Polymerase Chain Reaction (PCR)

3- Applications of the PCR

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Applications of the PCR

Genetic counseling.

= Genetic diseases

- parents tested for being genetic carriers
- their children tested for actually being affected by a disease

Medicine:

* Diagnostic tests for genetic, bacterial or viral diseases.

* diagnose of early stages of bacterial and viral infections.

forensic medicine:

* Fatherhood (Paternity testing)
* Criminal…

A short tandem repeat (STR) in DNA (GATC)

= over 10,000 published STR sequences in the human genome.

= STR analysis, prevalent method for determining: genetic profiles (fingerprinting) forensic cases

= The FBI has chosen 13 specific STR loci to serve as the standard for genetic fingerprinting (Si= 1 in 1 billion or greater.).

- Genetic Fingerprinting
  - * Specific genes
  - * STR
- Detecting pathogens using genome specific primers.
- Screening specific genes for unknown mutations
- DNA sequencing possible after PCR

- **Bio-diversity (species variation).**

  * Detection of specific micro-organisms.

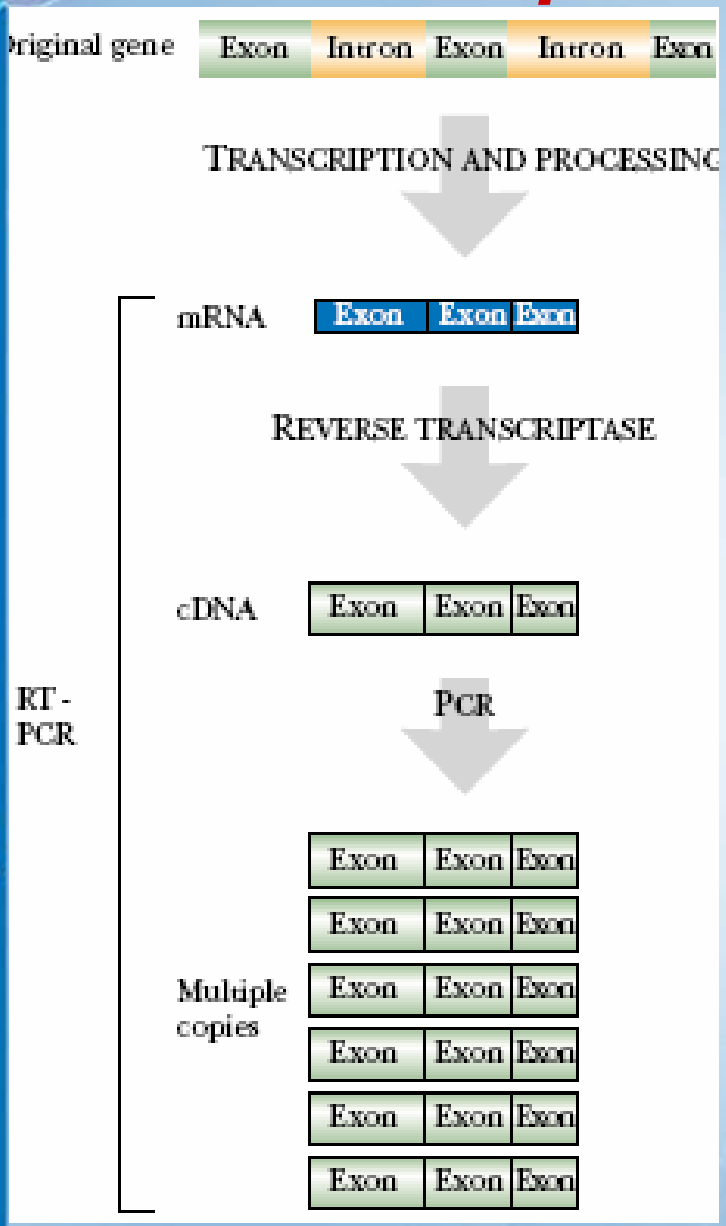
- **Cloning of genes**

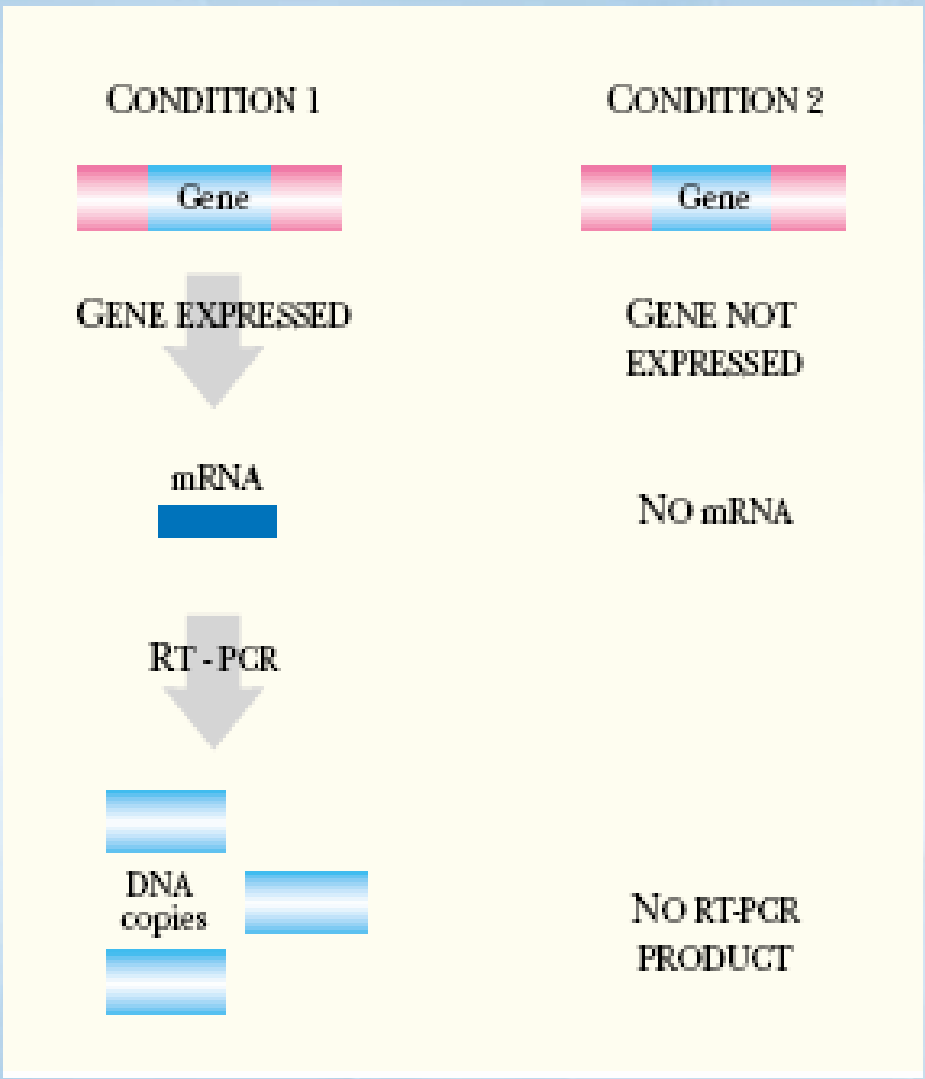
- **Gene expression**

  Reverse Transcriptase based PCR (RT-PCR)

  Start Template : mRNA

RT-PCR for Gene





RAPD-PCR

RAPD = Random Amplified Polymorphic DNA

- Amplify unknown DNA sequences using single, short (10-12 bases), and random primers.

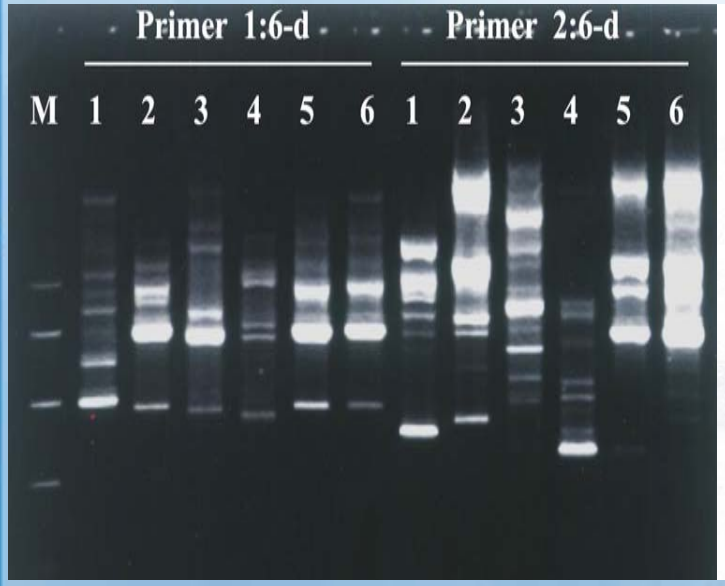
Example:

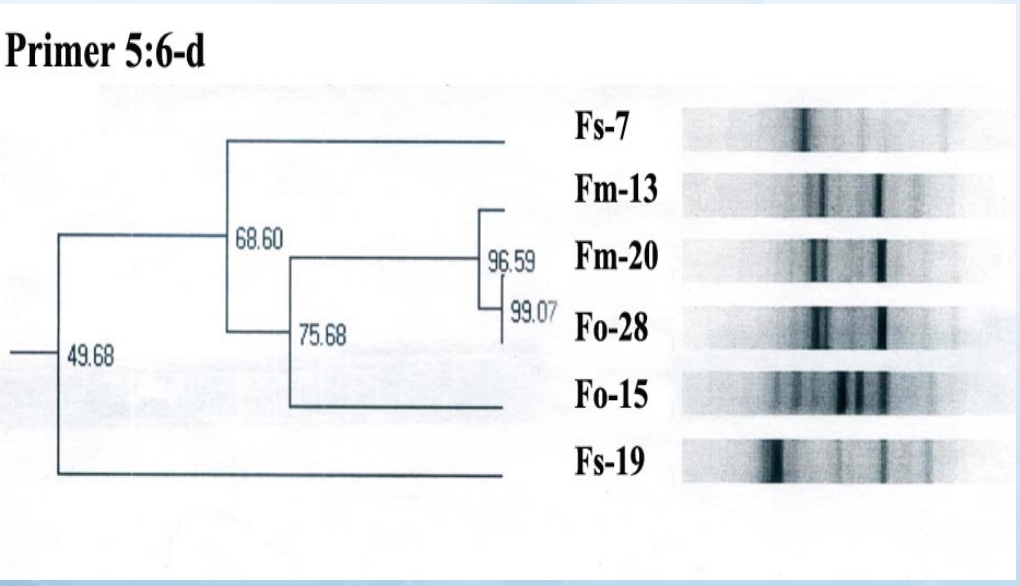
OPO-13	5`-GTC AGA GTC C-3`
OPO-14	5`-AGC ATG GCT C-3`
OPO-16	5`-TCG GCG GTT C-3`
OPO-18	5`-CTC GCT ATC C-3`

- Small number of primers could be used to generate a very large number of fragments.
- These fragments are usually generated from different regions of the genome and hence multiple loci might be examined very quickly.
- Fast, easy and cheap.
- Commercial primer sets are available.

- **Quite useful genetic markers in:**

- **breeding programs.**
- **Determination of genetic variability and fingerprint.**
- **detection of variation between closely related cultivars or strains.**






Thank you
Prof. Dr. Hamdy El-Aref

References

1. Saiki RK et al. "Enzymatic Amplification of β-globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia" Science vol. 230 pp. 1350-54 (1985).
2. Quill E "Blood-Matching Goes Genetic" Science Magazine (14 March 2008) pp. 1478-1479.
3. Kwok S et al. "Identification of HIV sequences by using in vitro enzymatic amplification and oligomer cleavage detection." J. Virol. vol. 61(5) pp. 1690-4 (1987).

- Boehnke M et al. "Fine-structure genetic mapping of human chromosomes using the polymerase chain reaction on single sperm." Am J Hum Genet vol. 45(1) pp. 21-32 (1989).