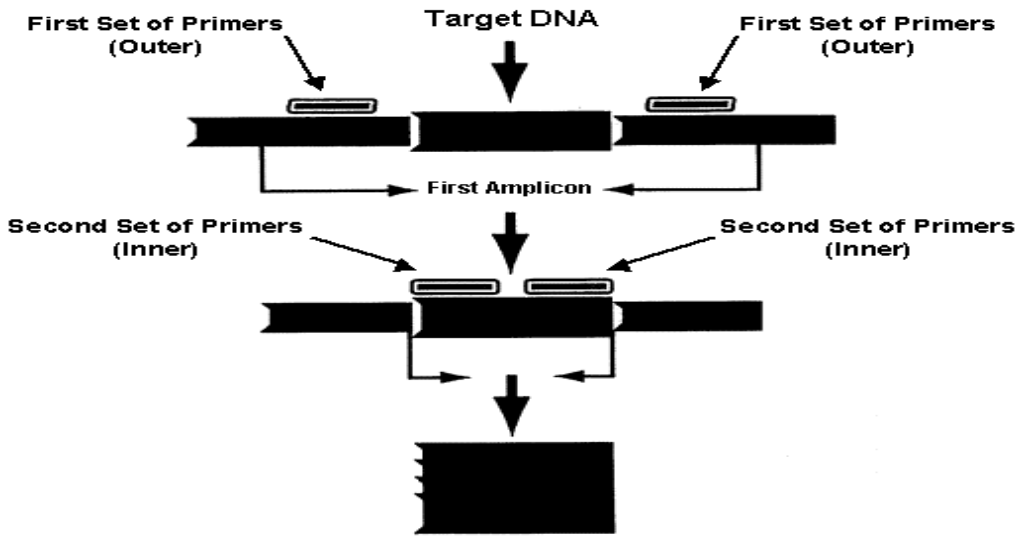
PCR Based Assays: Types of PCR

Week 7

Nested PCR

- In Nested PCR, there are two different PCRs
- Two different primer pairs
- In the first PCR, a longer sequence of DNA including the sequence of target DNA is amplified. The amplicons are used as template DNA in the second PCR reaction!
- In the second PCR, only the target sequence is amplified.
- Thus, the sensitivity of overall PCR assay is increased!



Specific Amplification of the Target DNA

RT-PCR

- Reverse transcriptase polymerase chain reaction (RT-PCR)
- Used for the detection of viruses containing RNA genome / used for the identification of RNA transcripts
- The first step is the isolation of RNA!

Method:

FIG. 1. RNA template. Prior to initiating reverse transcription the template RNA must be isolated from the sample to be tested. This figure shows a polyadenylated mRNA.

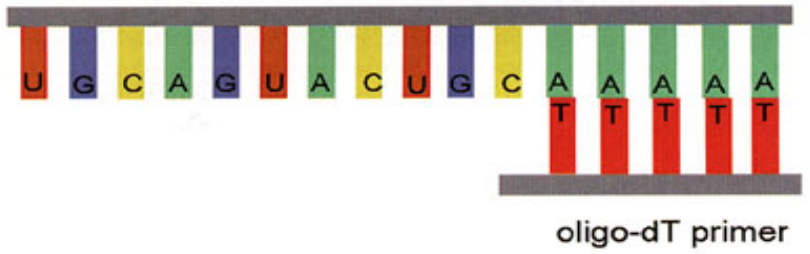


FIG. 2. Priming for reverse transcription. To generate cDNA using the enzyme reverse transcriptase (RT), a primer is annealed to the template RNA. The primer can be gene specific primers, random primers or oligo-dT primers for mRNA. In this example, oligo-dT primers are used to initiate cDNA synthesis from mRNA.

Then by the help of reverse transcriptase enzyme, for cDNA synthesis reverse primer binds to single stranded template RNA!

RT-PCR

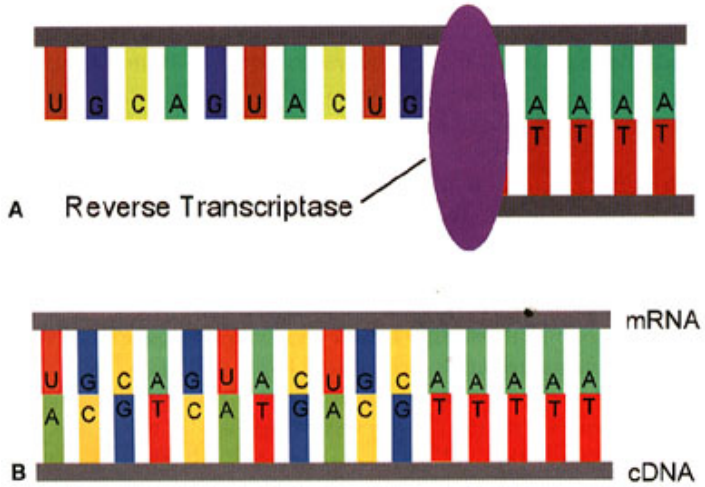


FIG. 3. First strand synthesis. The first strand of cDNA is synthesized using RT. Beginning at the primer annealing site (A), RT adds complementary nucleotide bases to the mRNA strand creating a strand of cDNA (B).

RT enzyme synthesize cDNA by adding appropriate complementary nucleotides on 3' end of the reverse primer!

For this purpose;

•Spesific primers (reverse primer)

•Random hexamers

•Oligo dT primers are used!

RT-PCR

FIG. 4. Removal of RNA. The template strand of RNA is removed by treatment with RNAse H. The cDNA can now be used for amplification by PCR.

Later on RNA template is excluded with RNAse H enzyme!

Now cDNA can be used in PCR amplification as DNA template!

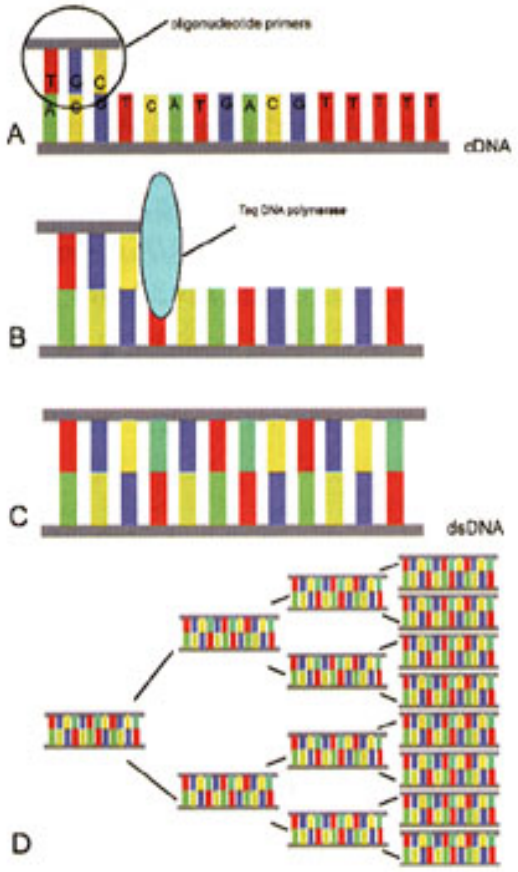


FIG. 5. The PCR reaction. The oligonucleotide primer is allowed to anneal to the template cDNA (A). *Taq* polymerase adds complementary nucleotides beginning at the primer annealing site (B). The resultant product is a double stranded cDNA (C). The three step process of denaturation, primer annealing and extension are repeated to yield a detectable PCR product (D). The product can be visualized on an ethidium bromide stained agarose gel following electrophoresis.

All the procedures after this step is very much the same with standard PCR reaction!

Advantages and Disadvantages of RT-PCR

Advantages

- High sensitivity
- High specificity
  Especially when the specific reverse primer is used for cDNA synthesis!!!
- Results are obtained in 1-2 days and even in hours!!!.

Disadvantages

- Same with disadvantages of PCR
- RT-PCR does not detect functional proteins but rather transcripts!!!

Touchdown PCR

- **In this method, primer annealing temperatures are decreased by 1^0C (or 0.1-1^0C) in each second step of every cycle. Thus, more specific primer annealing is aimed!**
- In the very first cycles of Touchdown PCR high annealing temperatures are preferred (i.e. 60^0C). In these temperatures a more specific binding occurs between the targeted template sequence and the primers. However, the sensitivity of these bindings are low!
- In the later cycles, primer annealing temperatures are decreased gradually until the optimal binding temperature for the primers!
- By the help of this strategy, non-specific annealing to the targets is hindered!
- In the following cycles, with the temperatures for optimal binding of primers are reached, much more sensitive annealing of primers to their target sequences occur which increases the sensitivity!
- In this technique, non-specific sequences are excluded due to a race depending on primer annealing temparatures
- Advantage of the method is high sensitivity
- Disadvantage is the need for special thermal cyclers

RAPD-PCR

- **R**andom **A**mplification of **P**olymorphic **D**NA
- This is the type of PCR where DNA sequence segments are randomly amplified!
- In RAPD, only one short primer of 8-12 nucleotide length are used!
- These short primers binds to corresponding sequences of an whole genome of bacterial strains
- More than one (actually 8-12) DNA bands are obtained in the method. By the analysis of these band patterns / profiles the strains are characterized molecularly

RAPD-PCR Electrophooresis Results