



SNP detection by PCR

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What is Single Nucleotide Polymorphism ?

- A SNP is small genetic change or variation that occur within a person's DNA sequence. The genetic code is specified by the four nucleotide letters i.e. A, T, G or C. SNP variation occurs when a single nucleotide, such as an A replaces one of the other three letters i.e.C, G, or T.
- e.g. after single point mutation, in seq. AAGGTTA the seq. changes to ATGGTTA
- In human population, most of the SNP through mutations are found only in about 3- 5 % of the genome.
- Mutations, found in coding region, are more likely to alter the biological function of a protein.
- Classical example: Sickle cell anaemia

Gene and alleles:simplified



Beta globin gene exists in two forms : β^a and β^s

Hb AA (Normal hemoglobin)

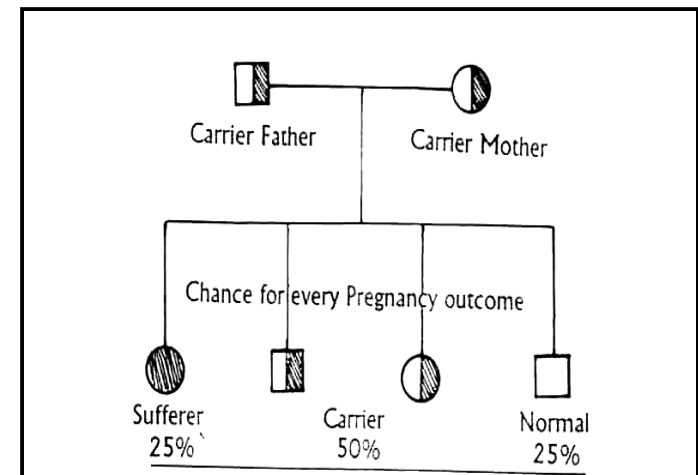
Both the alleles are normal

Hb SS (Sickle hemoglobin)

Both the alleles are abnormal

Hb AS (Sickle cell carrier)

One allele normal, one allele abnormal



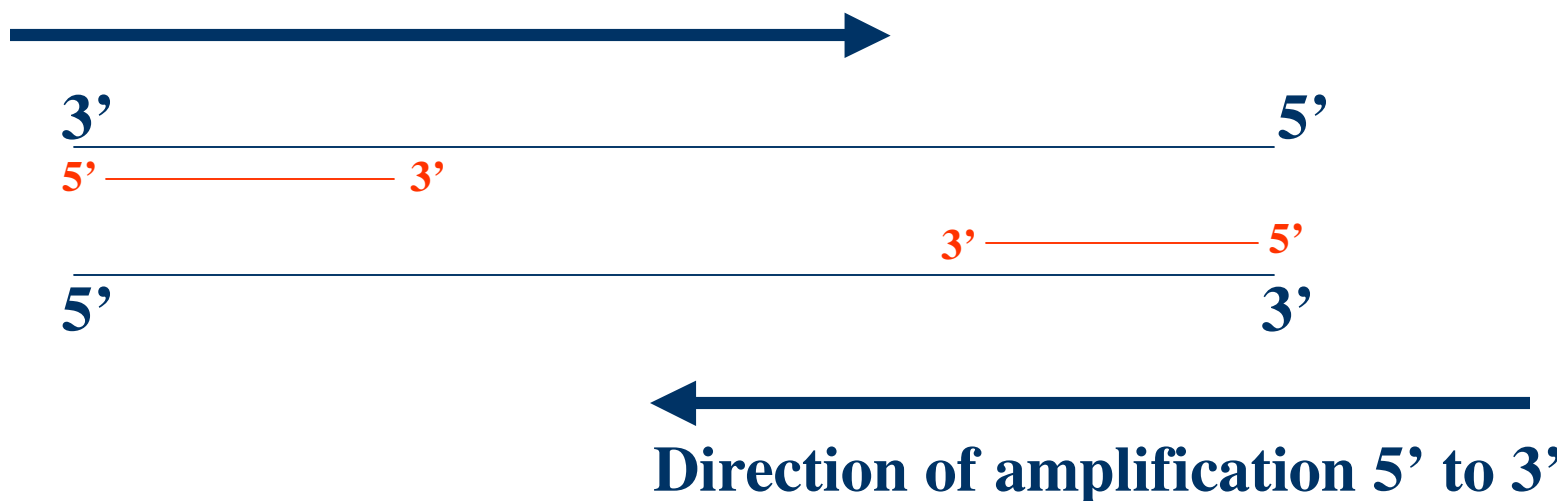
Sickle Cell Disease (SCD)



- 600 Hemoglobin variants
- One of the prominent Hemoglobinopathies
- Globin chains are produced with abnormal amino acid sequence
- Hb A (6) Glut⁶ Hb S (6)Val⁶
- Hemoglobins Polymerization is very concerted reaction and depends on the effective concentration of Hb S where
- Val β^6 hydrophobic interaction with Phe β^{85} and Leu β^{88}
- Hb AA Normal
- Hb AS Carrier
- Hb SS Sufferer

Simple standard PCR: Principle

Direction of amplification 5' to 3'



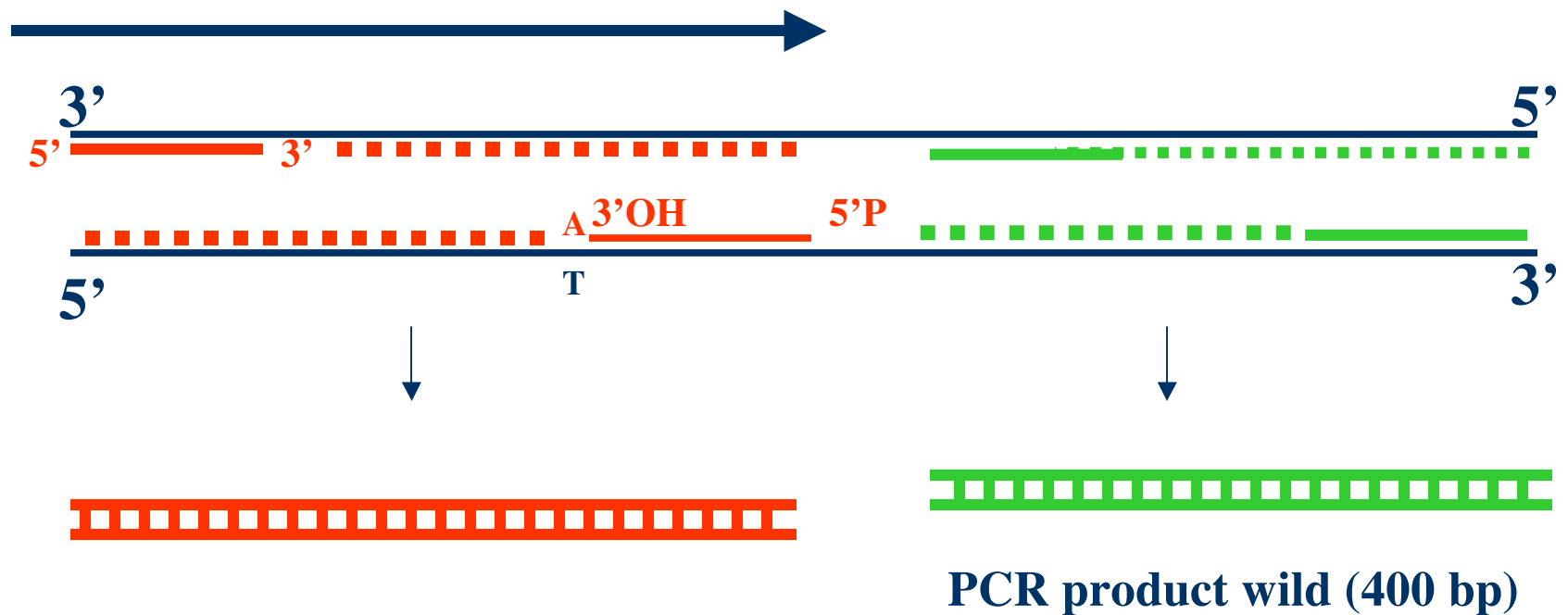
ASA: Allele specific amplification: cont...



e.g. 1000 bp DNA genomic region

Amplification, if base at the 3'OH is correct

Direction of amplification 5' to 3'



PCR product mutant (600 bp)

We get two products of 600 bp and 400 bp in single tube with four primers

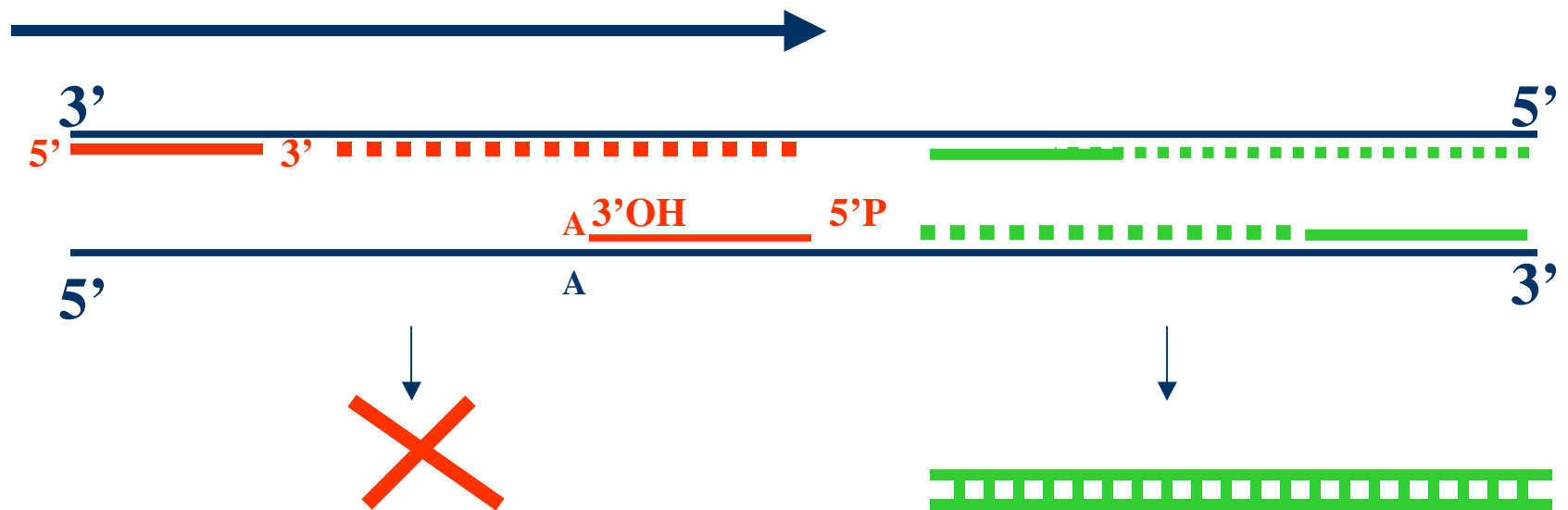
ASA: Allele specific amplification: cont...



e.g. 1000 bp DNA genomic region

Amplification, if base at the 3'OH is correct

Direction of amplification 5' to 3'

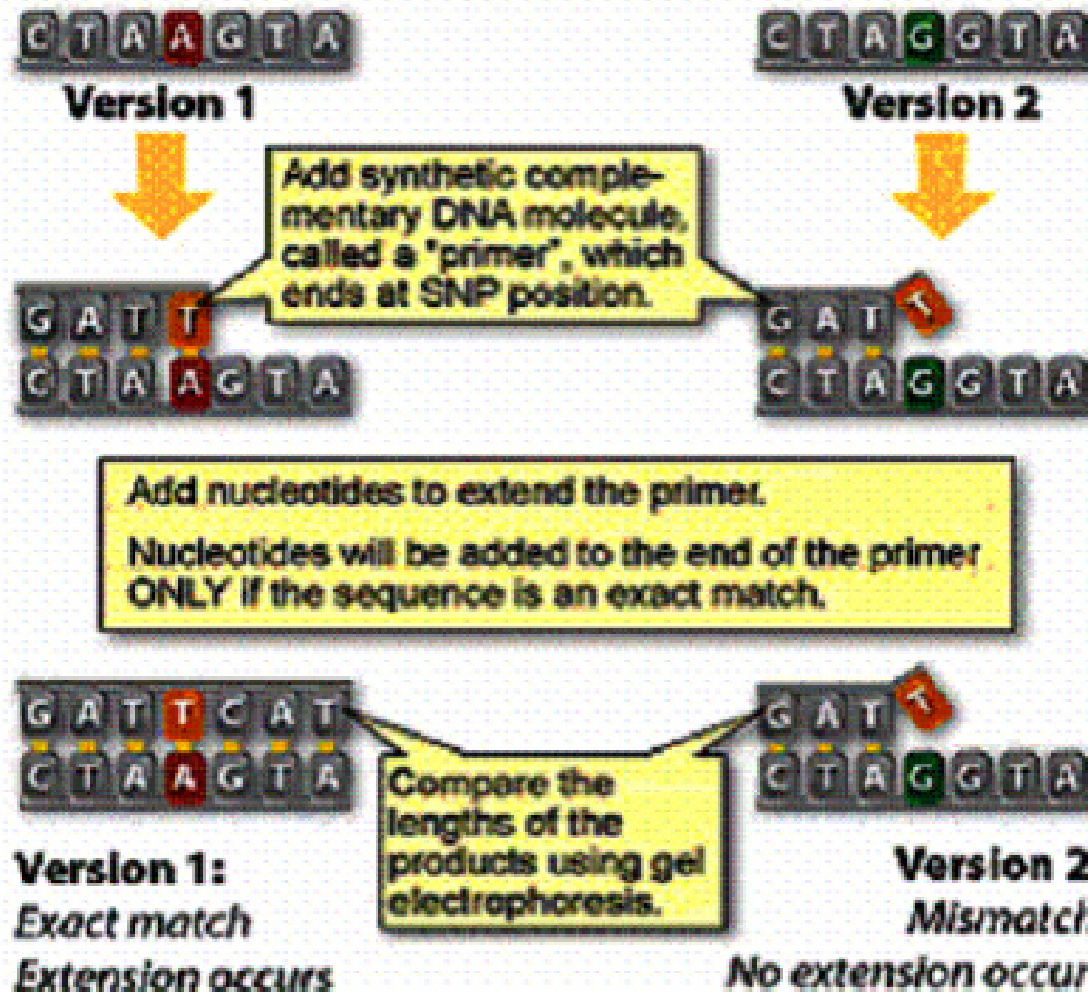




No amplification, because the primers were designed for mutated allele specific

PCR product wild (400 bp)

We get only one PCR product of 400 bp in single tube with four primers if there is no mutation

Using Primer Extension to Identify SNPs:





Visualization of PCR products by Agarose gel electrophoresis

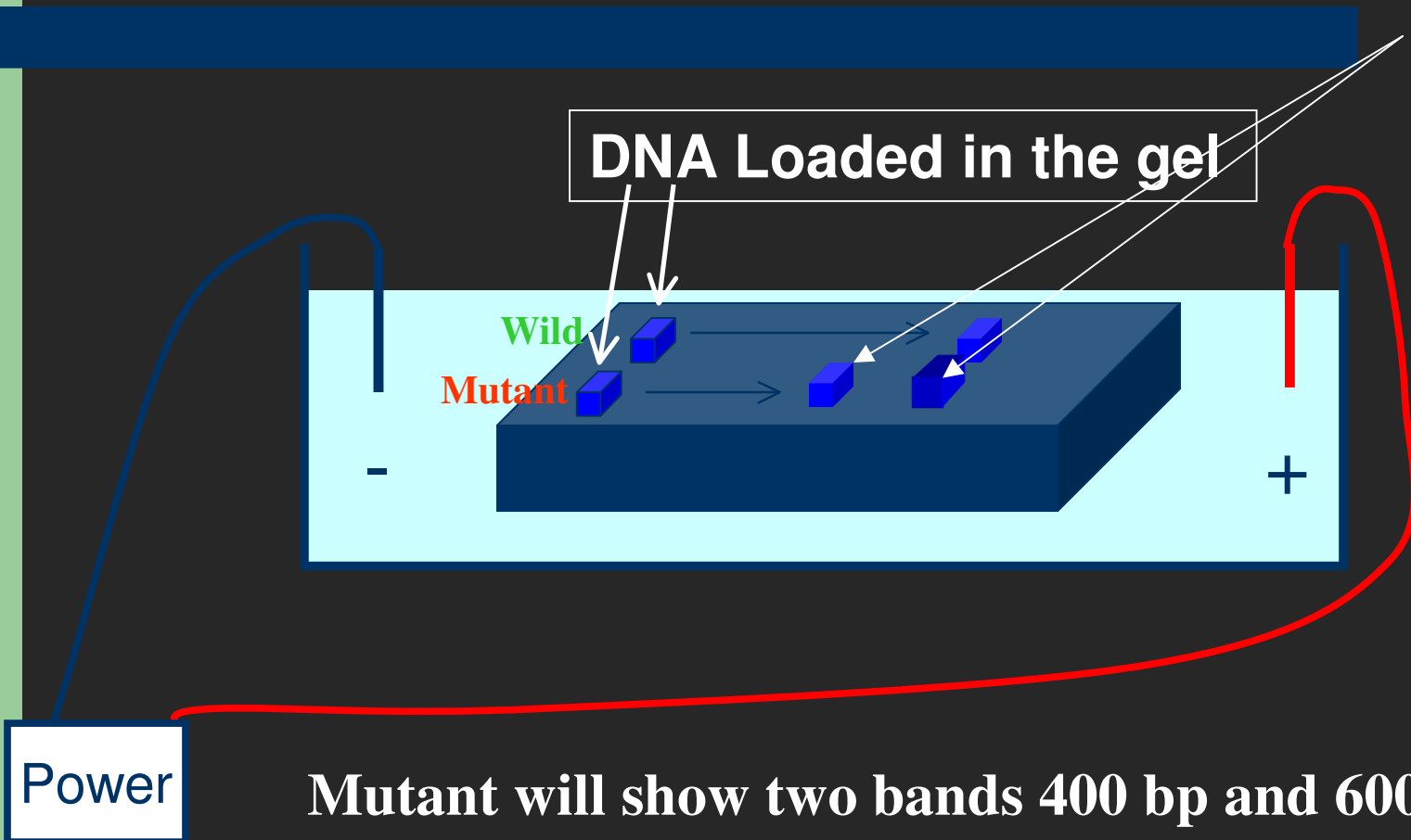


Separation and Visualization of PCR products by electrophoresis

One mutant specific larger

and

Wild smaller DNA product can be seen in mutant DNA sample



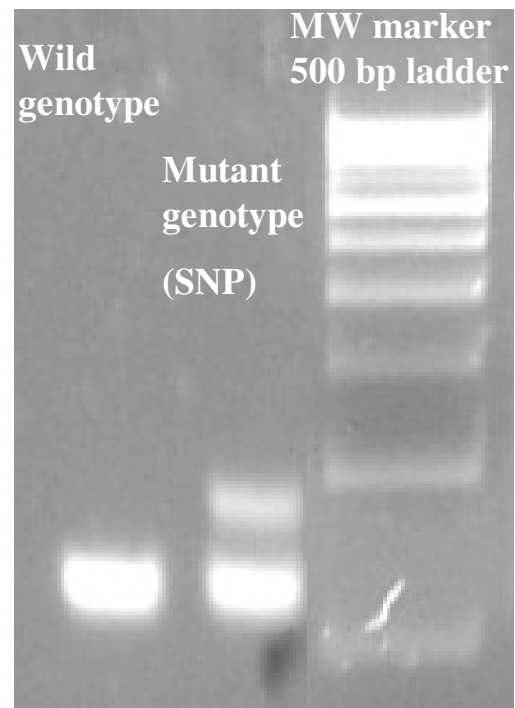
Power

Mutant will show two bands 400 bp and 600 bp
Wild will show only one band of 400 bp

Result



Agarose gel electrophoresis (1% gel)



Summary SNP detection by ASA

- Primers designed for coding strand of mutated DNA. (Mutant allele specific PCR), so as to get mutant specific PCR amplification
- Primers designed for coding strand of non mutated region near (at least 200 base apart) the site of mutant specific primers to get PCR product from wild as well as mutant sample