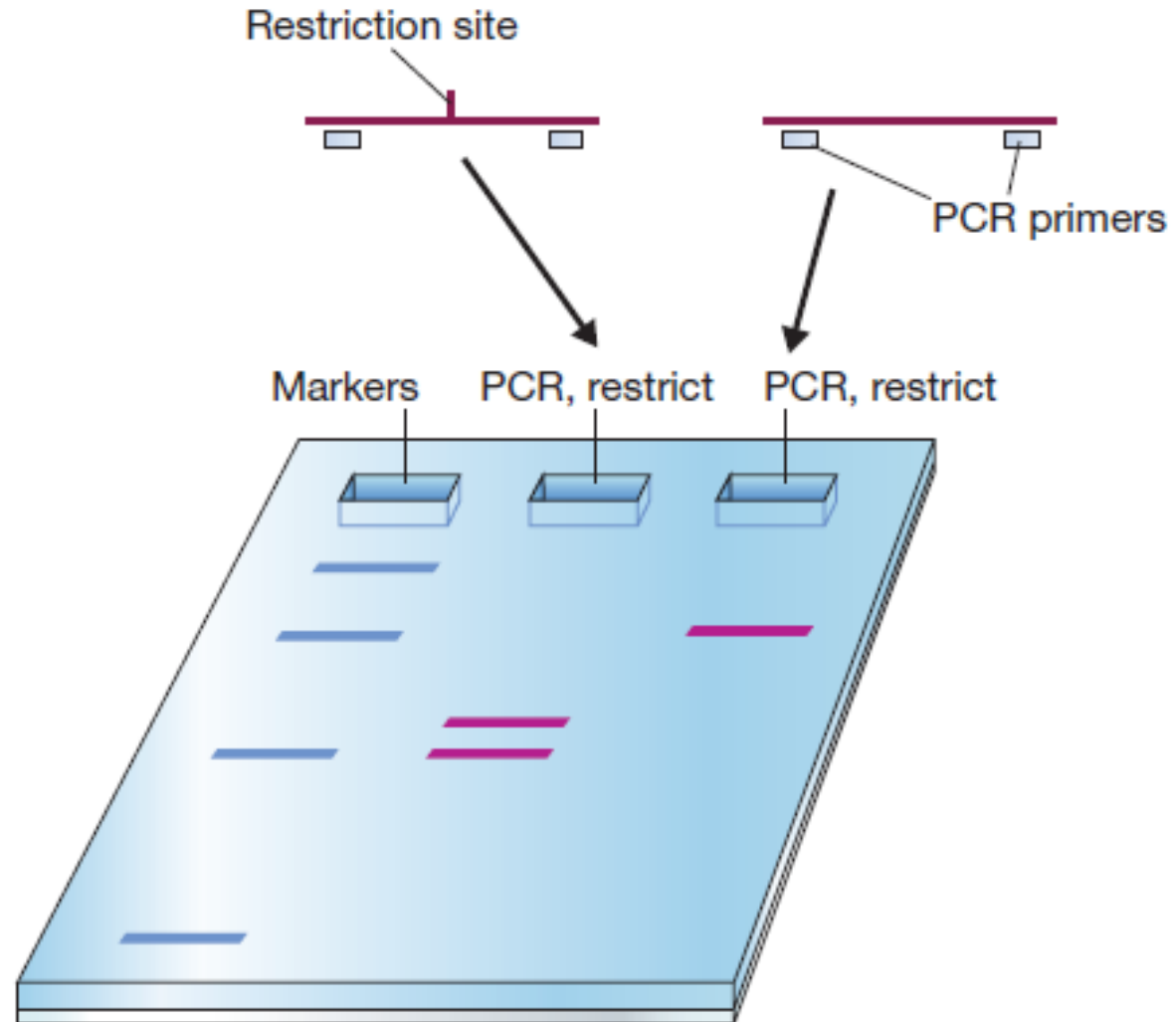


...ATAGAC**C**ATGGCAA...
 ...ATAGAC**T**ATGGCAA...
 |
 SNP



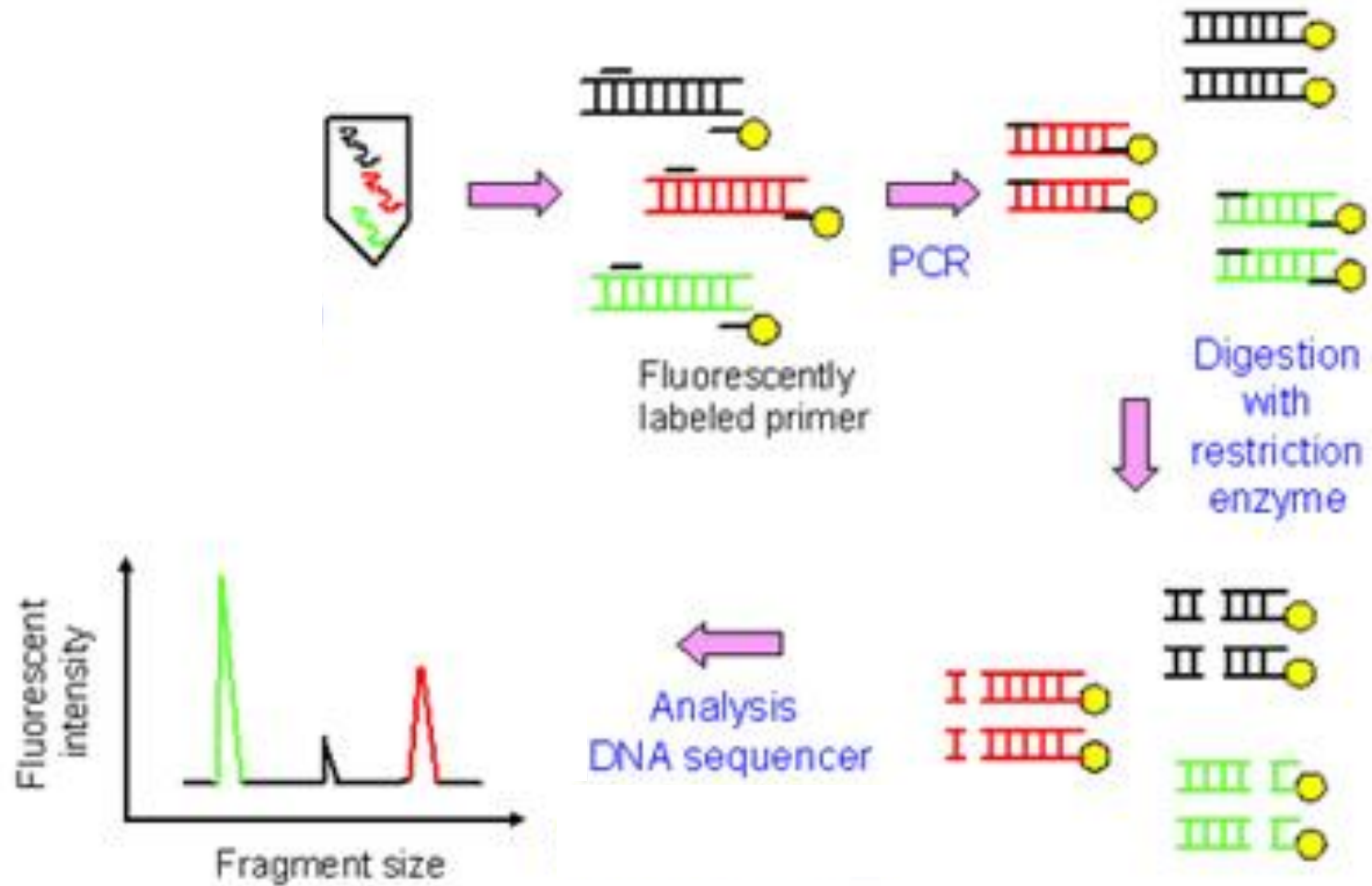
Frederick Sanger

Conventional PCR-RFLP



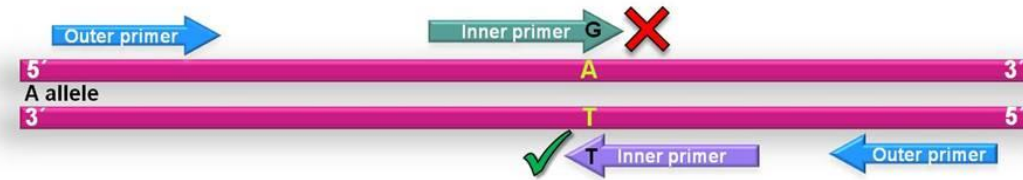
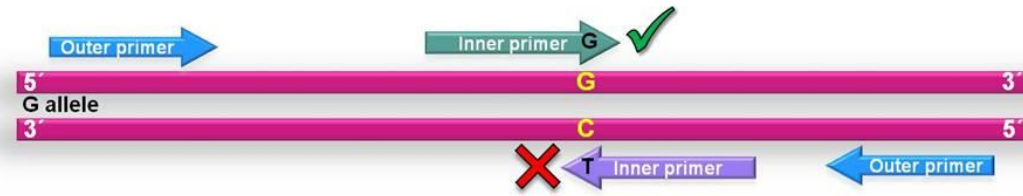
PCR-RFLP (New)

T-RFLP analysis

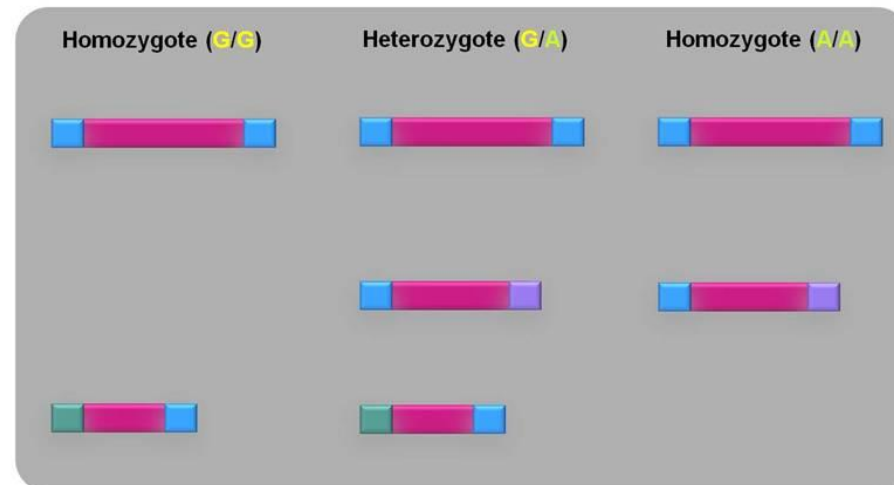


T-RFLP : Terminal Restriction Fragment Length Polymorphism

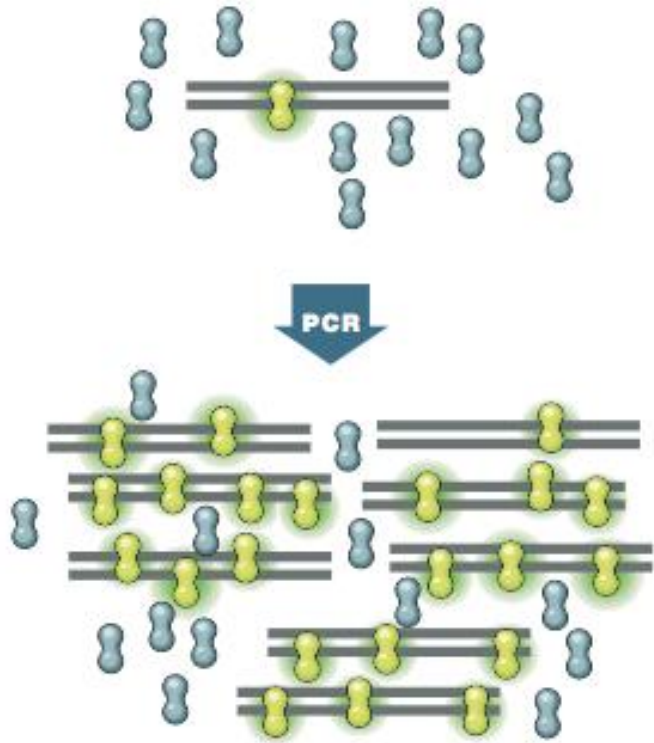
ARMS



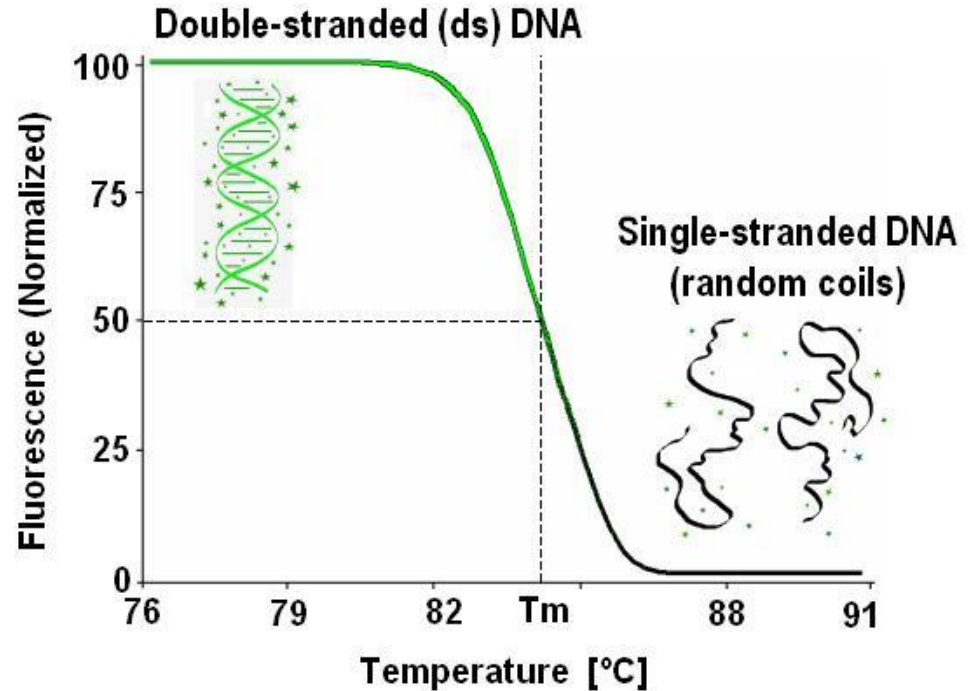
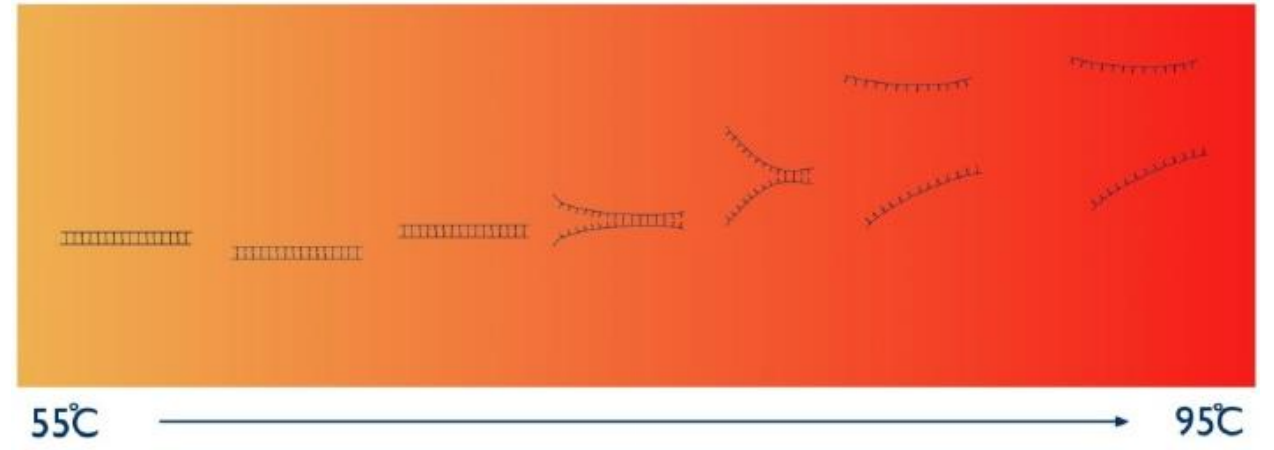
Gel electrophoresis



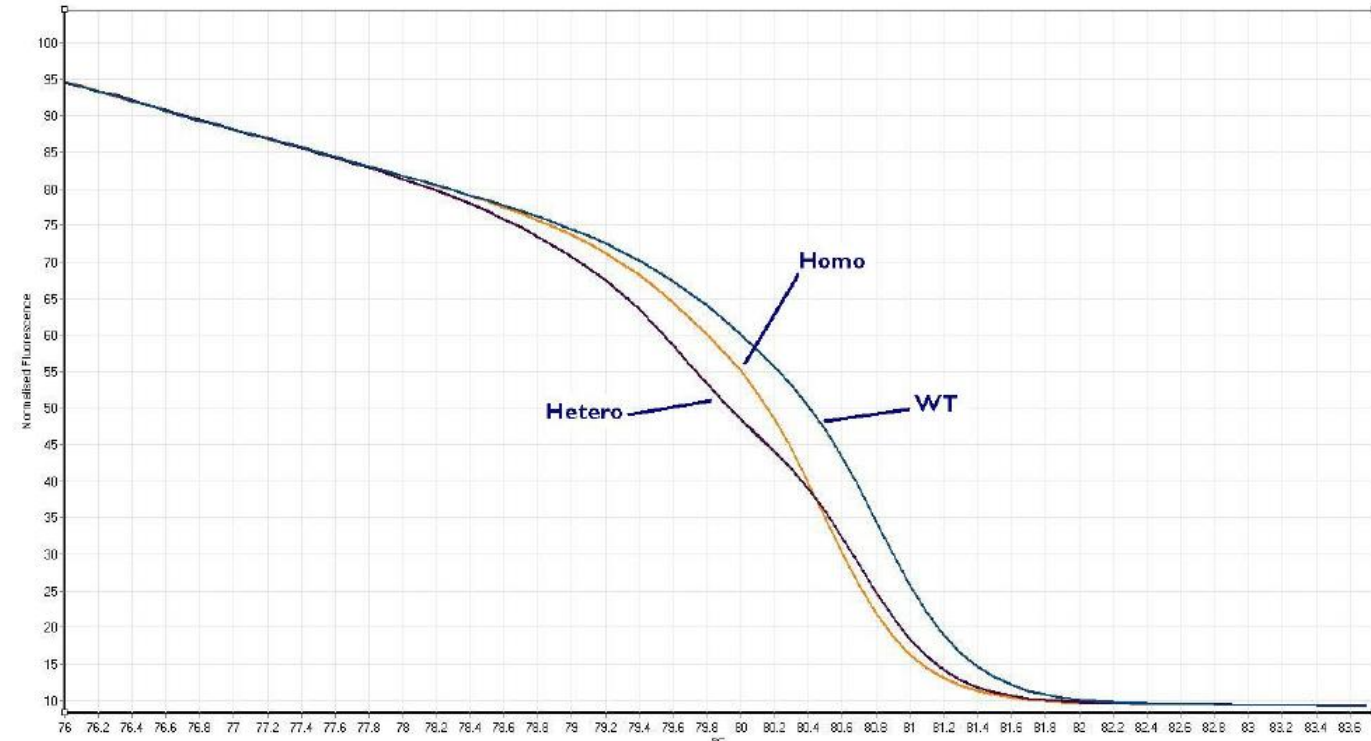
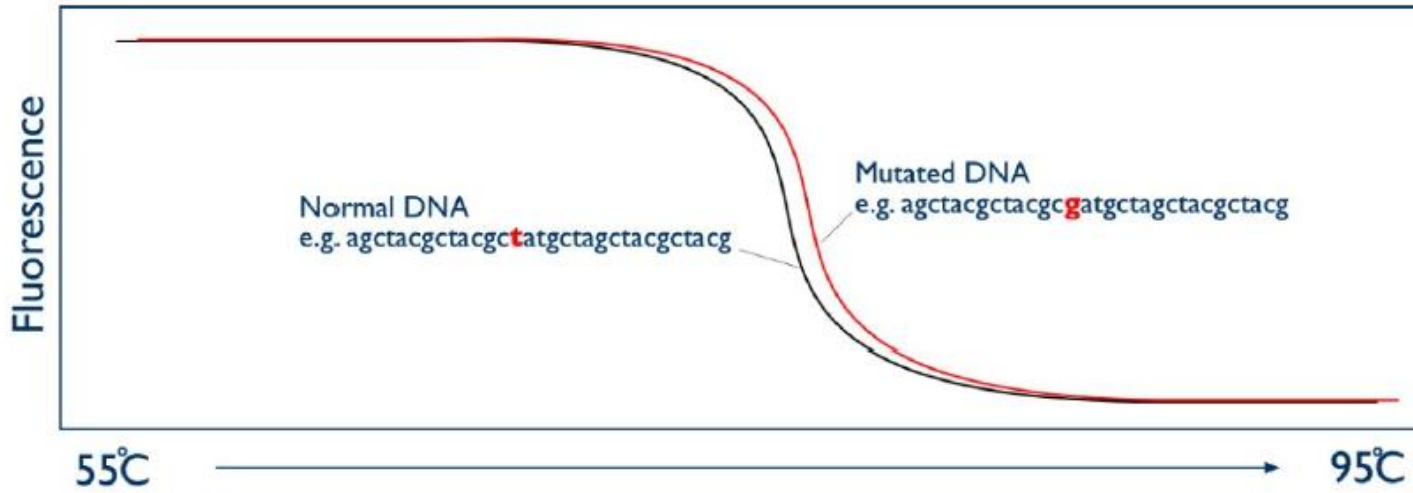
PCR-HRM



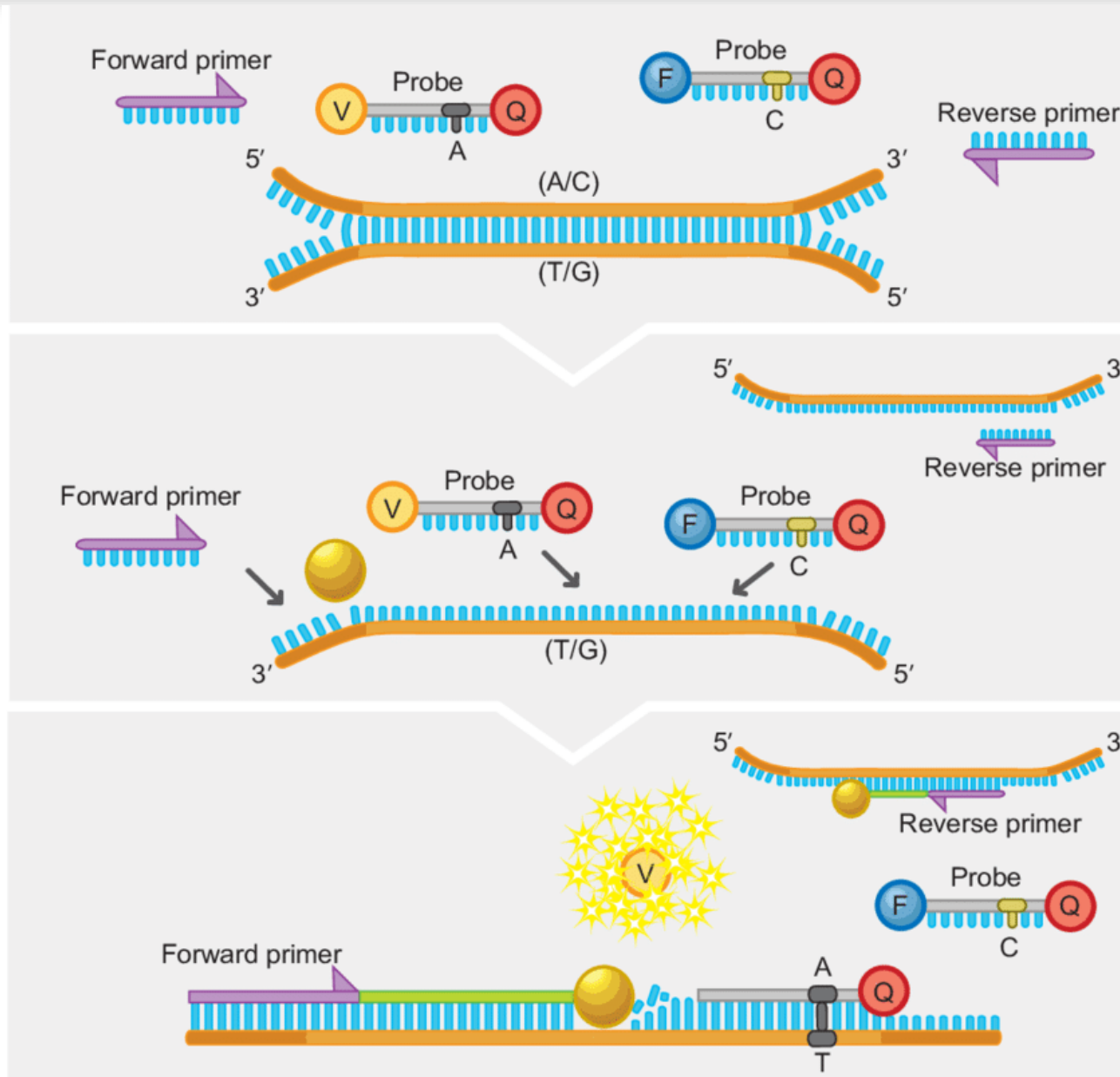
Unbound SYBR Green I
Bound SYBR Green I



PCR-HRM

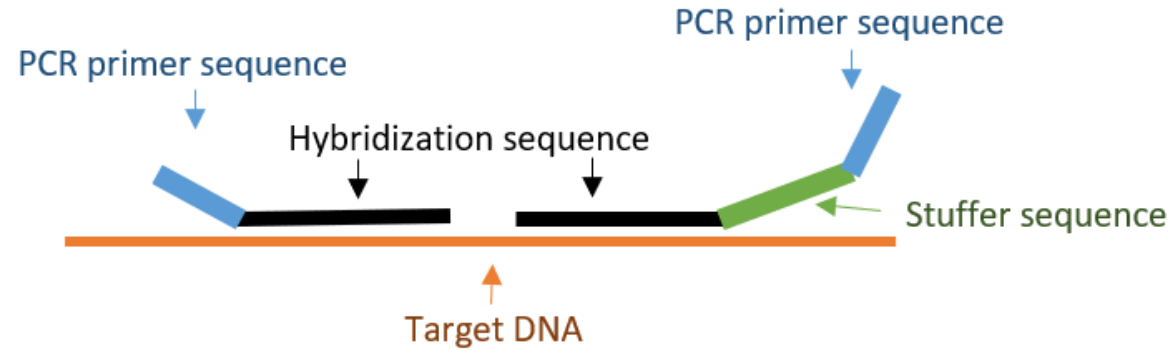


TaqMan Genotyping

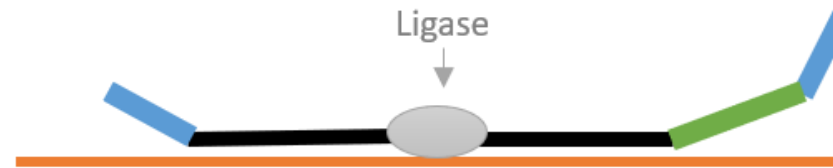


MLPA

1 – Denaturation; 2 – Hybridization



3 – Ligation

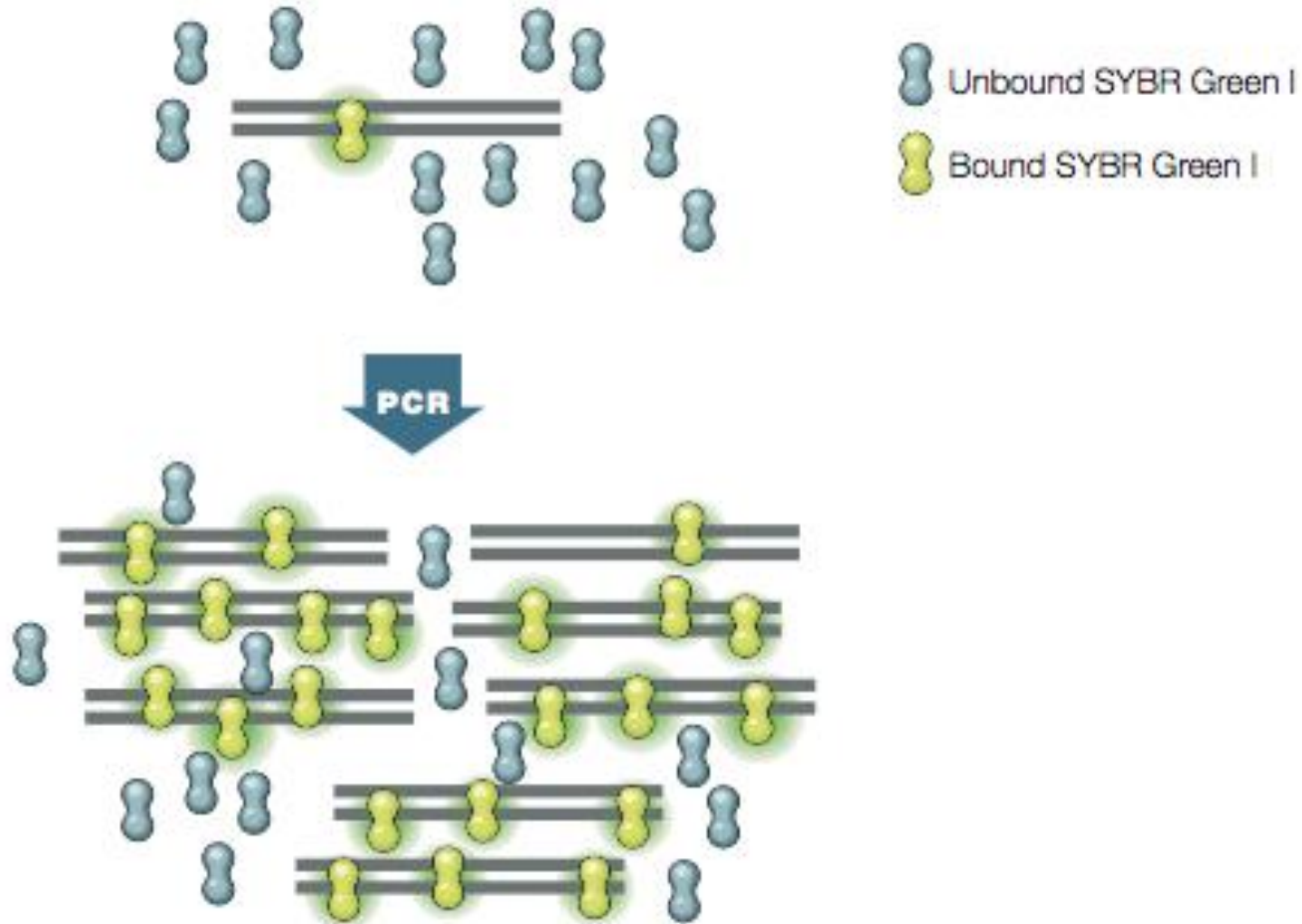


4 – Amplification

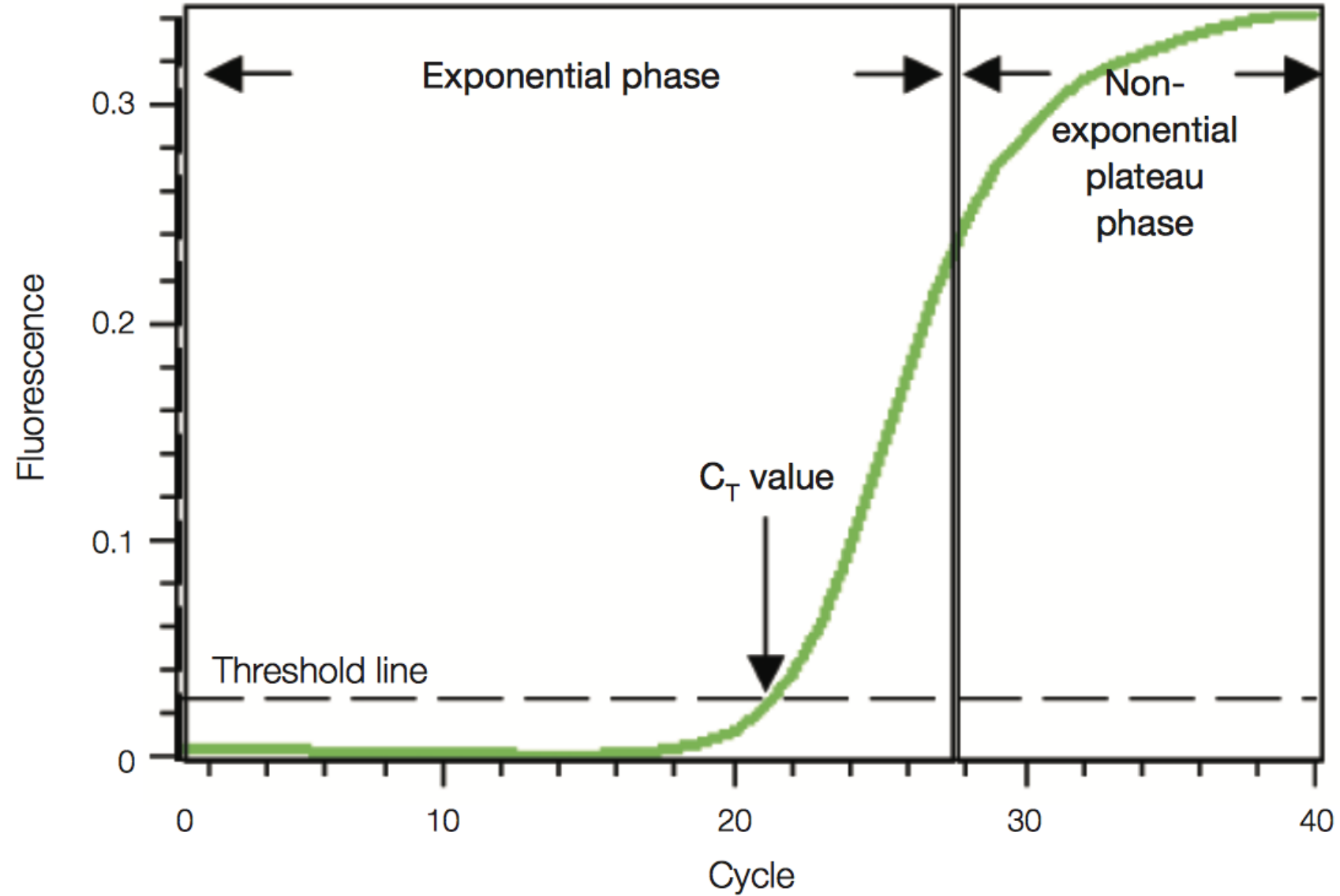


5 – Fragment separation and Data analysis

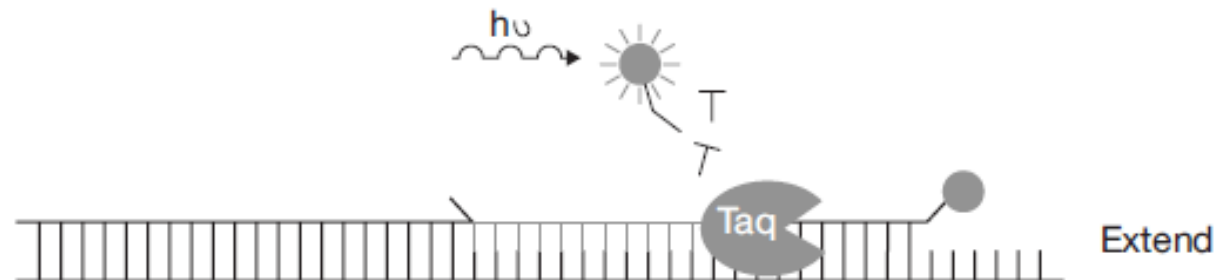
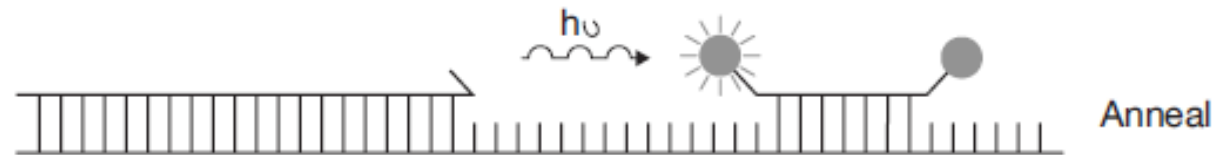
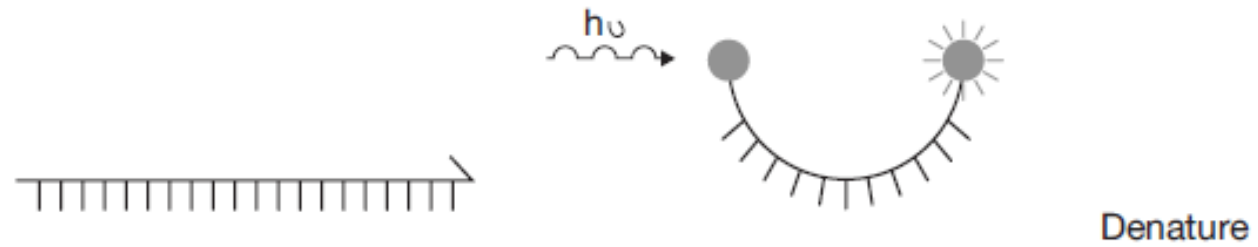
Real-time qPCR (SyberGreen)



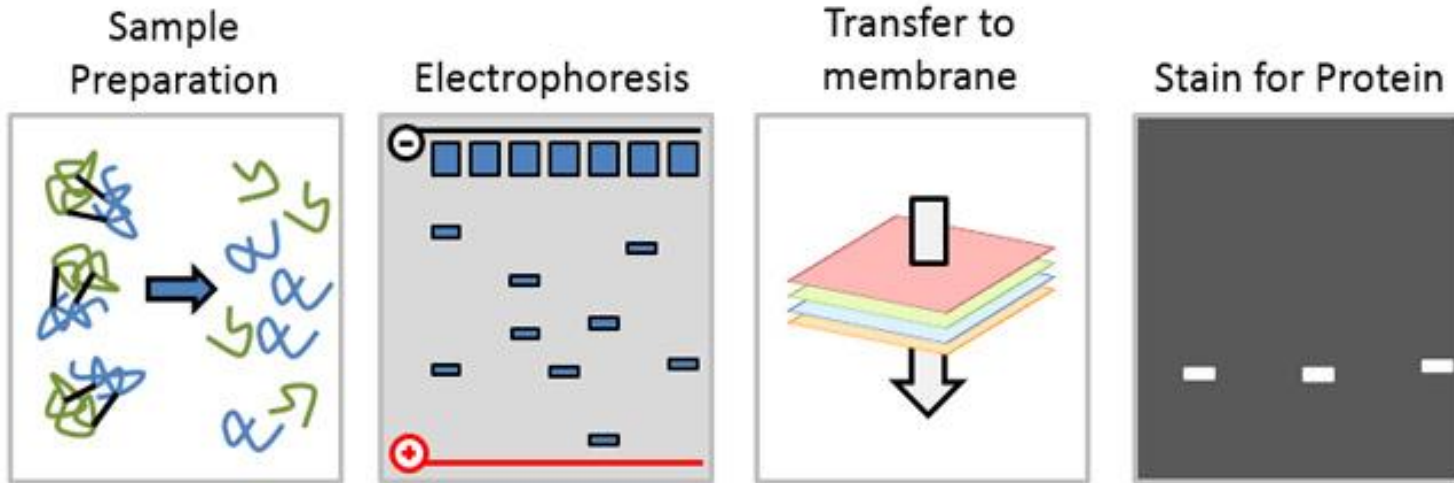
Fluorescent Signals after qPCR



Real-time qPCR (TaqMan)

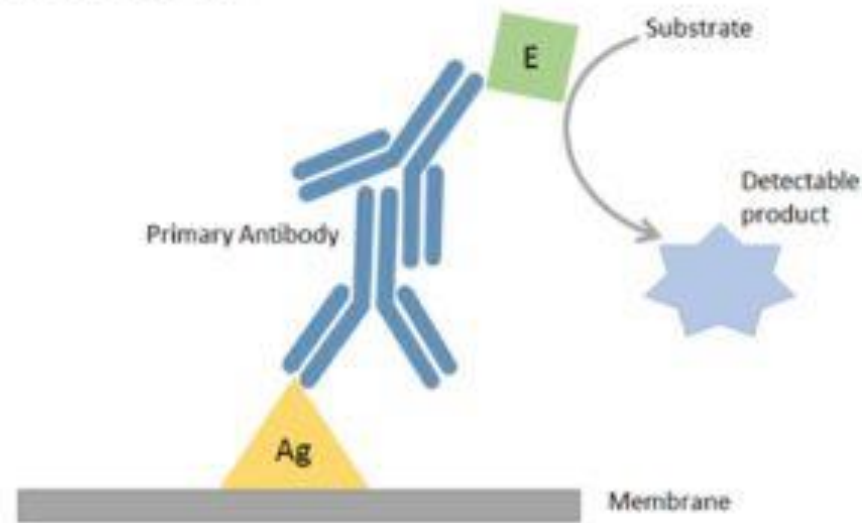


Western Blot (Immunoblotting)

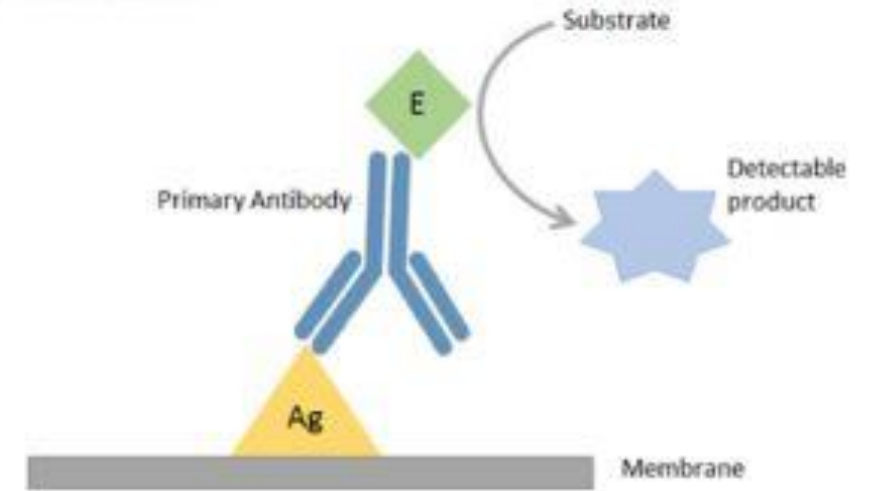


<https://www.genscript.com/Western-Blot-Antibody-Troubleshooting.html>

Indirect detection

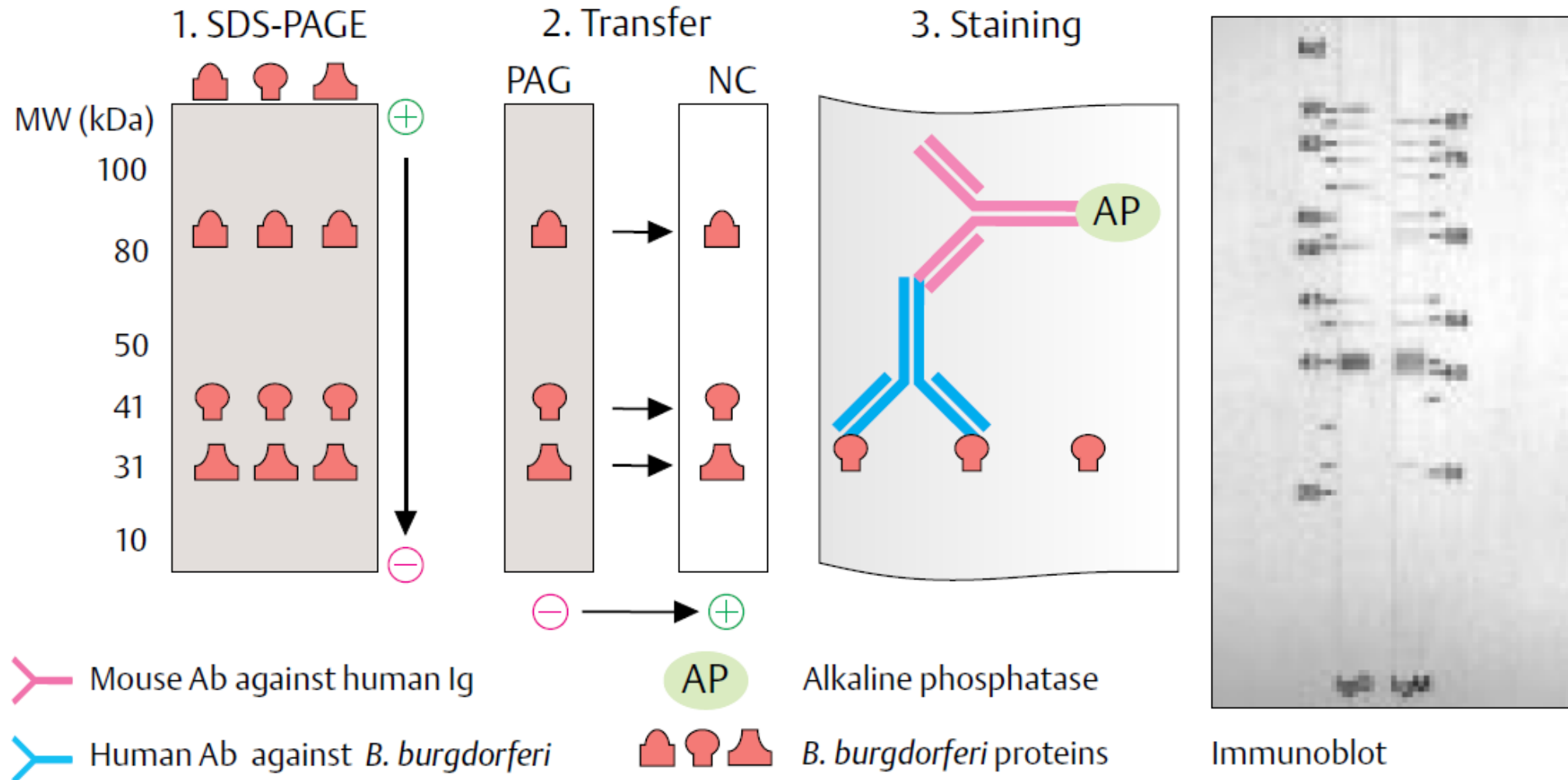


Direct detection



<https://www.expedeon.com/resources/applications/western-blot/>

Western Blot (Immunoblotting)



D. Immunoblotting (Western blot)

Many Thanks
