

The Rapid Detection of Unknown Mutations in Nucleic Acids

By:

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Methods for Mutation Detection

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graph TD; A[Methods for Mutation Detection] --> B[Detection of known mutation]; A --> C[Detection of unknown mutation]; C --> D[Large gene alterations]; C --> E[Single base alterations & small insertions/deletions];
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The diagram is a hierarchical flowchart. At the top is a box labeled 'Methods for Mutation Detection'. A vertical line descends from this box and splits into two horizontal lines. From the left horizontal line, an arrow points down to a box labeled 'Detection of known mutation'. From the right horizontal line, an arrow points down to a box labeled 'Detection of unknown mutation'. From the 'Detection of unknown mutation' box, a vertical line descends and splits into two horizontal lines. From the left horizontal line, an arrow points down to a box labeled 'Large gene alterations'. From the right horizontal line, an arrow points down to a box labeled 'Single base alterations & small insertions/deletions'. All boxes have a red border and yellow text on a blue background.

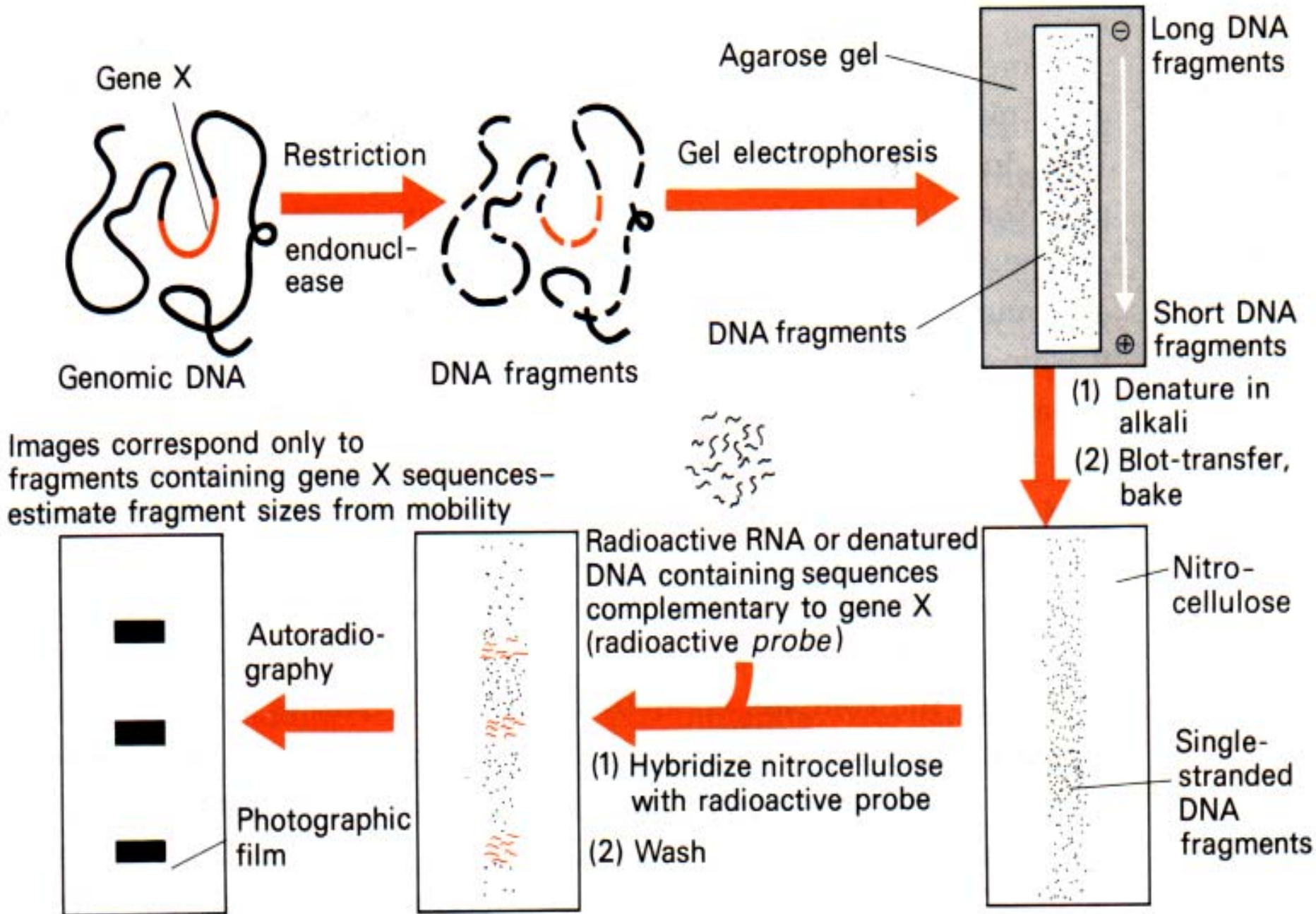
**Detection of
known mutation**

**Detection of
unknown mutation**

Large gene alterations

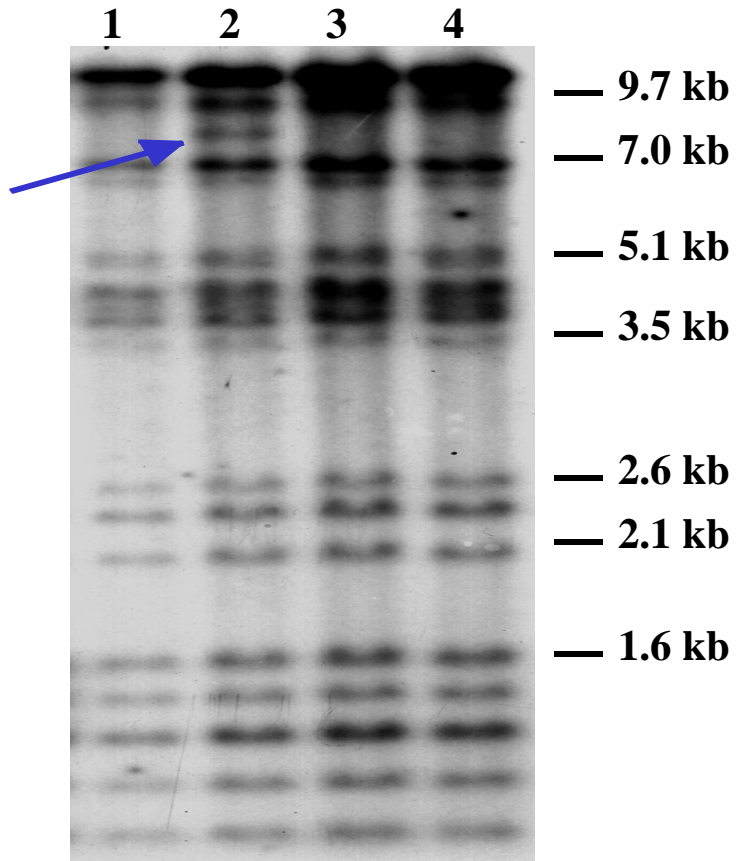
**Single base alterations &
small insertions/deletions**

Principal of Southern blotting (Old & Primrose, 1993)

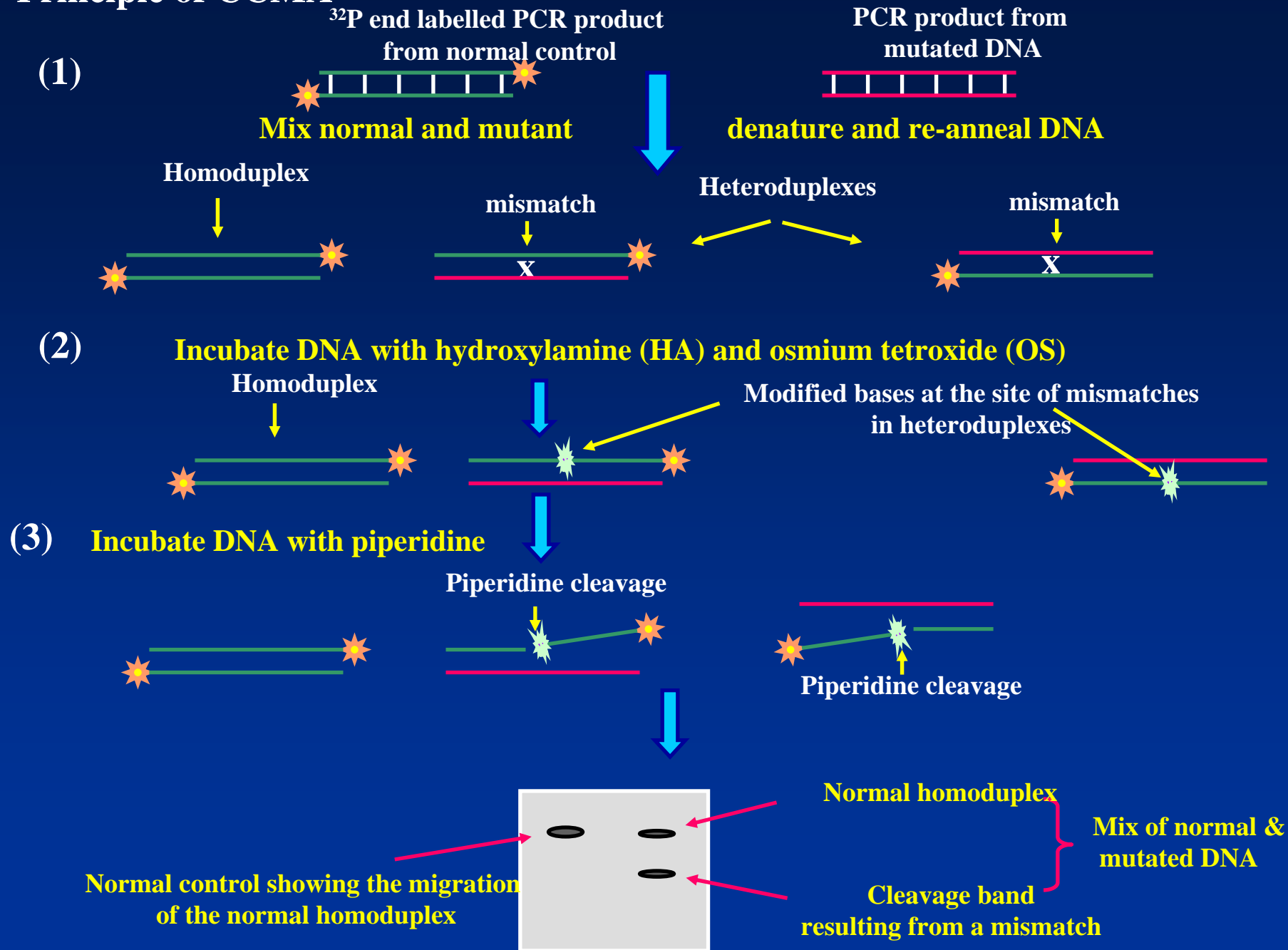


Southern Blot for VWF Gene

A novel ~8 kb band resulted from the deletion of exons 17&18

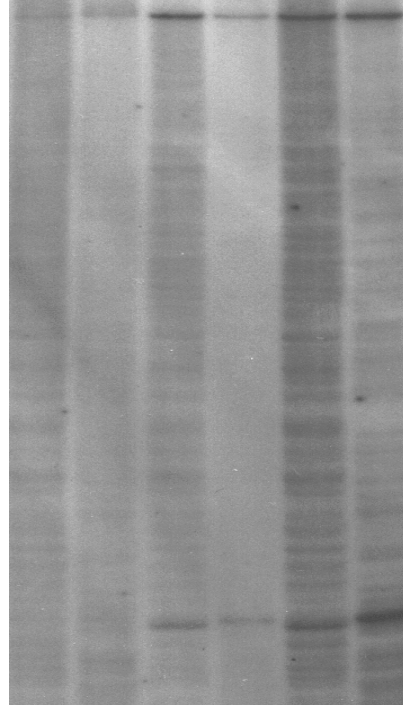


Principle of CCMA



CCMA

Negative
control I:1 II:2
 ┌───┐ ┌───┐ ┌───┐
 OS HA OS HA OS HA



← Cleavage bands due to C→T transition
Exon 36, VWF gene

Principle of CSGE

PCR product from normal control

PCR product from mutant DNA

(1)

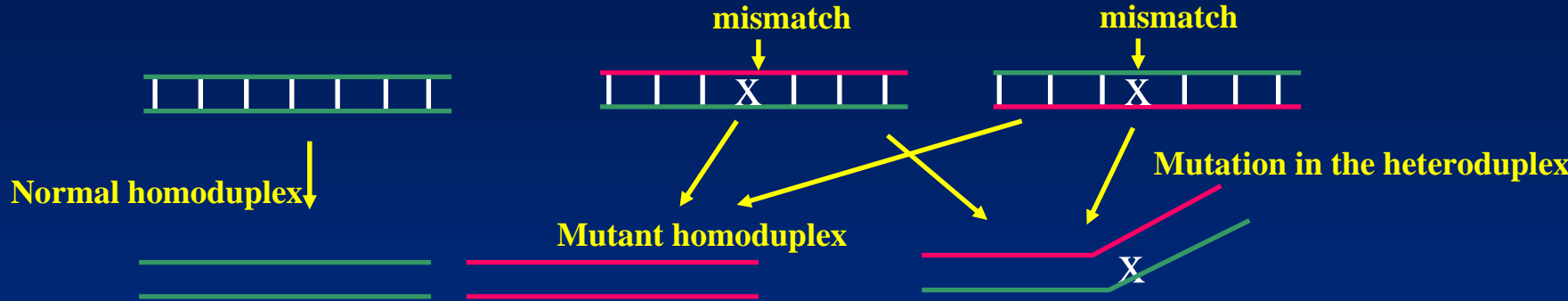


A- Mix equal volumes and heat to denature DNA



B- incubate the denatured DNA at 65 °C for 30 min for heteroduplex formation*

(2)



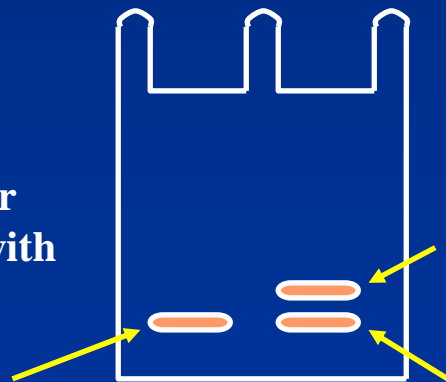
(3)

Load the DNA mix onto polyacrylamide gel in mildly denaturing solvent system, electrophorese for 17hr at 400 volts

Conformational change as a result of the mismatch

(4)

Bands can be viewed under UV after staining the gel with ethidium Bromide

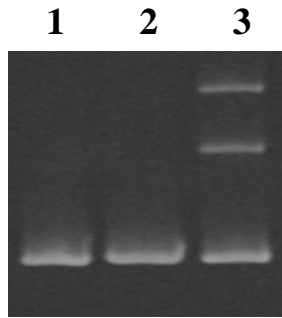


Mutated heteroduplex

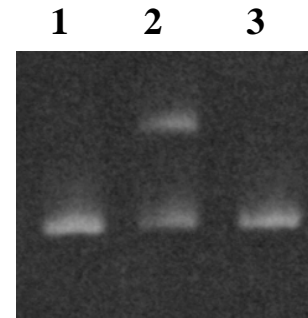
Normal and mutant homoduplexes

Mix of normal & mutated DNA

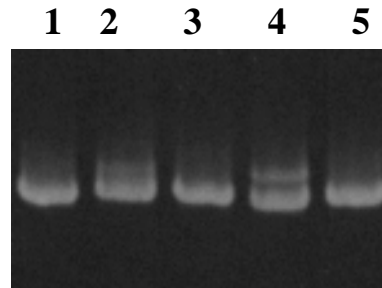
Normal control showing the migration of normal homoduplex



↑
AG nucleotides deletion
Exon 26 VWF gene

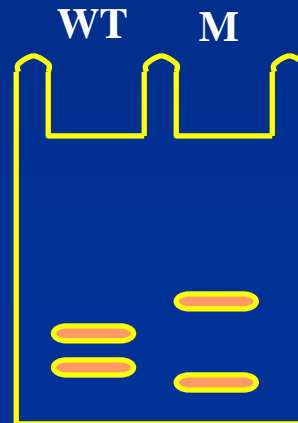
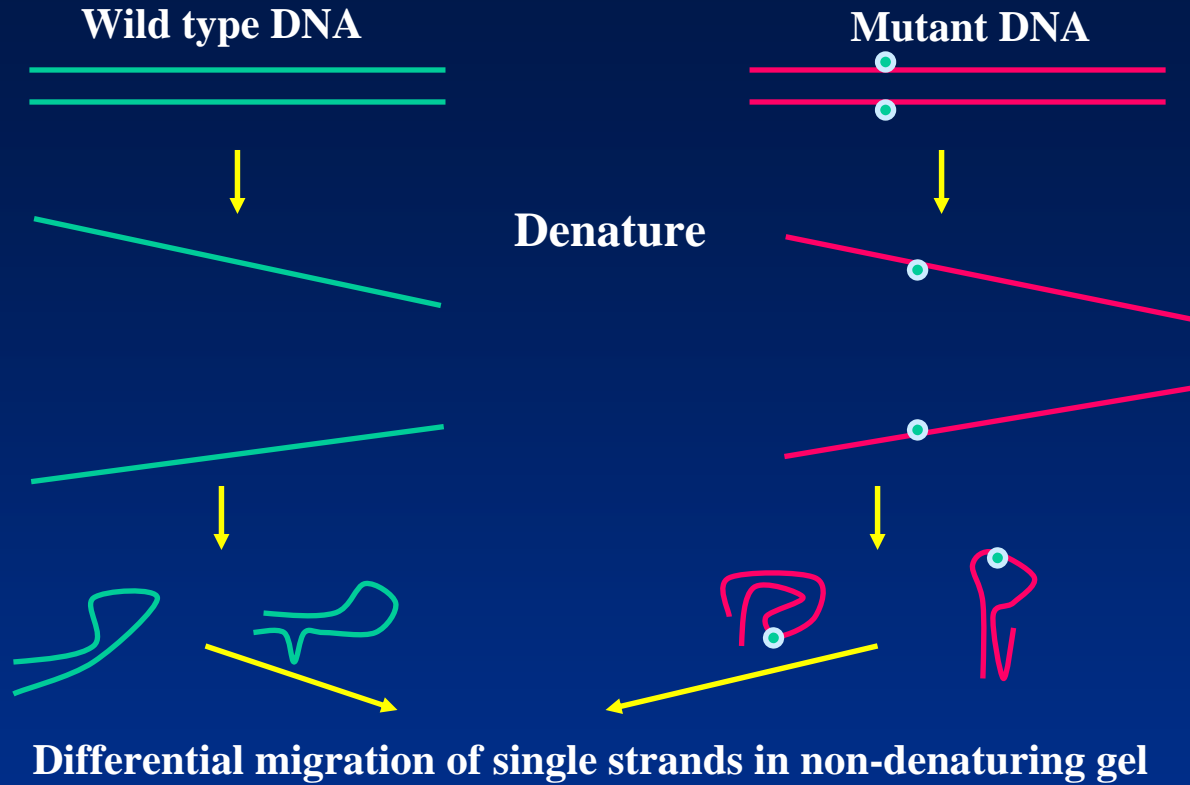


↑
Single T Insertion
Exon 21 VWF gene



↑
Single base substitution (T→A)
Exon 28 VWF gene

Principle of SSCP:



Principle of DGGE

Wild type DNA



Mutant DNA



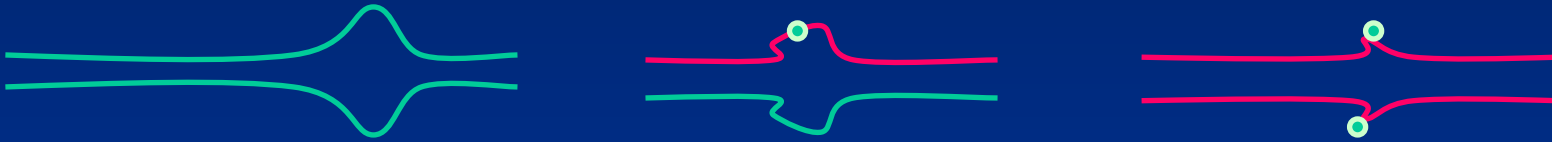
Denature and mix



Hetero- and Homoduplex DNA

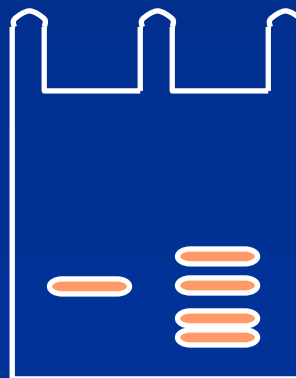


Electrophoresis in denaturing gradient

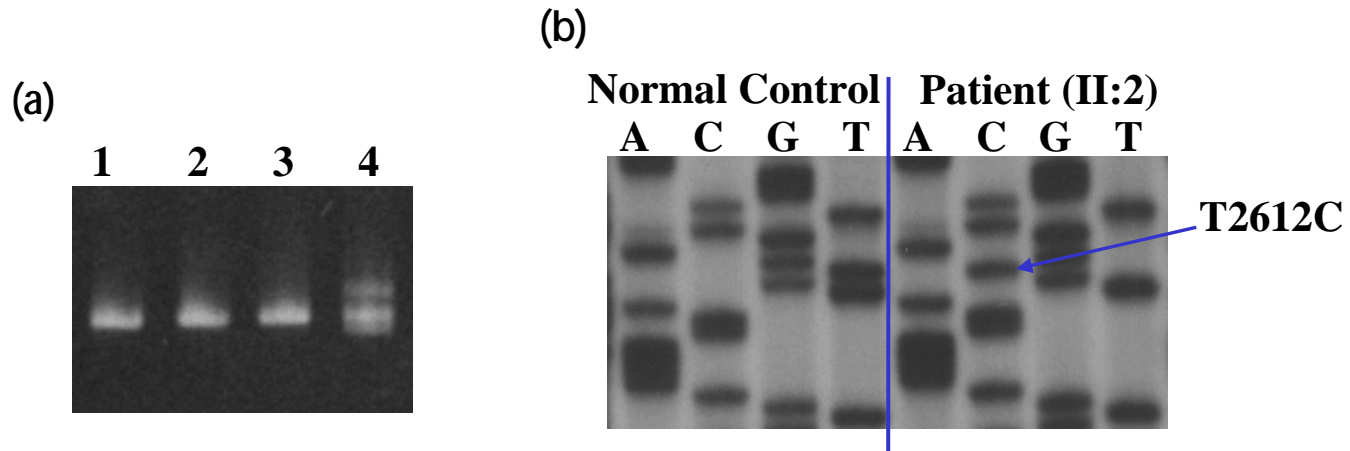


Differential melting behavior

WT WT+M



CSGE & Direct DNA Sequencing



exon 18 analysis by CSGE and direct DNA sequencing. (a) CSGE gel showing in lane 4 a positive pattern detected in sibling II:2. Lane 1 shows a negative control and lanes 2 and 3 show negative results from index cases of other families. (b) Direct DNA sequencing showing the homozygote (patient II:2) for the T2612C mutation.