Course: M.Sc. Biotechnology

Paper: BIOT4009: Genetic Engineering and Gene Therapy

UNIT – III

POLYMERASE CHAIN REACTION-7

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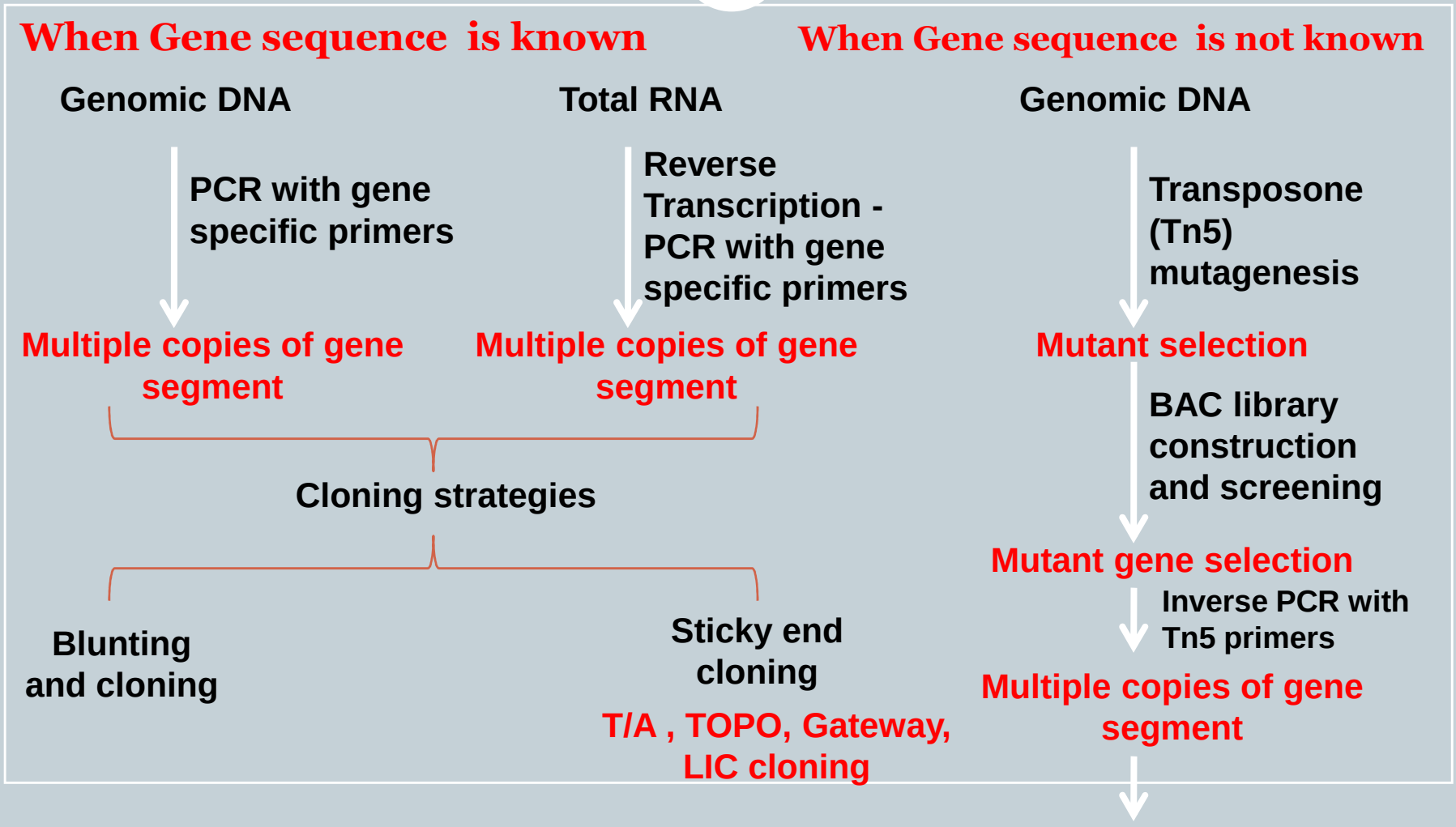
Strength of PCR

- Accuracy
- Sensitivity
- Ease of operation
- Robustness
- Cost efficacy
- Automation
- Innovation
- Diversity
- Speed of detection/ operation
- Availability of raw material

Important Applications of PCR

Gene cloning (simple strategies)



Important Applications of PCR

Diversity assessment (simple strategies)

RAPD

Genomic DNA samples

↓ PCR with arbitrary primers

PCR products

↓ Agarose gel electrophoresis

PCR Amplicon pattern

↓ Similarity index

Phylogenetic tree/ dendro/ cladogram

PCR-Restriction digestion

DNA samples

↓ PCR with gene specific primers

PCR products

↓ Restriction digestion with marker RE

Fragments of PCR product

↓ Agarose/ Polyacrylamide gel electrophoresis

Analysis of band pattern

In other methods, SSR, SST, ISSR, VNTR etc. primer based methods are also available

Important Applications of PCR

Gene Expression analysis (simple strategies)

Reverse Transcription -PCR

RNA from different tissues

↓ Reverse Transcription in separate tubes

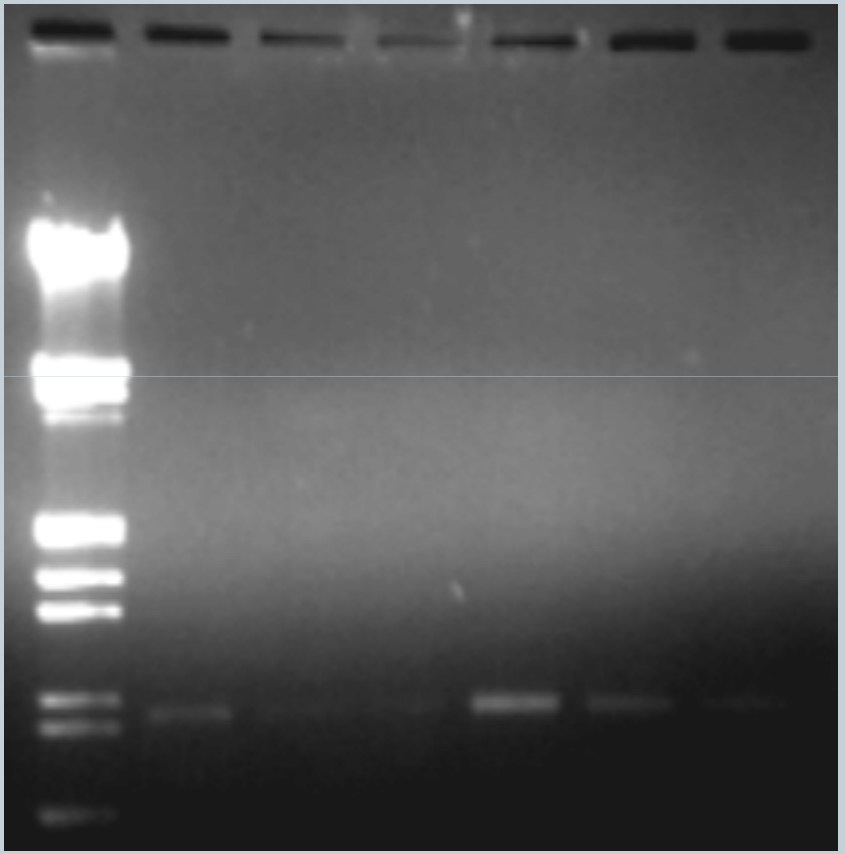
cDNA in different tubes

↓ PCR with gene specific primers

Amplicons in different tubes

↓ Agarose gel electrophoresis of product in different wells

Compare band number, size and intensity



M (E/H) C 50 100 200 500 L

Important Applications of PCR

Gene Expression analysis (simple strategies)

Real Time PCR (Semi quantitative)

RNA from different tissues

↓ Reverse Transcription in separate tubes

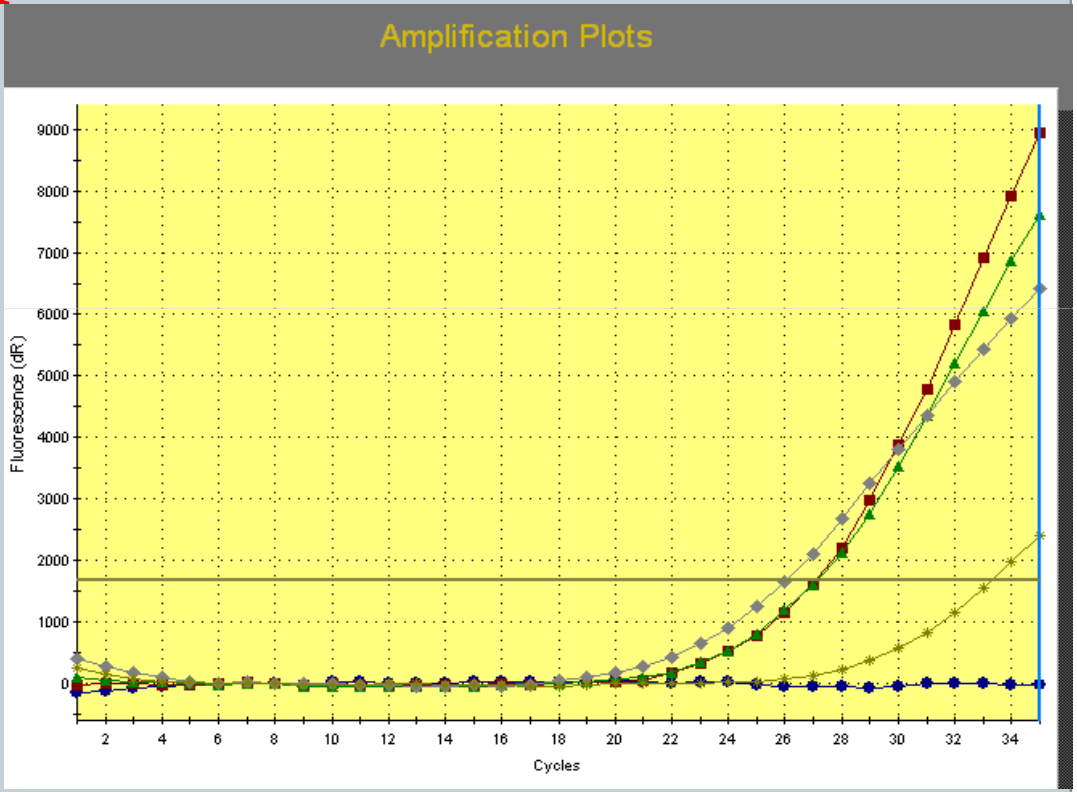
cDNA in different tubes

↓ Real-Time PCR with gene specific primers with SYBR Green master mix

Real time monitoring of amplification and Ct value estimation

↓

Semi quantitative estimation



Other Important Applications of PCR

- Mutation screening: SSR, VNTR, RAPD, Restriction-PCR
- Drug discovery
- Classification of organisms
- Genotyping
- Molecular Archaeology
- Molecular Epidemiology
- Molecular Ecology

Other applications contd.

- **Bioinformatics**
- **Genomic cloning**
- **Site-directed mutagenesis**
- **Gene expression studies**
- **Genetic matching**
- **Detection of pathogens**
- **Pre-natal diagnosis**
- **DNA fingerprinting**
- **Gene therapy**

Other applications

- **Comparative estimation of microbial population diversity**
- **Array verification**
- **Quality control and assay validation**
- **Biosafety and genetic stability testing**
- **Drug therapy efficacy / drug monitoring**
- **Viral quantitation**
- **Pathogen detection**
- **DNA damage (microsatellite instability) measurement**

Other applications contd.

- Radiation exposure assessment
- In situ PCR
- Mitochondrial DNA studies
- Methylation detection
- Detection of inactivation at X-chromosome
- Detection of apoptosis
- Quarantine assemment
- Adulteration
- Pedigree analysis
- Genotyping (allelic discrimination)
- Epigenetic assessment

Thanks

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