Polymerase Chain Reaction

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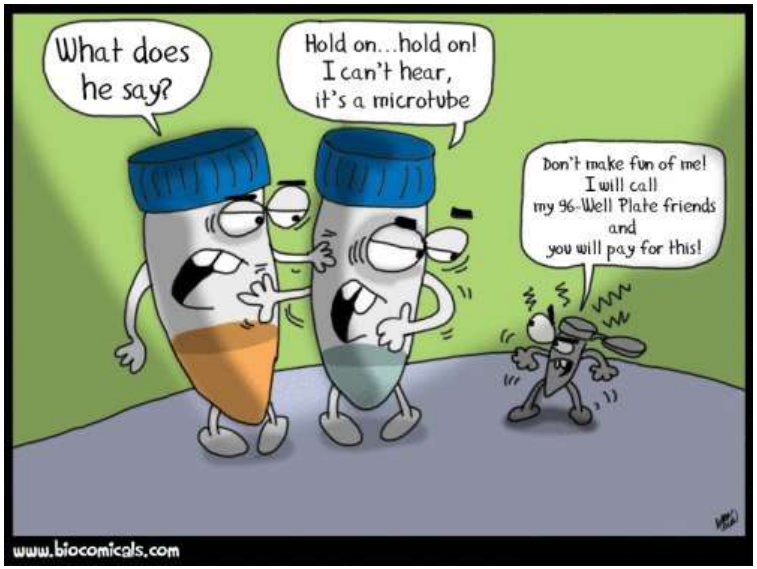
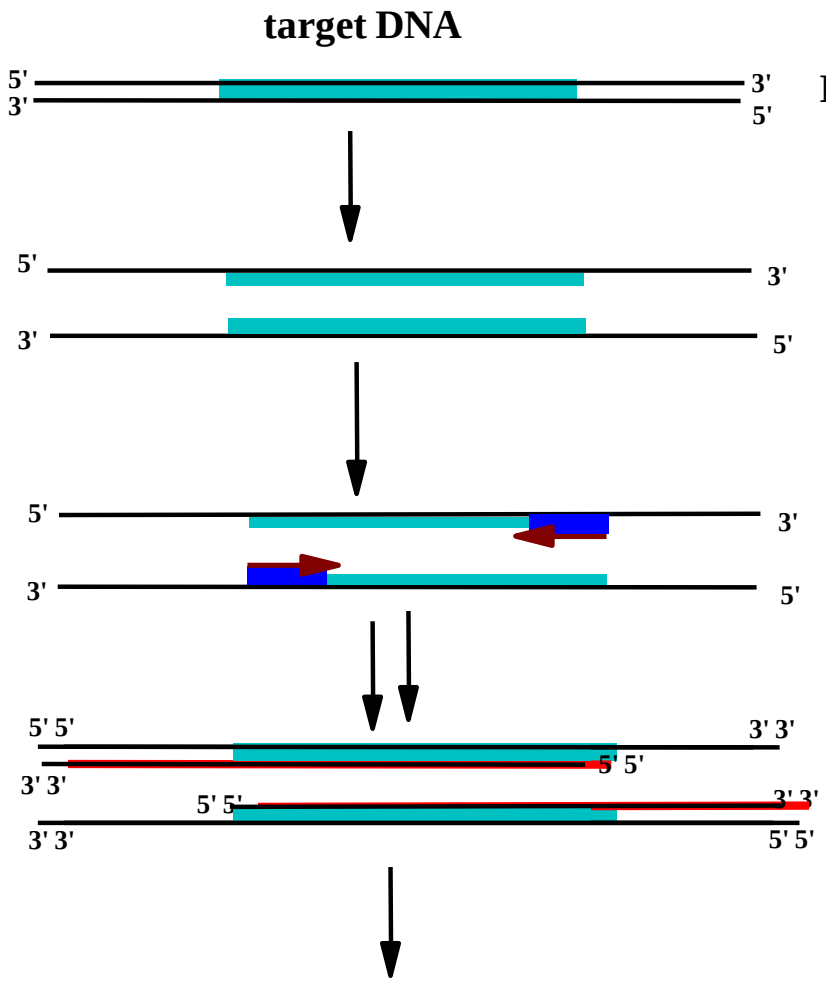
What is PCR?

- PCR is an exponentially progressing synthesis of the defined target DNA sequences **in Vitro**.

- It was invented in 1983 by Dr. Kary Mullis, for which he received the Nobel prize in chemistry in 1993.



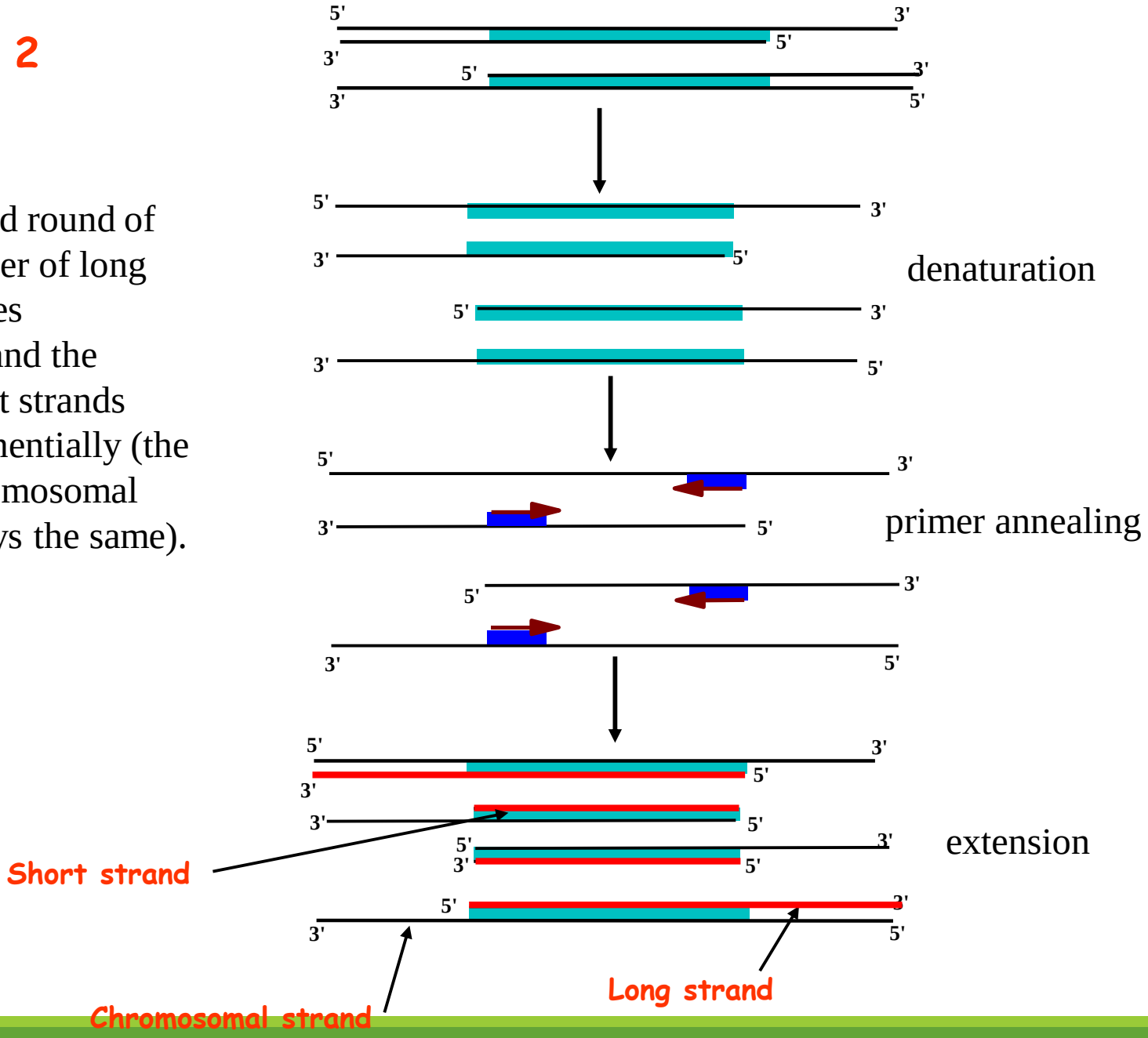
PCR Round 1



DNA polymerase always adds nucleotides to the 3' end of the primer

PCR Round 2

After the second round of PCR, the number of long strands increases arithmetically and the number of short strands increases exponentially (the number of chromosomal strands is always the same).



Theoretical Yield Of PCR

Theoretical yield = 2^n x y

Where y = the starting number of copies and n = the number of thermal cycles

If you start with 100 copies, how many copies are made in 30 cycles?

2^n x y

= 2^{30} x 100

= 1,073,741,824 x 100

= 107,374,182,400

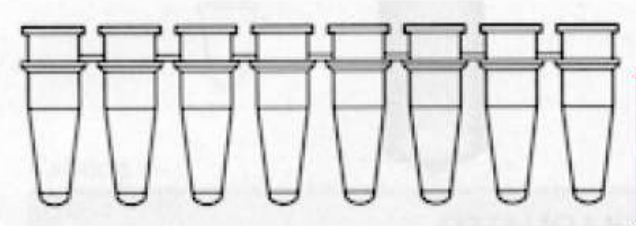
The PCR Reaction Components

1. Target DNA
2. Pair of Primers
3. dNTPs
4. Thermostable DNA Polymerase.
5. Mg^{++} ions
6. Buffer solution.

PCR method – second step:

Component	1X	20X
Sterile Water	38.0 ul	760 ul
10X PCR Buffer	5.0 ul	100 ul
MgCl2 (50mM)	2.5 ul	50 ul
dNTP's (10mM each)	1.0 ul	20 ul
PrimerFWD (25 pmol/ul)	1.0 ul	20 ul
PrimerREV	1.0 ul	20 ul
DNA Polymerase	0.5 ul	10 ul
DNA Template	1.0 ul	--
Total Volume	50.0 ul	980 ul

Aliquot
49 ul



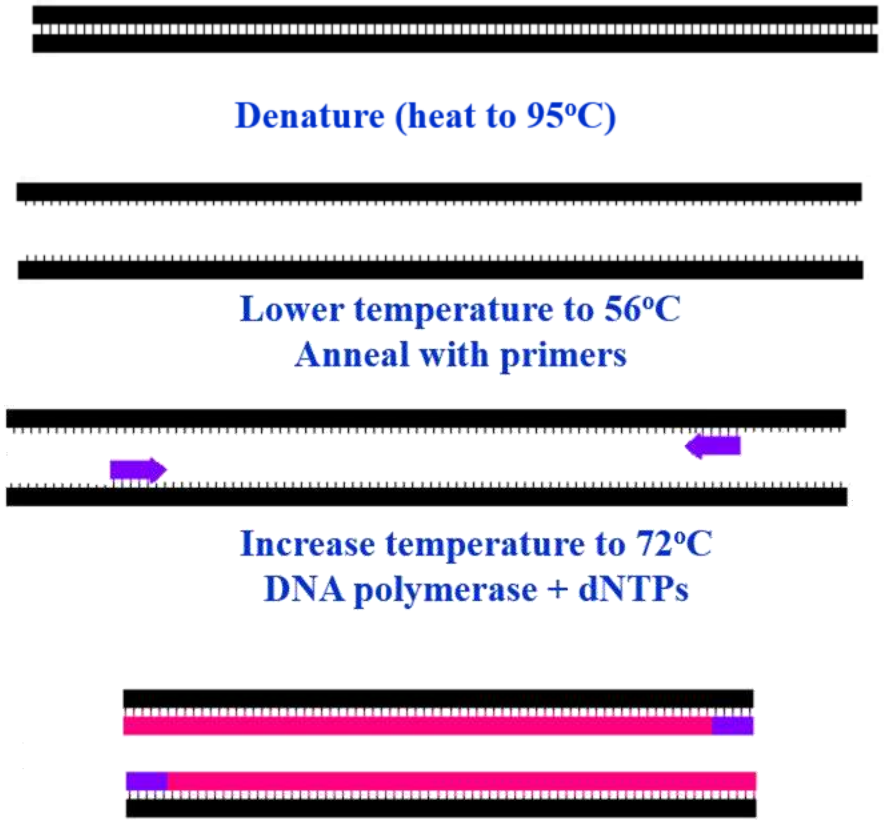
Add DNA
as last step

PCR tube

THERMOCYCLER

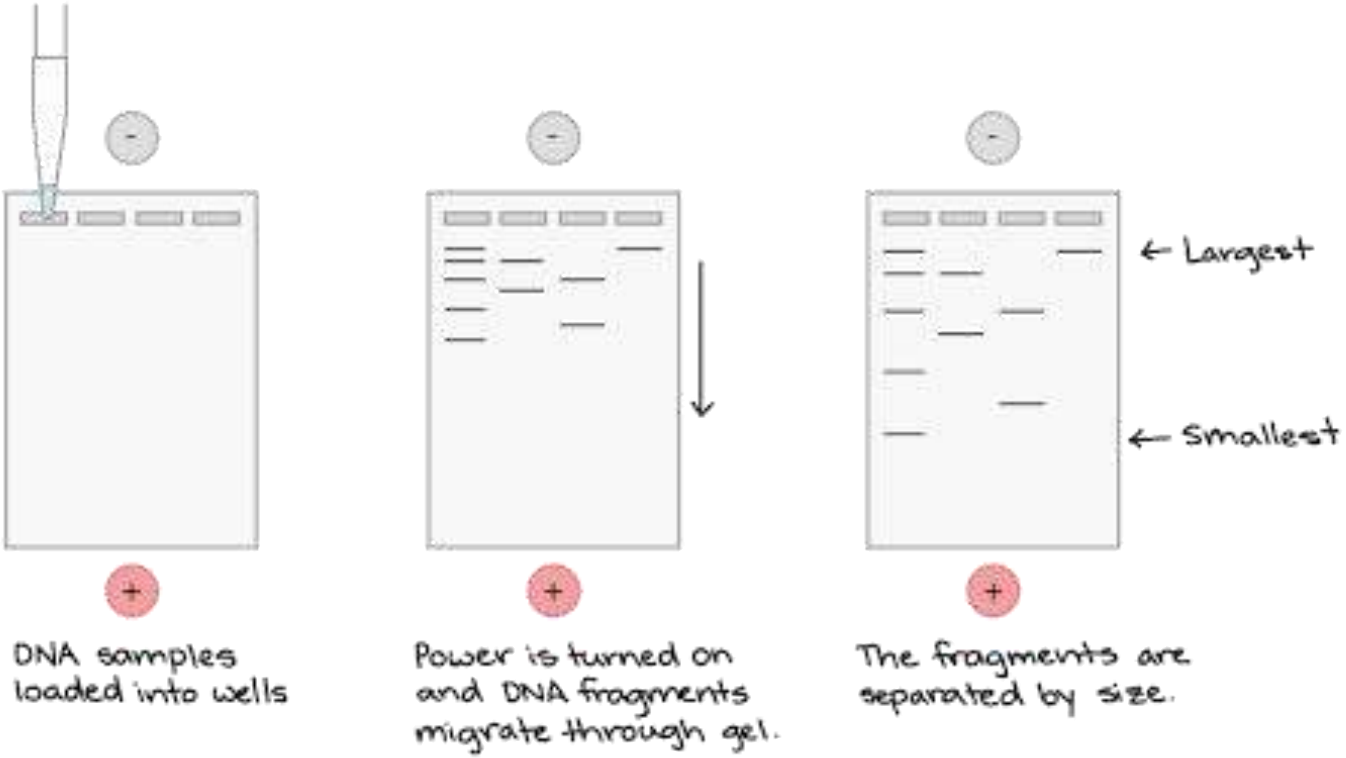
1. Initial Denaturation	95 C	4 min
2. DNA Denaturation	95 C	1 min
3. Primer Annealing	56 C	1 min
4. Primer Extension	72 C	1 min
5. Go to step #2, repeat 29 more times		
6. Hold at 4 C		
7. End		

PCR Reaction

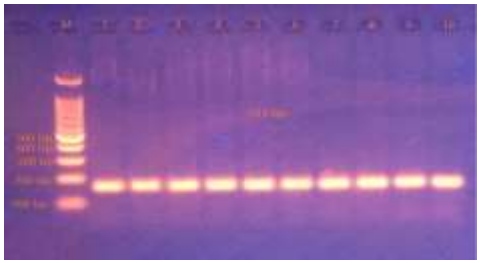


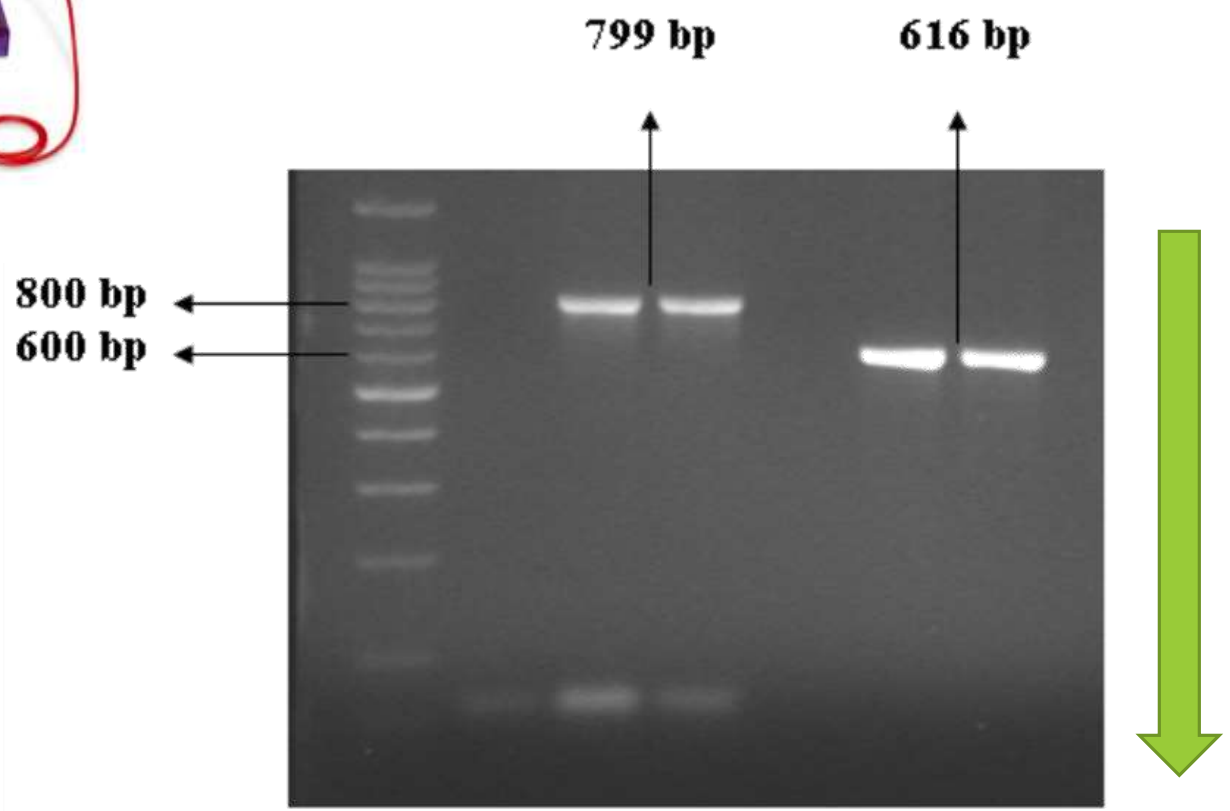
Third Step: Gel electrophoresis

Detection and identification of amplified target DNA fragments based on molecular size

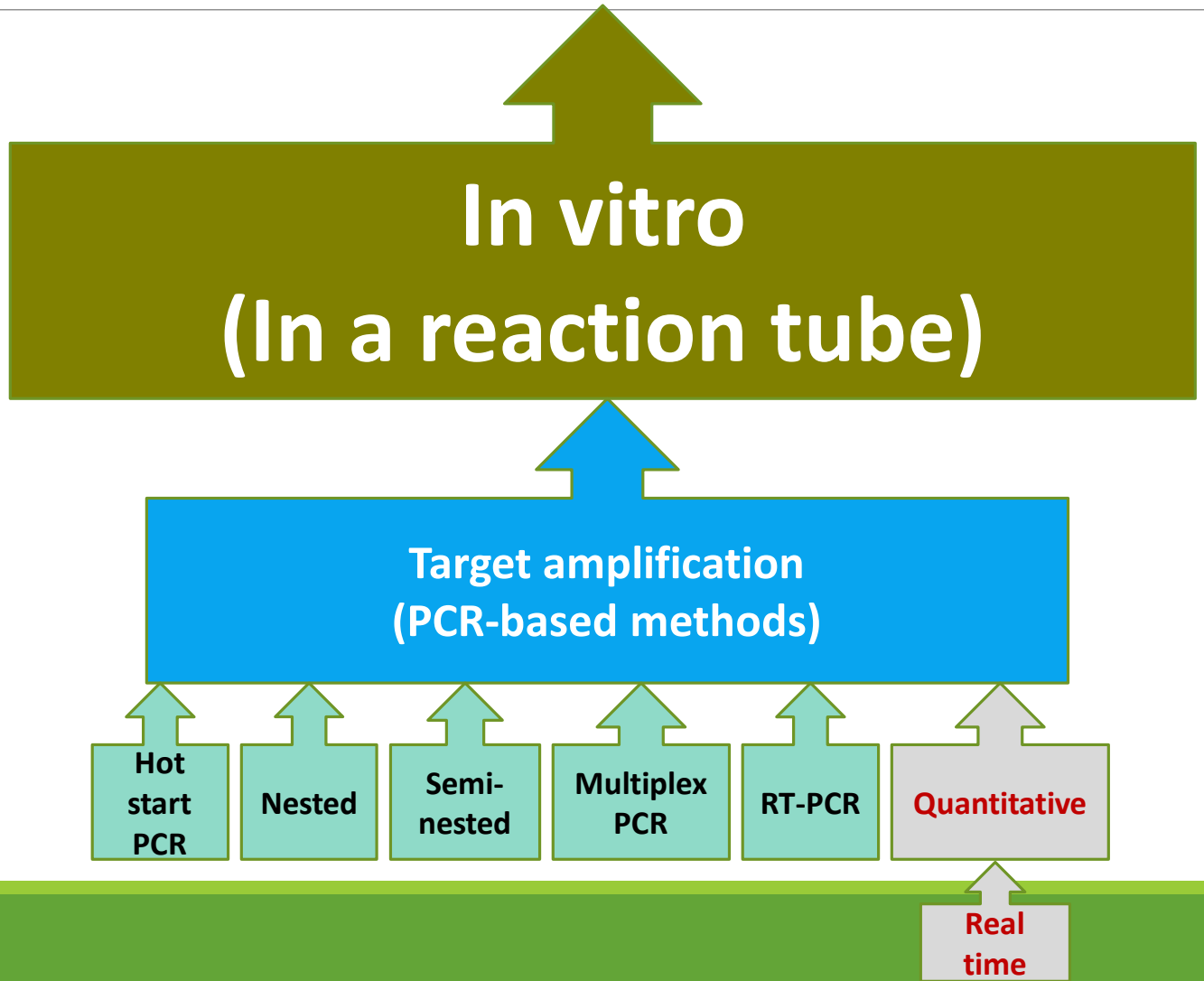


Third Step: Gel electrophoresis





Types of PCR


In vitro
(In a reaction tube)
Target amplification
(PCR-based methods)
Hot start PCR
Nested
Semi-nested
Multiplex PCR
RT-PCR
Quantitative
Real time

Hot start PCR

Hot start PCR often eliminates production of primer dimers caused by primer annealing at low temperature before the start of thermocycling.

- **Mechanical hot start PCR:** all components of PCR are added to the PCR vial except for the DNA polymerase enzyme which will be added just at the first denaturation step.
- **Non mechanical hot start PCR:**

- The use of a form of Taq DNA polymerase, for example, Amplitaq Gold which is activated only if the reaction mixture is heated at about 94°C (the first denaturation step).
- Other method depends on covalent linking of the polymerase enzyme to certain inhibitors. The enzyme becomes dissociated from these inhibitors at the first denaturation step.

Nested & seminested PCR

- **In nested PCR**, two amplification reactions occur with two different sets of primers.
  - The first round PCR: external primers are used which target the flanking regions of a specific gene.
  - The second round PCR: internal primers are used which target a sequence within the amplified product of the first reaction.
- **Seminested PCR** is similar to nested PCR but: the second round PCR uses one of the first round PCR primers and one new internal primer.
  - Advantages: nested PCR increases specificity of the reaction because the internal primers anneal only if the first round PCR occurred correctly. Disadvantages: more time and cost & increased possibility of contamination (so, single tube method is now used).

Multiplex PCR

- Whereas standard PCR usually uses one pair of primers to amplify a specific sequence, multiplex PCR uses multiple pairs of primers to amplify many sequences simultaneously.
- The presence of many PCR primers in a single tube could cause many problems, such as the increased formation of primer dimers and non specific amplified products.
- Primers used in multiplex reactions must be designed carefully to have similar annealing temperatures.
- Multiplex PCR is also used to amplify an internal control with one set of primers and the target DNA sequence with a second set of primers.
- The internal control is included to verify the integrity of the PCR. A positive result with the internal control primers demonstrates that all conditions of PCR were favorable.

RT-PCR

- RT-PCR is an abbreviation of reverse transcriptase PCR.
- RT-PCR is used to amplify RNA targets.
- RT-PCR has two steps:
  - First convert RNA to cDNA by reverse transcriptase enzyme.
  - Amplification of the DNA.
- RT-PCR in microbiology is used to study RNA viruses like HIV & HCV.

Advantages of PCR:

1. Quick
2. Reliable
3. Sensitive
4. Specific
5. Relatively easy

Disadvantage of PCR:

1. Need for equipment, not automatable & capacity building needed.
2. Need for Internal control
3. Taq polymerase is expensive.
4. Cross-reaction and unspecific amplification can occur, false-positive results and false-negative results.
5. Enrichment steps in (contaminated) samples.
6. No indication of pathogen viability, confirms presence of nucleic acid but not infection.
7. Uses intercalating dye requiring double-stranded DNA, carcinogenic chemical (EtBr)

Thank You