Familial Hypercholesterolaemia

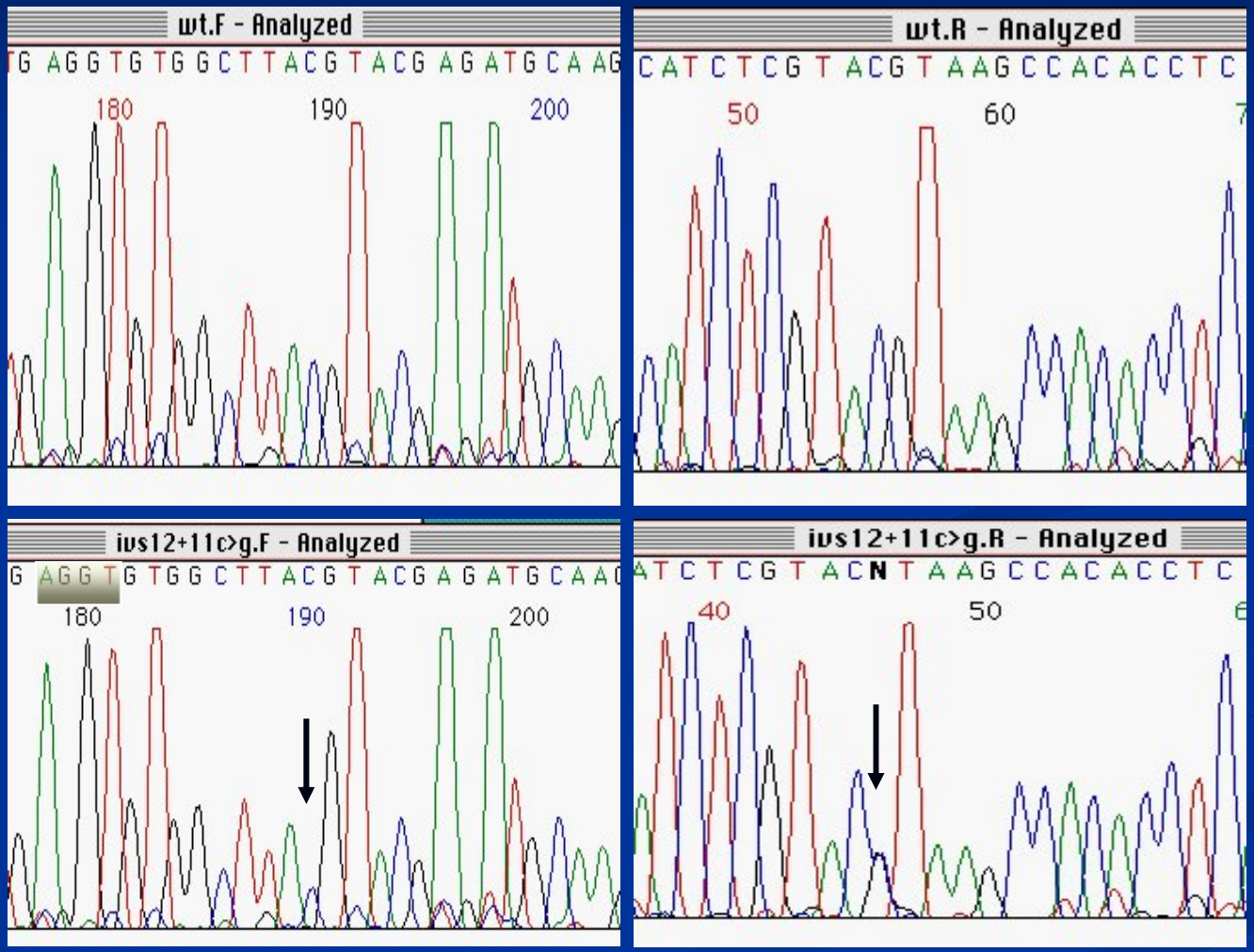
Splicing defects account for around 15% of mutations in LDLR gene

Reference	Population	No. of different point mutations	No. of splice defects	%
Hobbs et al. 1992[19]	*Mixed*	*105*	*3*	*3*
Day et al. 1997[20]	*UK*	*51*	*1*	*2*
Graham et al. 1999[5]	*N Ireland*	*23*	*1*	*4*
Lombardi et al. 2000[11]	*Dutch*	*51*	*8*	*16*
Ansellem et al [in press][12]	*France*	*54*	*13*	*24*
Graham et al 2005	*N Ireland & N England*	*44*	*6*	*14*

Splicing defects in LDLR causing Familial Hypercholesterolaemia

- 15/97 families with defined mutations causing FH have splicing defects in LDLR
- 6 separate mutations
  - c.313+1g>a
  - c.313+2t>c
  - c.621c>t
  - c.1587-1g>a
  - c.1706-1g>a – 4 families
  - c.1845+11c>g – 7 families

Sequence screen ex12 / intron12



LDLR ivs12+11C>G
LDLR c.1845+11C>G

- **Creates cryptic splice site**

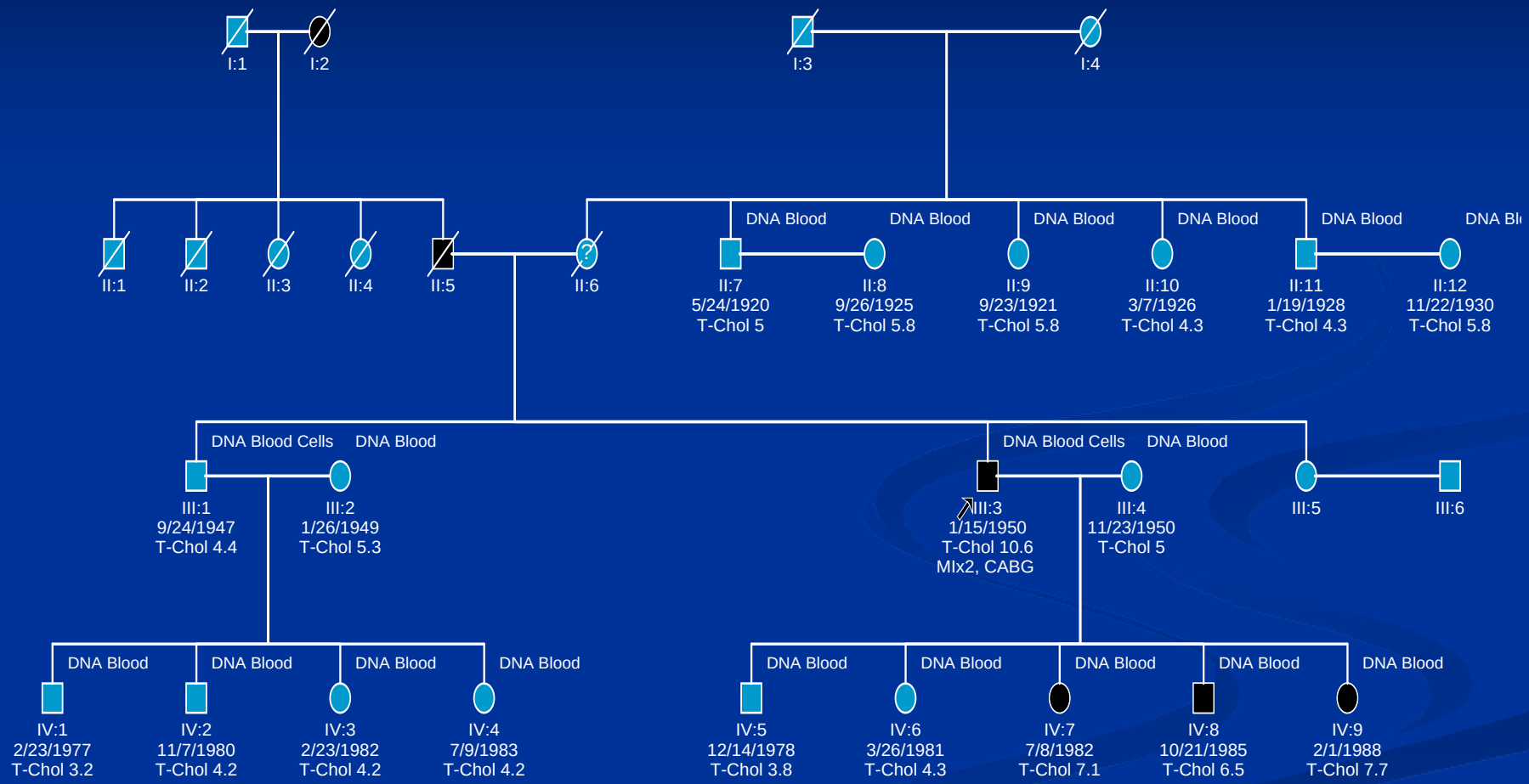
Exon --- A G | G T A A G T consensus donor splice site
% 64 73 100 100 62 68 84 63

Exon 12 --- A G | G T G T G G C T T A C / G G T A

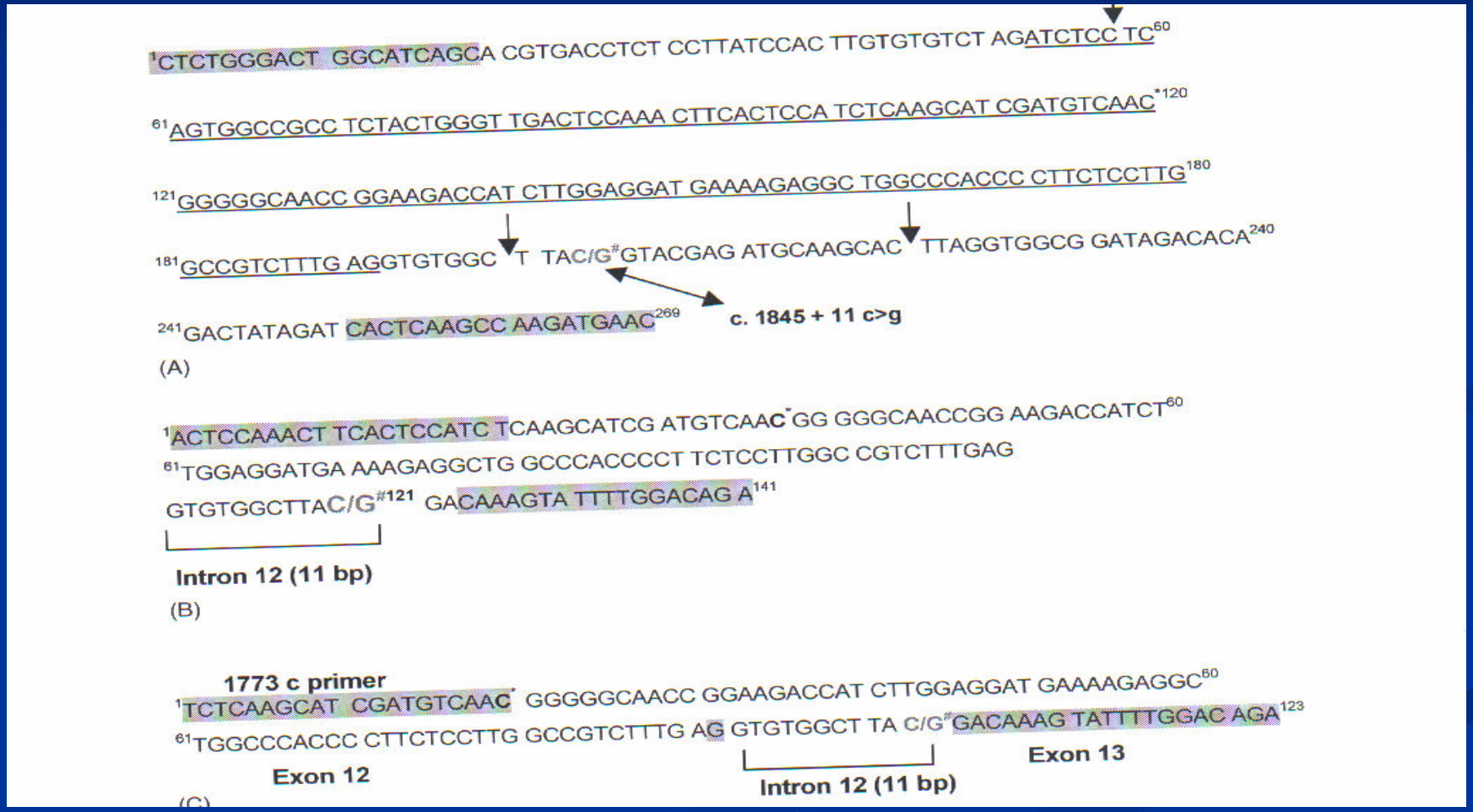
c.1845 +11 --- A G | G T A C G A

- **Not in 200 normal chromosomes**
- **Not found in any unaffected family members**

Family with LDLR 1845+11c>g splice mutation.

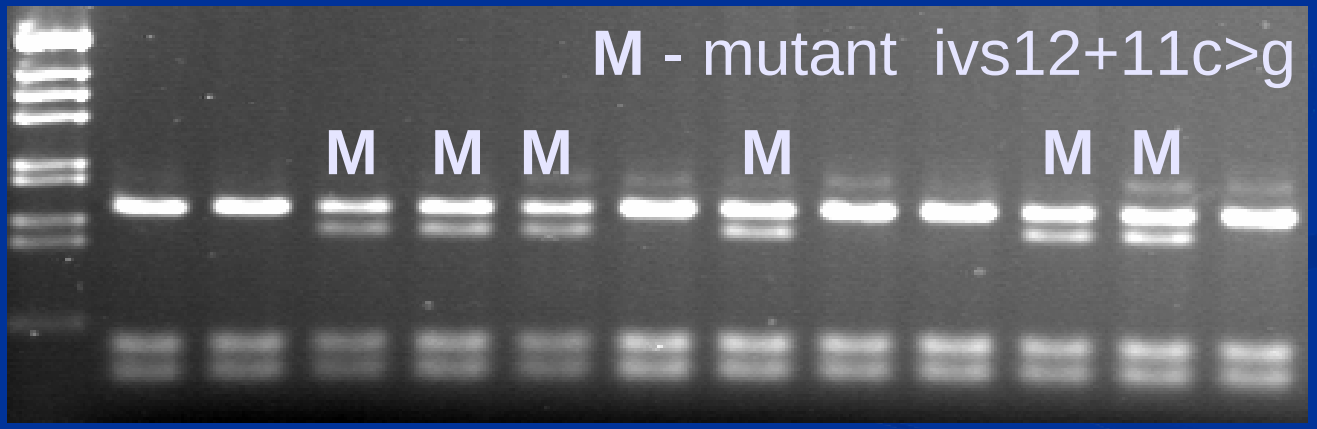


LDLR exon 12/intron 12 sequence



Restriction enzyme assay for ivs12+11c>g mutation.

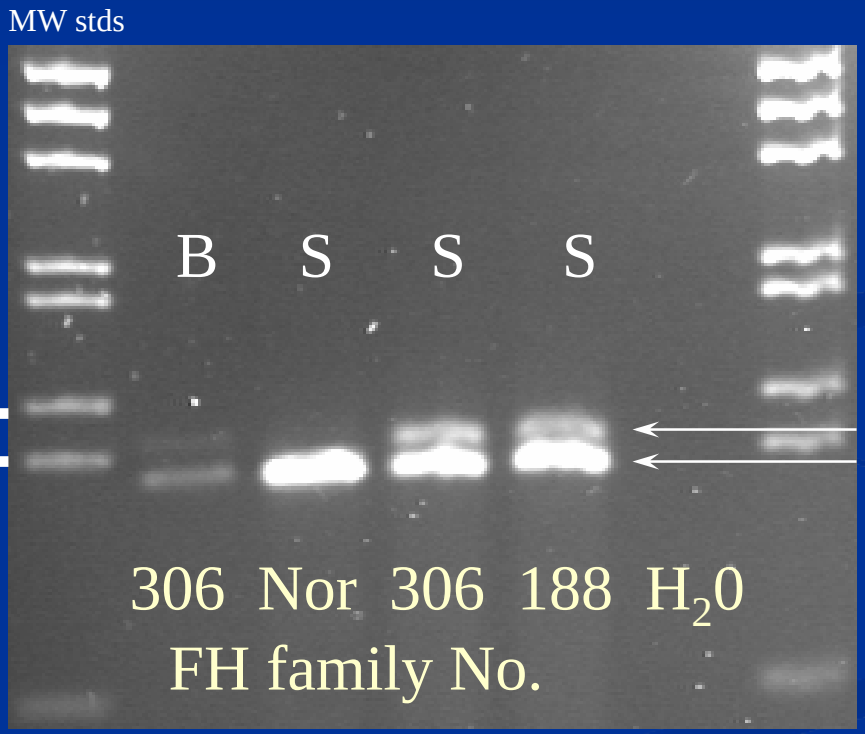
LDLR exon 12 PCR product digested with Dde.I



2% Nusieve + 1% BRL agarose.

Assay A

RT-PCR assay specific for LDLR exon 12 splice site on Blood and Skin Fibroblast RNA



Tissue RNA B - blood
S - skin

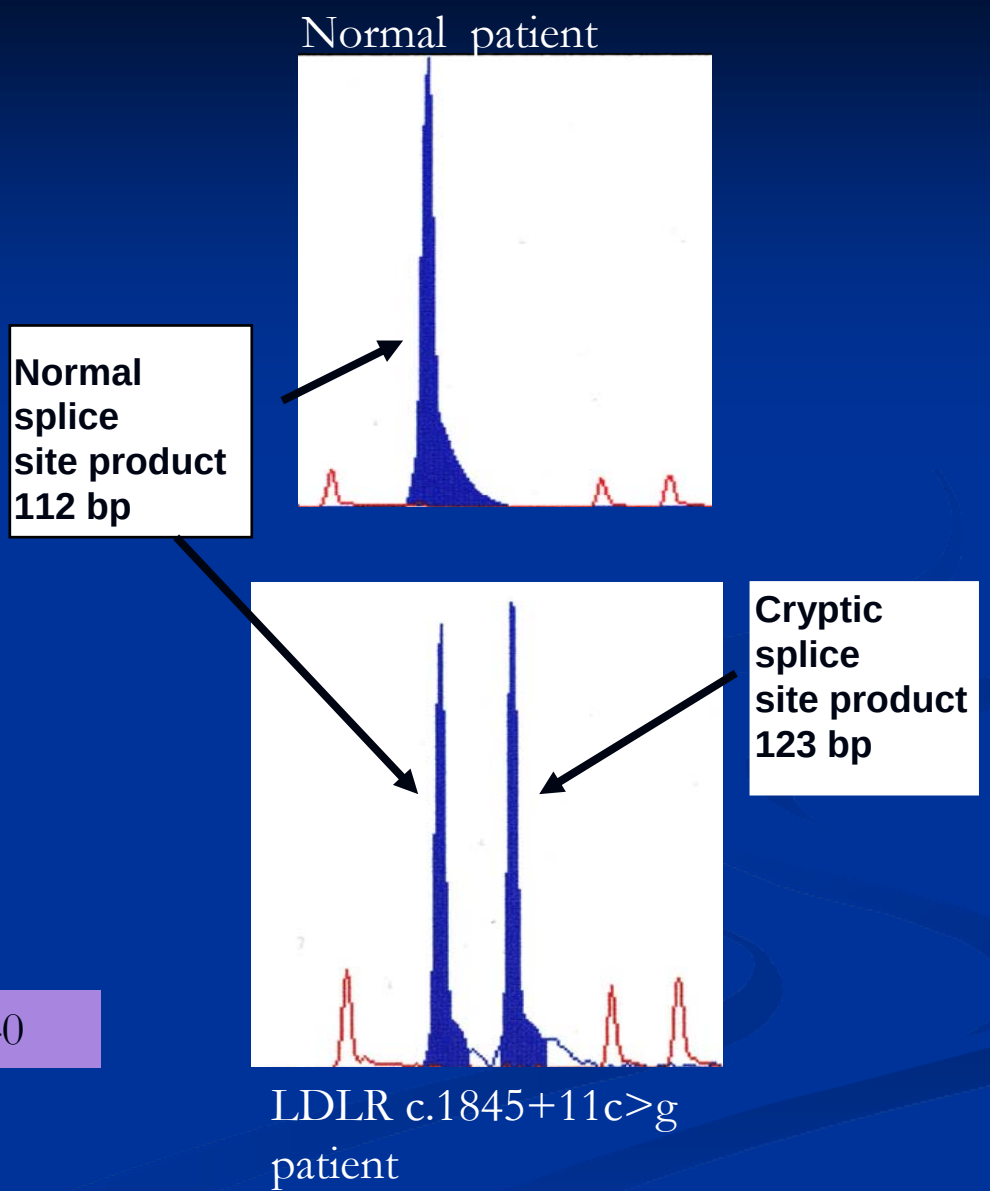
141 bp mutant
130 bp normal

Assay B

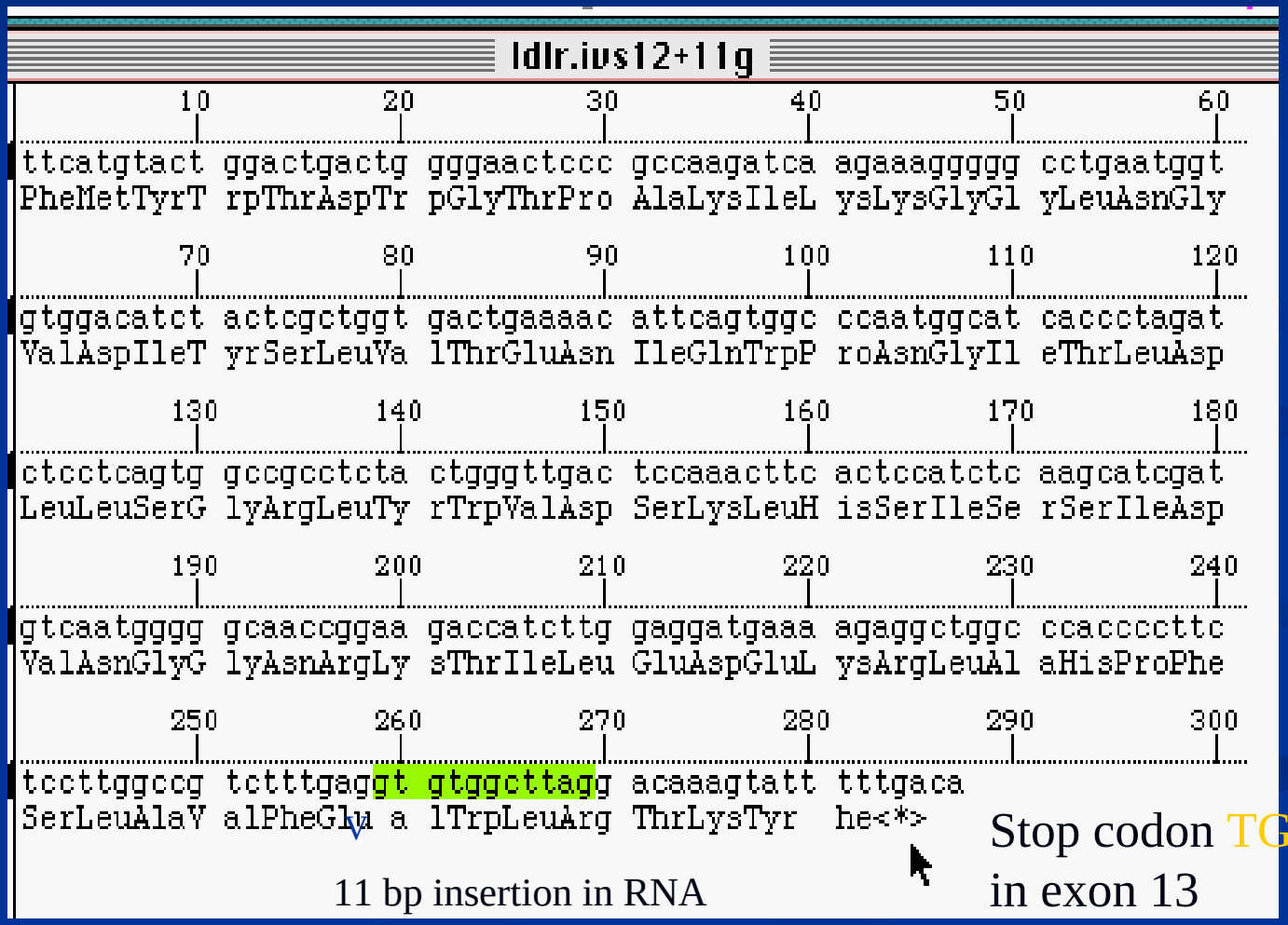
Allele specific fluorescent RT-PCR

Assay C

Atherosclerosis 182 (2005) 331-340



c.1845+11c>g splice mutation creates a premature stop in exon 13 and a truncated LDLR protein is predicted.



11 bp insertion in RNA

Stop codon TGA in exon 13