# Molecular Diagnosis: Sickle cell Anemia

Roll no- 21/4642

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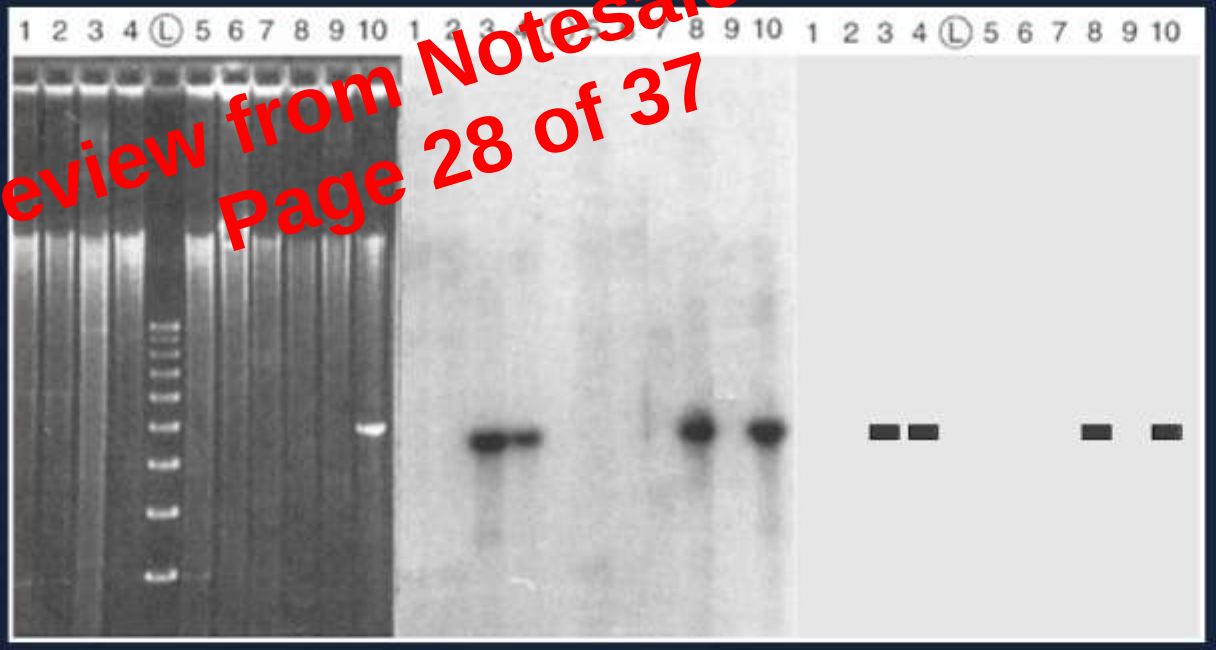
**2. Bi-directional allele-specific amplification (ASA)**: Allele specific PCR is a technique based on allele primers, which can be used to analyze single nucleotide polymorphism (SNP) effectively including the transition, tranversion and insertion/deletion polymorphism. Here, the point mutation of sickle cell anemia is used as the SNP model.

**Hot start system**: Hot Start PCR is a more sensitive technique than standard PCR that allows amplification of low-abundance targets and single-copy genes while reducing PCR background problems.

Thermophilic DNA polymerases are unfortunately active at room temperature, which can result in amplification of unspecific targets due to random primer annealing events. Hot start enzymes are completely inactive during room-temperature reaction setup and become active only after heating. Thus, misprimed amplification products and primer–dimer formation do not occur, diminishing PCR background.

**3. Polymerase chain Reaction-Oligonucleotide ligation assay(PCR-OLA)**:
Polymerase Chain Reaction-Oligonucleotide Ligation Assay (PCR-OLA) is a method to diagnose hereditary diseases caused by mutation not affecting restriction endonuclease sites.
This method combines Polymerase Chain Reaction with the Ligation Assay. PCR-OLA distinguishes between the ligation and the absence of ligation of two oligonucleotides. A single nucleotide mismatch (at the site of hybridized oligonucleotides) prevents ligation and hence we can distinguish between the wild and mutant genotype. PCR-OLA is a sensitive, rapid and highly specific method.

**4. Restriction fragment length polymorphism(RFLP) or Southern blotting technique**: Restriction fragment length polymorphism (RFLP) is used to detect sickle cell disease based on restriction enzymes, which remove the recognition site at the βs mutated gene. For example, MstII is one of the first described restriction enzymes; it cuts the DNA in the sequence CCTNAGG (where N represents any nucleotide). Therefore, when thymine replaces the adenine, it removes the recognition site for MstII restrictase.




Electrophoretic gel (with Ethidium bromine)

Southern Blot

Autoradiogram