The Role Of Mutation Analysis in Porphyria.

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Mutation Analysis in the Acute Porphyrias

- Family studies
- Identify relatives who are at risk
- Avoid known precipitants

  Sex hormones

  Unsafe drugs

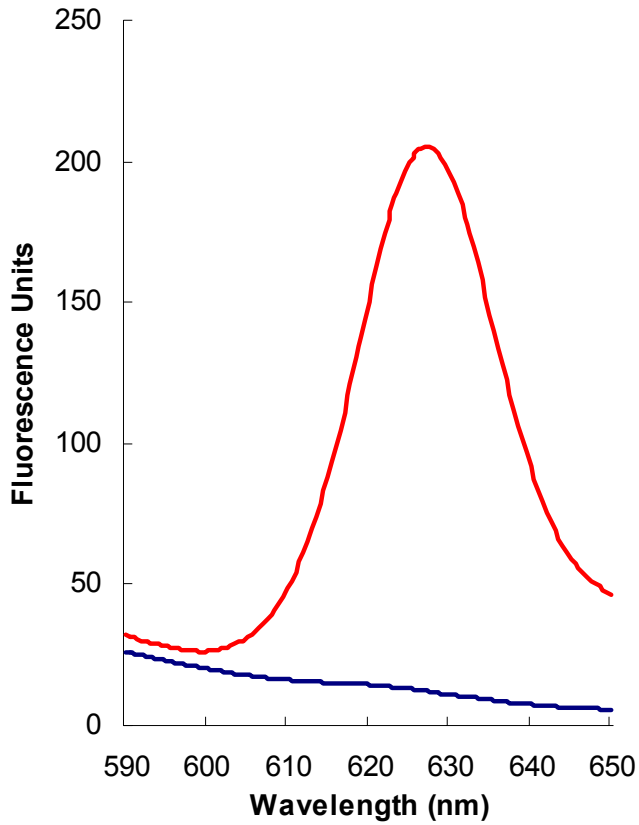
  Alcohol, infection, dieting

Mutation Analysis

- A patient with active porphyria can be diagnosed using biochemical methods.
- In these cases mutation analysis is not needed.
- Asymptomatic family members may have normal biochemistry even if they carry porphyria.

Biochemical Diagnosis in a Presymptomatic Relative

VP

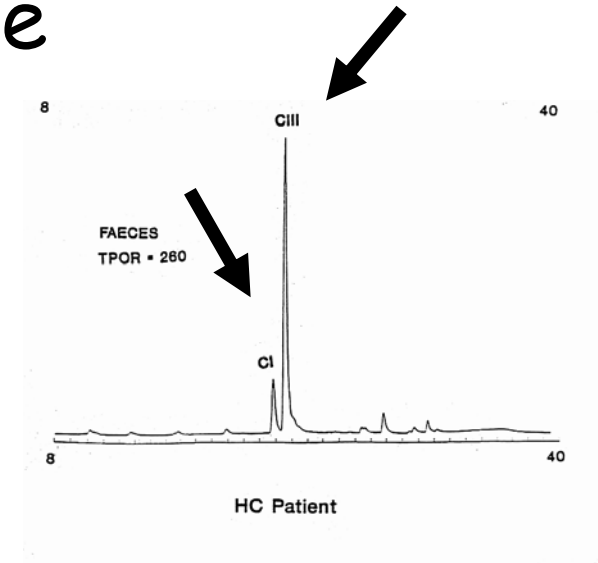


Plasma fluorescence
@ 628nm
(age >14 yrs)

100% specific but only present in 62% of those with VP

Biochemical Diagnosis in a Presymptomatic Relative

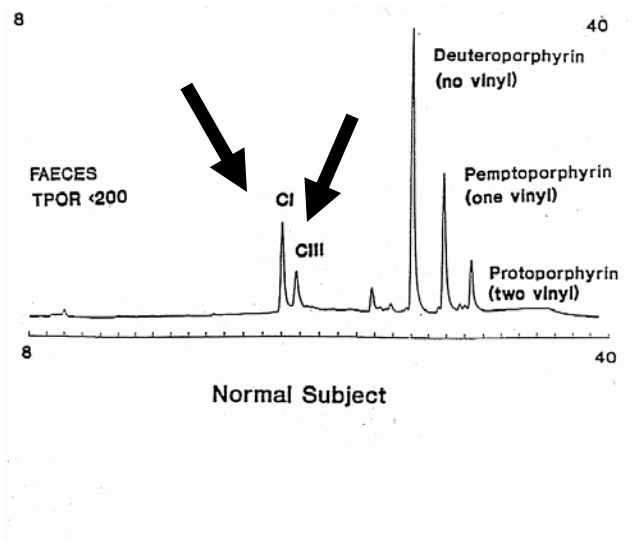
HC



HC Patient

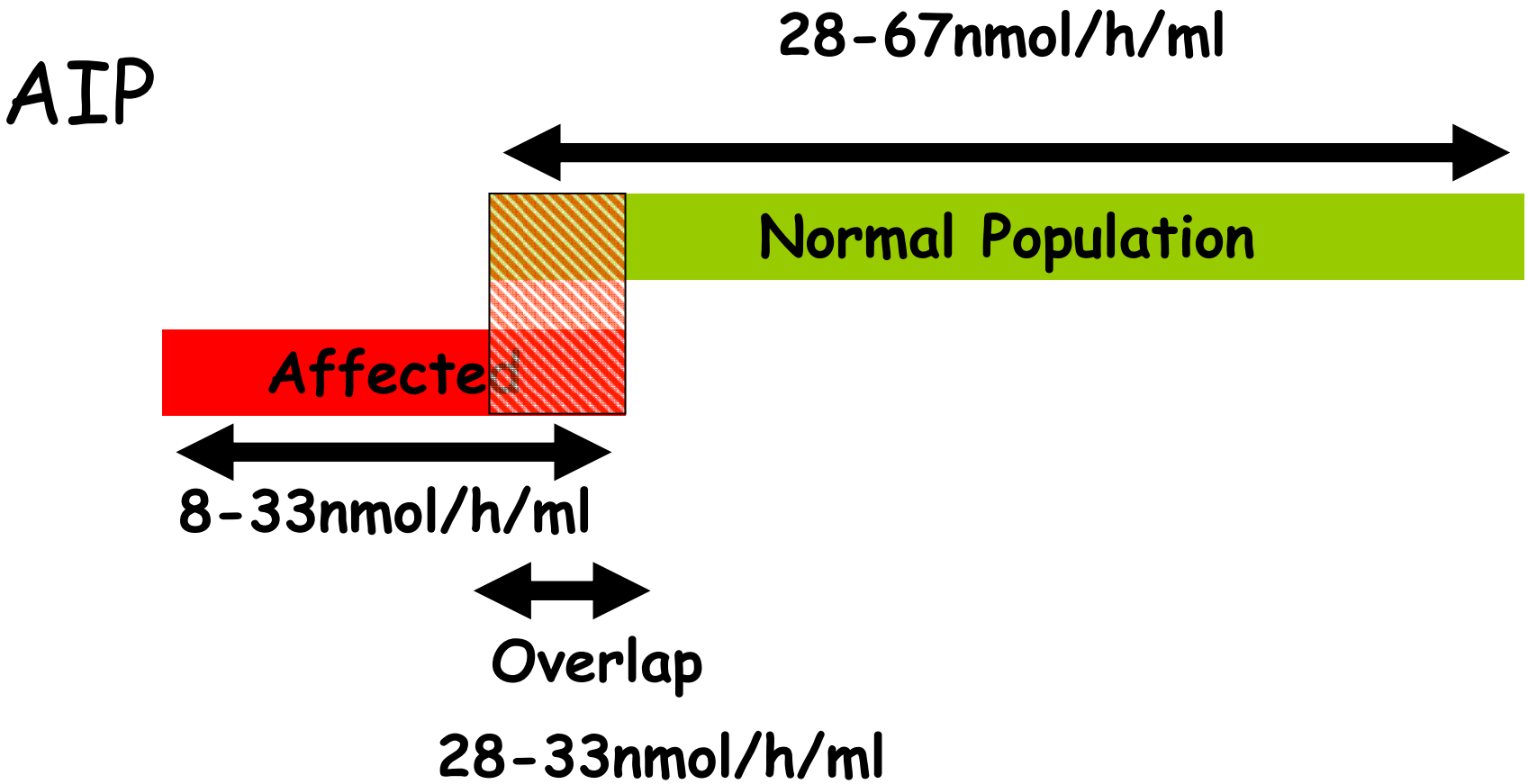
Faecal Copro
isomer ratio III:I <1.4
(age>6 yrs)

100% specific but only present in 64% of those with a mutation



Normal Subject

Porphobilinogen deaminase activity



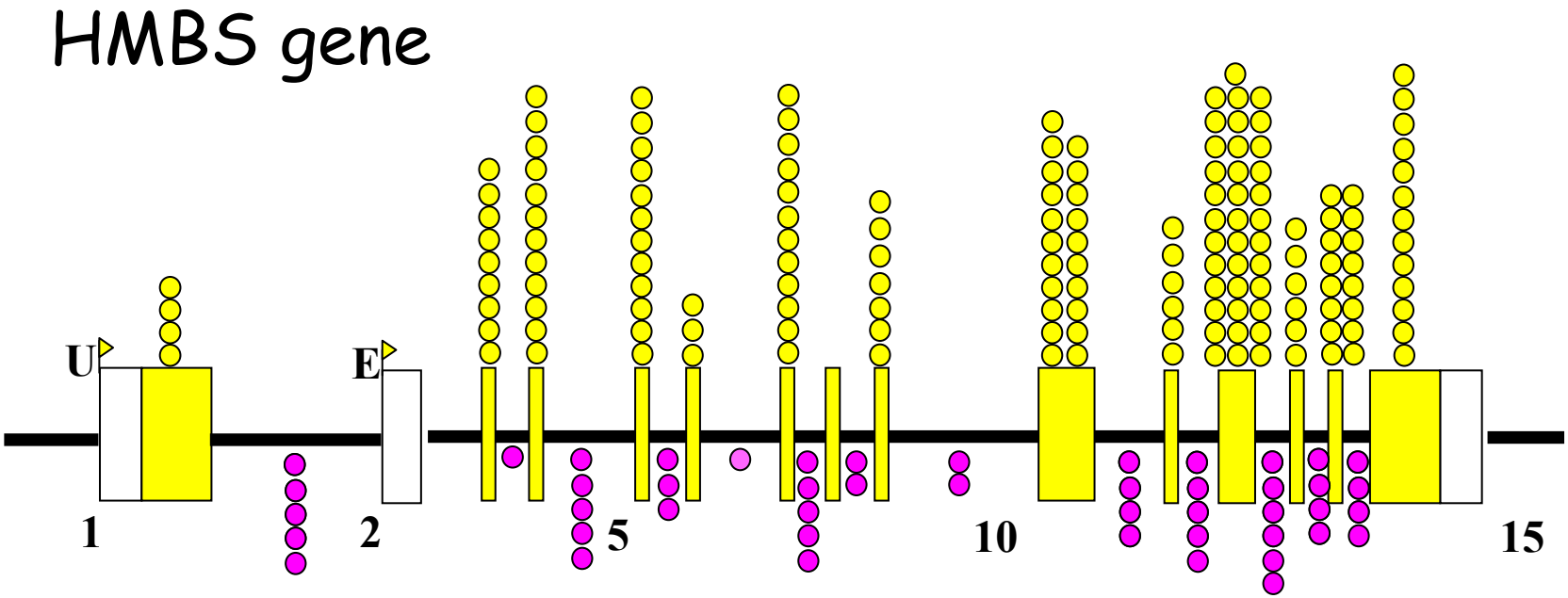
Biochemical Diagnosis in a Presymptomatic Relative

- Biochemistry usually normal before puberty

Mutation analysis in the acute porphyrias

- No common mutations
- Each family tends to have private mutation
- Entire gene needs to be analysed.

Mutations in the Porphyria Genes

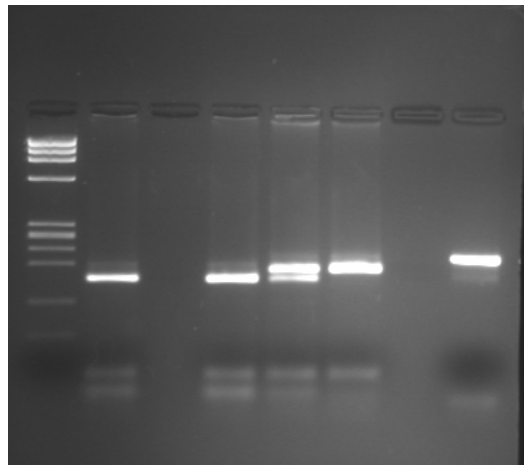


Over 270 mutations have been identified throughout the gene

Procedure for mutation analysis

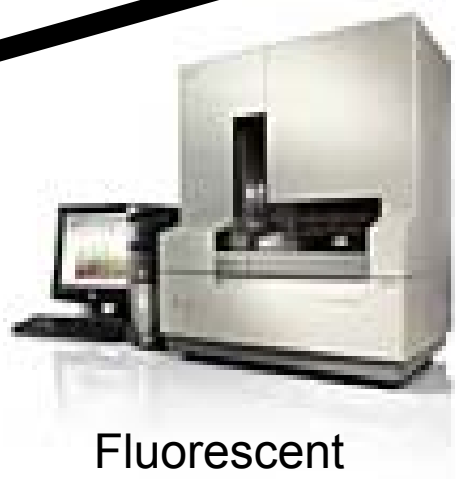
DNA isolation

PCR



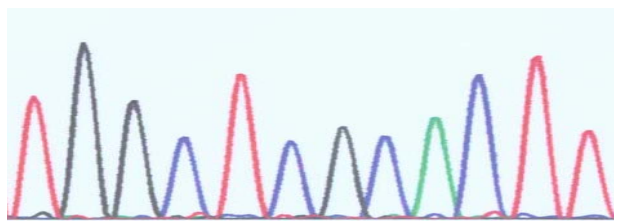
Electrophoresis
and gel extraction

Sequencing reaction

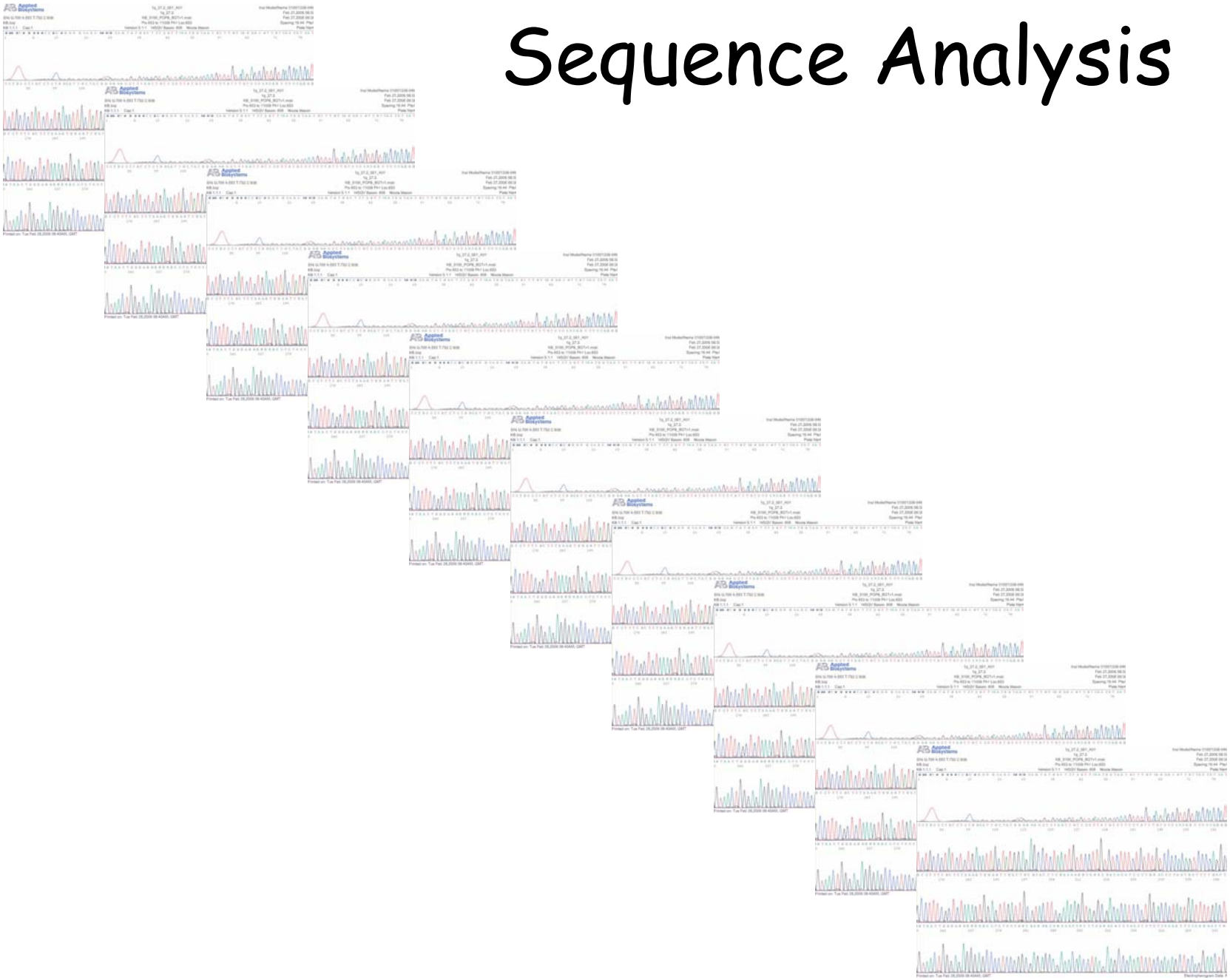


Fluorescent
sequencing

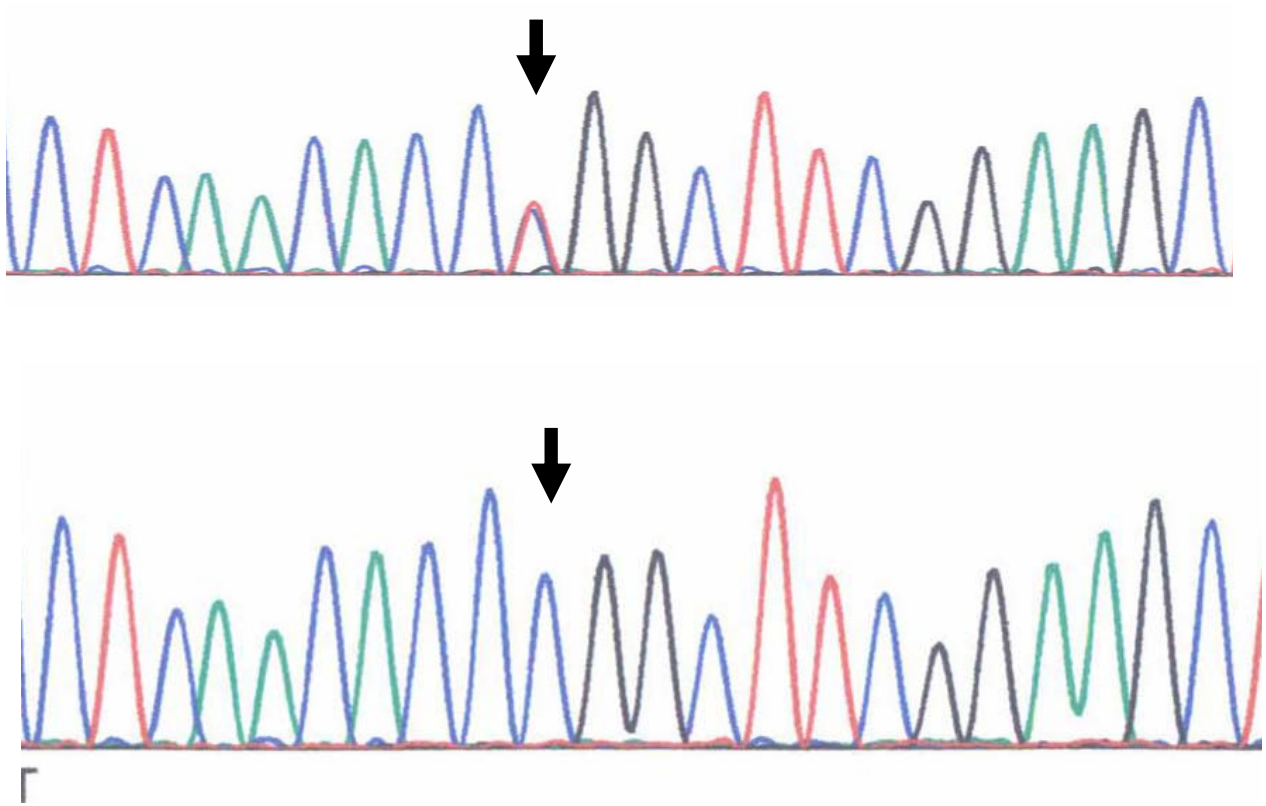
Sequence
analysis



Sequence Analysis



Missense mutations

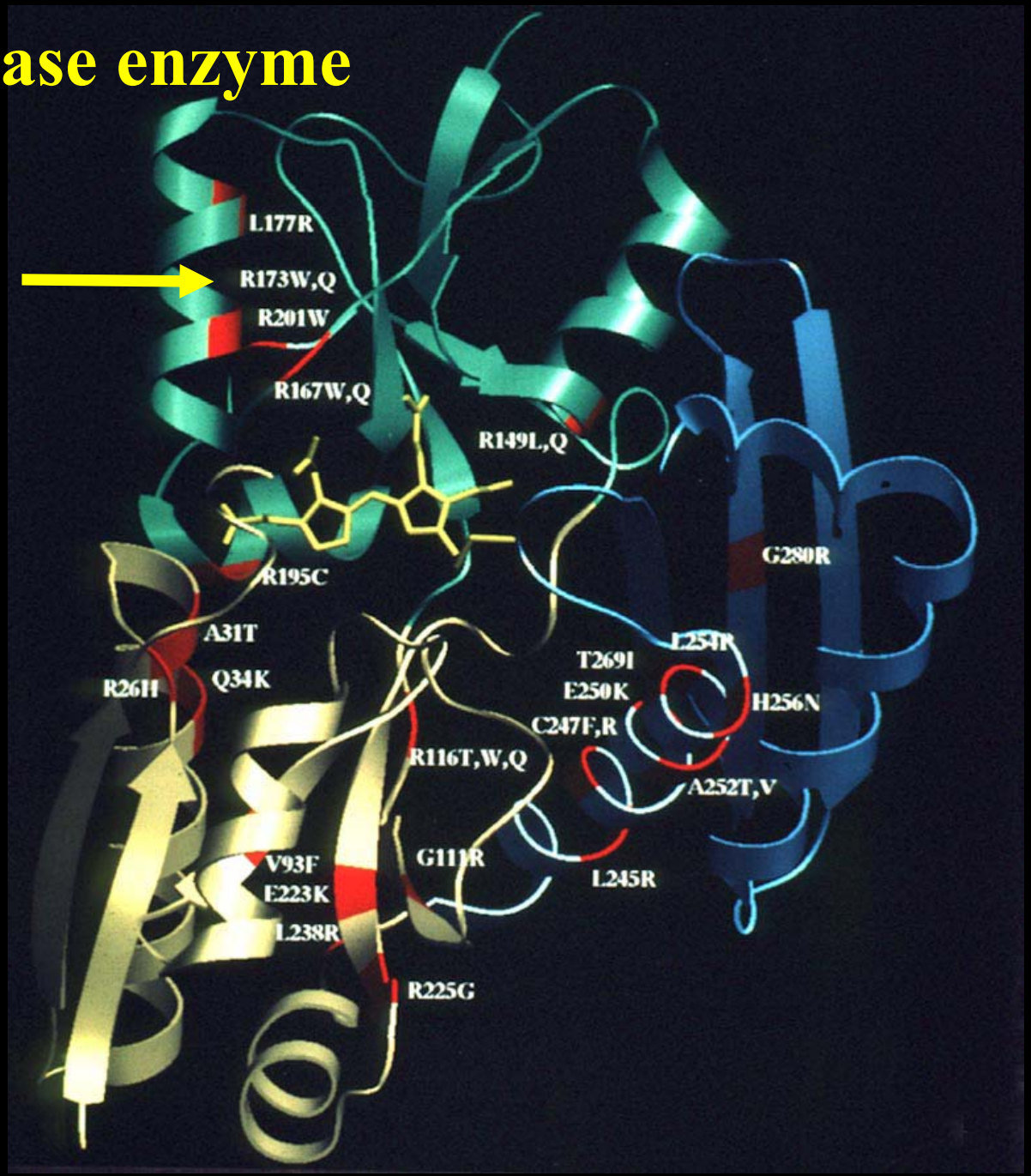


c.517C>T R173W

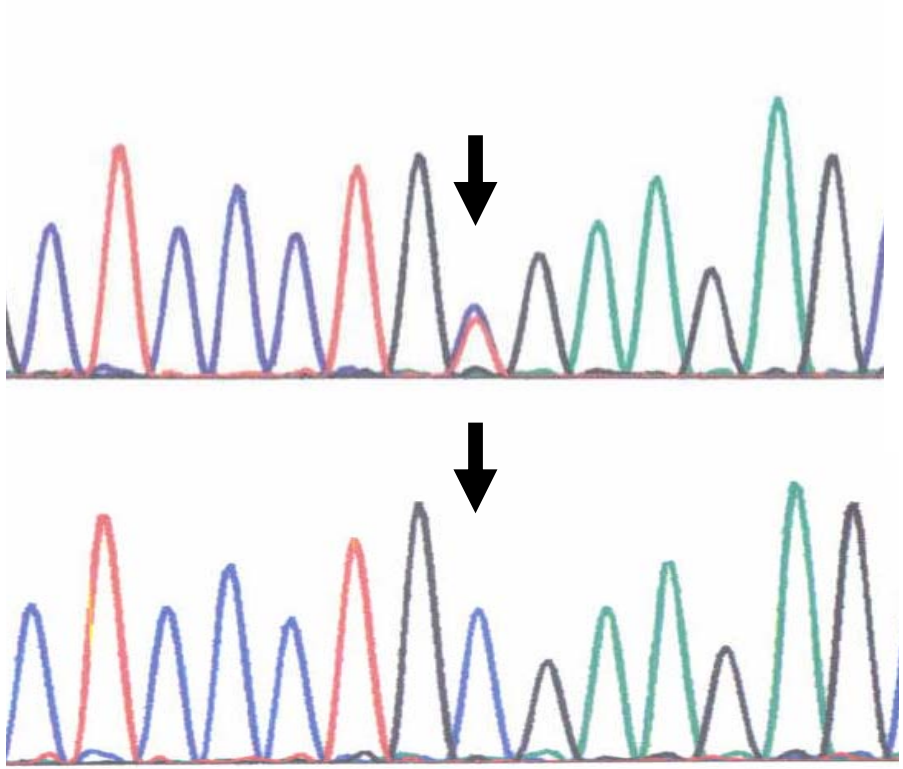
PBG deaminase enzyme

Substitution of a T for a C alters codon 173 from an arginine to a tryptophan

R173 is essential for interaction with the cofactor and substrate of the enzyme



Nonsense mutations



c.445C>T, R149X

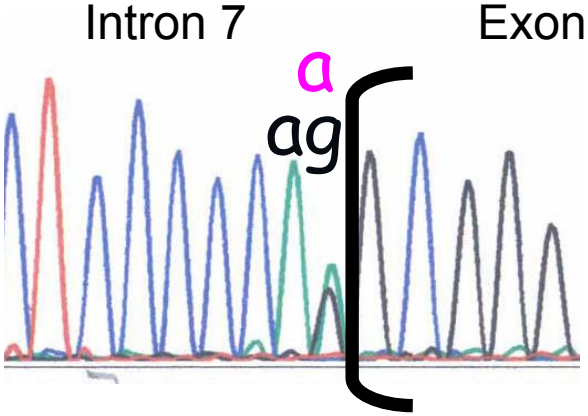
Base substitution C>T

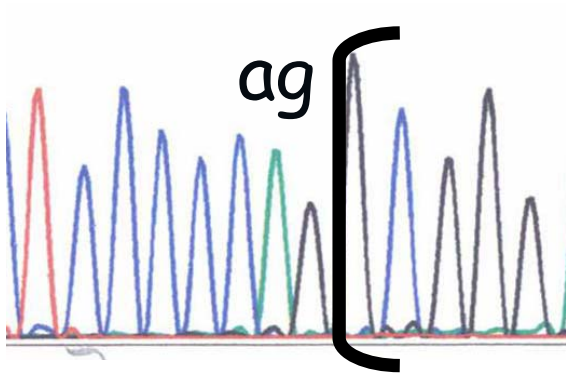
Amino acid CGA > TGA ar

Stop codons TAA TGA TAG

Transcription of the RNA will stop to produce either a stable RNA that will be translated into a truncated protein or an RNA that will be degraded

Splice site mutations





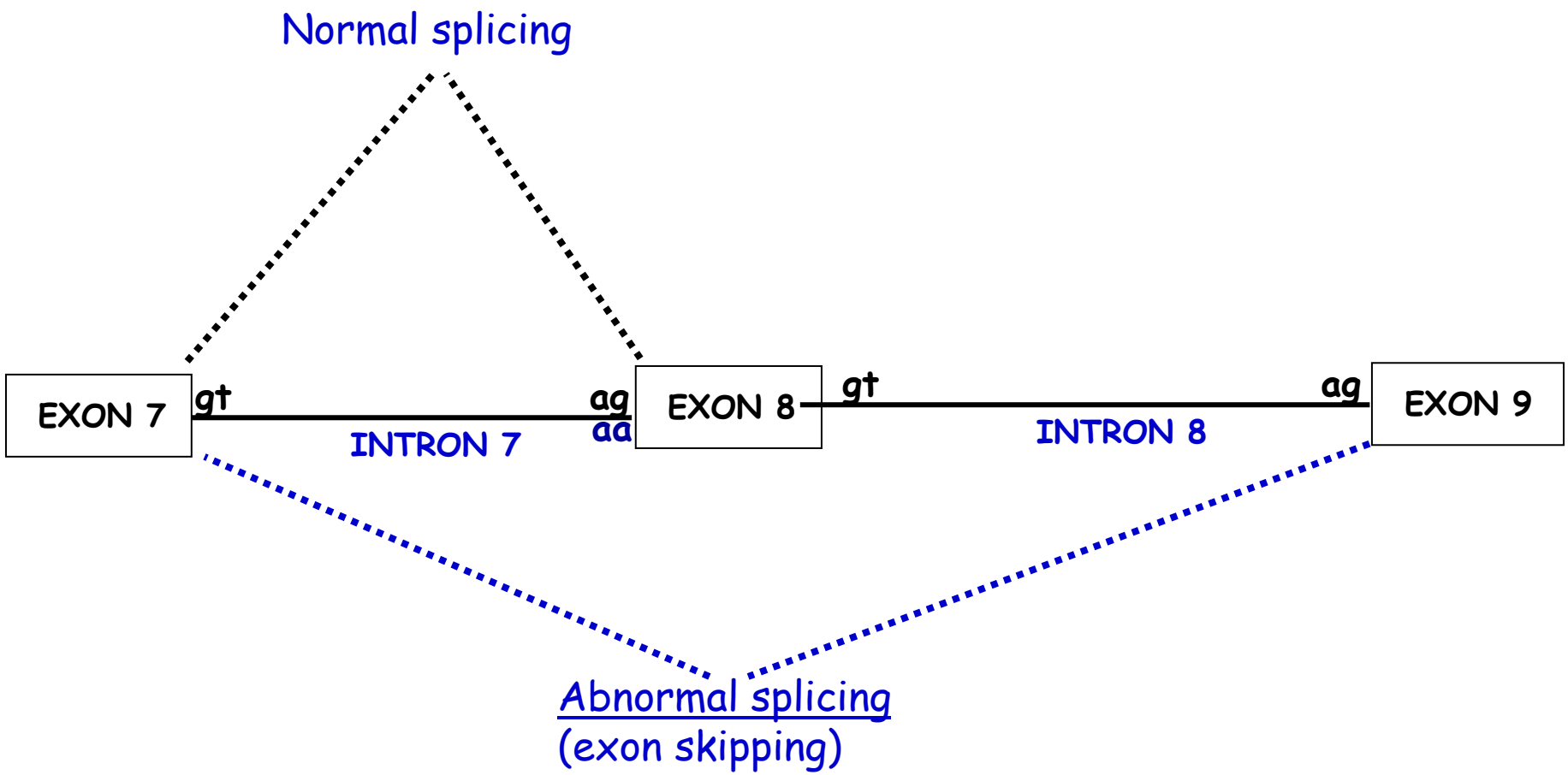
Alteration of the consensus splice site sequence

Invariable ag[exon]gt

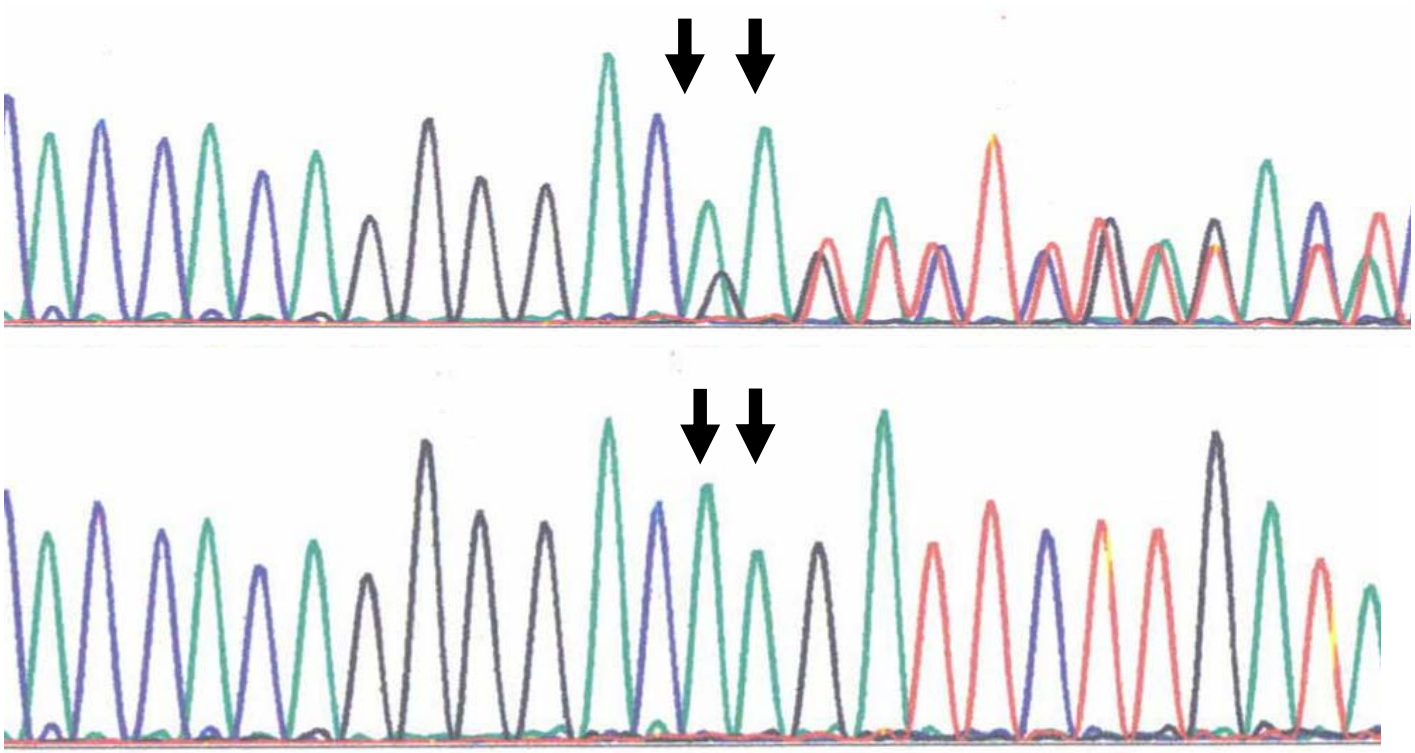
aa[exon]gt

Mutations in the consensus splice site sequence either abolish or reduce the efficiency of splicing.

Effect on splicing



Frameshift mutations



c.184-185 delAA

Lead to a stop codon

Mutation Types

Mutation Type	HMBS	PPOX	CPO
Missense	31%	26%	60%
Nonsense	14%	12%	13%
Frameshift	28%	38 %	17%
Splice	24%	22%	5%
Complex	2%	2%	0%

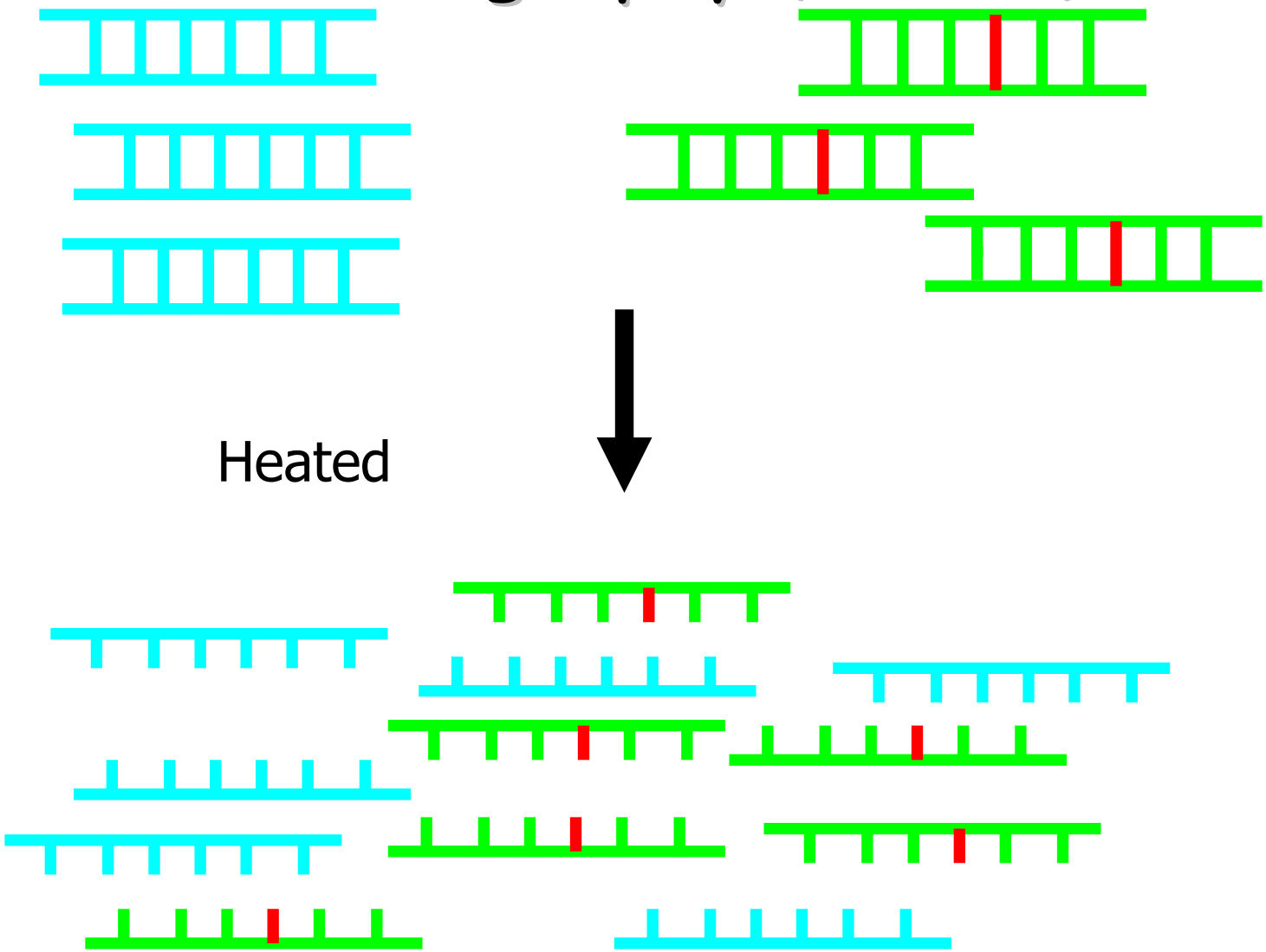
Sequencing

- Gold standard for mutation detection
- Technically demanding
- Labour intensive
- Costly

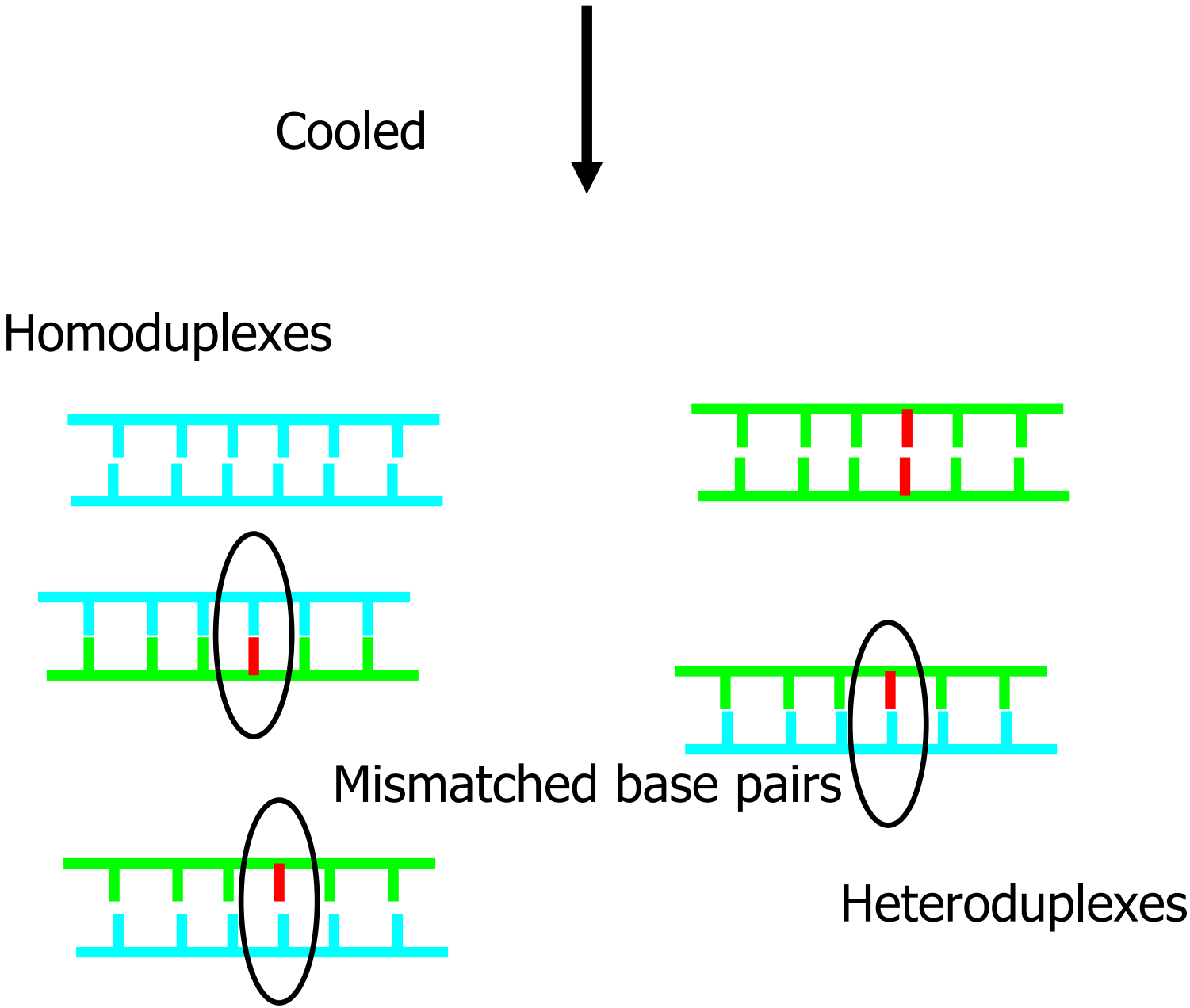
Screening method

- Reduce cost
- Improve efficiency
- Reduce turn around time.

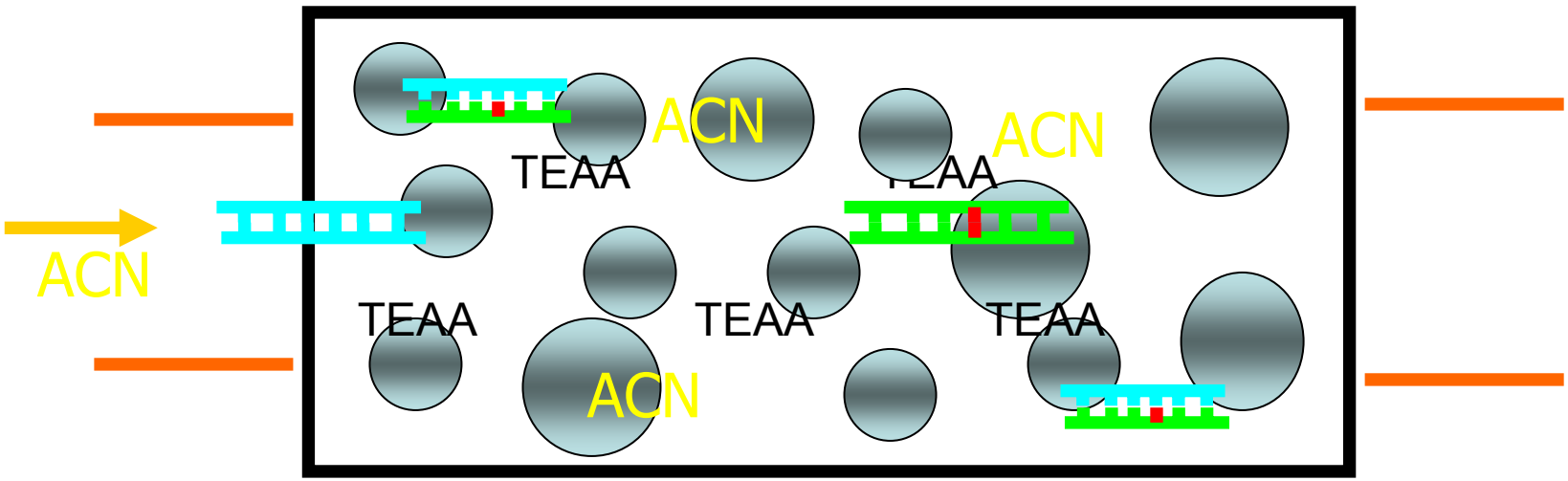
Denaturing high performance liquid chromatography (dHPLC)



Cooled



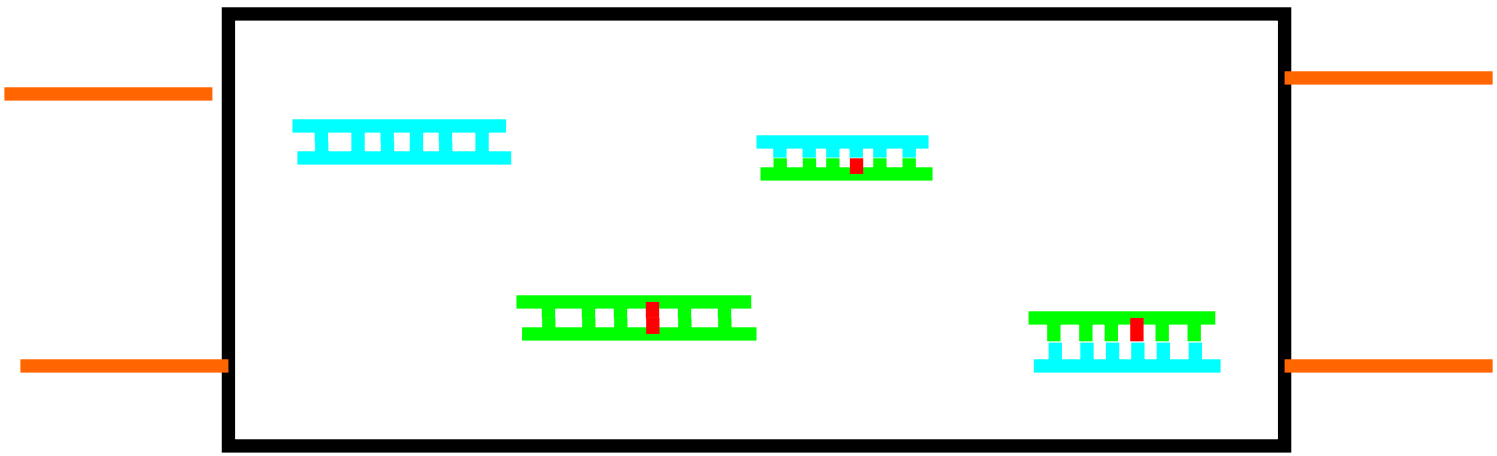
Cartridge



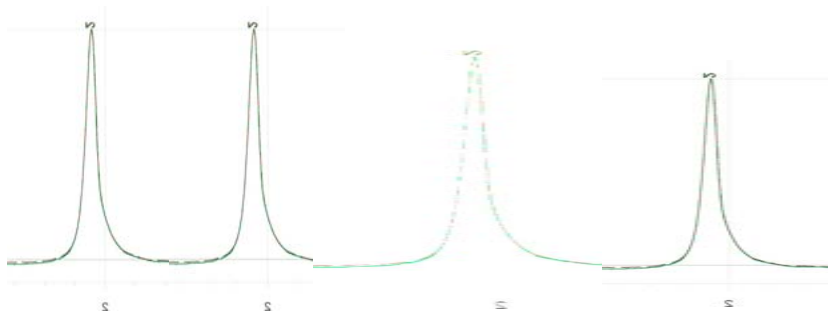
The heteroduplexes with mismatched basepairs at the point of mutation elute off the cartridge first

Then the homoduplexes

U.V. Detector



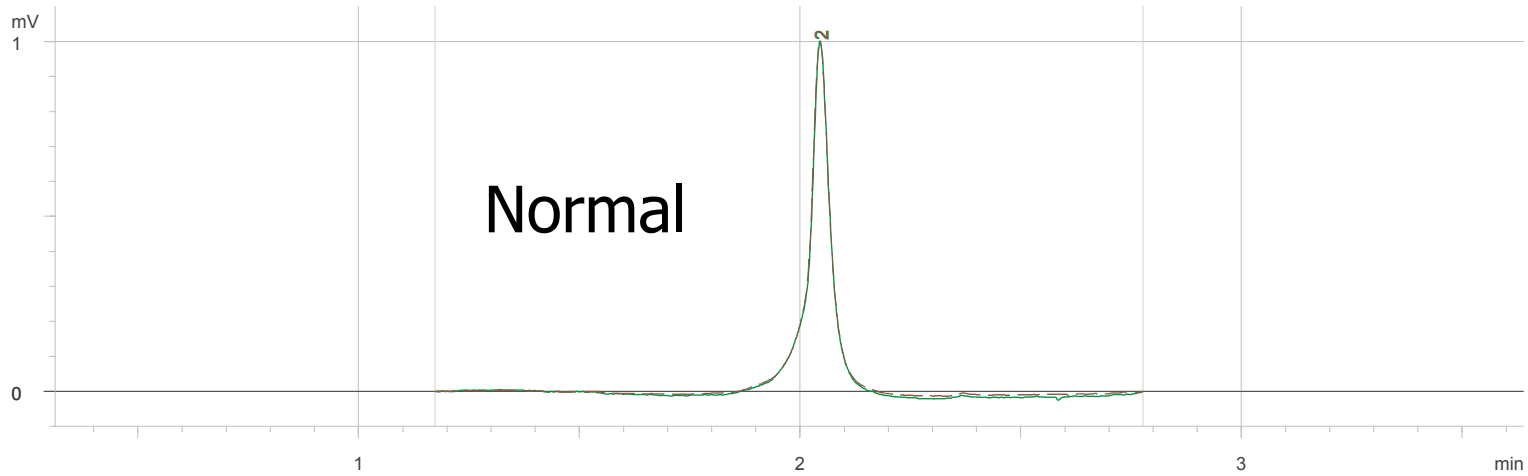
The DNA fragments are detected by a uv detector

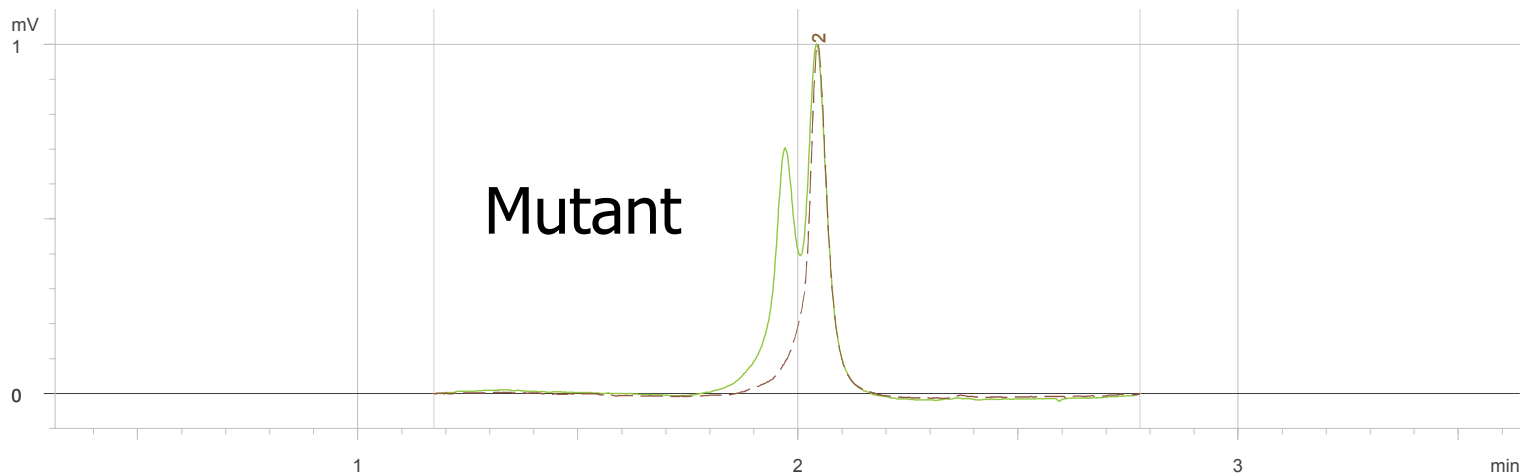


Heteroduplexes

Homoduplexes

dHPLC Traces





dHPLC

- Reduces the amount of sequencing
- Identifies polymorphisms
- Any shifts found with dHPLC have to be confirmed by sequencing.

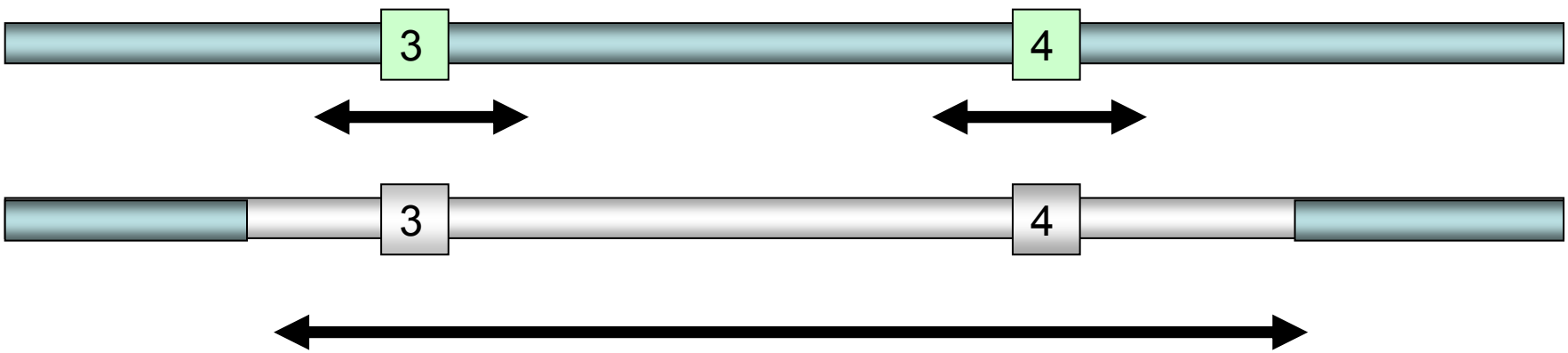
Mutation Analysis

	No of probands	Number with mutations	Sensitivity
AIP (raised PBG)	209	202	97%
VP (Peak @ 628nm)	139	139	100%
HC (Copro ratio >1.4)	30	27	90%

*Unequivocal biochemical diagnosis

Unidentified mutations

i. Deletion of whole or part of the gene.



Deletion of exons 3 and 4

Quantitative PCR

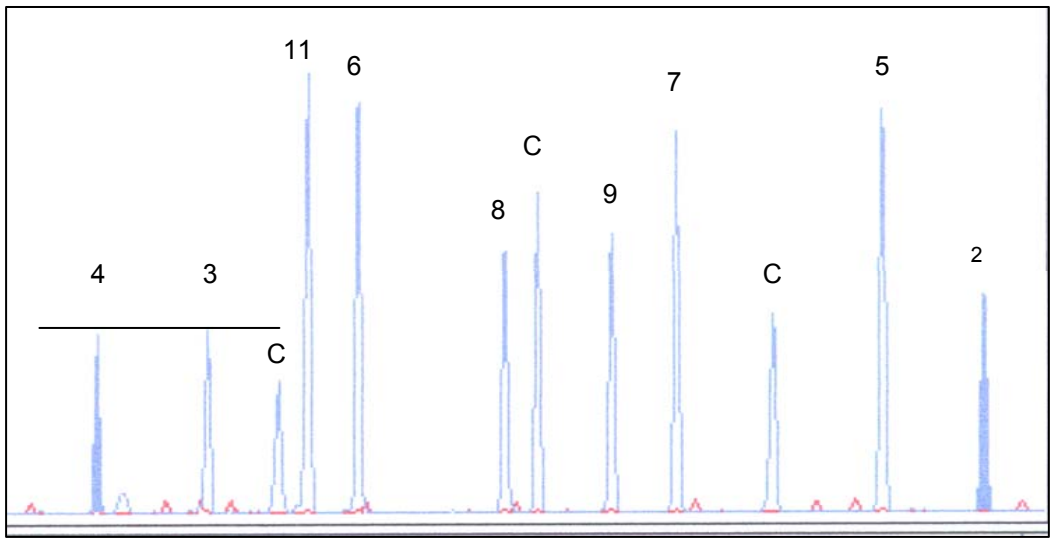
- Dosage of an allele can be detected by quantitative PCR using fluorescent labels.

- The amount of product produced during the linear part of the reaction is compared with controls from another gene.

Quantitative PCR

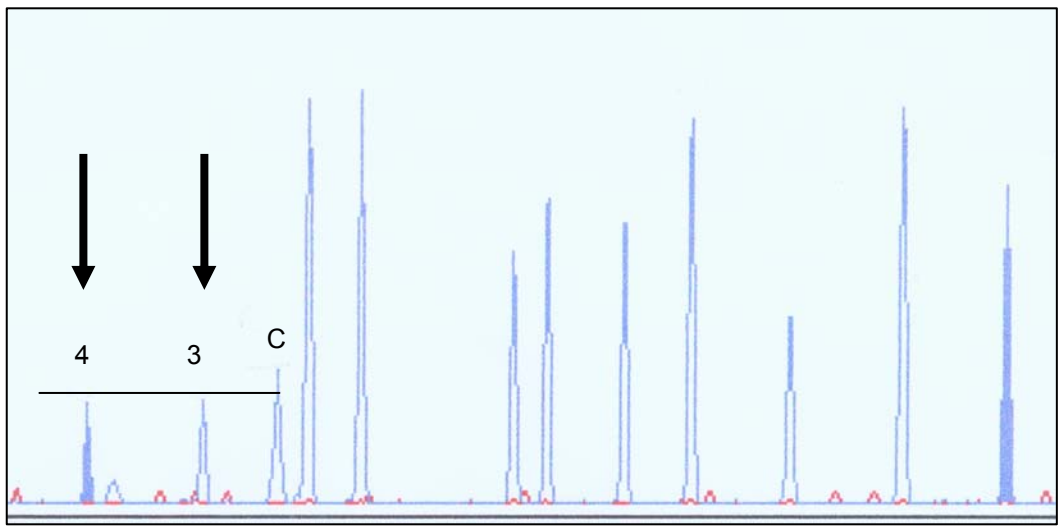
- A number of exons along with controls are amplified in the same reaction.
- If only one allele is present the signal will be half that normally obtained.

Fluorescent dosage analysis



Normal
C = control

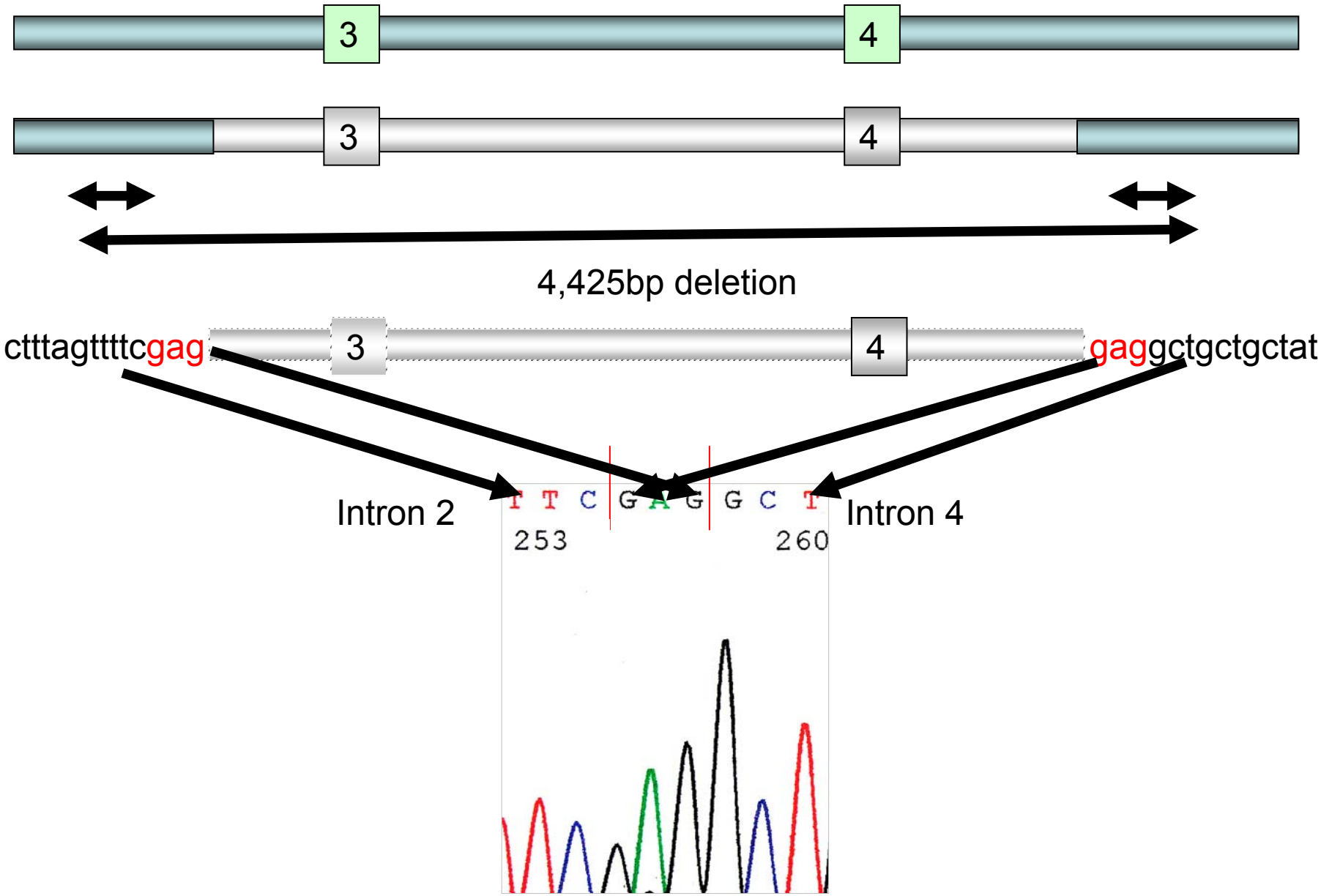
Number = exon



Patient

Fluorescent dosage analysis

	exon 4	exon 3	Control 1	exon11	exon 6	exon 8	Control 2	exon 9	exon 7	Control 3	exon 5	exon 2
exon 4	1.00	1.07	2.19	2.07	2.11	2.08	2.03	2.11	2.00	1.97	2.01	2.13
exon 3	0.93	1.00	2.05	1.93	1.97	1.94	1.89	1.97	1.87	1.83	1.88	1.99
Control 1	0.46	0.49	1.00	0.94	0.96	0.95	0.92	0.96	0.91	0.90	0.92	0.97
exon11	0.48	0.52	1.06	1.00	1.02	1.00	0.98	1.02	0.97	0.95	0.97	1.03
exon 6	0.47	0.51	1.04	0.98	1.00	0.98	0.96	1.00	0.95	0.93	0.95	1.01
exon 8	0.48	0.52	1.06	1.00	1.02	1.00	0.98	1.02	0.97	0.95	0.97	1.03
Control 2	0.49	0.53	1.08	1.02	1.04	1.02	1.00	1.04	0.99	0.97	0.99	1.05
exon 9	0.47	0.51	1.04	0.98	1.00	0.98	0.96	1.00	0.95	0.93	0.96	1.01
exon 7	0.50	0.54	1.10	1.03	1.06	1.04	1.01	1.05	1.00	0.98	1.01	1.06
Control 3	0.51	0.55	1.12	1.05	1.07	1.06	1.03	1.07	1.02	1.00	1.02	1.08
exon 5	0.50	0.53	1.09	1.03	1.05	1.03	1.01	1.05	0.99	0.98	1.00	1.06
exon 2	0.47	0.50	1.03	0.97	0.99	0.97	0.95	0.99	0.94	0.92	0.95	1.00


3
4
3
4
4,425bp deletion
ctttagttttcgag
3
4
gaggctgctgctat
Intron 2
Intron 4
T T C G A G G C T
253
260

Mutation Analysis of Acute Porphyrias

- Screen dHPLC
- Sequence
- Quantitative PCR

Cutaneous porphyrias

- DNA analysis only relevant in certain circumstances

Congenital Erythropoietic Porphyria (CEP)

- Very rare
- Clinical Manifestations
  - Extreme photosensitivity, scarring, mutilation
  - Hypertrichosis
  - Erythrodontia
  - Haemolytic anaemia

Mutation Analysis in CEP

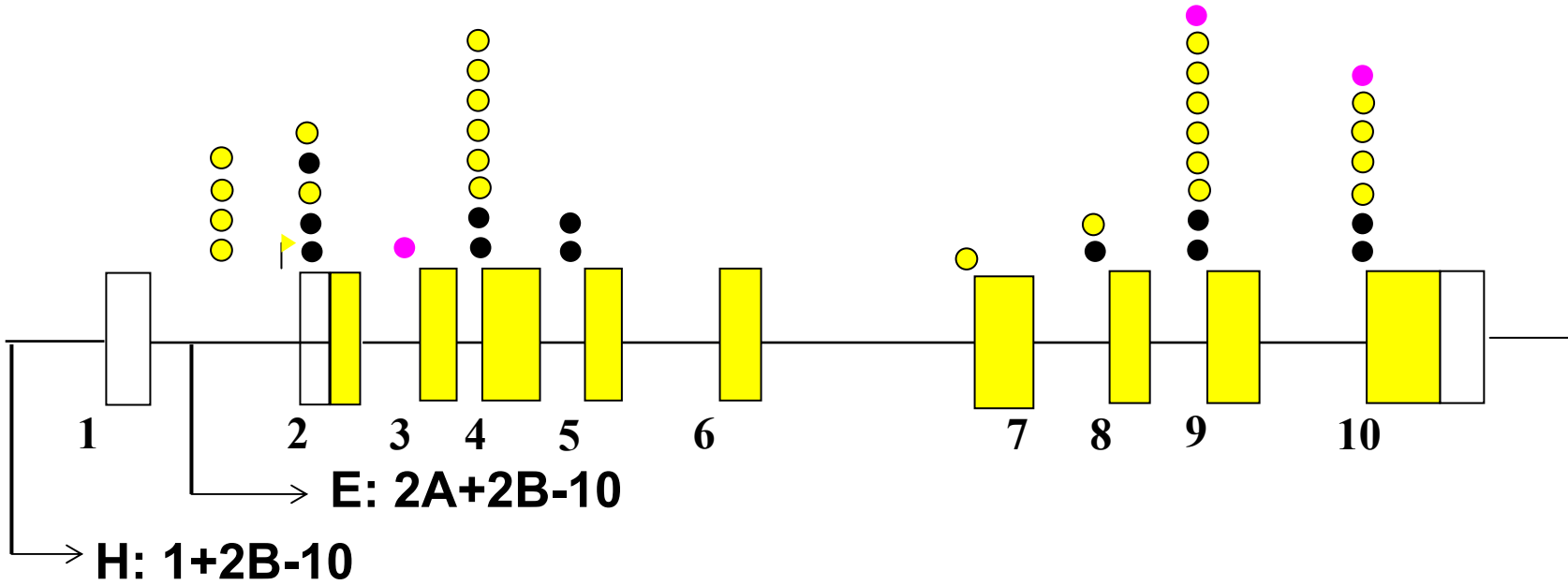
- One of the treatments for this condition is bone marrow transplantation
- High risk procedure
- Some genotype phenotype correlation
- Mutation analysis may help to decide whether to carry out this procedure

CEP: Genotype-Phenotype

Residual Activity*	Mutations	
Low (<1.5%)	Missense Nonsense Frameshift	V3F, Y19C, P53L, T63A, A69T, C73R, H173Y, Q187R, S212P, G225S, T228M, P248Q, Q249X All
Intermediate (2-8%)	Missense	L4F, V99A, A104V, G188W
High (10-35%)	Missense Splice	A66V E81D, V82F, (IVS8-23A>G)
* In vitro luciferase reporter assay		

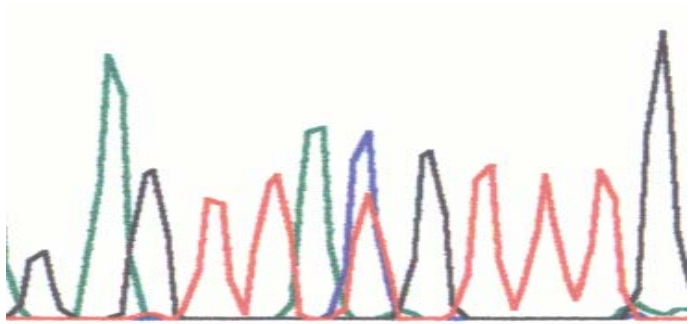
Phenotype	Genotype
Hydrops fetalis/Severe disease	2 x low activity
Moderate disease	Intermediate +low
Mild disease	Low/intermediate + high

Uroporphyrinogen III synthase



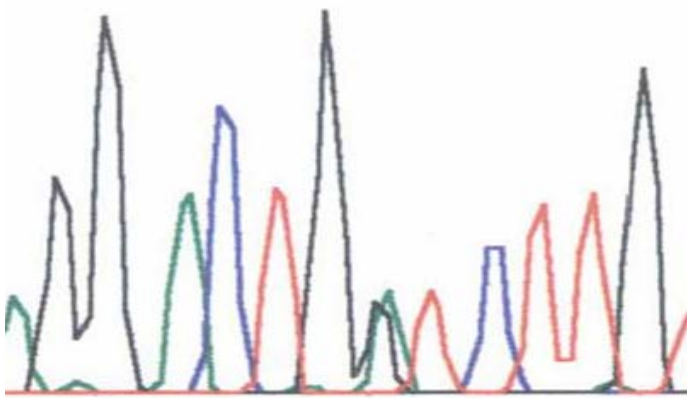
- Autosomal recessive
- Mutations throughout gene

Genotype



C73R

Severe mutation



IVS8-23 A>G

Mild mutation

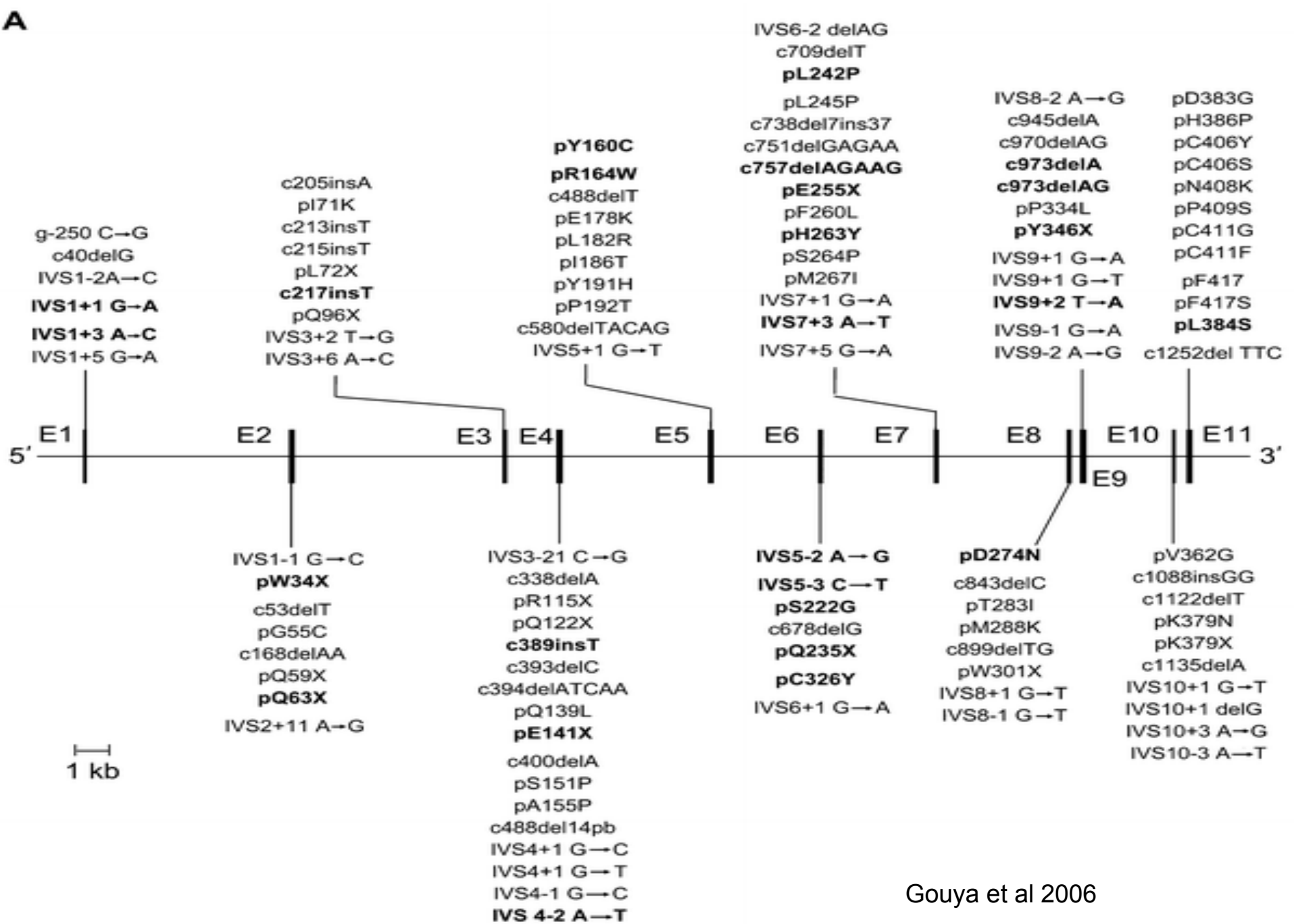
Moderate disease

Mutational Analysis

- Bone marrow transplantation
- Preconceptual counselling
- Prenatal diagnosis

Erythropoietic Protoporphyria

- EPP is a cutaneous porphyria
- It presents in childhood
- Photosensitivity
- 1-2% severe liver disease

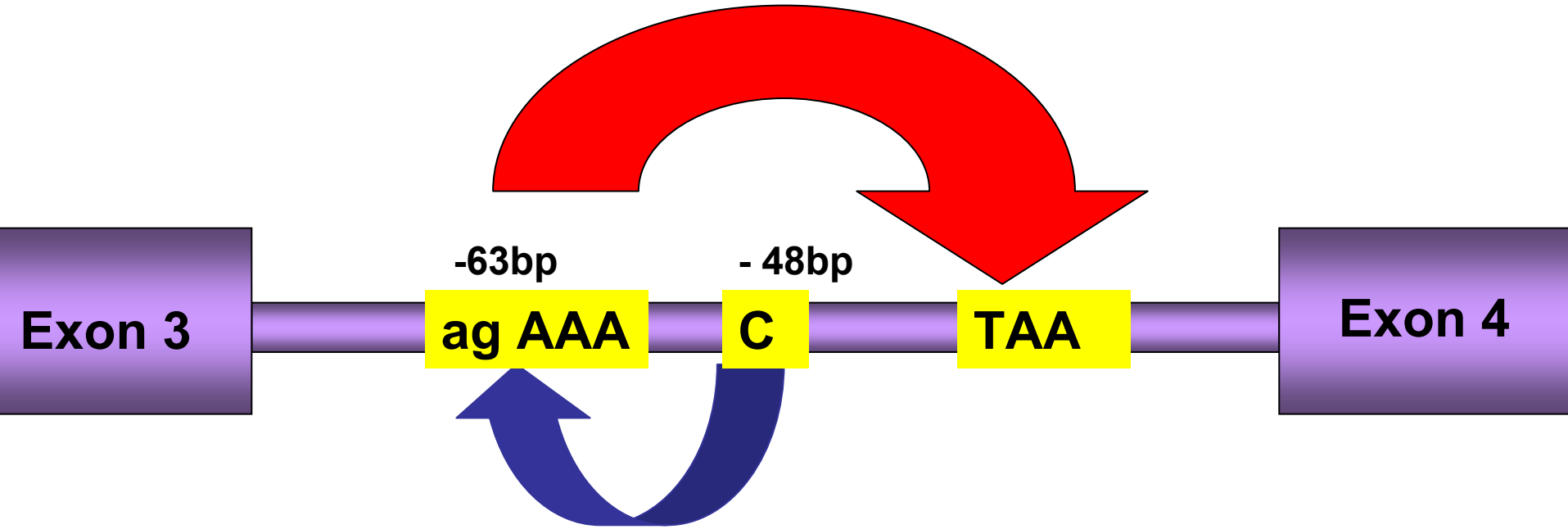


Gouya et al 2006

Genetics of EPP

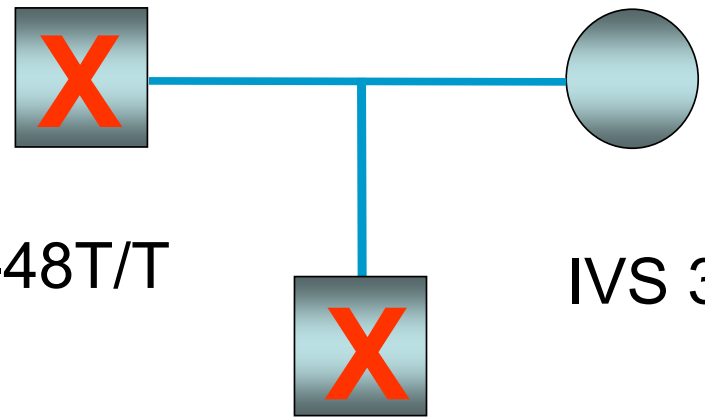
- A single mutation that reduces FECH activity by about 50% does not cause photosensitivity.
- Photosensitivity requires a reduction in FECH activity below a threshold of about 35%.
- A single nucleotide polymorphism present in 13% of the British population causes low expression of the *FECH* RNA.

The IVS3-48 T/C Polymorphism Modulates Splicing Efficiency



IVS3-48 T to C creates a
“splicing enhancer”

Expression of EPP



Mutation

50% FECH activity

IVS 3-48T/T

IVS 3-48C/T

Low expression allele

85% FECH activity

IVS 3-48C/T

Erythropoietic Protoporphyria

35% FECH activity

Mutation Analysis

- This can be useful in preconceptual counselling.
- The partner of a patient with EPP can be tested for the low expression allele to determine the risk for a future child.

Role Of Mutational Analysis In The Porphyrias

- Acute Porphyrias – required for preventative counselling including safe drug administration

- Cutaneous Porphyrias –
  - CEP - Prenatal Diagnosis and management options including bone marrow transplantation
  - EPP - risk calculation

Acknowledgments

Molecular Lab

- Nicola Mason
- Hannah Withers