RNA splicing– the Exeter experience

Sian Ellard, Mike Bulman, Lorna Harries, Melissa Sloman, Martina Owens, Jon Locke, Anna Murray, Beth Young

(1) Cryptic splice acceptor site mutation in *HNF1A* causing MODY

Diabetologia (2002) 45:1463–1467
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Diabetologia

Abnormal splicing of hepatocyte nuclear factor 1 alpha in maturity-onset diabetes of the young

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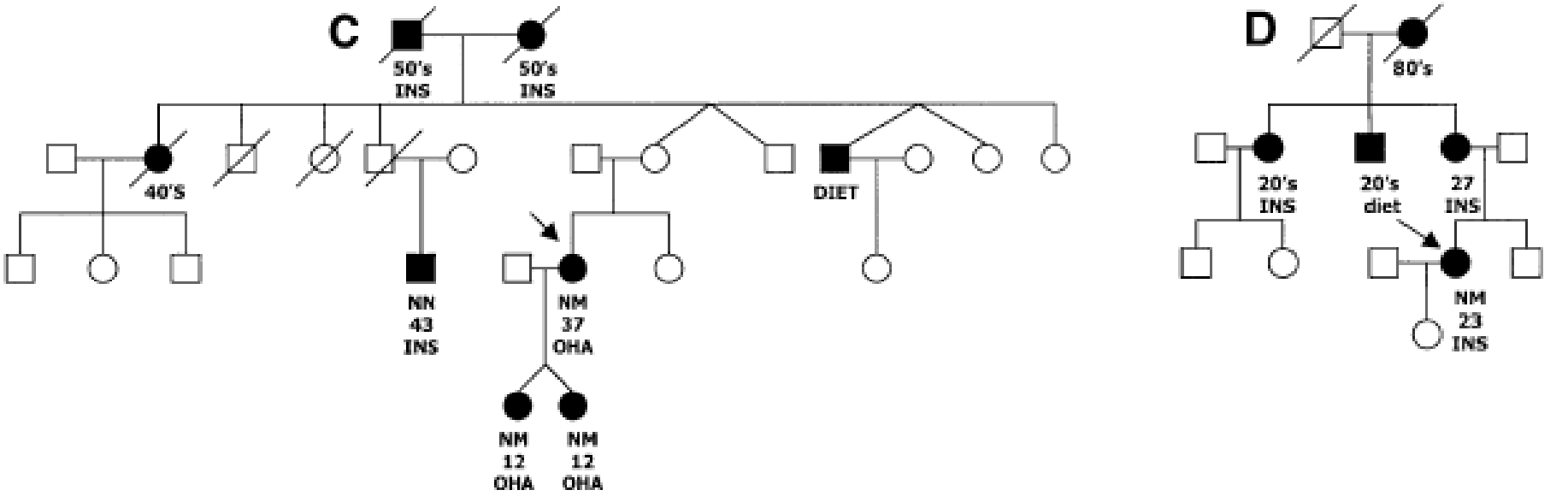
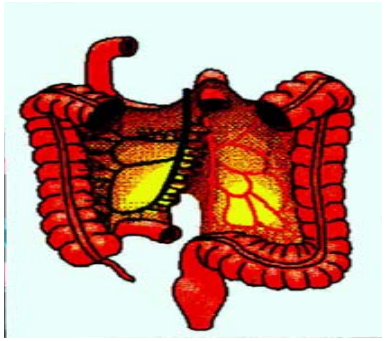
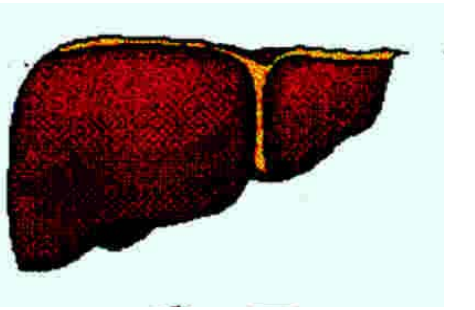
HNF1A IVS7nt-6G>A in two MODY families

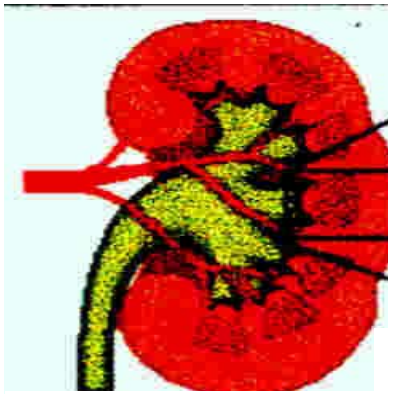


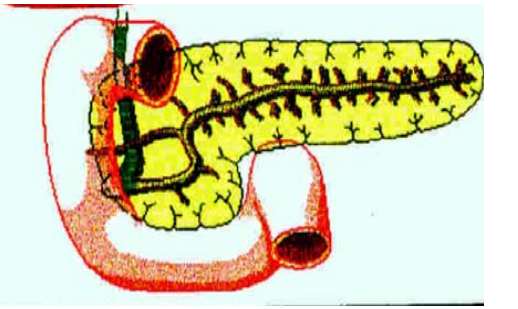
Expression profile of *HNF1A*





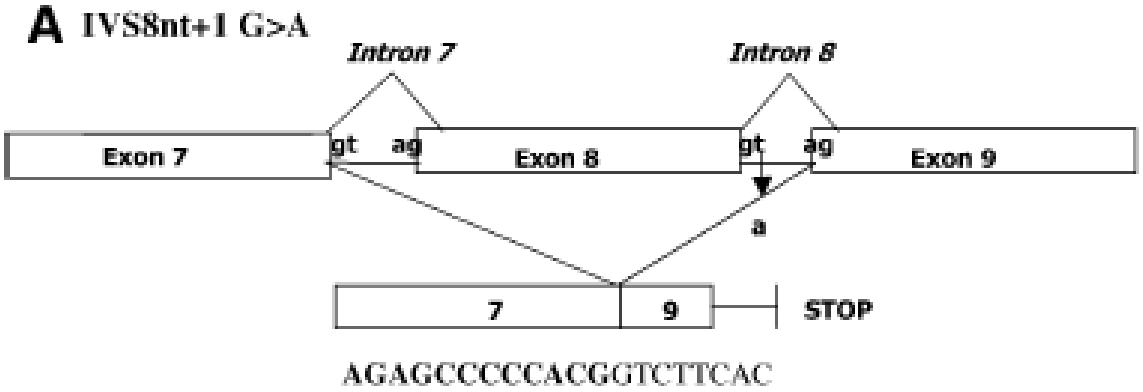
HNF1A



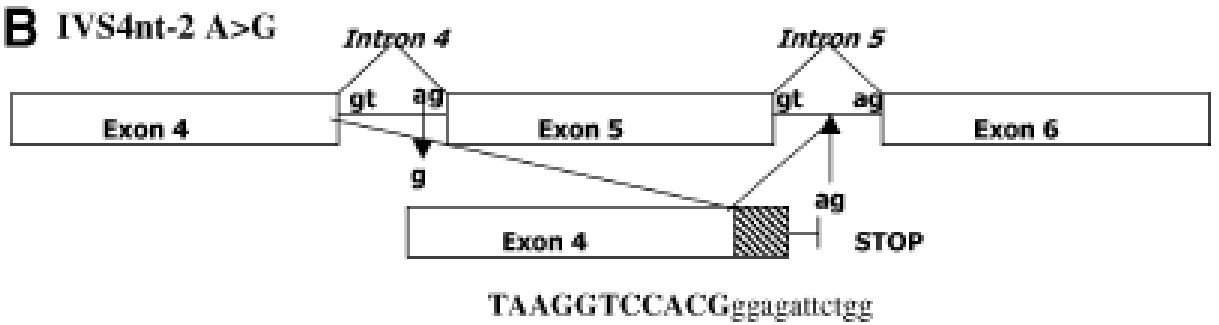


Ectopic transcripts

HNF1A mutations affecting conserved splice sites

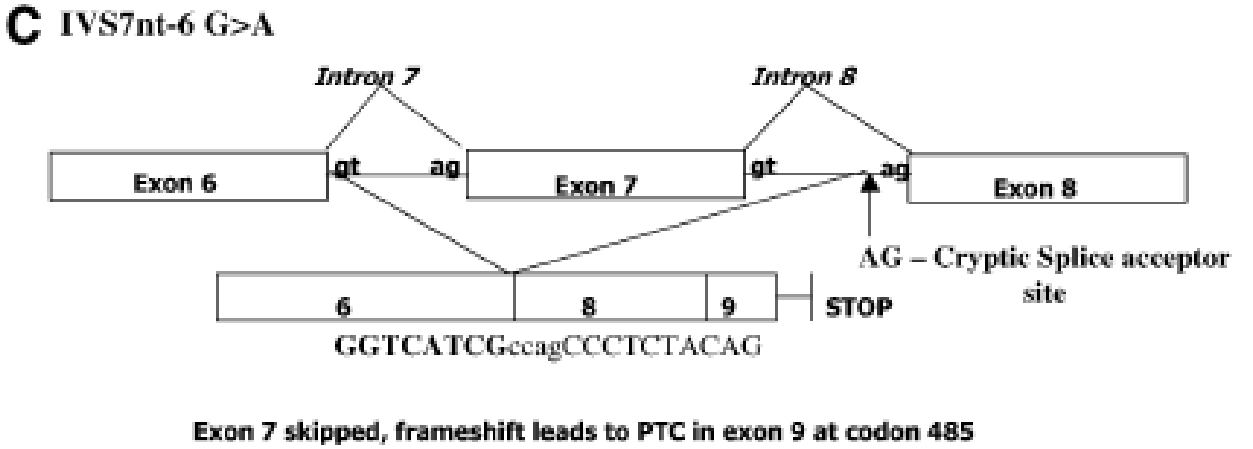


Exon 8 skipped, frameshift leads to PTC in exon 9 at codon 507



Exon 5 skipped & novel splice acceptor site recruited at nt3019 prior to PTC at codon 327.

HNF1A IVS7nt-6G>A mutation



- Aberrant transcripts in 1/3 RT-PCRs; presumed NMD

(2) *HNF1B* mutations causing renal cysts and diabetes (RCAD)

HNF1B splicing mutations causing renal cysts and diabetes (RCAD)

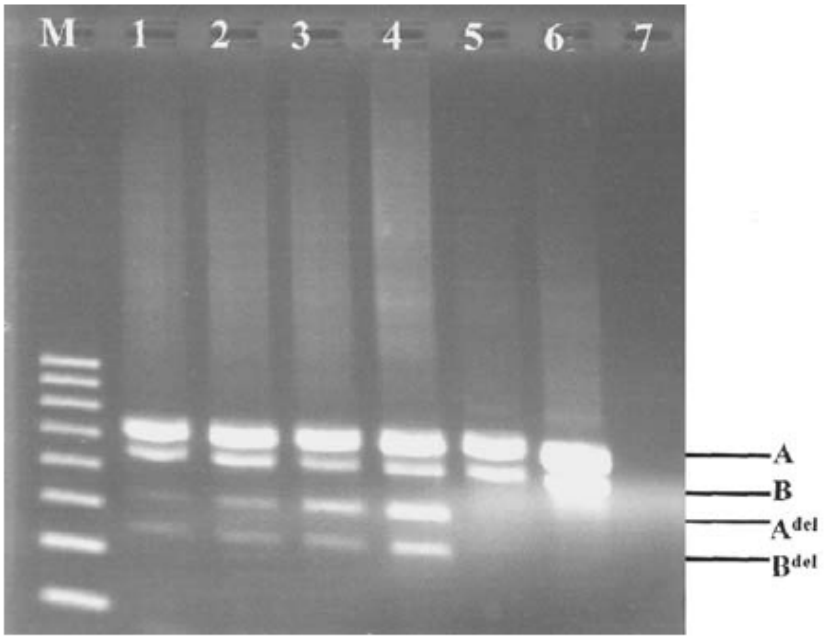
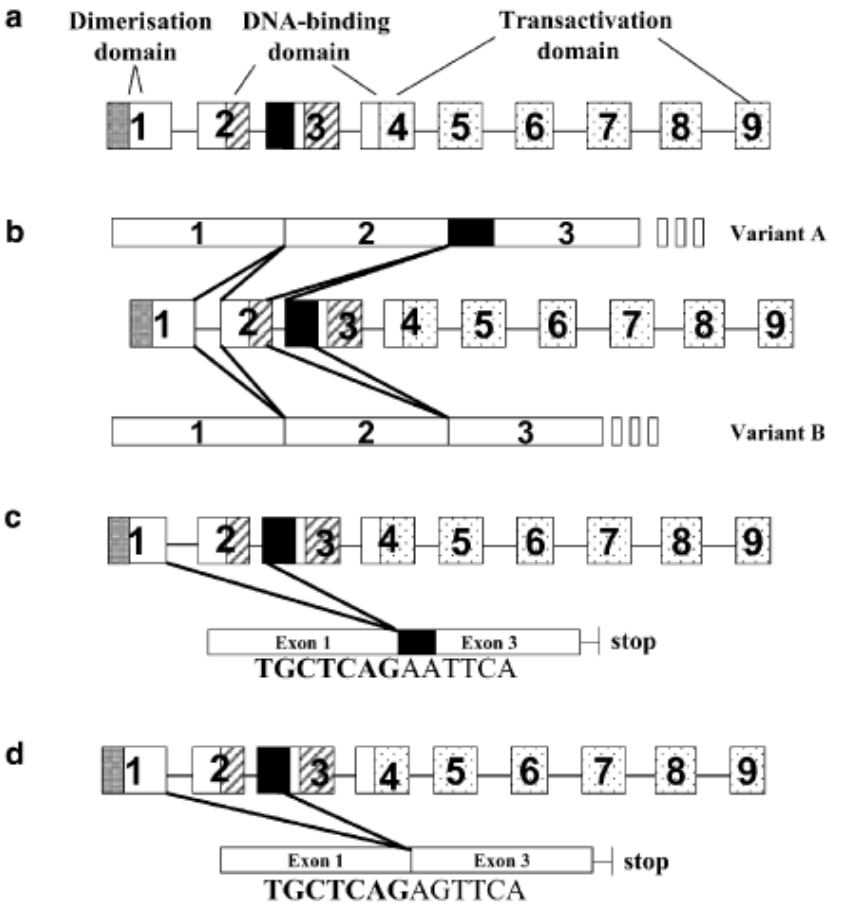


Fig. 2. Splice variants produced by the IVS2+1G>T and IVS2nt+2insT mutations. This figure shows the amplicons produced by RT-PCR of transcripts produced by IVS2nt+1G>T mRNA for exons 1–4. Size markers (100 bp ladder) are represented by 'M'. *Lanes 1 and 2* are HNF-1β RT-PCR products amplified from proband DUK350. *Lanes 3 and 4* are RT-PCR products amplified from the proband of family DUK504. *Lanes 5 and 6* are RT-PCR products from unaffected controls. The water (negative) control is in *lane 7*. A and B are wild-type transcripts; A^{del} and B^{del} are mutated transcripts



Harries et al 2004 Diabetologia

Hum Genet (2005)
DOI 10.1007/s00439-005-0023-y

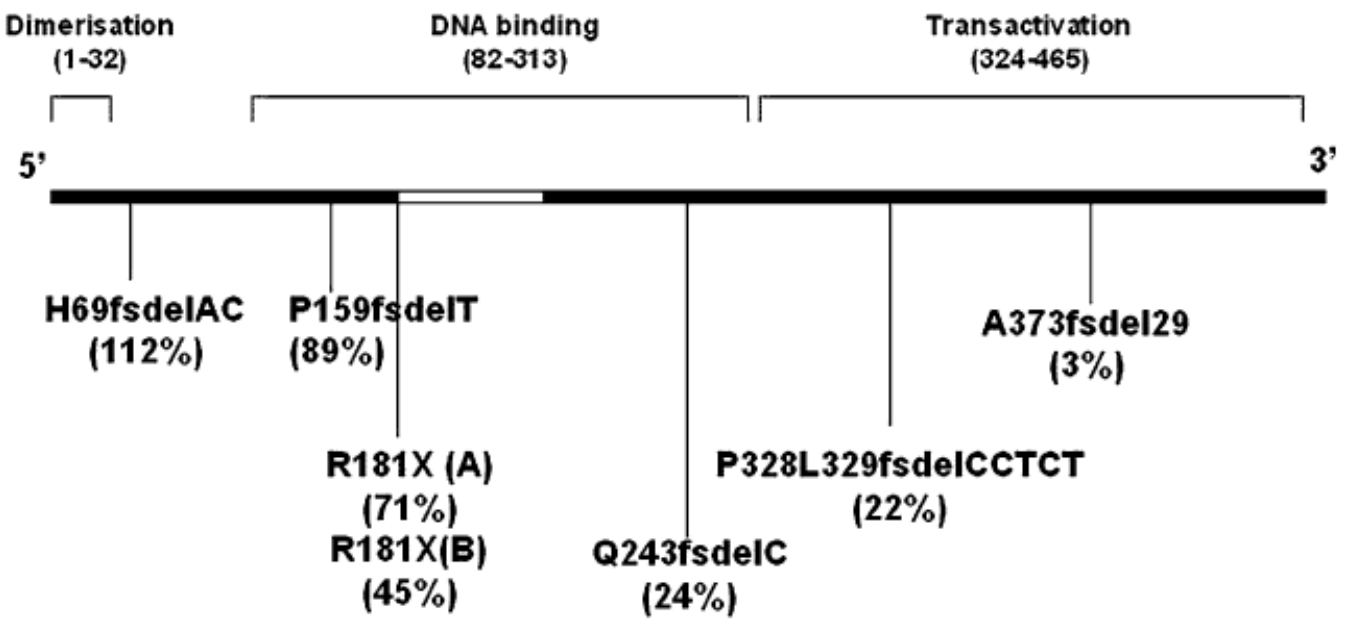
ORIGINAL INVESTIGATION

L. W. Harries · Coralie Bingham
Christine Bellanne-Chantelot · A. T. Hattersley
Sian Ellard

The position of premature termination codons in the hepatocyte nuclear factor −1 beta gene determines susceptibility to nonsense-mediated decay

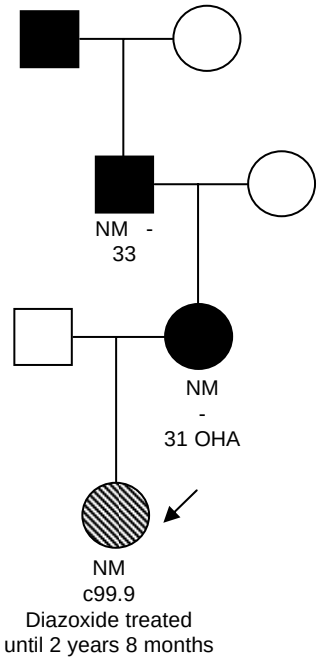
- Relative transcript levels quantified by real-time PCR
- Results validated from renal tubule cells (overnight urine collection) of one patient
- NMD susceptibility investigated in EBV transformed cell lines ± cycloheximide

Susceptibility to NMD depends on the location of the *HNF1B* mutation



(3) *HNF4A* branch site mutation causing neonatal hypoglycaemia and MODY

HNF4A branch site mutation causing neonatal hypoglycaemia and MODY



Wild type splice predictor

Acceptor site predictions for 144.173.84.201.8336.0 :

Start	End	Score	Intron	Exon
20	60	0.60	accatccaaagccctcccc**ag**	atttagccggcagtgcgtgg

IVS2-21A>G

Acceptor site predictions for 144.173.84.201.8429.0 :

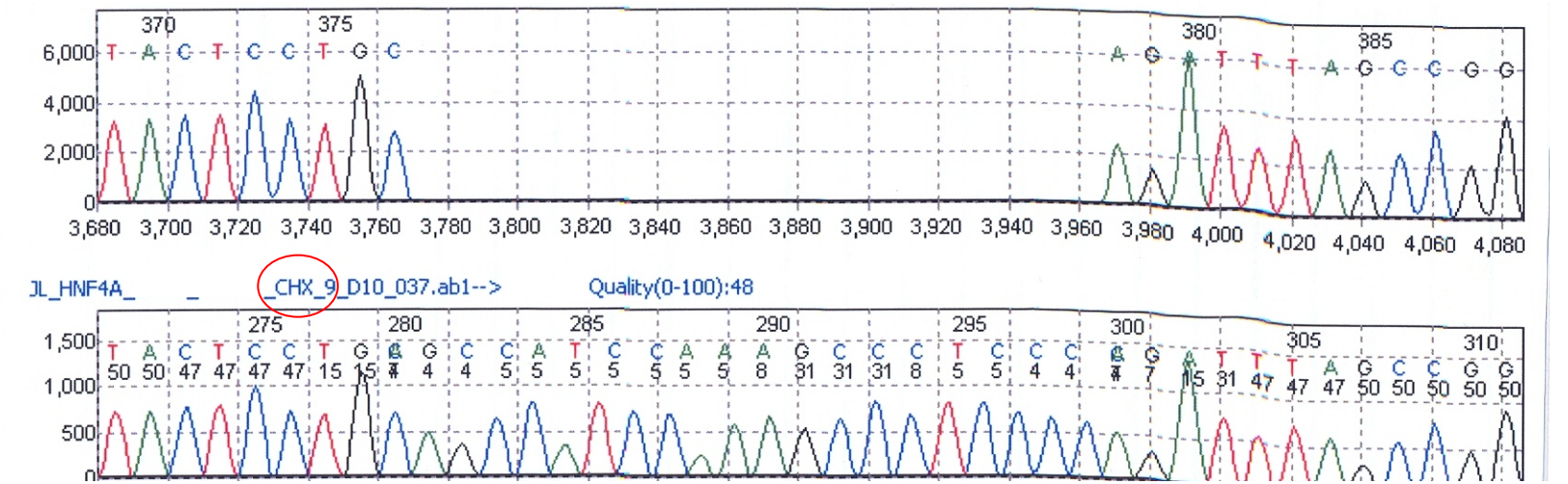
Start	End	Score	Intron	Exon
32	72	0.41	agttgtgtcttctccatcc**ag**	ccatccaaagccctccccag
52	92	0.61	gccatccaaagccctcccc**ag**	atttagccggcagtgcgtgg

http://www.fruitfly.org/seq_tools/splice.html

Mutation creates a cryptic splice acceptor site with a lower score than the normal acceptor site

c.264-21A>G

mRNA analysis confirmed this prediction

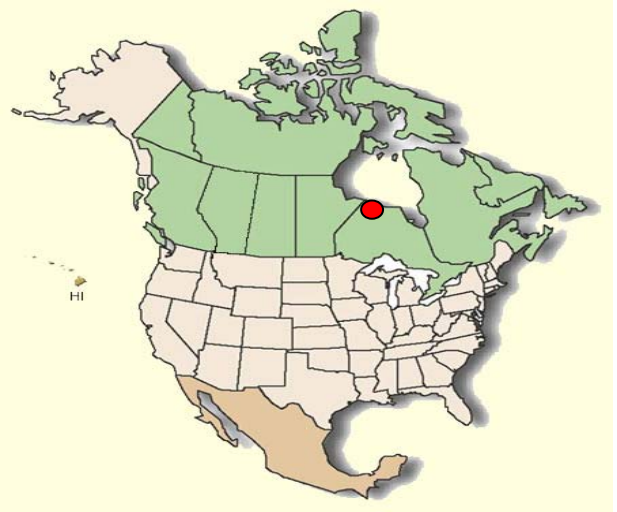


Aberrant transcript with 20 additional bases from intron 2 leading to a premature termination codon in exon 3

Kapoor et al 2008 Diabetes

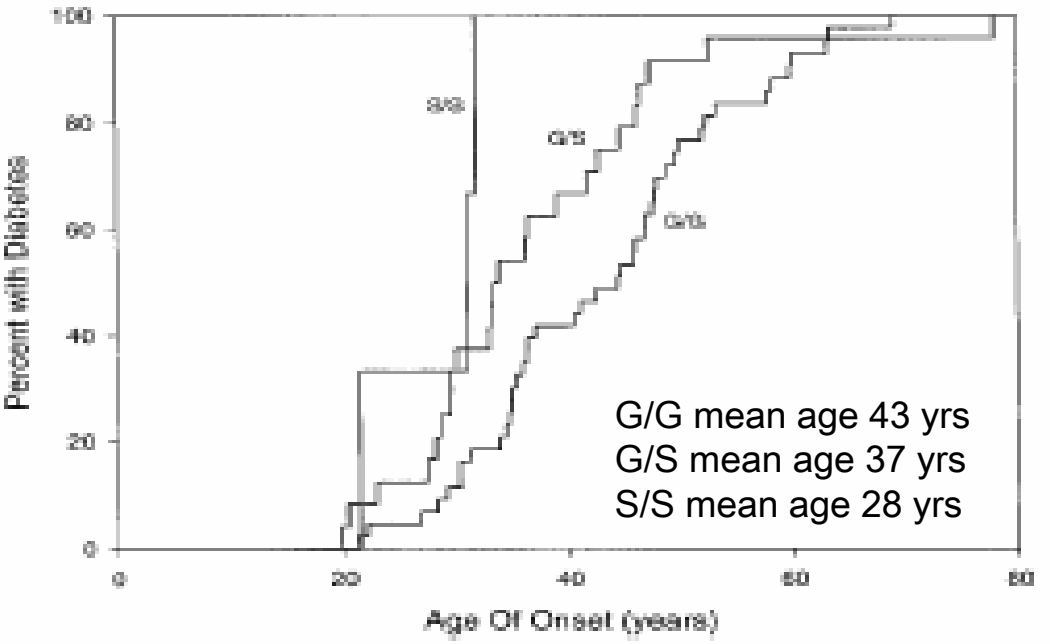
(4) *HNF1A* variant associated with type 2 diabetes

HNF1A variant (G319S) associated with susceptibility to type 2 diabetes



- ~40% Oji-Cree adults have type 2 diabetes
- Third highest prevalence in the world
- 40% with diabetes have *HNF1A* G319S (c.955G>A) variant

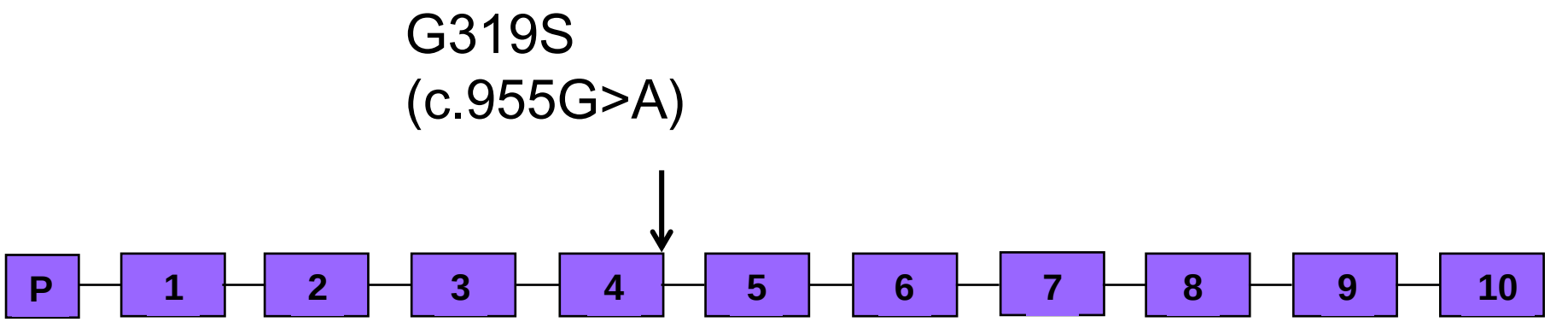
- Heterozygotes have odds ratio of 1.97
- Homozygotes have odds ratio of 4



Pathophysiology of G319S *HNF1A* variant associated with type 2 diabetes

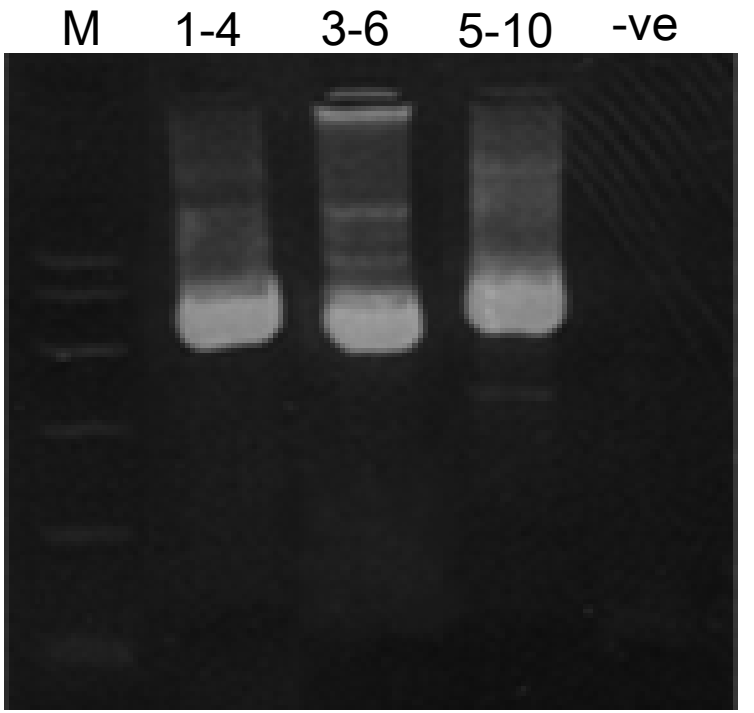
- In vitro functional analysis of G319S protein showed 54% reduced transactivation activity (Triggs-Raine et al 2002)

- But the variant is located within a conserved splice donor site

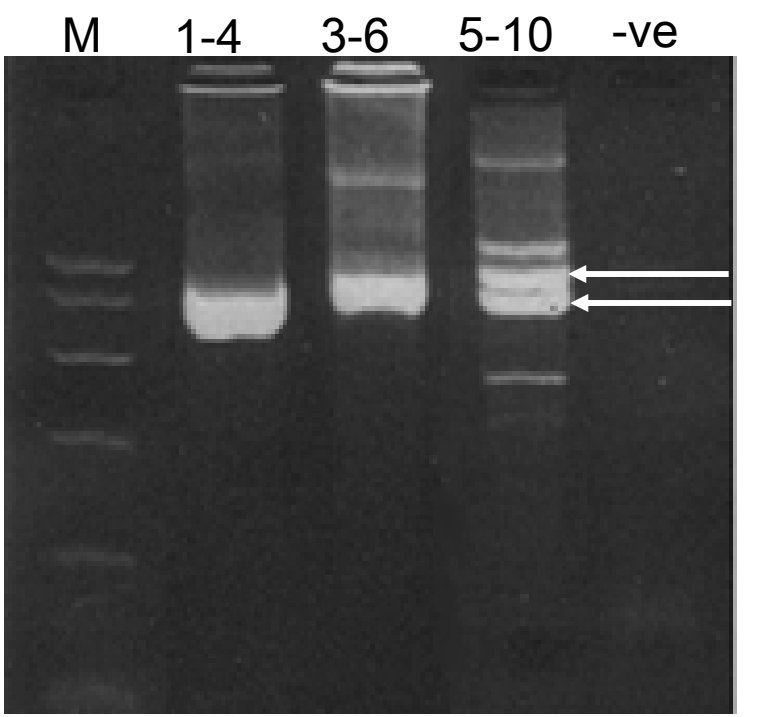


mRNA analysis of a G319S homozygote

Normal

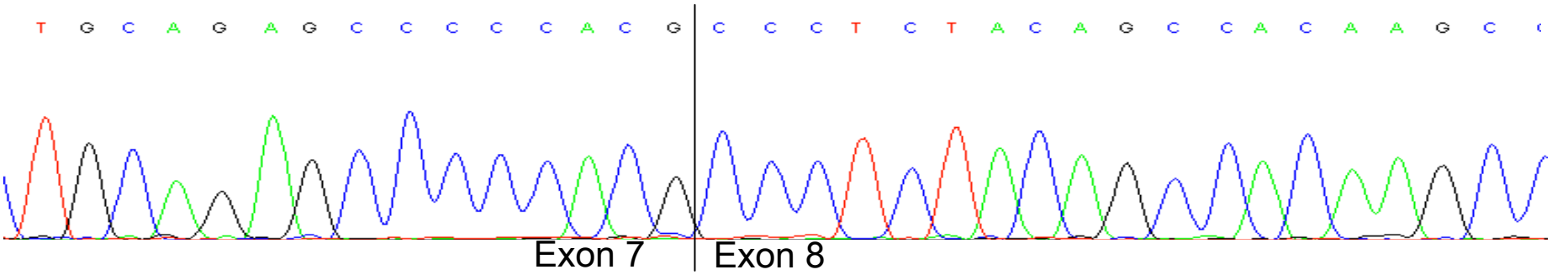


G319S

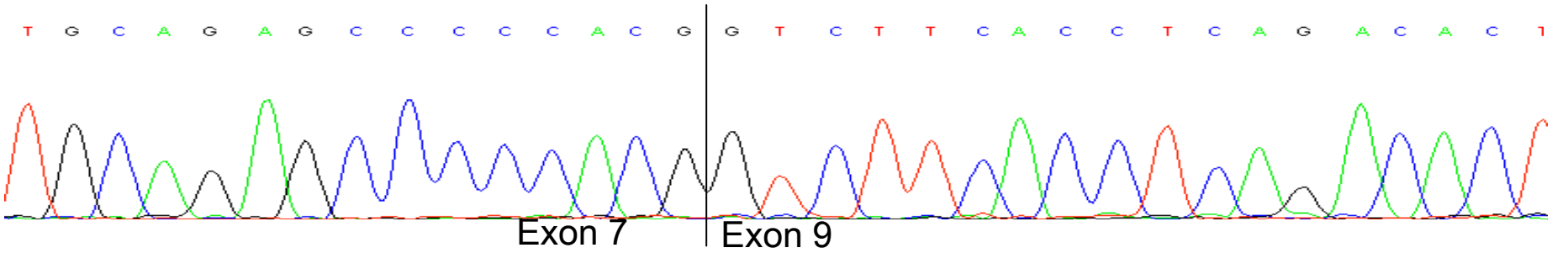


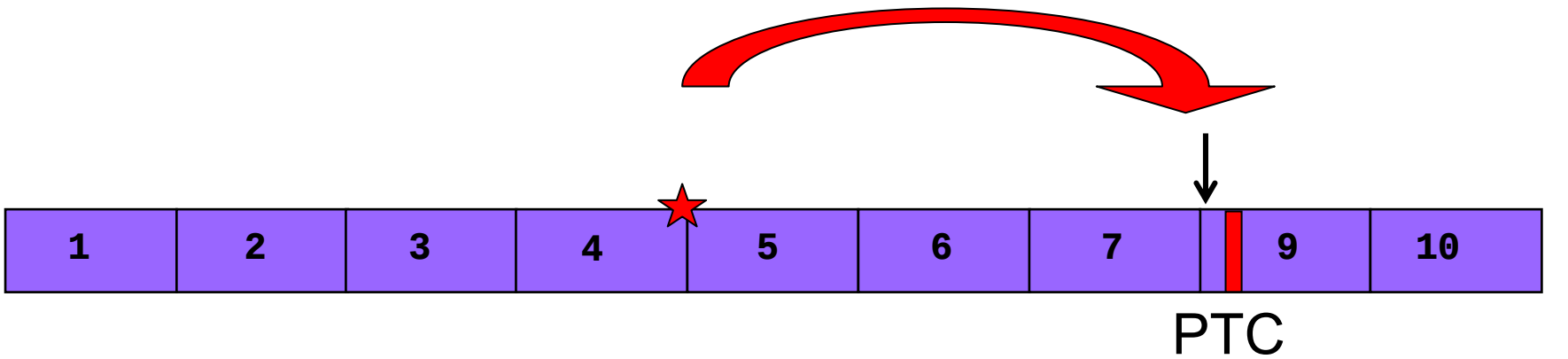
G319S causes exon 8 skipping

Normal

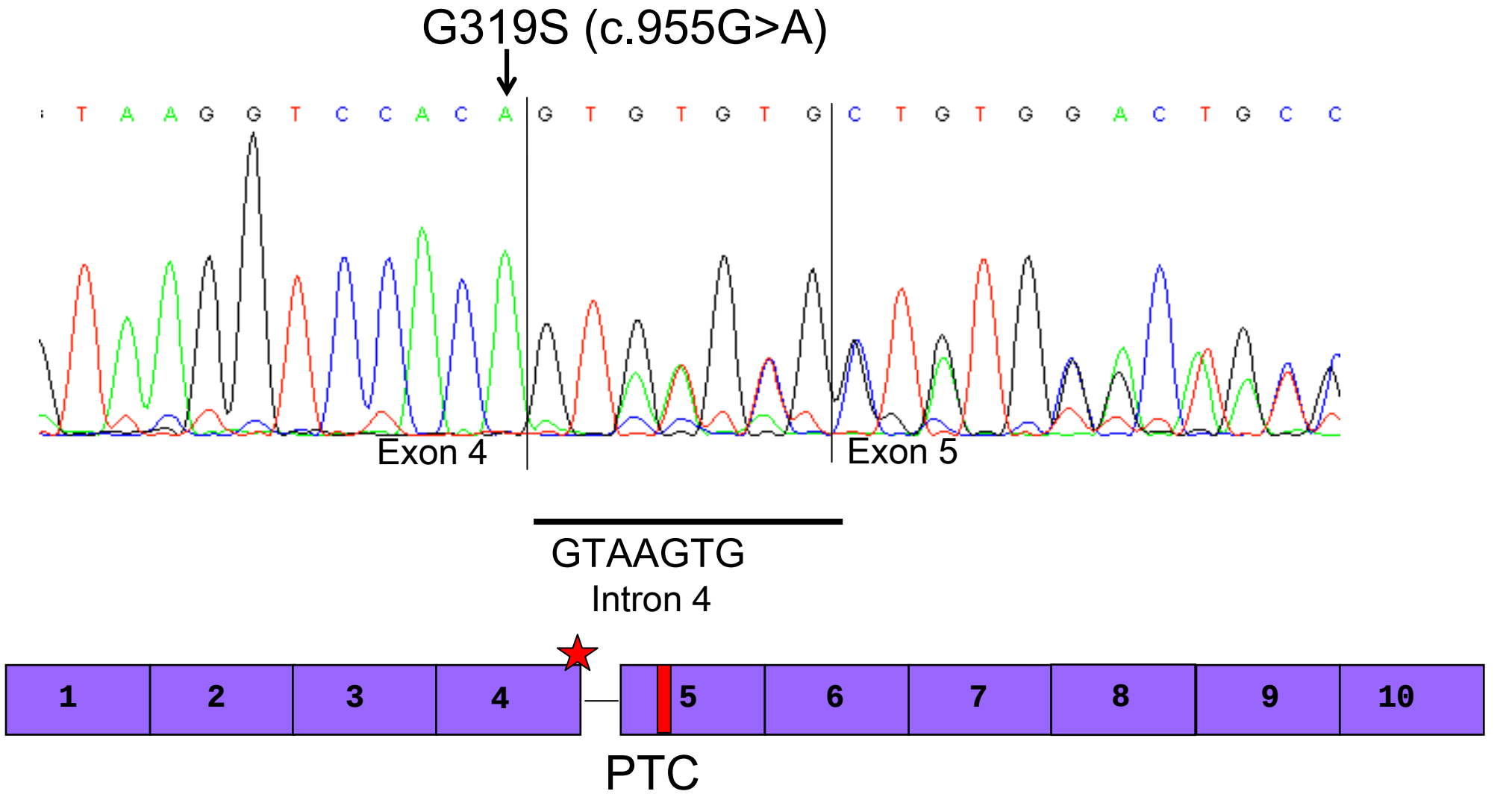


Mutant

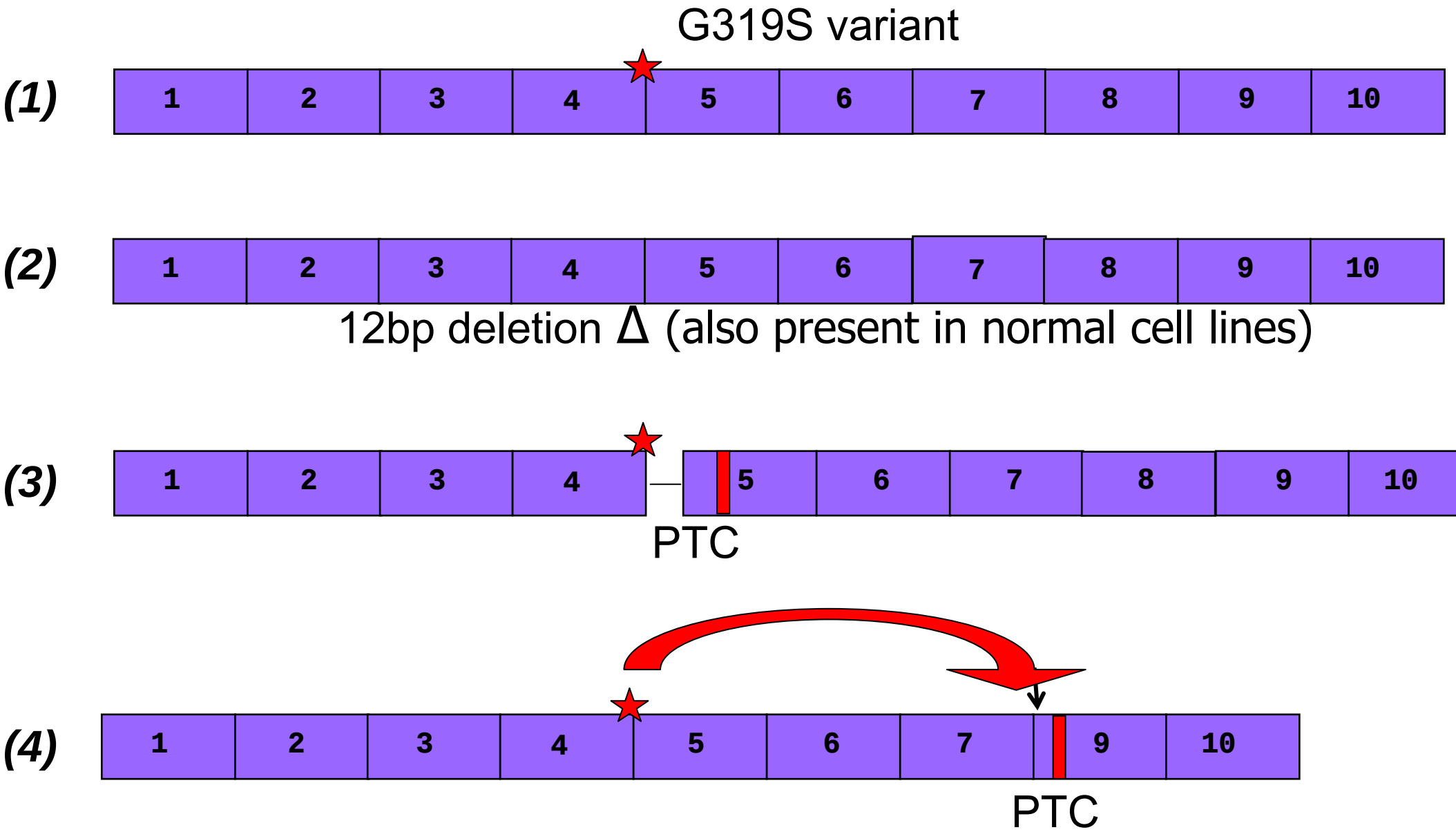




G319S also causes intron retention

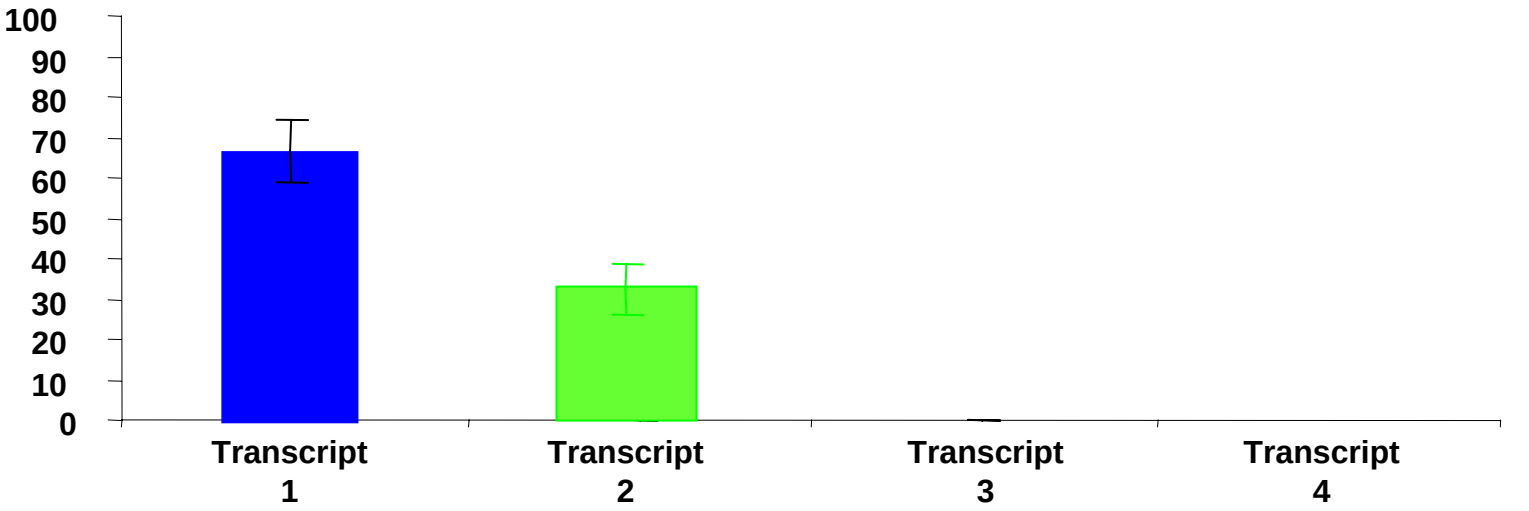


Four different transcripts identified

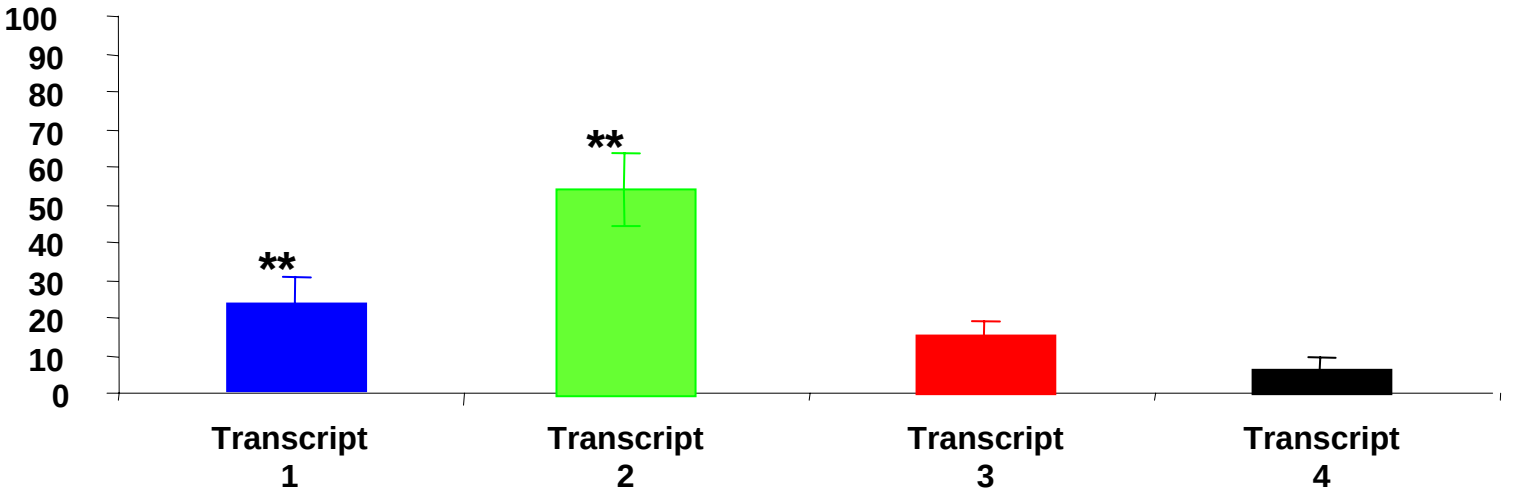


Quantitative analysis of mRNA transcripts

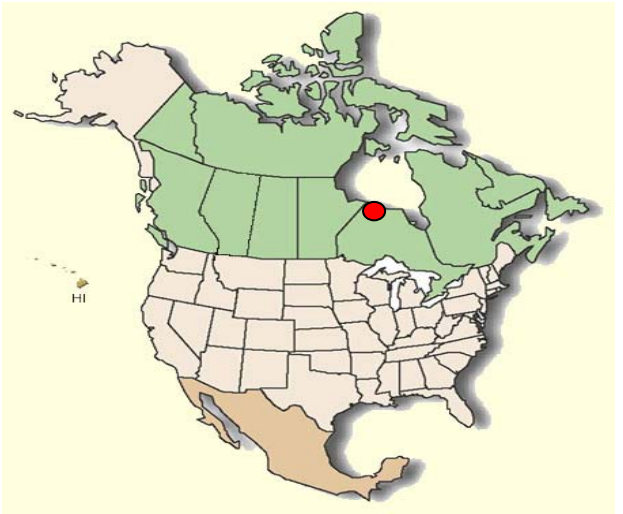
Normal



G319S



HNF1A variant (G319S) associated with susceptibility to type 2 diabetes



- A combination of reduced protein activity and mRNA expression underlies the susceptibility to type 2 diabetes in the Oji-Cree
- Homozygotes estimated to have ~66% normal HNF-1α protein activity; heterozygotes ~83% (vs 50% in MODY)

(5) Analysis of *RET* gene splicing in Hirschsprung disease

RET mutations identified in HSCR

Patient	Mutation	Exon	Phenotype	Occurrence	Reference
1	c.432delC	3	TCA	Sporadic	Novel
2	p.R360W (c.1078C>T)	6	TCA	Sporadic	Bolk et al, 2000
3	p.C585Y (c.1754G>A)	9	TCA	Sporadic	Novel
4	p.C620R (c.1858T>C)	10	L-HSCR	Familial	Angrist et al, 1995
5	p.V757M (c.2269G>A)	12	Unknown	Familial	Novel
6	c.2546delC	14	L-HSCR	Familial	Novel
7	c.2607+5G>A	Intron 14	L-HSCR	Sporadic	Auricchio et al, 1999
8	c.2801+3del4	Intron 16	S-HSCR	Familial	Novel
9	c.2939+10G>T	Intron 17	Unknown	Familial	Novel

Total number tested: 30 patients for exons 10, 11, 13-16 and 11 patients tested for exons 1-20

Familial cases: n=19, Sporadic cases: n=22

RET mutations identified in HSCR

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6	c.2546delC	14	L-HSCR	Familial	Novel
7	c.2607+5G>A	Intron 14	L-HSCR	Sporadic	Auricchio et al, 1999
8	c.2801+3del4	Intron 16	S-HSCR	Familial	Novel
9	c.2939+10G>T	Intron 17	Unknown	Familial	Novel

Total number tested: 30 patients for exons 10, 11, 13-16 and 11 patients tested for exons 1-20

Familial cases: n=19, Sporadic cases: n=22

NNSPLICE: wt 0.98, mutation 0.52 – skipping of exon 14

www.fruitfly.org/seq_tools/splice

RET mutations identified in HSCR

Patient	Mutation	Exon	Phenotype	Occurrence	Reference
1	c.432delC	3	TCA	Sporadic	Novel
2	p.R360W (c.1078C>T)	6	TCA	Sporadic	Bolk et al, 2000
3	p.C585Y (c.1754G>A)	9	TCA	Sporadic	Novel
4	p.C620R (c.1858T>C)	10	L-HSCR	Familial	Angrist et al, 1995
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6	c.2546delC	14	L-HSCR	Familial	Novel
7	c.2607+5G>A	Intron 14	L-HSCR	Sporadic	Auricchio et al, 1999
8	c.2801+3del4	Intron 16	S-HSCR	Familial	Novel
9	c.2939+10G>T	Intron 17	Unknown	Familial	Novel

Total number tested: 30 patients for exons 10, 11, 13-16 and 11 patients tested for exons 1-20

Familial cases: n=19, Sporadic cases: n=22

RNA analysis: skipping of exon 16

www.fruitfly.org/seq_tools/splice

RET mutations identified in HSCR

Patient	Mutation	Exon	Phenotype	Occurrence	Reference
1	c.432delC	3	TCA	Sporadic	Novel
2	p.R360W (c.1078C>T)	6	TCA	Sporadic	Bolk et al, 2000
3	p.C585Y (c.1754G>A)	9	TCA	Sporadic	Novel
4	p.C620R (c.1858T>C)	10	L-HSCR	Familial	Angrist et al, 1995
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6	c.2546delC	14	L-HSCR	Familial	Novel
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Total number tested: 30 patients for exons 10, 11, 13-16 and 11 patients tested for exons 1-20

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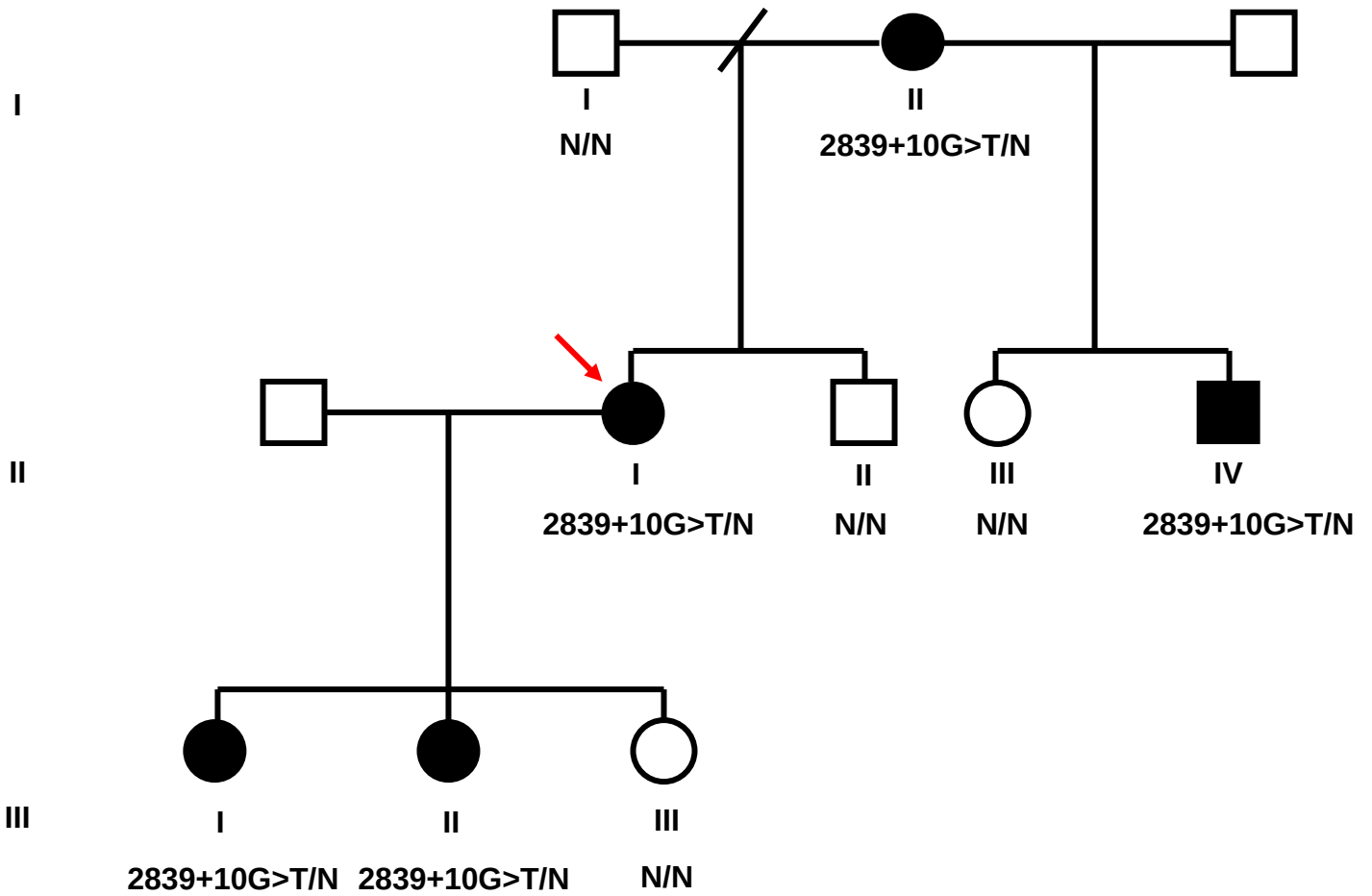
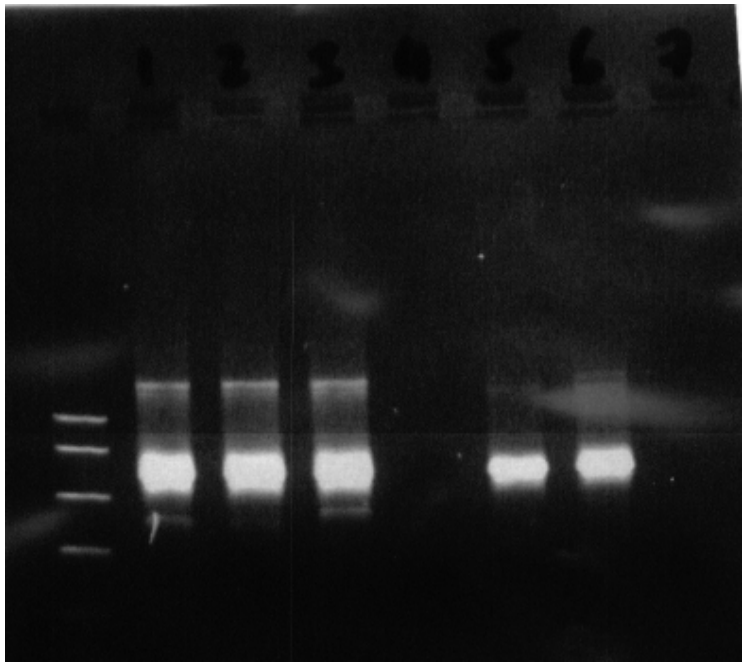
NNSPLICE: additional splice donor site in intron 17

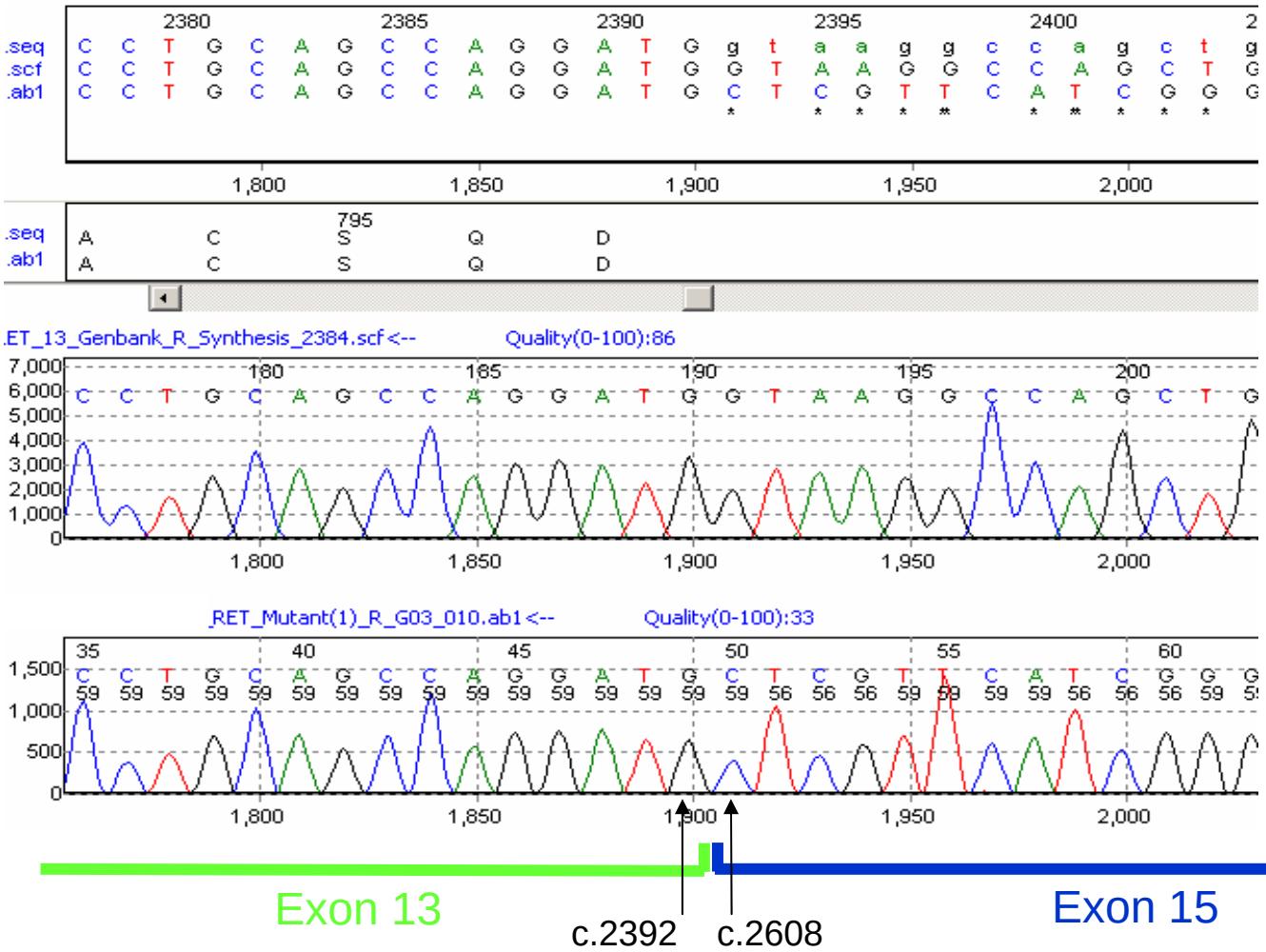
www.fruitfly.org/seq_tools/splice

Co-segregation of c.2939+10G>T *RET* mutation



mRNA analysis of c.2939+10G>T *RET* mutation





Key points

- Choice of cell type
- Multiple replicates required
- Use of cycloheximide in lymphoblastoid cell lines to suppress NMD
- Knowledge regarding normal isoforms
- Aberrant splicing may (or may not) be predicted by in silico software.