Primer Design

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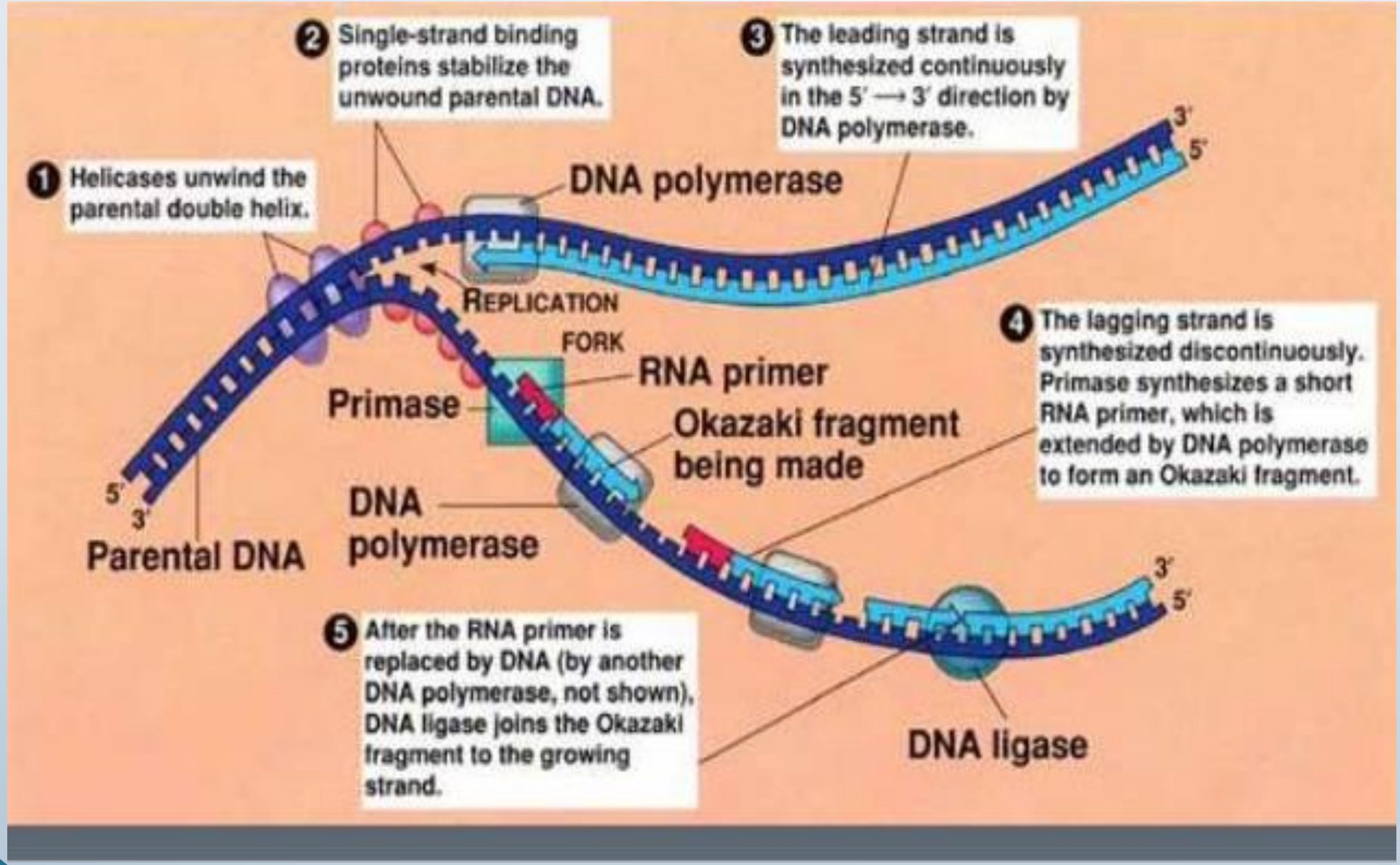
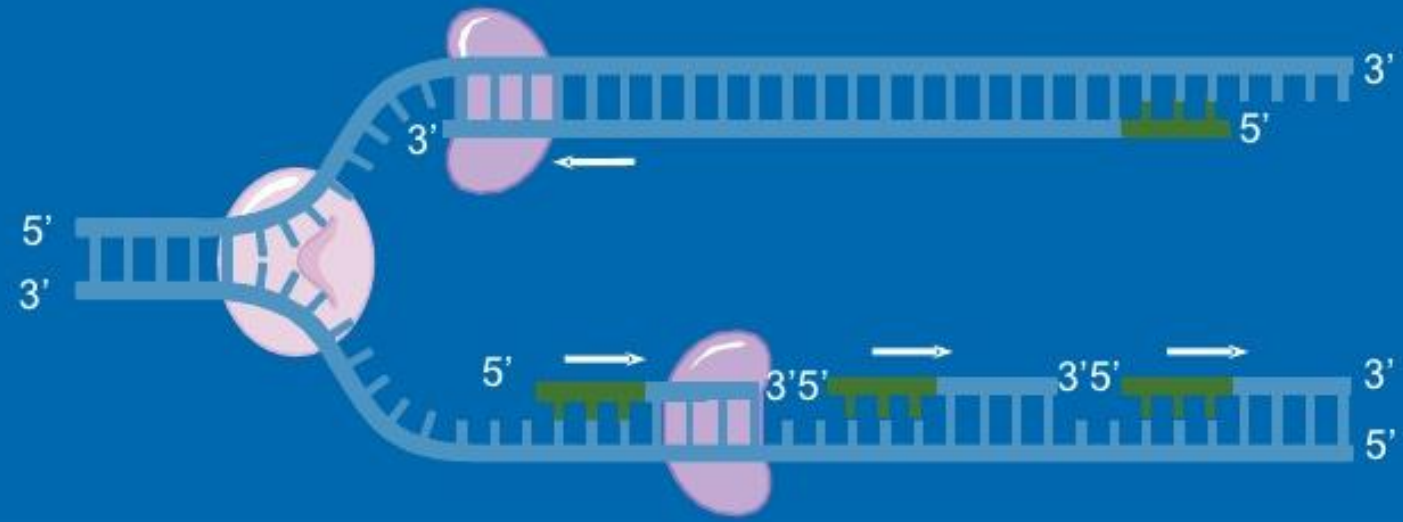
2019

DNA Replication (in vivo)



https://www.pathwayz.org

Replication

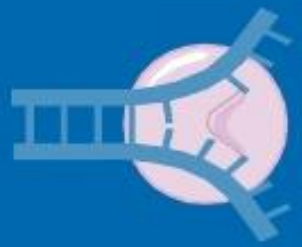


Leading strand synthesis continues in a 5' to 3' direction.

Discontinuous synthesis produces 5' to 3' DNA segments called Okazaki fragments.

https://www.pathwayz.org

Enzymes in DNA replication



Helicase unwinds parental double helix

Binding proteins stabilize separate strands

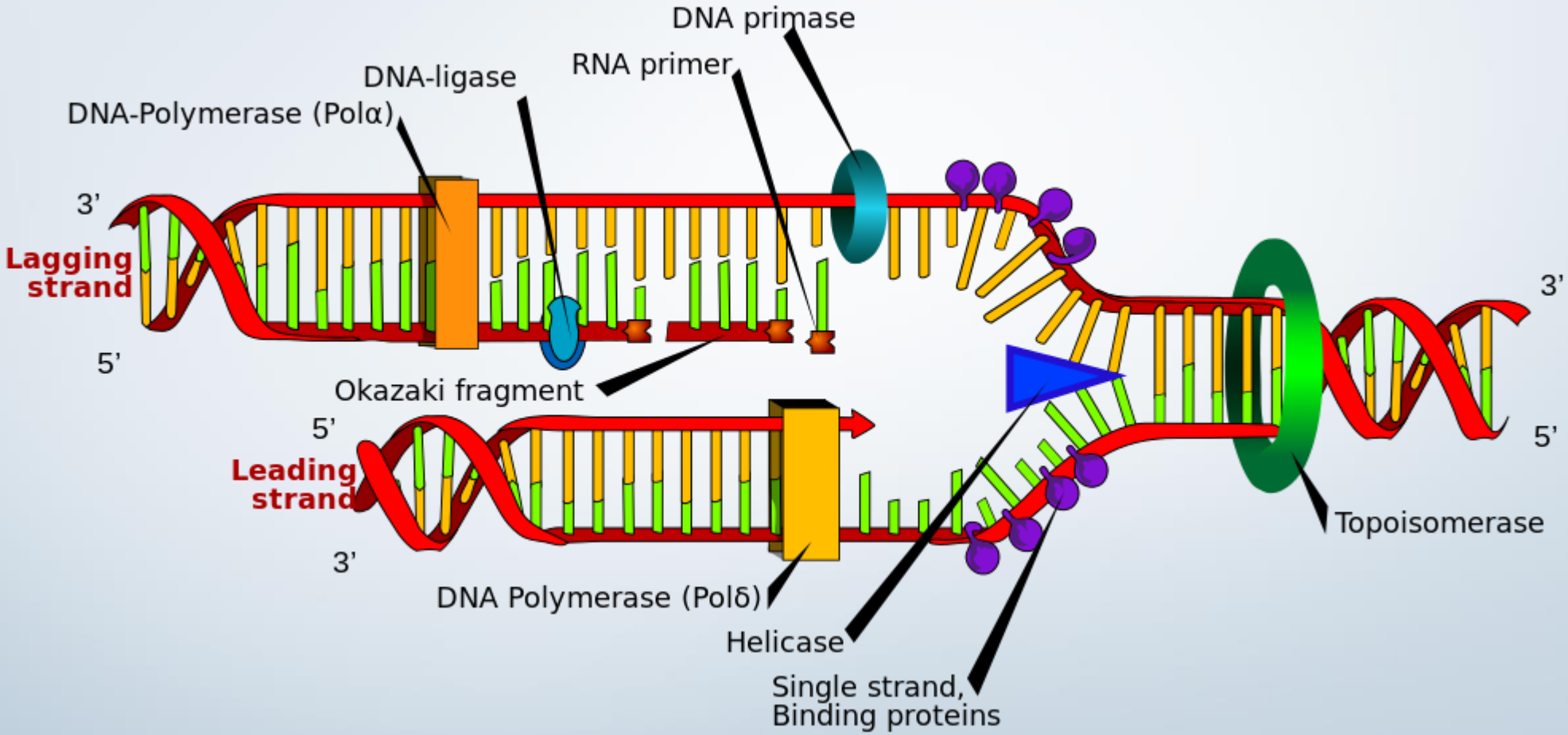
Primase adds short primer to template strand

DNA polymerase binds nucleotides to form new strands

Exonuclease removes RNA primer and inserts the correct bases

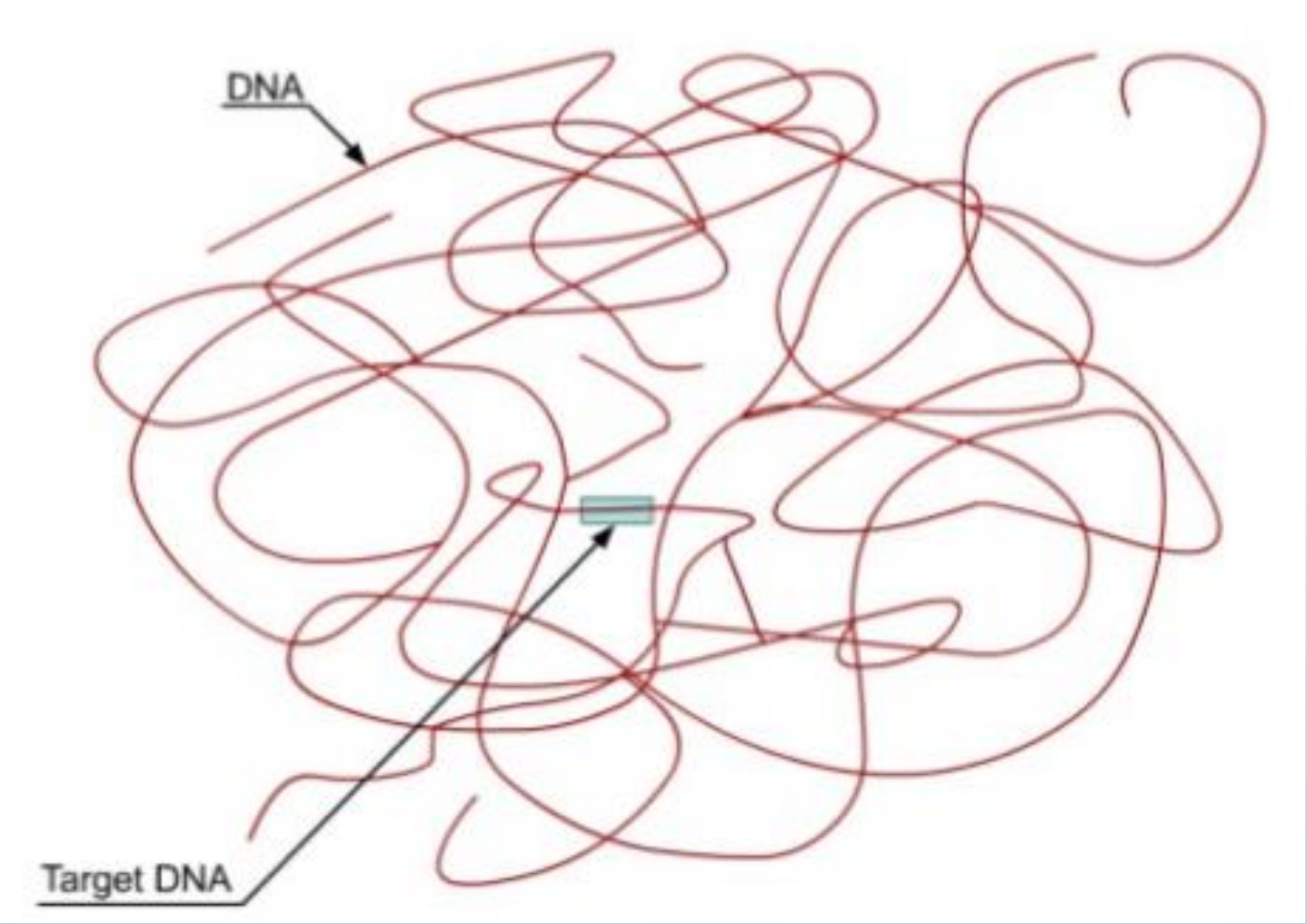
Ligase joins Okazaki fragments and seals other nicks in sugar-phosphate backbone

https://www.pathwayz.org



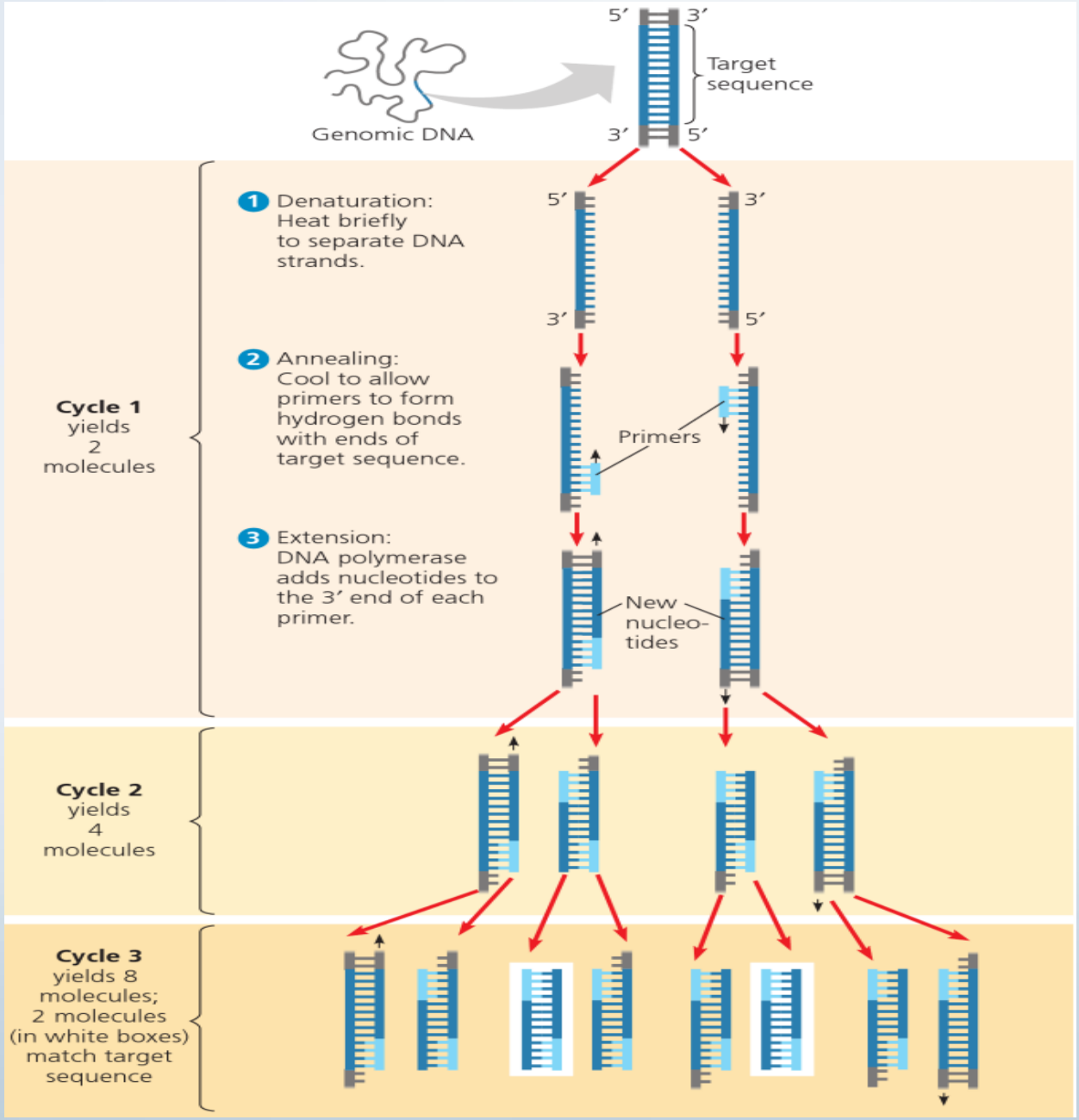
https://www.pathwayz.org

Primer Design: Why?



https://www.pathwayz.org

PCR Overview



http://laboratoryinfo.com/polymerase-chain-reaction-pcr/

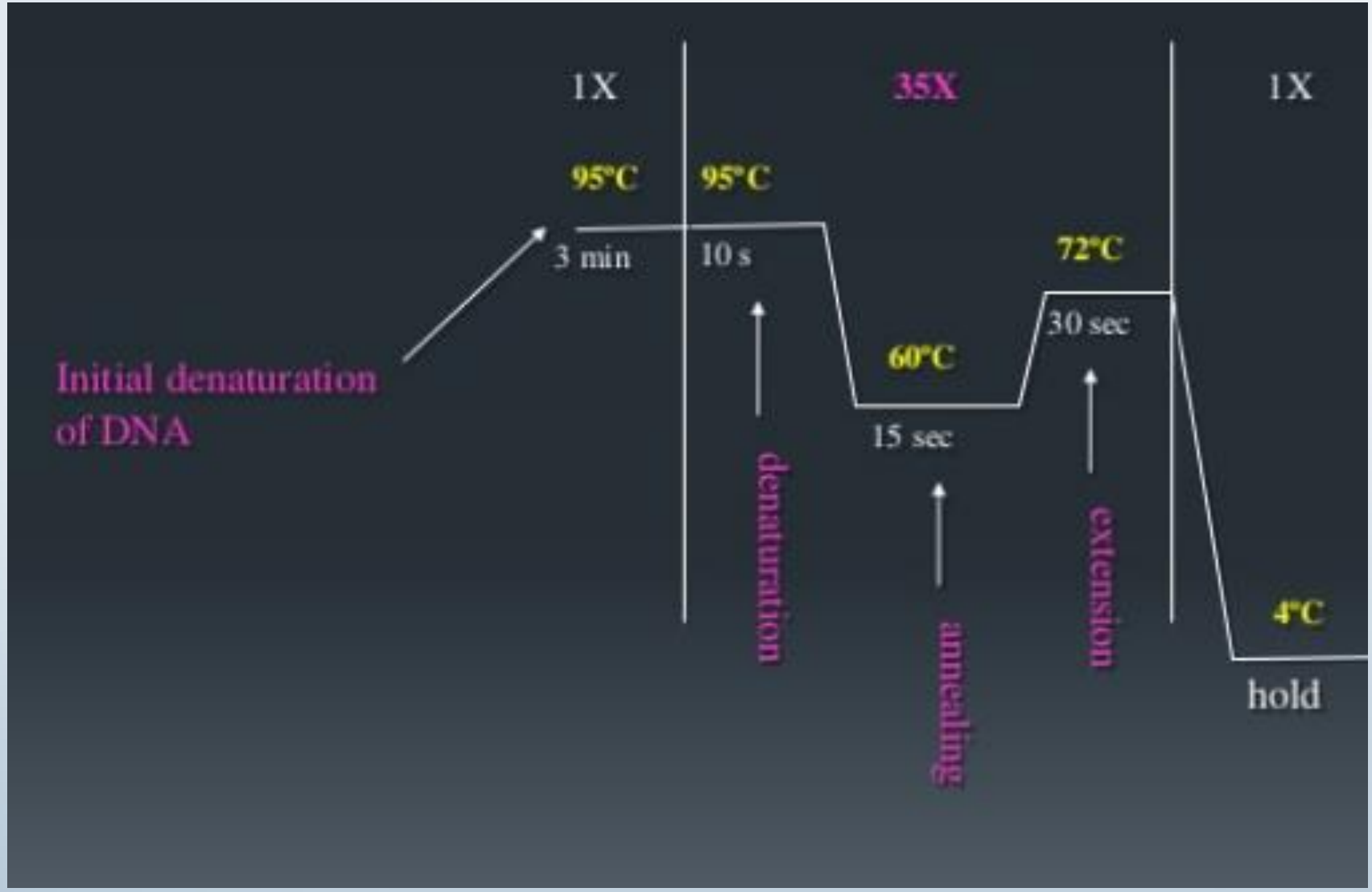
Polymerase Chain Reaction (PCR)

Is a method in which multiple repetitions of DNA replication are performed in a test tube.

Mix in test tube:

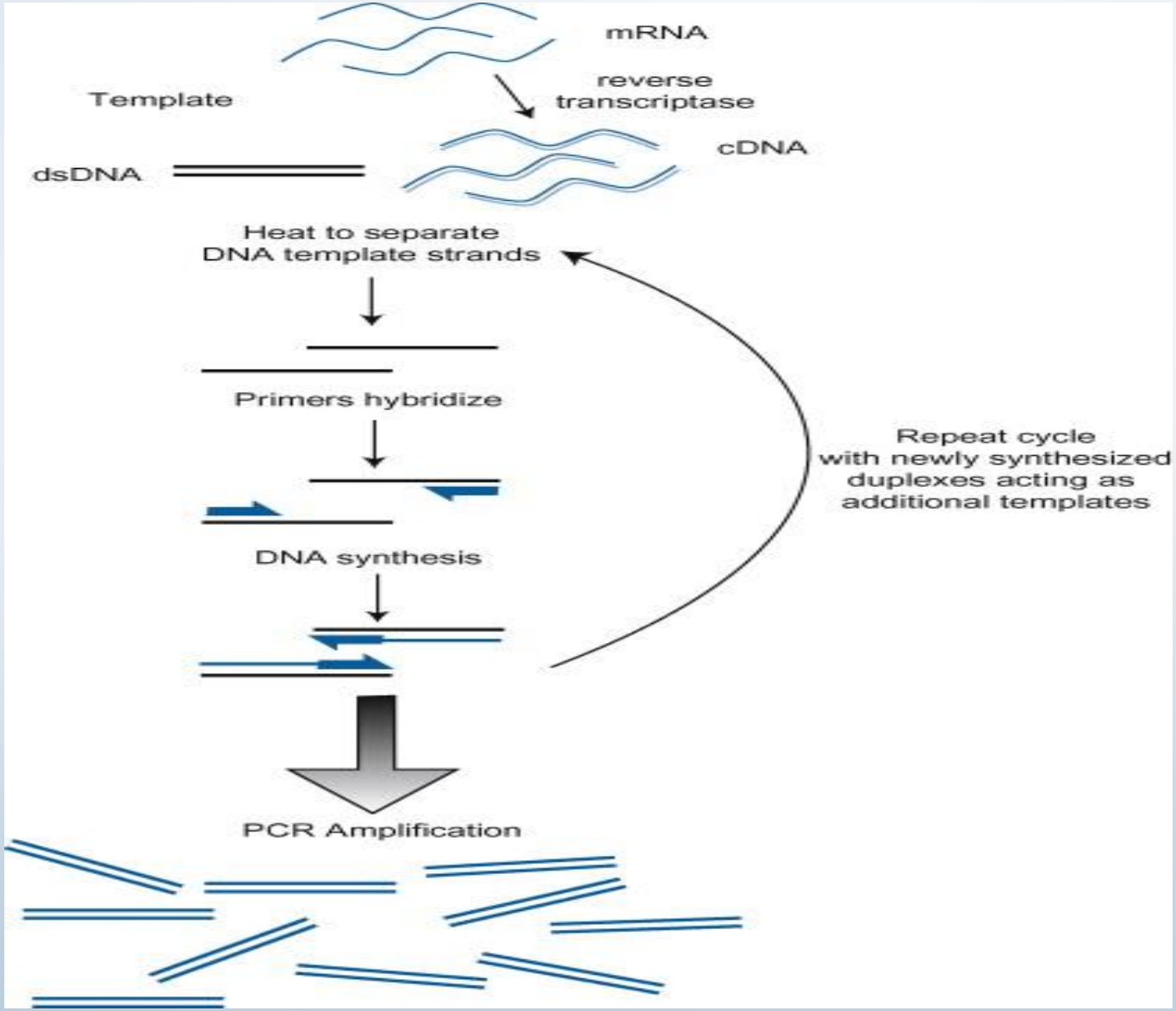
DNA template	DNA to be amplified
Primers	one complementary to each strand
Nucleotides	dATP,d GTP, dCTP, and dTTP
DNA polymerase	heat stable form from thermophilic bacteria

PCR Protocol



http://www.genscript.com/pcr-protocol-pcr-steps.html

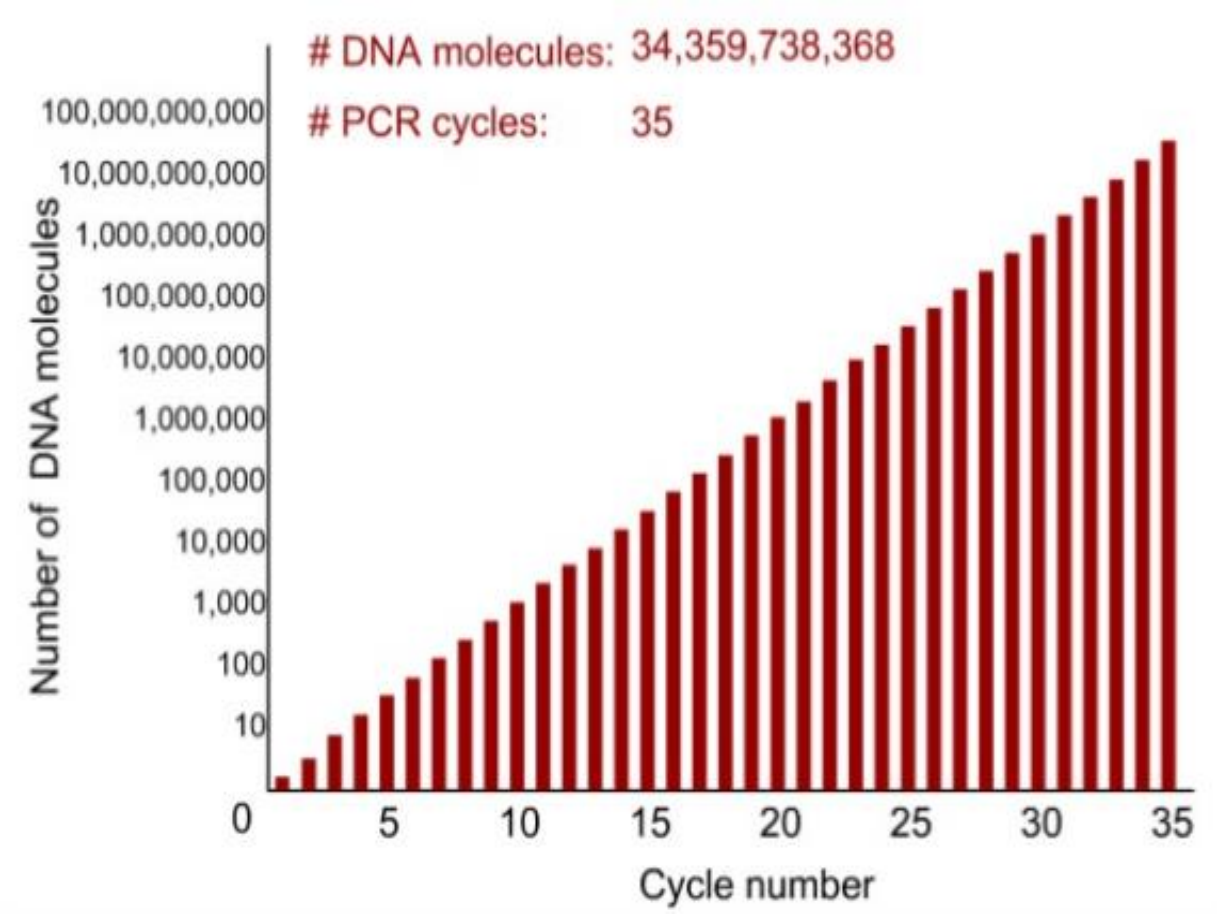
RT-PCR

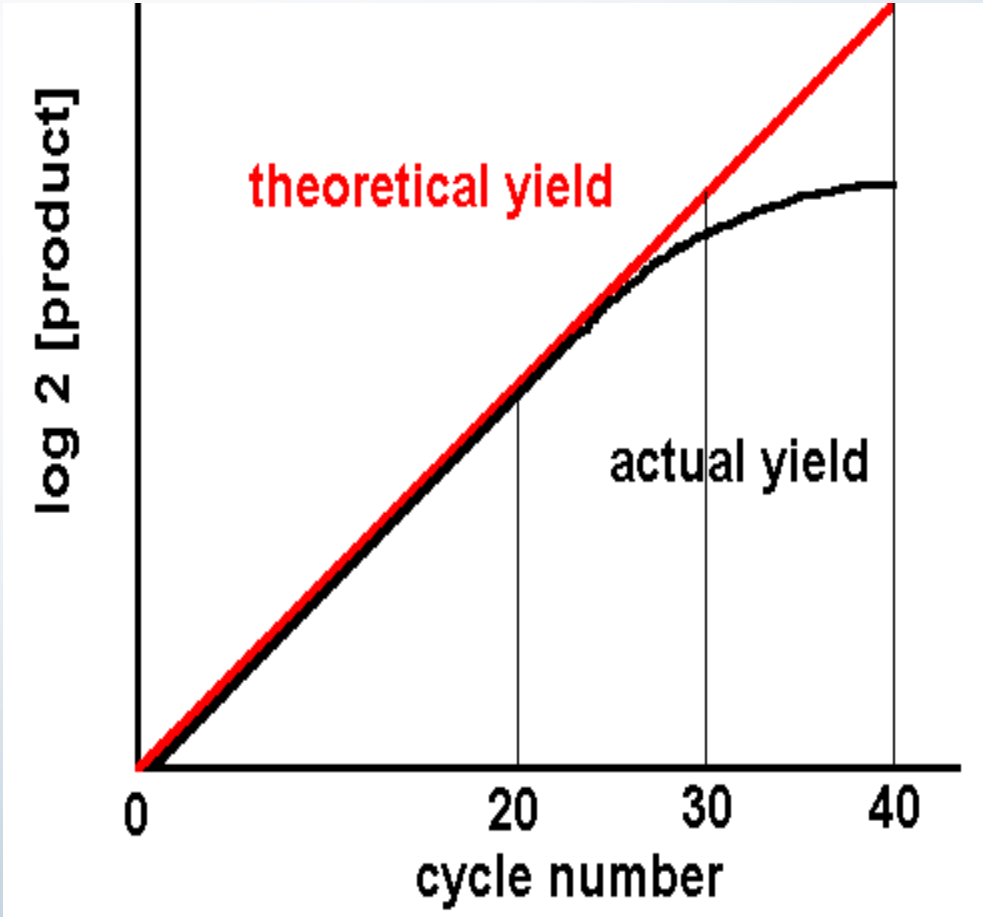


http://ars.els-cdn.com

PCR Cycles: Theory vs Experiment

Plateau Effect





http://www.mcb.uct.ac.za/mcb/resources/pcr/primer

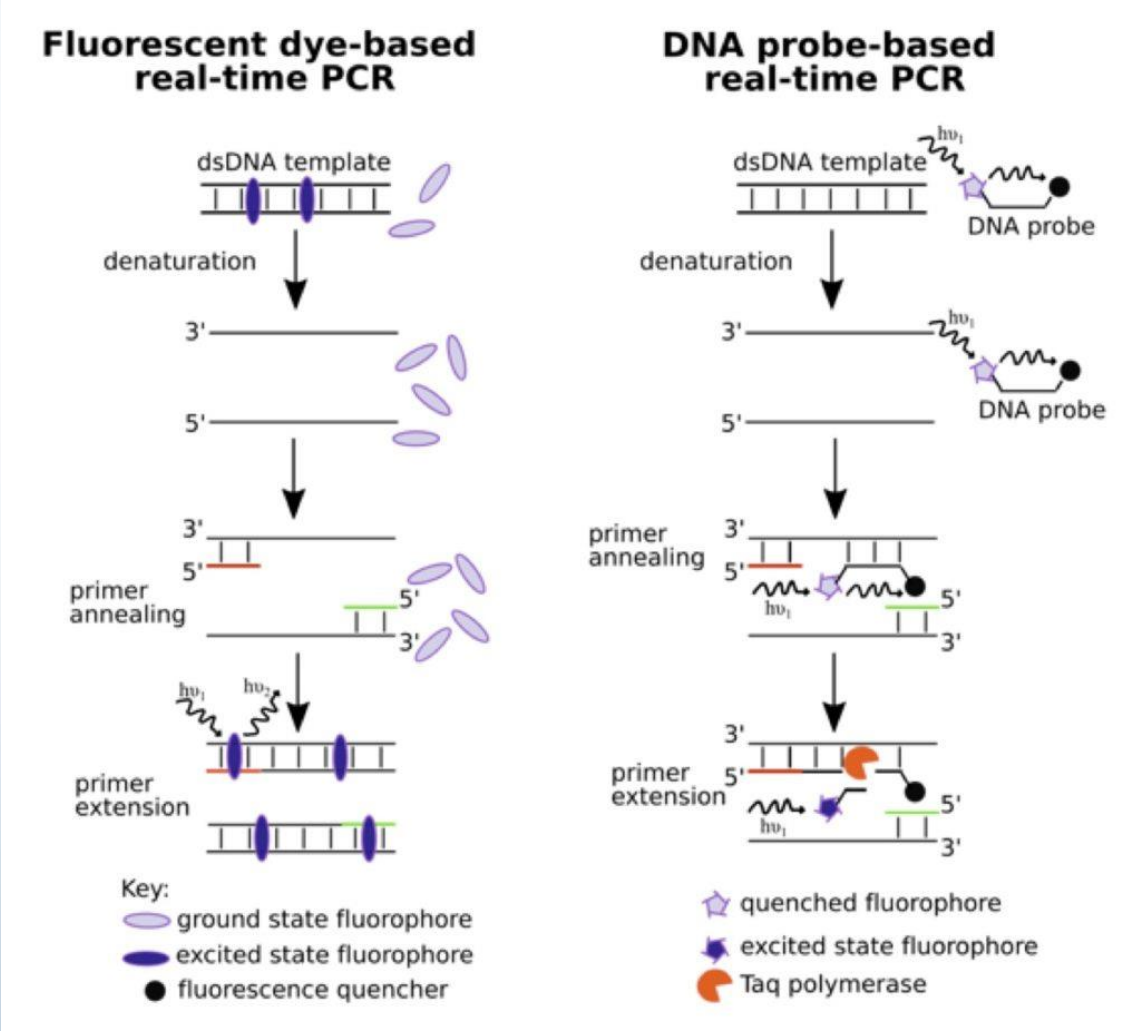
real-time PCR

real-time PCR monitors the amplification of a targeted DNA molecule during the PCR, i.e. in real-time, and not at its end, as in conventional PCR.

Two common methods for the detection of PCR products in real-time PCR are:

- non-specific fluorescent dyes that intercalate with any double-stranded DNA

- sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary sequence.



http://bitesizebio.com/29508/real-time-pcr-digest/

PCR Song (Bio-Rad)

https://www.youtube.com/watch?v=x5yPkxCLads

PCR Goals

1. Detection a segment of DNA (forensic medicine)
2. Detection a specific DNA sequence (pathogen detection)
3. Generating a library of DNA sequences
4. Mutation detection – polymorphism studies
5. Gene cloning
6. Gene expression studies
7. Genome analysis
8. DNA sequencing
9. Genetic (DNA/RNA) manipulation (gene knockouts, gene knockdowns, …)

PCR Goals - Classification

1. Detection
   I. Detection of a pathogen **(free)**
   II. Detection of a disease gene **(free / fixed)**
2. Quantification
   I. Quantification of a pathogen **(free)**
   II. Quantification of gene expression **(free)**
3. Production & Cloning
   I. Producing a protein **(fixed)**
   II. Cloning a segment of a gene **(fixed)**

Primer Design – different Scenarios

- fw → fixed rev → fixed
- fw → free rev → free
- fw → fixed rev → free
- fw → free (range) rev → free (range)