



Centurion
UNIVERSITY

Session 165: Types of PCR, cDNA synthesis and RT-PCR

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Polymerase Chain Reaction (PCR):

- ✓ It is a molecular technology aim to amplify a single or few copies of the DNA to thousands or millions of copies.
- ✓ Developed in 1983 by Kary Mullis, PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications. These include diagnosis of infectious diseases, DNA sequencing and DNA-based phylogeny.
- ✓ In 1993, Mullis was awarded the Nobel prize in Chemistry along with Michael Smith for his work on PCR.

Types of PCR:

- 1) Conventional (Qualitative)PCR
- 2) Multiplex PCR
- 3) Nested PCR
- 4) RT-PCR and qRT-PCR
- 5) Quantitative PCR
- 6) Hot-start PCR
- 7) Touchdown PCR
- 8) Assembly PCR
- 9) Colony PCR
- 10) Methylation-specific PCR
- 11) LAMP assay

1) Conventional (Qualitative)PCR:

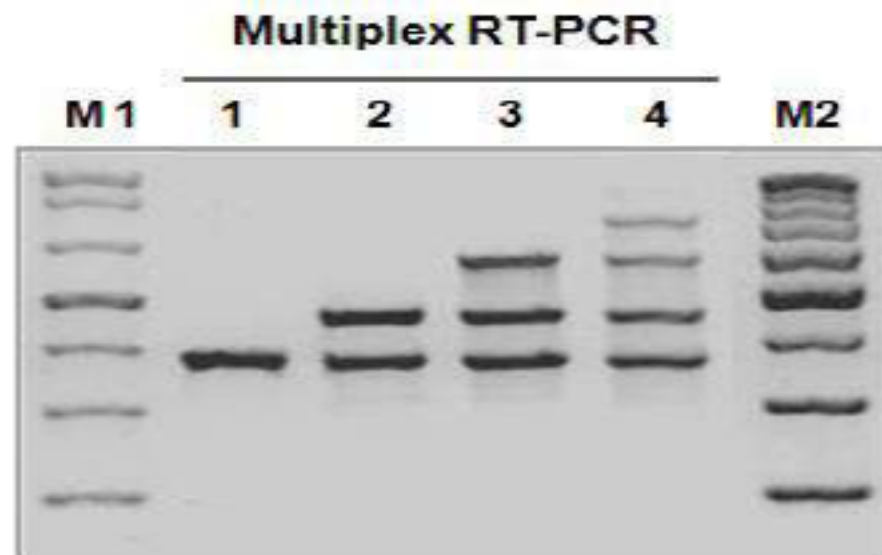
- The polymerase chain reaction(PCR) is a test tube system for DNA replication which allows a “target” DNA sequence to be selectively amplified several million folds in just a few hours.
- PCR enables the synthesis of specific DNA fragments using a DNA-polymerase enzyme, which takes part in the replication of the cellular genetic material.
- This enzyme synthesizes a complementary sequence of DNA, as a small fragment (primer) is connected to one of the DNA strands in the specific site chosen to start the synthesis.
- Primers limit the sequence to be replicated, and the result is the amplification of a particular DNA sequence with billions of copies.
- Conventional PCR is applied in selective DNA isolation, amplification and quantification of DNA, medical and diagnostic approaches, infectious disease diagnosis, forensic studies and research areas.

2) Multiplex PCR:

It is a special type of the PCR used for detection of multiple pathogens by using Multiple primers sets each one targets a particular pathogen.

Uses:

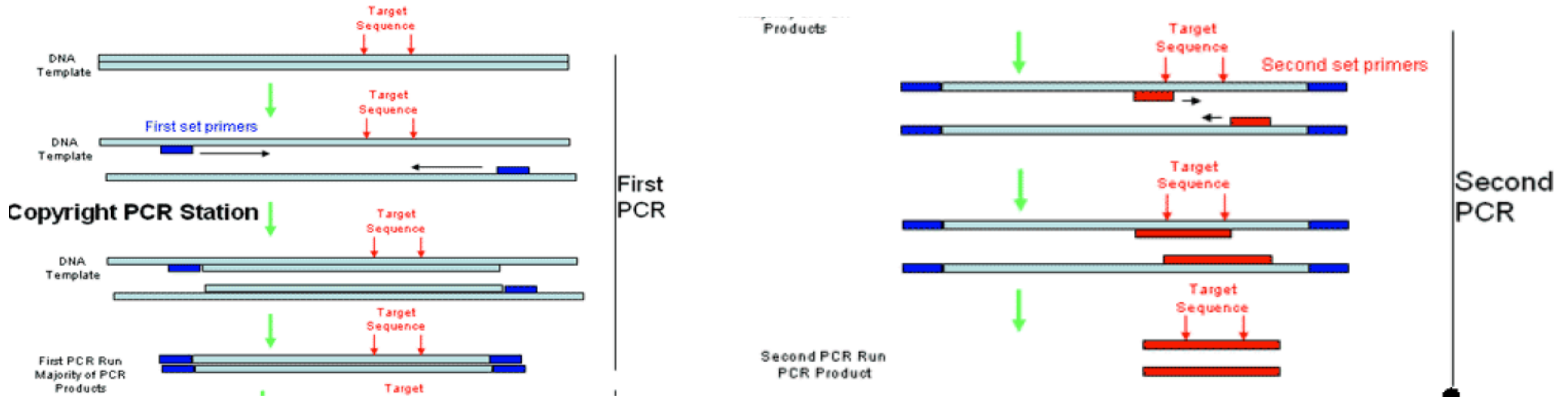
This permits the simultaneous analysis of multiple targets in a single sample.



3) Nested PCR

- Nested PCR is a useful modification of PCR technology where the specificity of the reaction is enhanced by preventing the non-specific binding with the help of the two sets of primer.
- The first set of primer binds outside of our target DNA and amplifies larger fragment while another set of primer binds specifically at the target site.
- In the second round of amplification, second set of primer amplifies only the target DNA.
- Nested PCR is a helpful method for the phylogenetic studies and detection of different pathogens.
- The technique has higher sensitivity; hence even if the sample contains lower DNA, it can be amplified which is not feasible in the conventional PCR technique.

3) Nested PCR uses: Pathogen detection



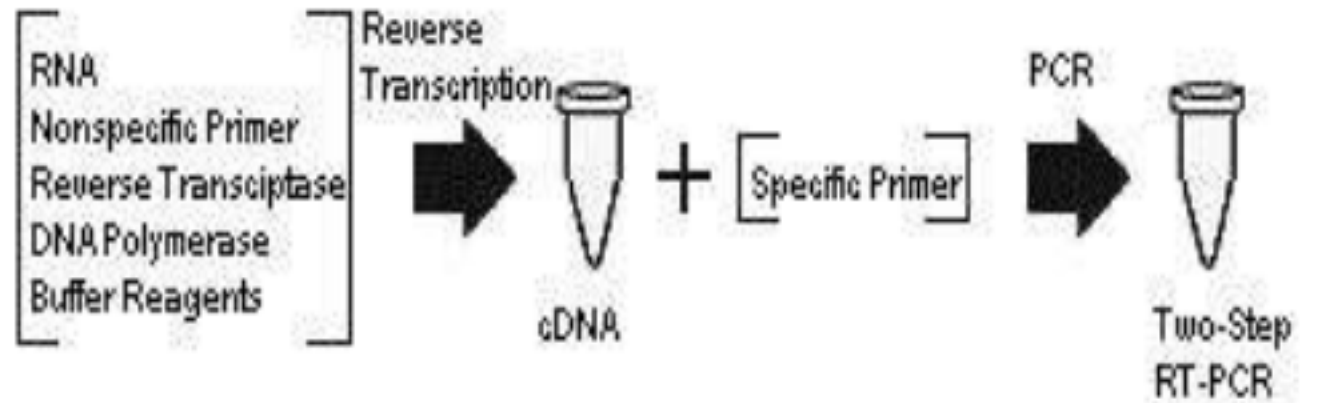
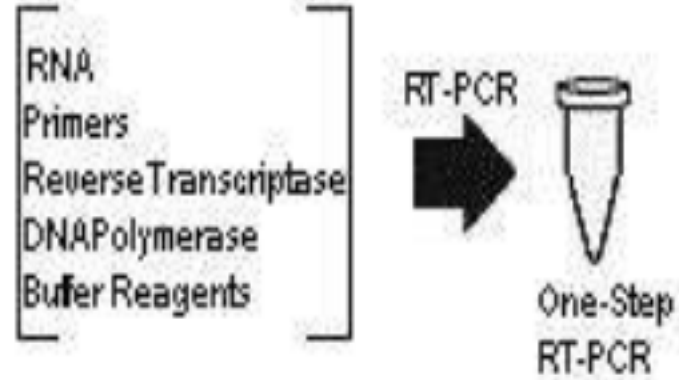
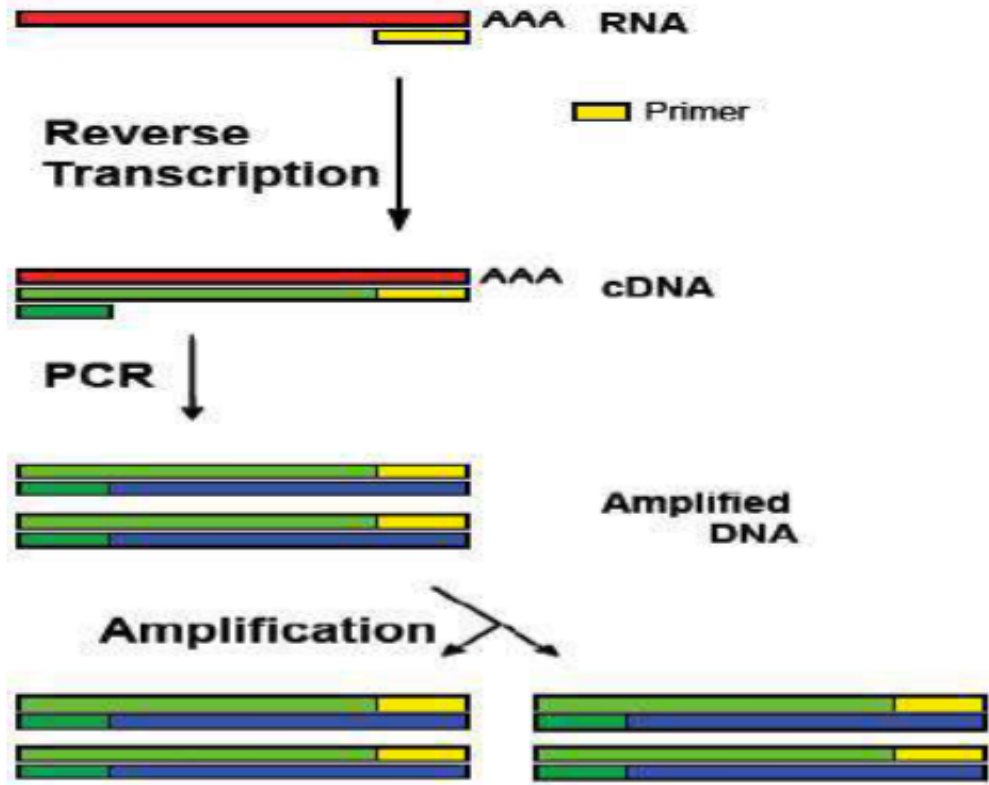
4) Reverse Transcription PCR:

- ✓ Used to reverse-transcribe and amplify RNA to cDNA.
- ✓ PCR is preceded by a reaction using reverse transcriptase, an enzyme that converts RNA into cDNA.
- ✓ The two reactions may be combined in a tube.

Uses:

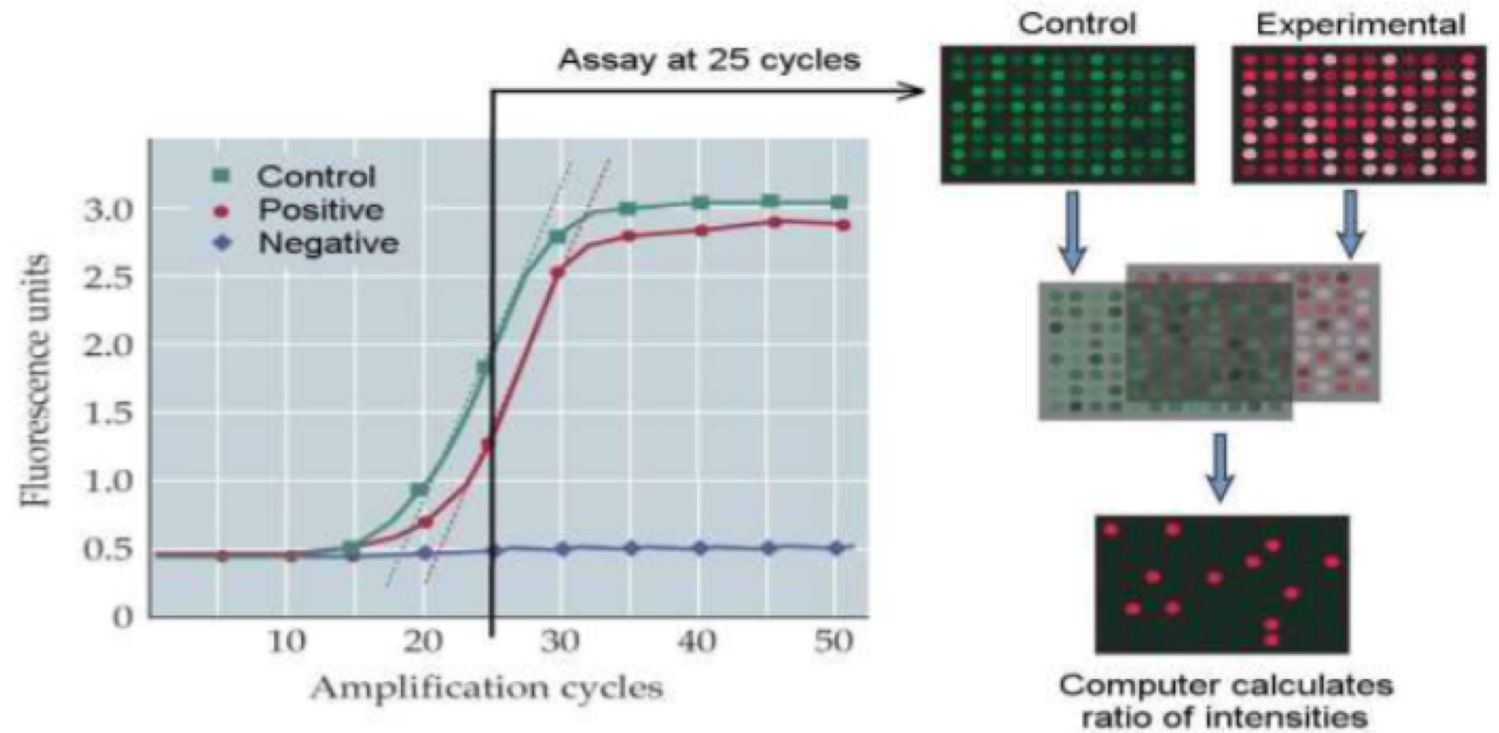
1. Detection of RNA virus like (HCV).
2. Detection of other M.O. through targeting of their Ribosomal RNA.

RT-PCR



5) Quantitative Real-Time PCR (qRT-PCR):

- Method use fluorescent dyes, such as Sybr Green, or fluorescence-containing DNA probes, such as TaqMan, to measure the amount of amplified product as the amplification progresses.
- Progress of DNA amplification during real time (RT-PCR) by measuring the release of fluorescent "flashes" during amplification. A computer measures the rate of "flashing" in 96 simultaneous experimental PCR reactions relative to a control reaction.



Quantitative - PCR Uses:

- ❖ Used to measure the specific amount of target DNA (or RNA) in a sample.
- ❖ By measuring amplification only within the phase of true exponential increase, the amount of measured product more accurately reflects the initial amount of target.
- ❖ Special thermal cyclers are used that monitor the amount of product during the amplification.

6) Hot-start PCR: It is a technique performed manually by heating the reaction components to the DNA melting temperature (e.g. 95°C) before adding the polymerase.

7) Touchdown PCR:

- ✓ In this type the annealing temperature is gradually decreased in later cycles.
- ✓ The annealing temperature in the early cycles is usually 3-5°C above the standard T_m of the primers used, while in the later cycles it is a similar amount below the T_m .
- ✓ The initial higher annealing temperature leads to greater specificity for primer binding, while the lower temperatures permit more efficient amplification at the end of the reaction.

8) Assembly-PCR (also known as Polymerase Cycling Assembly or PCA):

- ✓ In this type synthesis of long DNA structures by performing PCR on a pool of long oligonucleotides with short overlapping segments, to assemble two or more pieces of DNA into one piece.
- ✓ It involves an initial PCR with primers that have an overlap and a second PCR using the products as the template that generates the final full-length product.
- ✓ This technique may substitute for Ligation-based assembly.

dsDNA fragments with overlapping ends.



Add fragments to
Gibson Assembly
Master Mix.



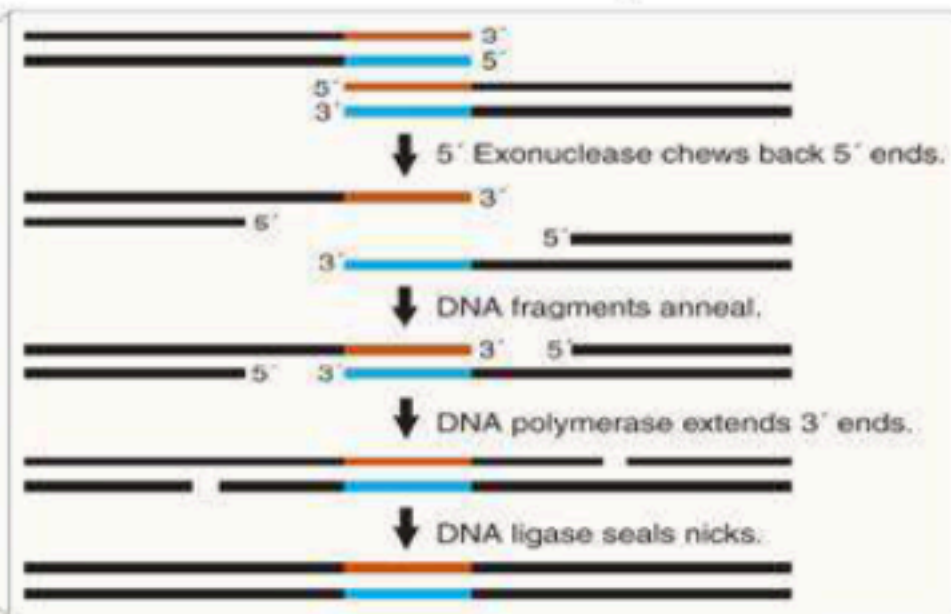
Incubate at 50°C
for 1 hour.

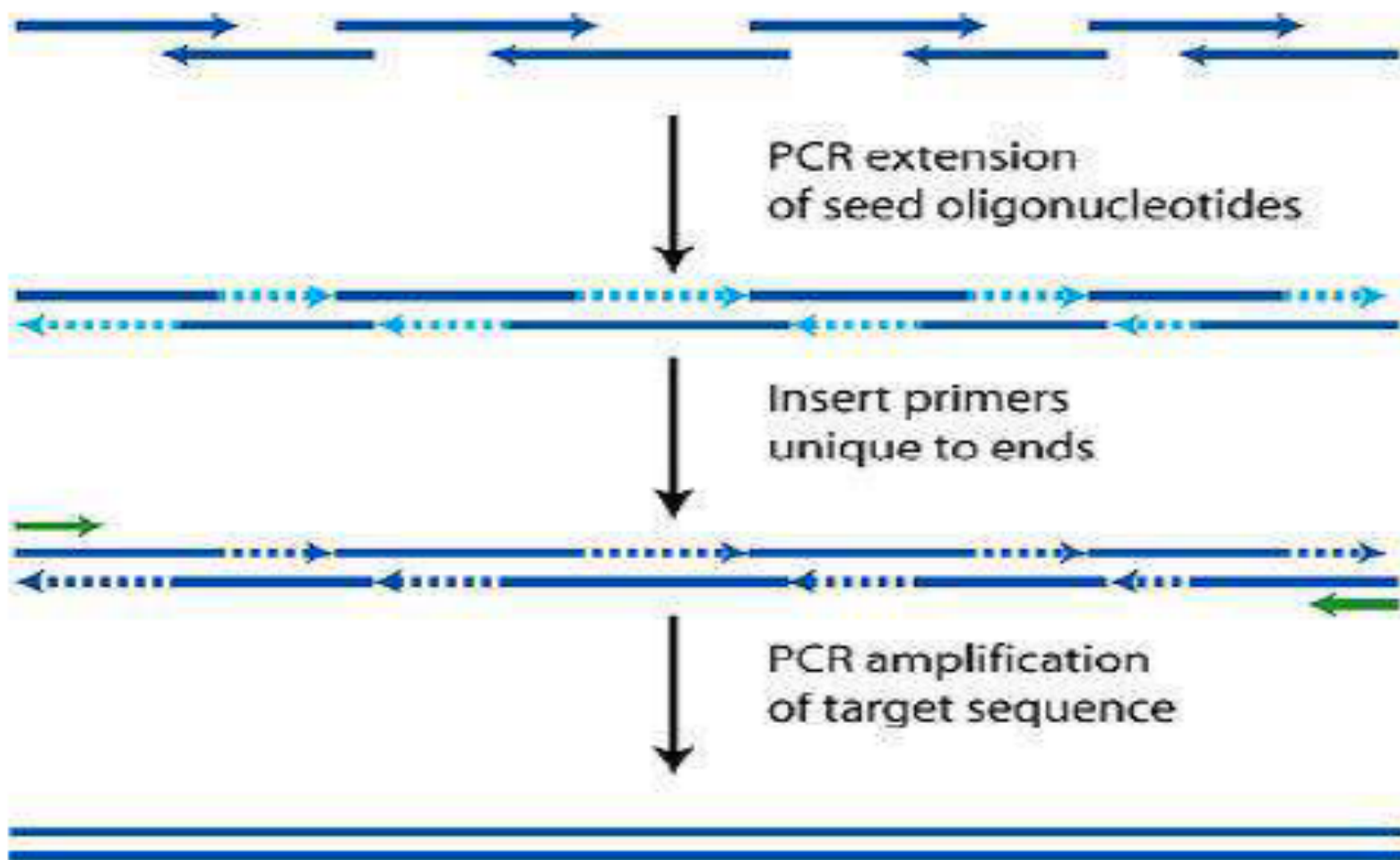


A + B

Fully Assembled DNA

Gibson Assembly





9) Colony PCR:

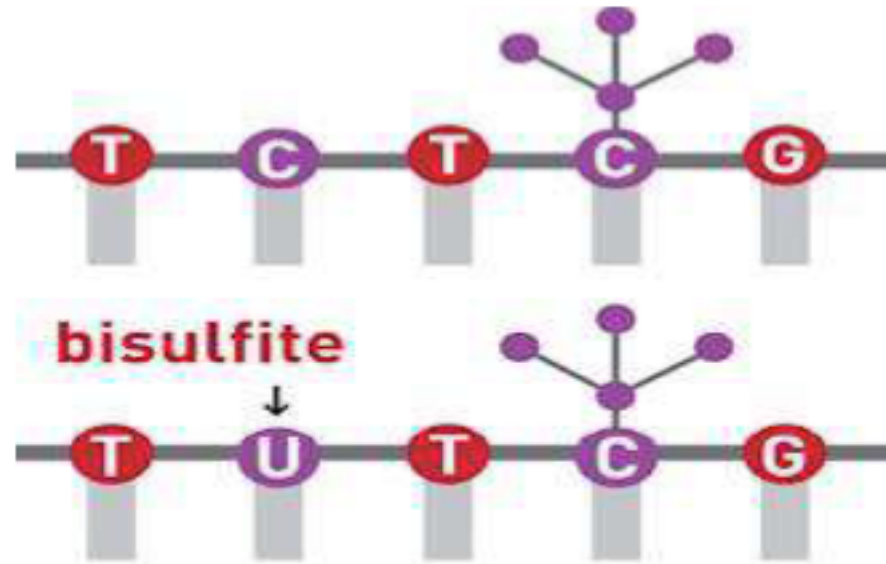
- ✓ Bacterial colonies are screened directly by PCR, for example, the screen for correct DNA- vector constructs.
- ✓ Colonies are sampled with a sterile pipette tip and a small quantity of cells transferred into a PCR mix.

10) Methylation-specific PCR (MSP):

- ✓ Used to identify patterns of DNA methylation at cytosine guanine islands (C&G islands) in genomic DNA. C&G islands, are concerned in regulation of gene expression in mammalian cells.
- ✓ Target DNA is first treated with sodium bisulfite, which converts unmethylated cytosine bases to uracil, which is complementary to adenosine in PCR primers.
- ✓ Two amplifications are then carried out on the bisulfite-treated DNA:

10) Methylation-specific PCR (MSP):

- ✓ One primer set anneals to DNA with cytosine (corresponding to methylated cytosine),
- ✓ The other set anneals to DNA with uracil (corresponding to unmethylated cytosine).
- ✓ MSP used in quantitative PCR provides quantitative information about the methylation state of a given C&G island.



11) LAMP assay: (Loop-mediated isothermal amplification):

- ✓ It is a Modified type of the PCR using 3:6 primers sets one of them is loop like primer.
- ✓ This test use Bst- polymerase enzyme (*Bacillus stearothermophilus* DNA Polymerase).
- ✓ Using only two temperatures (63°C for 45 min. then 85°C for 5 min.), may be carry out in water bath.

cDNA Synthesis & Reverse Transcriptases

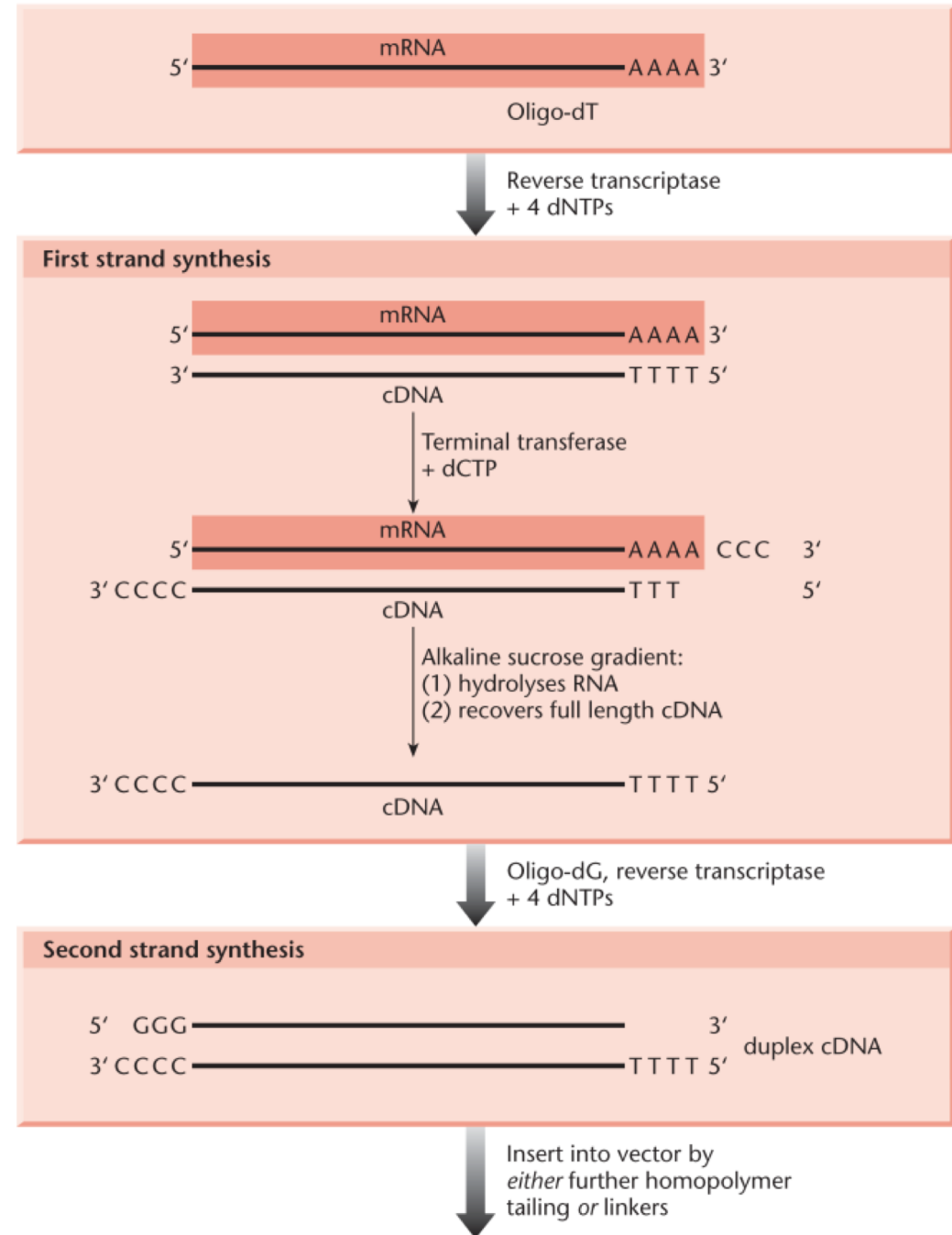
- The synthesis of DNA from an RNA template, via reverse transcription, produces complementary DNA (cDNA).
- Reverse transcriptases (RTs) use an RNA template and a short primer complementary to the 3' end of the RNA to direct the synthesis of the first strand cDNA, which can be used directly as a template for the Polymerase Chain Reaction (PCR).
- This combination of reverse transcription and PCR (RT-PCR) allows the detection of low abundance RNAs in a sample, and production of the corresponding cDNA, thereby facilitating the cloning of low copy genes.
- Alternatively, the first-strand cDNA can be made double-stranded using DNA Polymerase I and DNA Ligase. These reaction products can be used for direct cloning without amplification.
- In this case, RNase H activity, from either the RT or supplied exogenously, is required.

cDNA Library:

- ✓ It's a population of mRNAs
- ✓ Generated by *Reverse Transcription* of cellular mRNA, reveals expression profiles in different cell types and developmental stages
- ✓ Cloned eukaryotic cDNAs have their own special uses

Advantage:

Size of cDNA clone is significantly lower than the Genomic DNA library



References:

<http://Microbiology methods>