CH. 22: POLYMERASE CHAIN REACTION (PCR) e pCR is used in chromosome

Random PCR walking.

In this method, a synthetic DNA strand ontaining two or three internal primers is containing of more interest of two or more interest. Syndesigned and two or more internal primers is called nested primer.

In the second PCR, the synthetic DNA binds on the DNA strand to be amplified and

then polymerization proceeds. If one or two internal primers failed to anneal with the target DNA strand, polymerization does not take place. So the soughtafter DNA clone is selectively amplified in

this method.

Use

Random PCR is used to propagate a specific DNA strand in a pool of DNA fragments without amplifying the other strands. It is used in RAPD analysis.

RT-PCR

RT-PCR refers to reverse transcription followed by polymerase chain reaction. It is used to make cDNA clones from mRNA. Here,

reverse transcriptase and a primer are added to the conventional PCR reaction mixture.

The reverse transcriptase synthesizes cDNA strand to form a RNA-DNA hybrid. It is followed by PCR. After heat denaturation of the RNA-DNA hybrid, the second DNA strand is synthesized on the cDNA strand by Taq polymerase. As a result, cDNA clones are formed.

If the mRNA is primed with oligo (d)T primer, cDNAs are made from different kinds of mRNAs in the reaction mixture. Such cDNA clones are used to make cDNA library.

If the primer is nested one, it selectively forms cDNAs from a particular mRNA and amplifies the cDNA clones. This will help for rapid screening.

Rapid Amplification of cDNA Ends (RACE)

In this PCR, some sequence at 3' end or 5' end of the mRNA is made into cDNA and the latter is made to synthesize the second strand.

