Course: M.Sc. Biotechnology

Paper: BIOT4009: Genetic Engineering and Gene Therapy

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UNIT – III
POLYMERASE CHAIN REACTION-4

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Types and Applications of PCR

Important types (component dependent) and features

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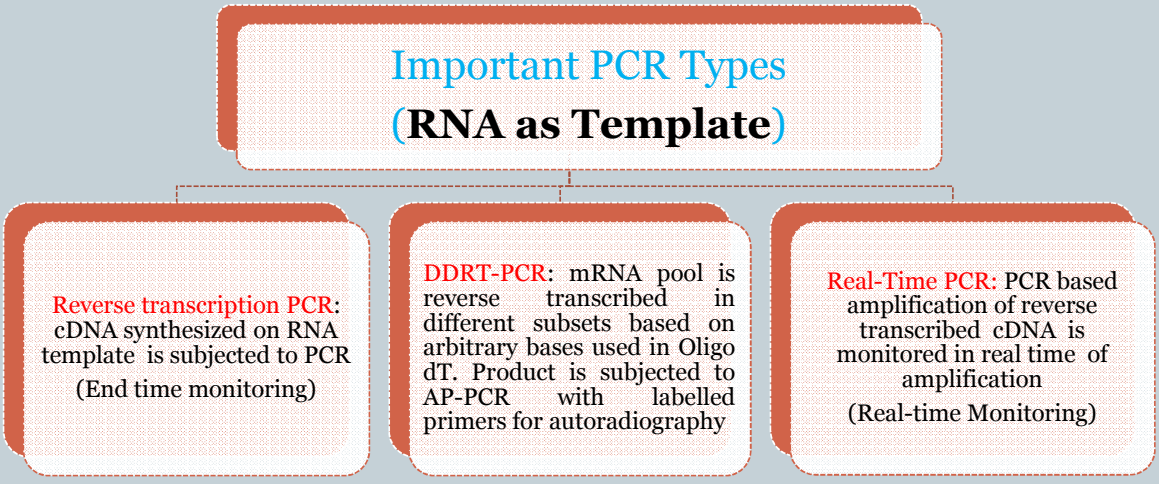

REVERSE TRANSCRIPTION PCR

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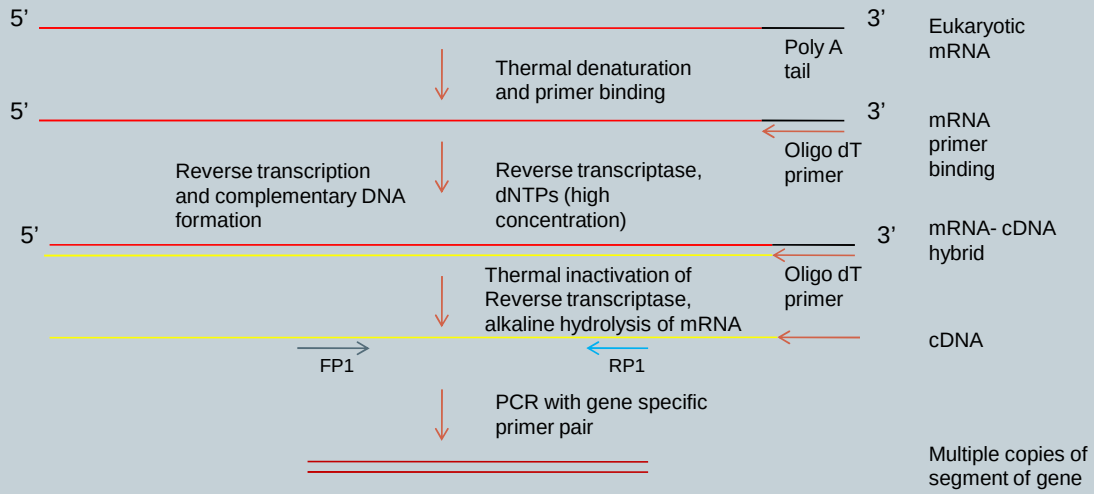
- mRNA is reverse transcribed by reverse transcriptase
- Complementary DNA thus formed in subjected to PCR with gene specific primer pairs (FP1 and RP1 may be homo or heterologous forward and reverse primers)



Reverse Transcription PCR contd.

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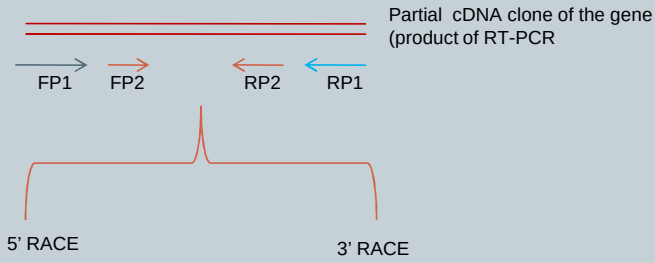
- A part of gene cloned by RT-PCR is ligated in vector and transformed in bacterial host cell and allowed to amplify with cell division.
- The cloned part is sequenced and new primers are designed from sequences internal to those corresponding to FP1 And RP1
- Internal primers FP2 and RP2 are used in Rapid Amplification of cDNA Ends (RACE) reactions to generate full 3' and 5' regions of the gene in two different reactions called 3' RACE and 5' RACE



3’ RACE

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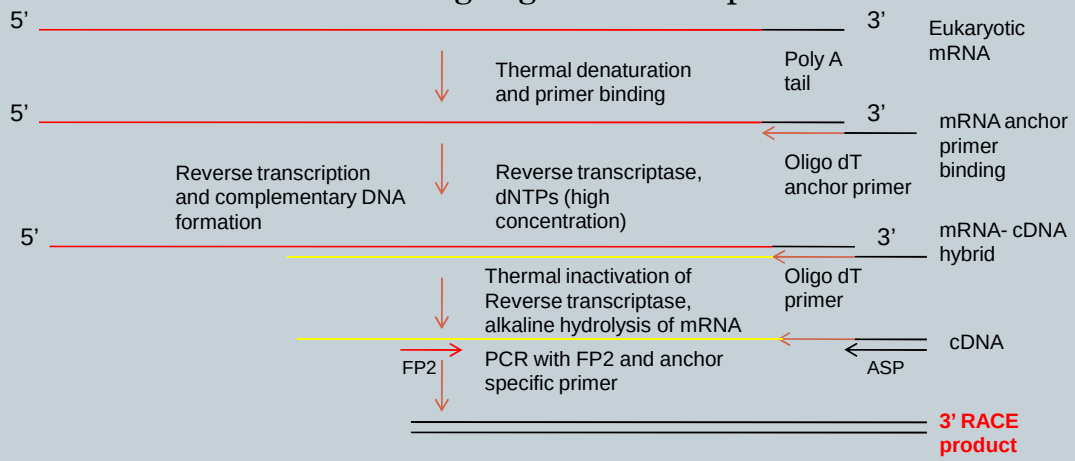
mRNA in reverse transcribed using oligo dT anchor primer



5'RACE

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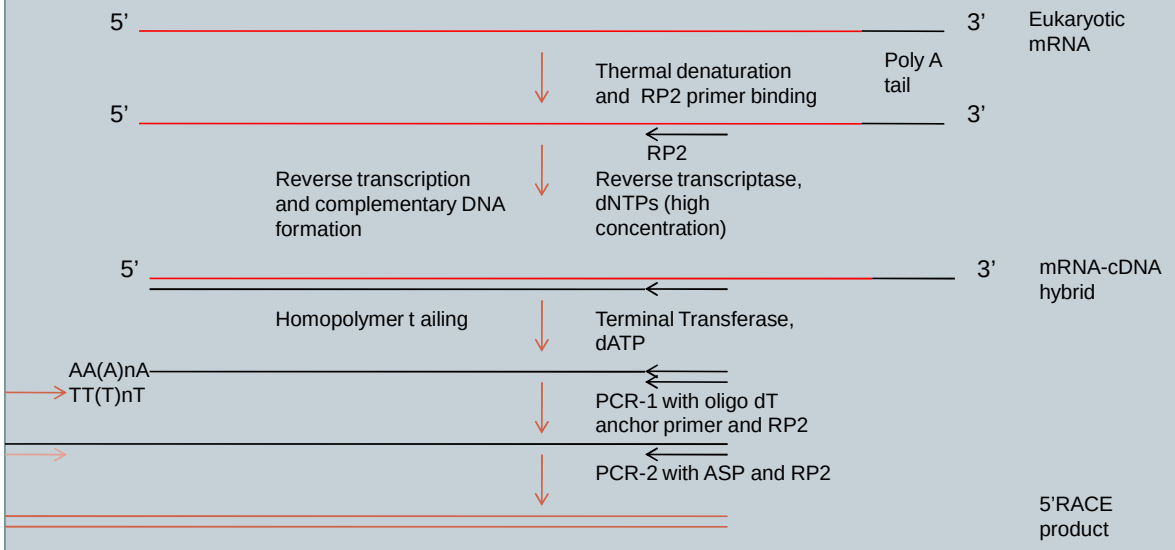
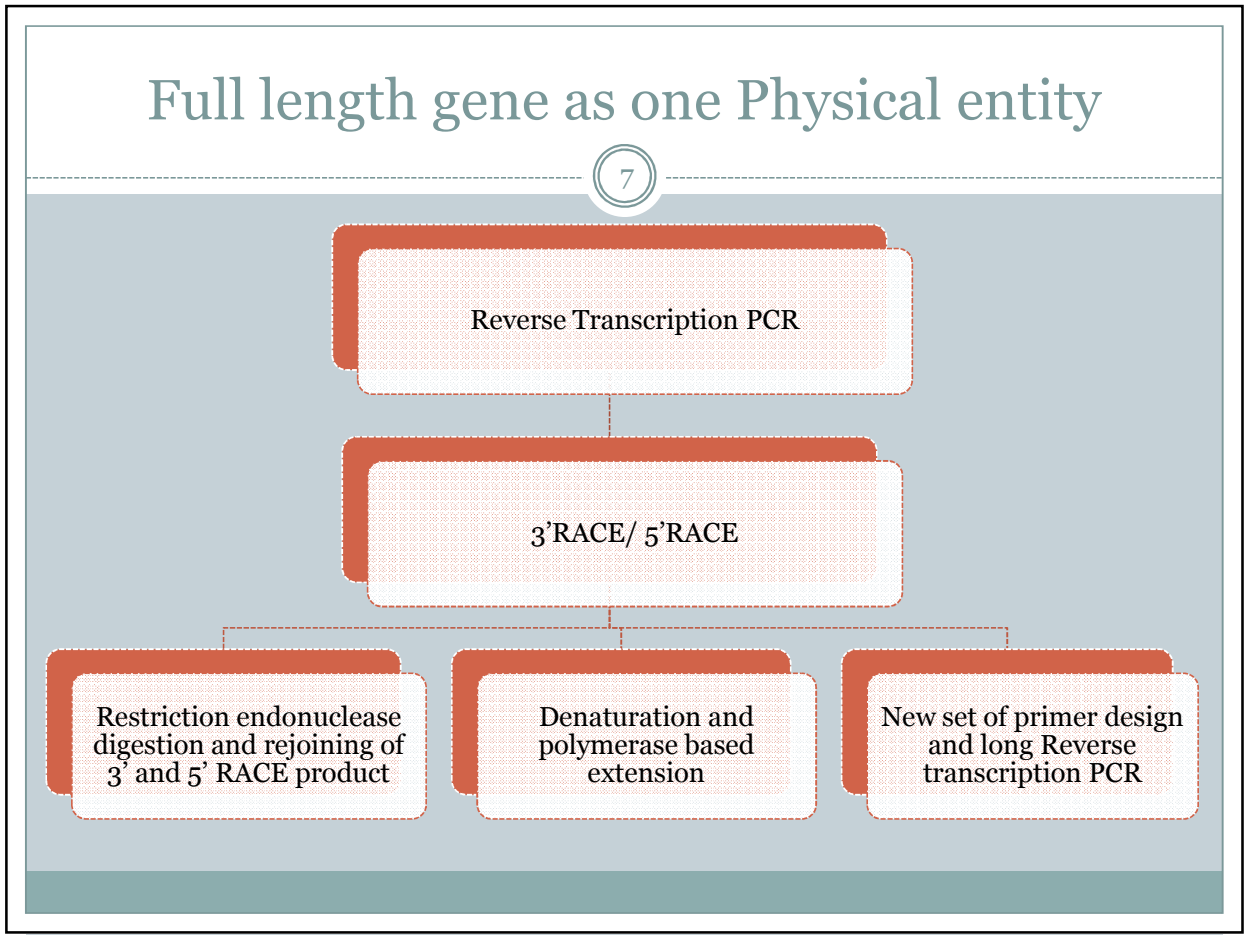
mRNA is reverse transcribed by RP1/ RP2




Full length gene as one Physical entity
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Reverse Transcription PCR
3'RACE/ 5'RACE
Restriction endonuclease digestion and rejoining of 3' and 5' RACE product
Denaturation and polymerase based extension
New set of primer design and long Reverse transcription PCR

DDRT-PCR

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For analyzing expression of many genes simultaneously in different samples

Sequence of events

- RNA of many samples are taken
- Selective reverse transcription of mRNA pool in different subsets
- Oligo dT primers with 2/3 arbitrary nucleotides at 3' end are used for selective reverse transcription
- All subsets are subjected to AP-PCR with radio-labeled primers
- Products are subjected to electrophoresis
- Bands are analyzed by autoradiography
- Differentially expressed bands may be identified, extracted from gel and cloned
- Cloned fragments may be sequenced and analyzed

Arbitrary Primed-PCR (AP-PCR)

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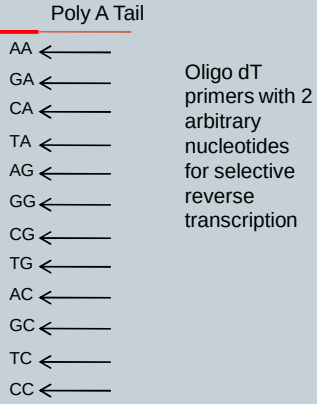
Method of selective reverse transcription

Using oligo dT primers with 2 arbitrary nucleotides 12 Reverse transcription reactions will be set. 1 with each primer and aliquot of RNA

Each primer reverse transcribe the mRNA base on 2 complementary nucleotides before polyA tail

Total cDNA is in the form of 12 subsets based on primers

Upon amplification in AP-PCR bands can be monitores if products divided in 12 subsets



Home assignment 2

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1. Discuss the important applications of Reverse Transcription-PCR in detection of Corona Patients.

2. How can AP-PCR be used to assess genetic similarity or differences in individuals of same population?

Note: Assignment, in the form of write up supported with diagram, is to be submitted to the e mail brijeshpandey@mgcub.ac.in by 25th April positively

Thanks

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TO BE CONTINUED