β-GLOBIN MUTATIONS AND SICKLE CELL DISORDER (SCD) - RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLP)

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SICKLE CELL ANAEMIA
OPEN EDUCATIONAL RESOURCES FOR
SICKLE CELL ANAEMIA AND THALASSEMIA

β-GLOBIN MUTATIONS AND SICKLE CELL DISORDER (SCD)

- Well over 700 abnormal forms of haemoglobin have been identified and characterised to some extent on the basis of the mutation responsible and/or clinical consequences.

- Over 1000 variants of the globin genes have been identified.

- A variety of mutation mechanisms are responsible for the abnormal haemoglobins. These mutations affect either haemoglobin structure or synthesis.

- The most common are point mutations in the β–globin gene resulting in a nucleotide substitution and a change of encoded amino acid which affects haemoglobin structure.

β-GLOBIN POINT MUTATIONS – Hb A, Hb S and HbC

Exon 1

```
          1   2   3   4   5   6   7   8   9  10
     atg gtg cat ctg act cct GAG gag aag tct gcc gtt act gcc ctg tgg ggc aag gtg
      M   V   H   L   T   P   E   E   K   S   A   V   T   A   L   W   G   K   V

                                    26
   aac gtg gat gaa gtt ggt ggt GAG gcc ctg ggc ag
    N   V   D   E   V   G   G   E   A   L   G   R
```

Exon 2

```
       g ctg ctg gtg gtc tac cct tgg acc cag agg ttc ttt gag tcc ttt ggg gat ctg tcc act cct
         L   L   V   V   Y   P   W   T   Q   R   F   F   E   S   F   G   D   L   S   T   P
 gat gct gtt atg ggc aac cct aag gtg aag gct cat ggc aag aaa gtg ctc ggt gcc ttt agt gat ggc
  D   A   V   M   G   N   P   K   V   K   A   H   G   K   K   V   L   G   A   F   S   D   G
 ctg gct cac ctg gac aac ctc aag ggc acc ttt gcc aca ctg agt gag ctg cac tgt gac aag ctg cac
  L   A   H   L   D   N   L   k   G   T   F   A   T   L   S   E   L   H   C   D   K   L   H
 gtg gat cct gag aac ttc agg
  V   D   P   E   N   F   R
```

Exon 3

```
                                                                          121
        ctc ctg ggc aac gtg ctg gtc tgt gtg ctg gcc cat cac ttt ggc aaa GAA ttc
         L   L   G   N   V   L   V   C   V   L   A   H   H   F   G   K   E   F

acc cca cca gtg cag gct gcc tat cag aaa gtg gtg gct ggt gtg gct aat gcc ctg gcc
 T   P   P   V   Q   A   A   Y   Q   K   V   V   A   G   V   A   N   A   L   A

cac aag tat cac taa
 H   K   Y   H   *
```

POINT MUTATIONS IN THE β–GLOBIN GENE AND SCD – Hb S

A.

Codons 1-10 of β–globin

```
 1   2   3   4   5   6   7   8   9  10
gtg cat ctg act cct GAG gag aag tct gcc
 V   H   L   T   P   E   E   K   S   A
```

```
                    5   6   7
B. Hb A (βA allele)    cct GAG gag
                       P   E   E
```

The "usual" allele is referred to as the β^A allele and produces Hb A

```
C. Hb S (βS allele)    cct GTG gag  20A>T mutation
                       P   V   E
```

The sickle cell mutation or β^S allele. In this mutation the 20th nucleotide is mutated from an "A" to a "T" which causes a change in the amino acid encoded by this codon from glutamic acid (E) to valine (V)

POINT MUTATIONS IN THE β–GLOBIN GENE AND SCD – Hb C

A.

Codons 1-10 of β–globin

1	2	3	4	5	6	7	8	9	10
gtg	cat	ctg	act	cct	GAG	gag	aag	tct	gcc
V	H	L	T	P	E	E	K	S	A

B. Hb A (β^A allele)

5	6	7
cct	GAG	gag
P	E	E

The "usual" allele is referred to as the β^A allele and produces Hb A

C. Hb C (β^C allele)

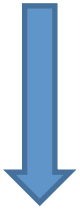
cct	AAG	gag
P	K	E

19G>A mutation

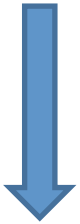
The Hb C mutation or β^C allele. In this mutation the 19th nucleotide mutates from a "g" to an "a" which causes a change in the amino acid encoded from glutamic acid (E) to lysine (K)

IDENTIFYING THE Hb S MUTATION USING THE POLYMERASE CHAIN REACTION (PCR) AND RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

MUTATION ABOLISHES RESTRICTION SITE

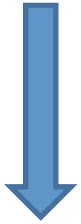


AMPLIFY PORTION OF β-GLOBIN GENE BY PCR



Firstly a portion of the β–globin gene is amplified by the PCR. The region of DNA amplified must contain the specific DNA sequence that is mutated and that mutation must introduce or abolish a restriction site.

DIGEST PRODUCTS WITH RESTRICTION ENZYME



The amplified DNA is then digested with the specific restriction endonuclease. Mutated and non-mutated amplicons will have different restriction sites in them, giving different patterns of bands on electrophoresis

ANALYSE PRODUCTS BY GEL ELECTROPHORESIS

RESTRICTION FRAGMENT LENGTH POLYMORPHISM

A.

```
 4   5   6   7   8
actcctGAGgagaag
tgaggaCTCctcttc
```

B.

***Mst*II = CC↓TNAGG**

C.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM

A.

```
  4   5   6   7   8
actcctGAGgagaag
tgaggaCTCctcttc
```

B.

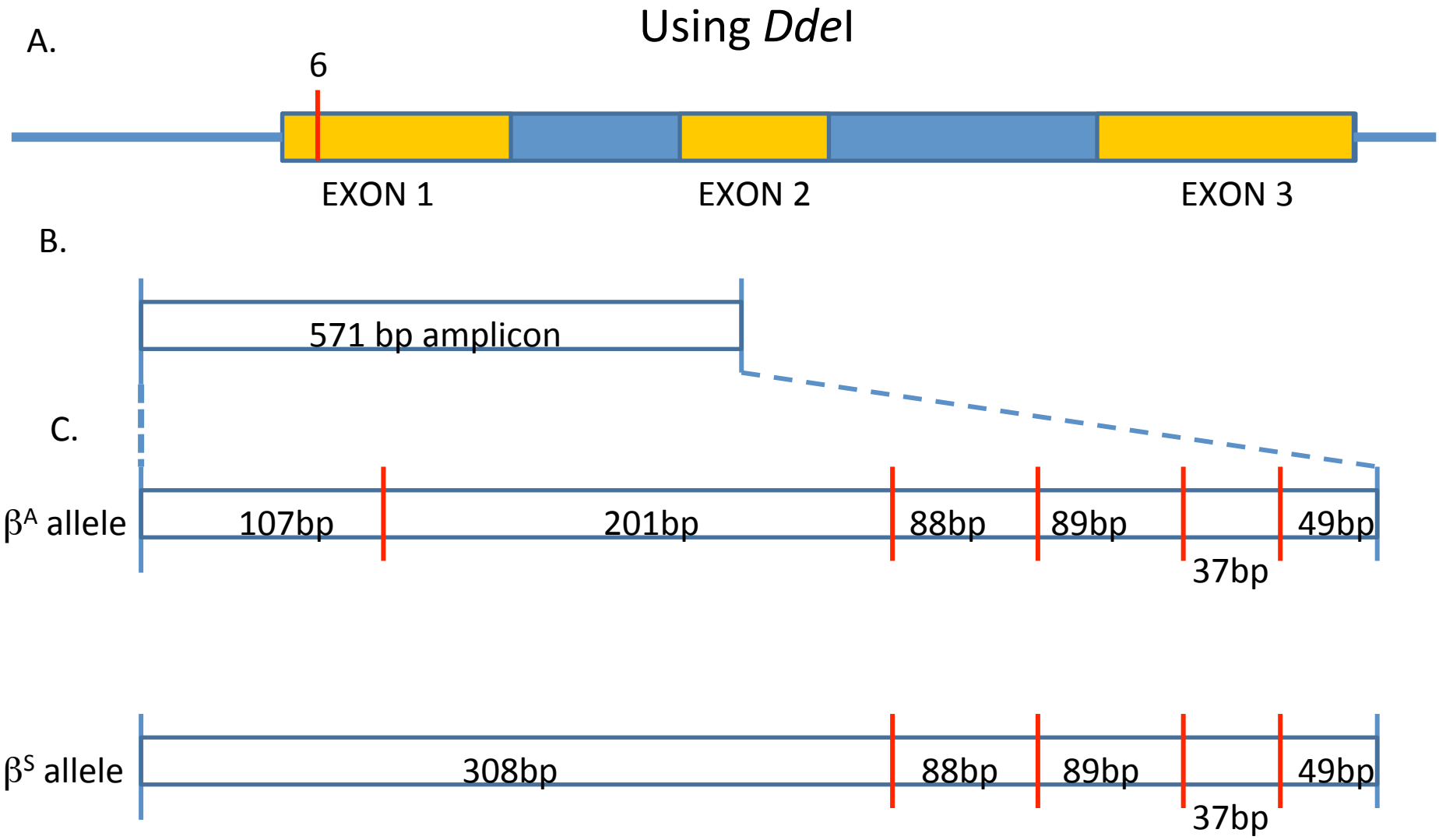
C↓TNAG = *DdeI*

C.

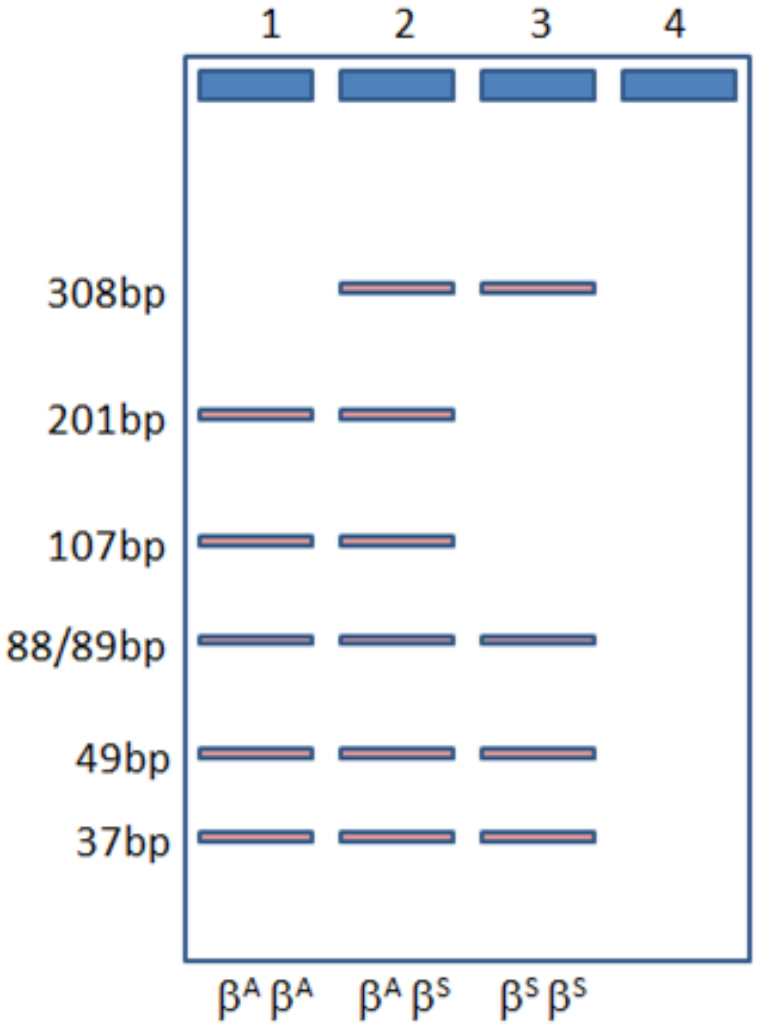
```
        ↓
actcctGAGgagaag
tgaggaCTCctcttc
        ↑
```

DdeI

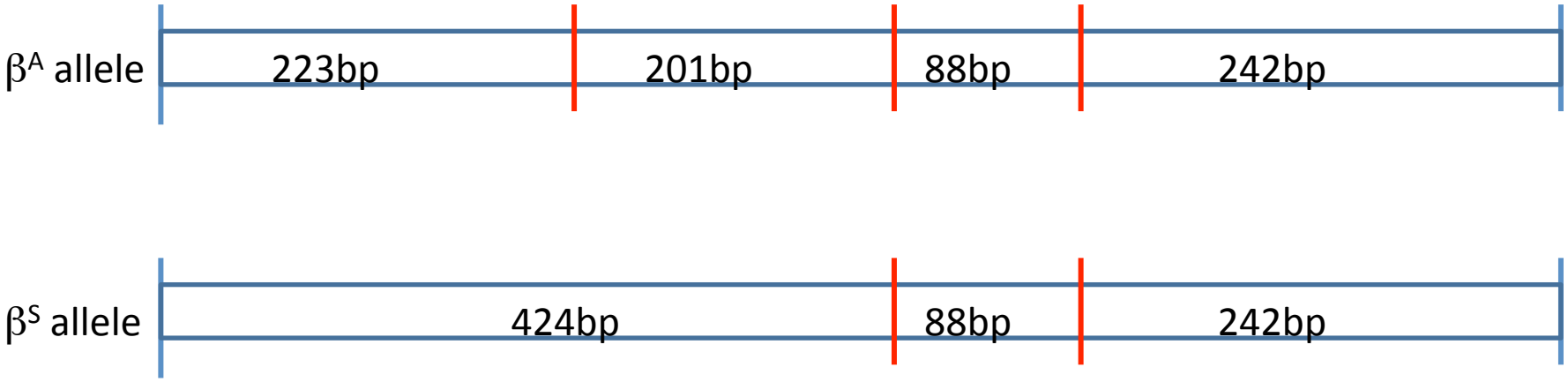
```
actcc     tGAGgagaag
tgaggaCT     Cctcttc
```


Using *Dde*I
A.
6
EXON 1
EXON 2
EXON 3
B.
571 bp amplicon
C.
βA allele
107bp
201bp
88bp
89bp
37bp
49bp
βS allele
308bp
88bp
89bp
37bp
49bp

Gel Electrophoresis



Using *Mst*II


β^A allele
223bp
201bp
88bp
242bp
β^S allele
424bp
88bp
242bp

Gel Electrophoresis