

Fallstricke bei der Etablierung von IL28B Tests Pitfalls in IL28B test development



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Test Development Procedures

Target Definition uses different „languages“ :

- **Historical or Trivial Names** e.g. Apolipoprotein E E2 E3 E4, HLA B27, HLA B5701, α 1 Antitrypsin Pi-S or Pi-Z
- **Street number or „Hausnummern“** approaches :
 - Amino Acid number and exchange e.g. C282Y in HFE gene
 - Nucleotide number and exchange e.g. G20210A 3' of FII gene
- **Relational approaches :**
 - RFLP e.g. VDR BsmI
 - rs nomenclature of dbSNP

Street number or „Hausnummern“ approach



Genes defined by chromosomes and „street numbers“ :

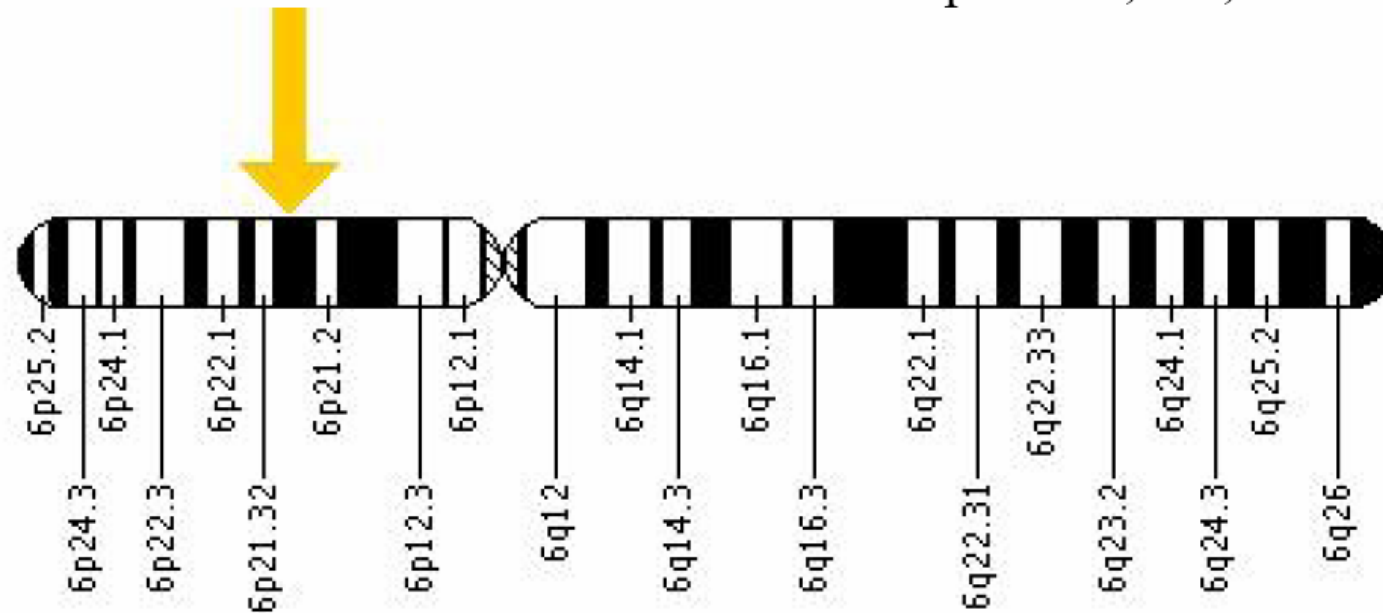
HFE



Genes defined by chromosomes and street numbers :

HFE

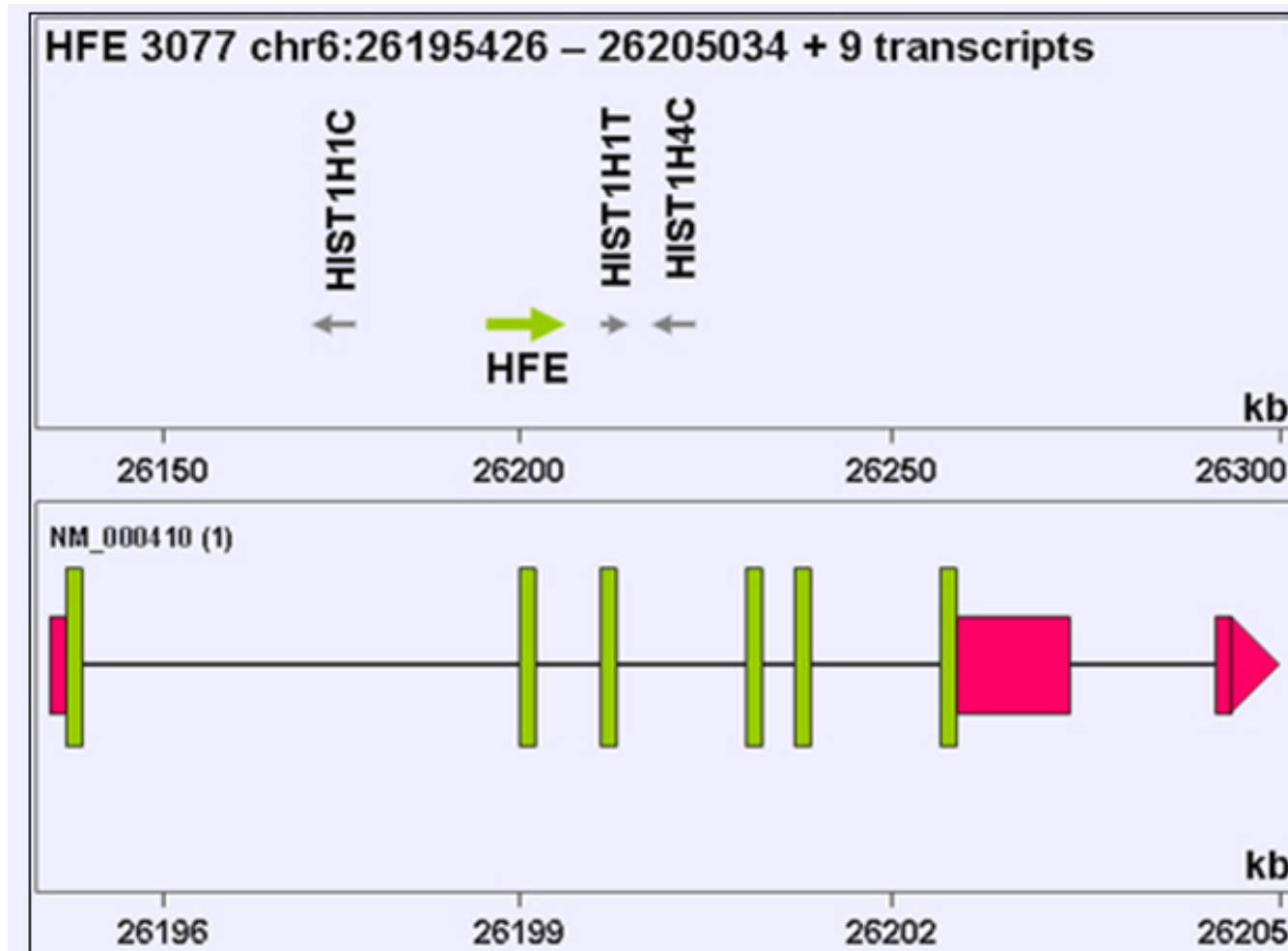
Molecular Location on chromosome 6: base pairs 26,087,447 to 26,097,058



The HFE gene is located on the short (p) arm of chromosome 6 at position 21.3.

Genes defined by chromosomes and street numbers :

HFE



Genes variants defined by street numbers :

HFE AA C282Y = nt G845A

TTTCCTGTCAAGTGCCTCCTTTGGTGAAGGTGACACATCATGTGACCTCTTCAGTGACCACTCT

ACGGTGTGCGGCCTTGAACACTACCCCCAGAACATCACCATGAAGTGGCTGAAGGATAAGCAG

CCAATGGATGCCAAGGAGTTCGAACCTAAAGACGTATTGCCCAATGGGGATGGGACCTACCAGG

GCTGGATAACCTTGGCTGTACCCCTGGGGAAGAGCAGAGATATACGT**G**CCAGGTGGAGCACC

CAGGCCTGGATCAGCCCCTCATTGTGATCTGGGGTATGTG TGAGA

AAATCTATTGGGGGTTGAGAGGAGTGCCTGAGGAGAGCCC TCATT

GGAGTCATCAGTGGAAATGCTGTTTTTGTGTCGTCATCTTGT AATAT



Gene variation definition by RFLP : VDR *Bsm* I polymorphism

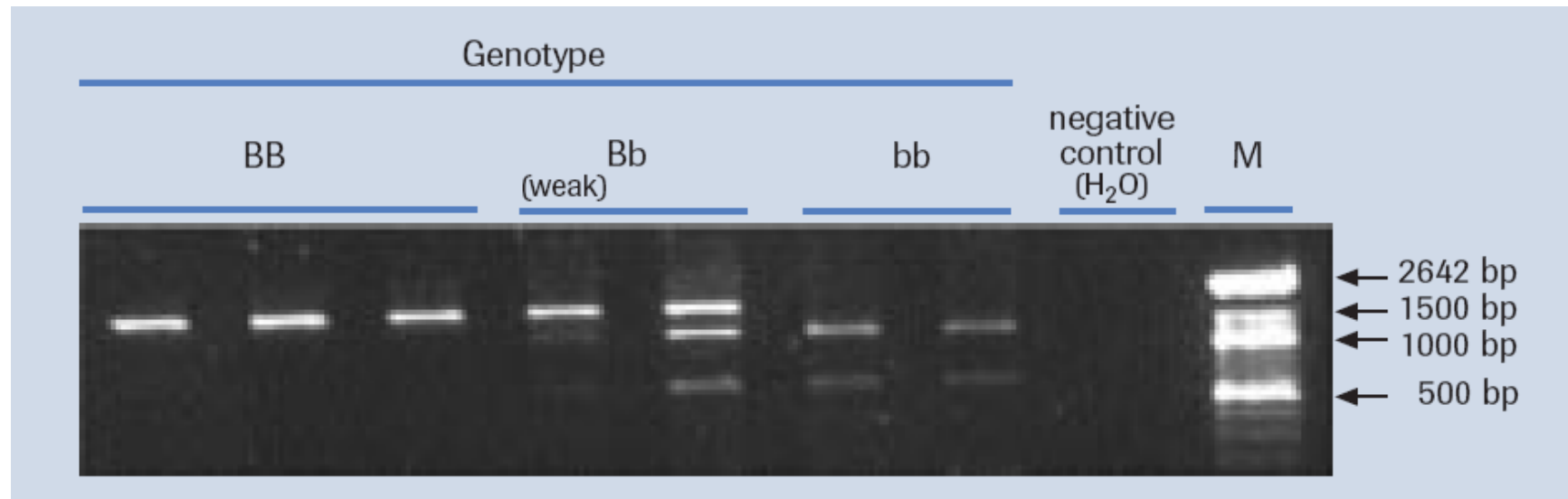


Figure 3: RFLP analysis. The classical RFLP approach was used to genotype the VDR *Bsm* I gene polymorphism. Samples were analyzed on a 1% agarose gel (M, DNA Molecular Weight Marker XIV).

From PCR RFLP to nt target

- *BsmI*

| Restriction Site Sequence: |
|----------------------------|
| ... GAATGCNN ... |
| ... CTTACGNN ... |

| Restriction Site Sequence After Cut: |
|--------------------------------------|
| ... GAATGCN N ... |
| ... CTTAC GNN ... |

By eye or with



TAGGGGGGATTCTGAGGAACTAgataagcagggttcctggggccacagacaggcctgcgcattccaatact
caggctctgctcttgctgaactgggctcaacattcctgttatttgaggtttcttGCGGGCAGGGTACAAAAC



TAGGGGGGATTCTGAGGAACTAgataagcagggttcctggggccacagacaggcctgc**Gcatt**ccaatac
tcaggctctgctcttgctgaactgggctcaacattcctgttatttgaggtttcttGCGGGCAGGGTACAAAAC

Gene variation definition by rs dbSNP :
IL28B C/T rs12979860

[rs12979860](#) [*Homo sapiens*]

5' CTGAAC CAGGGAGCTCCCCGAAGGCG
 [C/T]
GAACCAGGGTTGAATTGCACTCCGC 3'

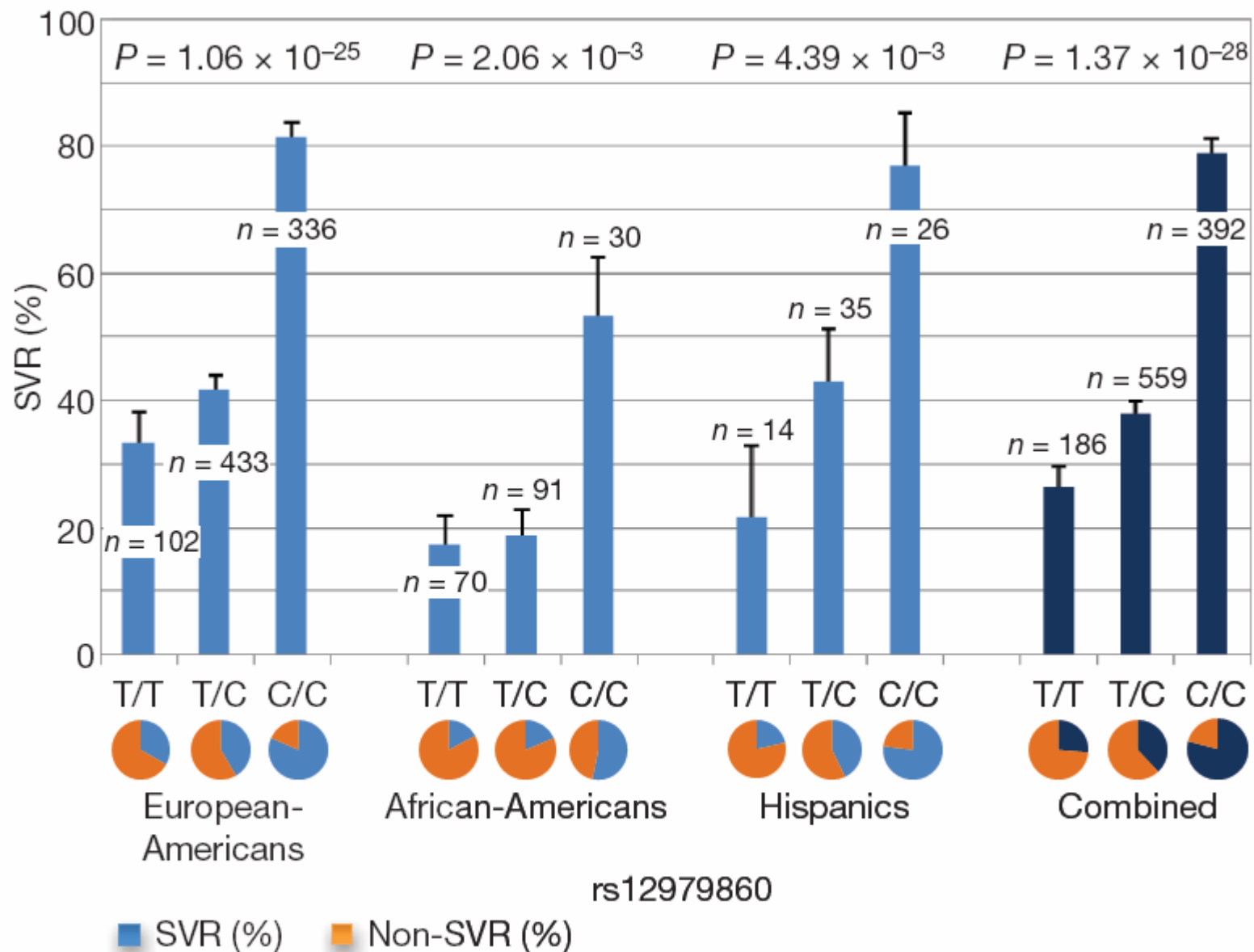


Figure 1 | Percentage of SVR by genotypes of rs12979860. Data are percentages + s.e.m.

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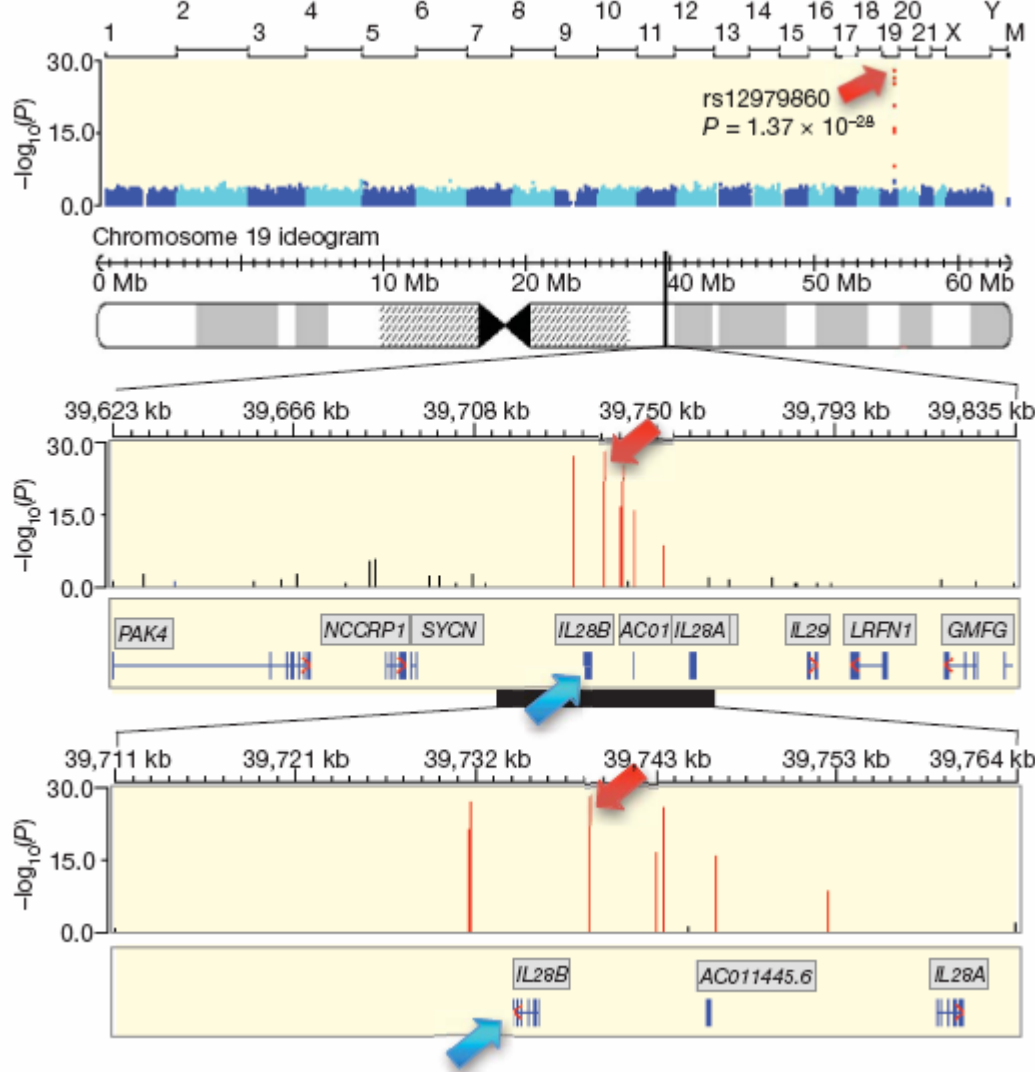
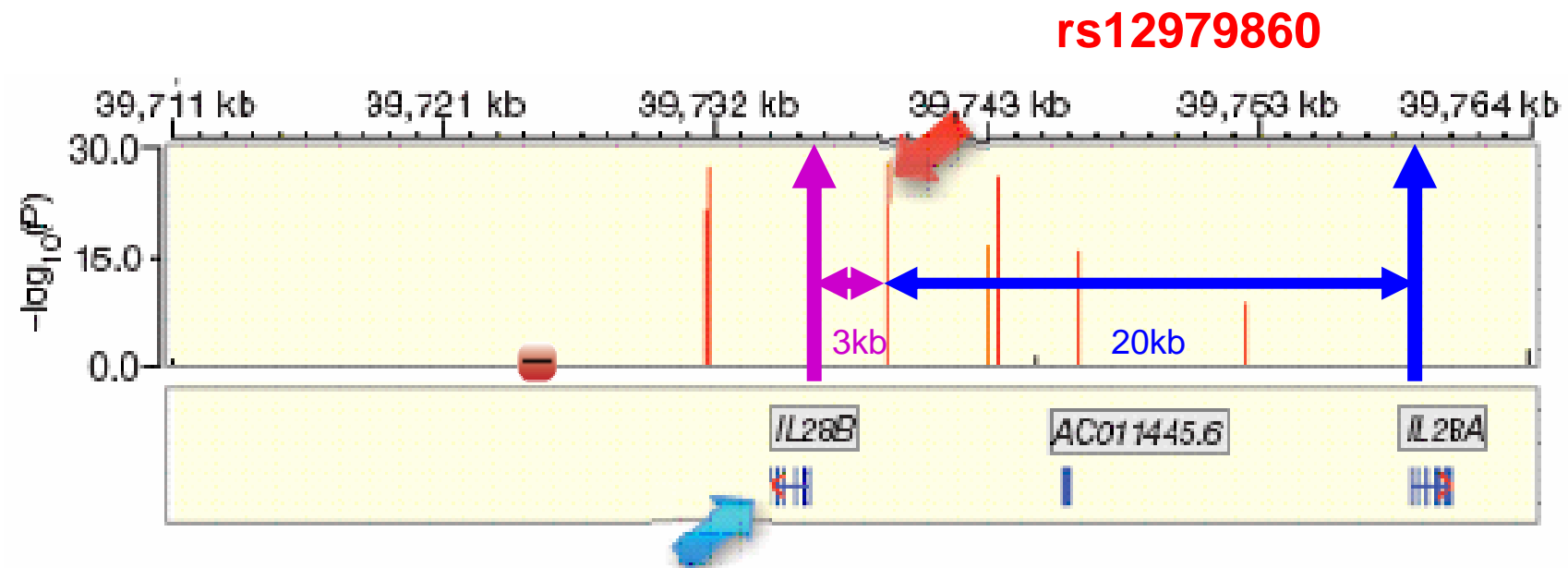


Figure 2 | Genomic overview of the region of 19q13.13 surrounding the genome-wide significant determinant of response to treatment and including the *IL28B* gene. The top panel shows a genome-wide view of the P values [$-\log_{10}(P)$]. Panels below show all genotyped SNPs in the region of significance and the structures of the surrounding genes. The SNPs that show genome-wide significant association with SVR are marked in red. The polymorphism rs12979860 (red arrow) is 3 kb upstream to the gene encoding IFN- λ -3 (*IL28B*, blue arrow). Other SNPs in the same region showing genome-wide significant P values largely reflect the same signal (Supplementary Information IX). The results were annotated using the WGAViewer software¹⁹.

rs12979860



CTGAACCAGGGAGCTCCCCGAAGGCG[C/T] GAACCAGGGTTGAATTGCACTCCGC

CTGAACCAGGGAGCTCCCCGAAGGCG

[C/T]

GAACCAGGGTTGAATTGCACTCCGC

Find this SNP in GenBank: AC011445.6

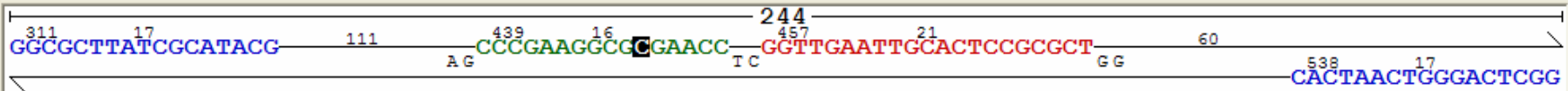
Homo sapiens chromosome 19 clone CTC-246B18, complete sequence



```
ccgccagcagctccaggatcgggcccggcggggagcagctccgagcgggtgcaggccgctgagcactgcctggg
cgtccgcgatgccccgggcacgtggcggagccgagcgcaggactgcggggacgagaggggcgttagagcgggccc
cgcgccgggcatgcctctcccgcccactccgggcccaccgatggccgcggaggatccctcctggggcgggaagg
agcagttgcgctgccccagctcagcgcctcttctcctcctgcgggacaagcggcgcttatcgcatacggctaggcc
ccctcggccagggccctaacctctgcacagctctgggatctcctggacgtggatgggtactggcagcgcacggctgt
gcctgtcgtgtactgaaccagggagctccccgaaggcgCgaaccagggttgaattgcaactccgcgctccccagc
aaagcccctcgcgccgacctggagccgagtcctcccggcaggggctcccttctgtgattgacctgagcctgcgtt
cgcgctgacgacggggactgcgggggtctcgtggtgggaattgtgggcgctgacataggagaggcgcctgctggg
cgctaggacgcaggacccttgggacaggaaagggtgtatgggaaccgggtggggccagggtcccagggggcaca
ggggctgggcgggtgacttaogtagcgggtccctcagcgccttggcagccgccagcgtccggggctccagcgcagcgg
tagtgcgagagcaggcagcgcggggggccttctgcgatcaccgtgcacaggaccacagcccccgggccactgc
ggcccagacactcggccgcactctctgctctgcagcaggc
```



IL28B - LightCycler Probe Design Software 2.0



Rank 1 Score -864 MaxScore -864

| | 5' Pos | 3' Pos | Length | GC% | Tm |
|---|--------|--------|--------|------|------|
| Forward Primer GGCGCTTATCGCATACG | 311 | 327 | 17 | 58.8 | 59.8 |
| Reverse Primer GGCTCAGGGTCAATCAC | 554 | 538 | 17 | 58.8 | 59.8 |
| Sensor CCCGAAGGCGGAACC-Fluorescein | 439 | 454 | 16 | 75.0 | 65.2 |
| Anchor LC Red 640-GGTGAATTGCACTCCGCGCT-Phosphate | 457 | 477 | 21 | 57.1 | 68.1 |

| | Position | Type | Match | Mismatch |
|----------|----------|--------|-------|----------|
| Mutation | 449 | C to T | wt | 53.8 |

Reaction Parameters

Buffer Name

[Mg++] (μM)

Search Type

Sequence

Analyzed From Analyzed To

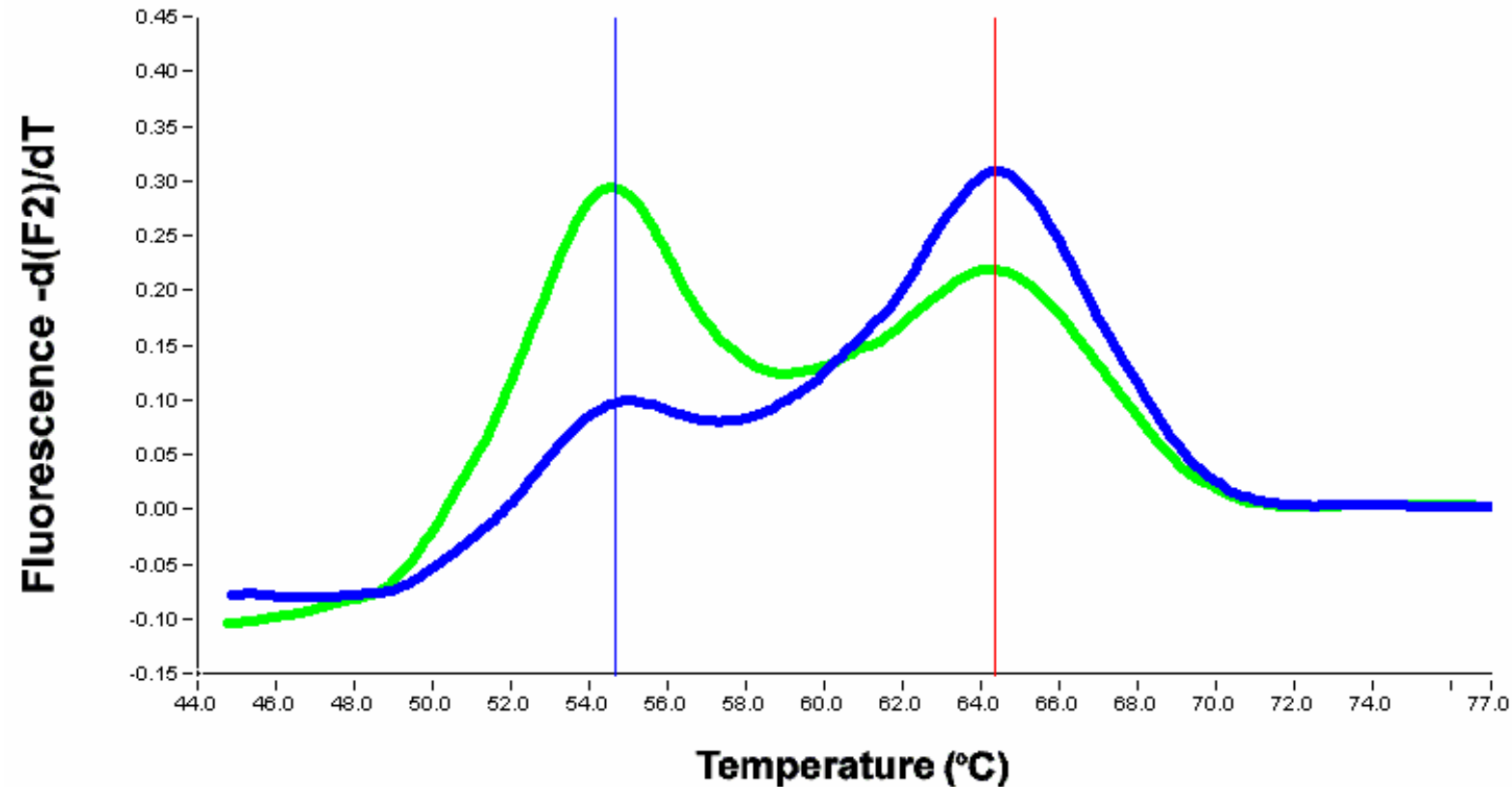
Length GC%

Region

Region Start Region End

Length GC%

First Trials : Everything has 2 peaks ! Why ?

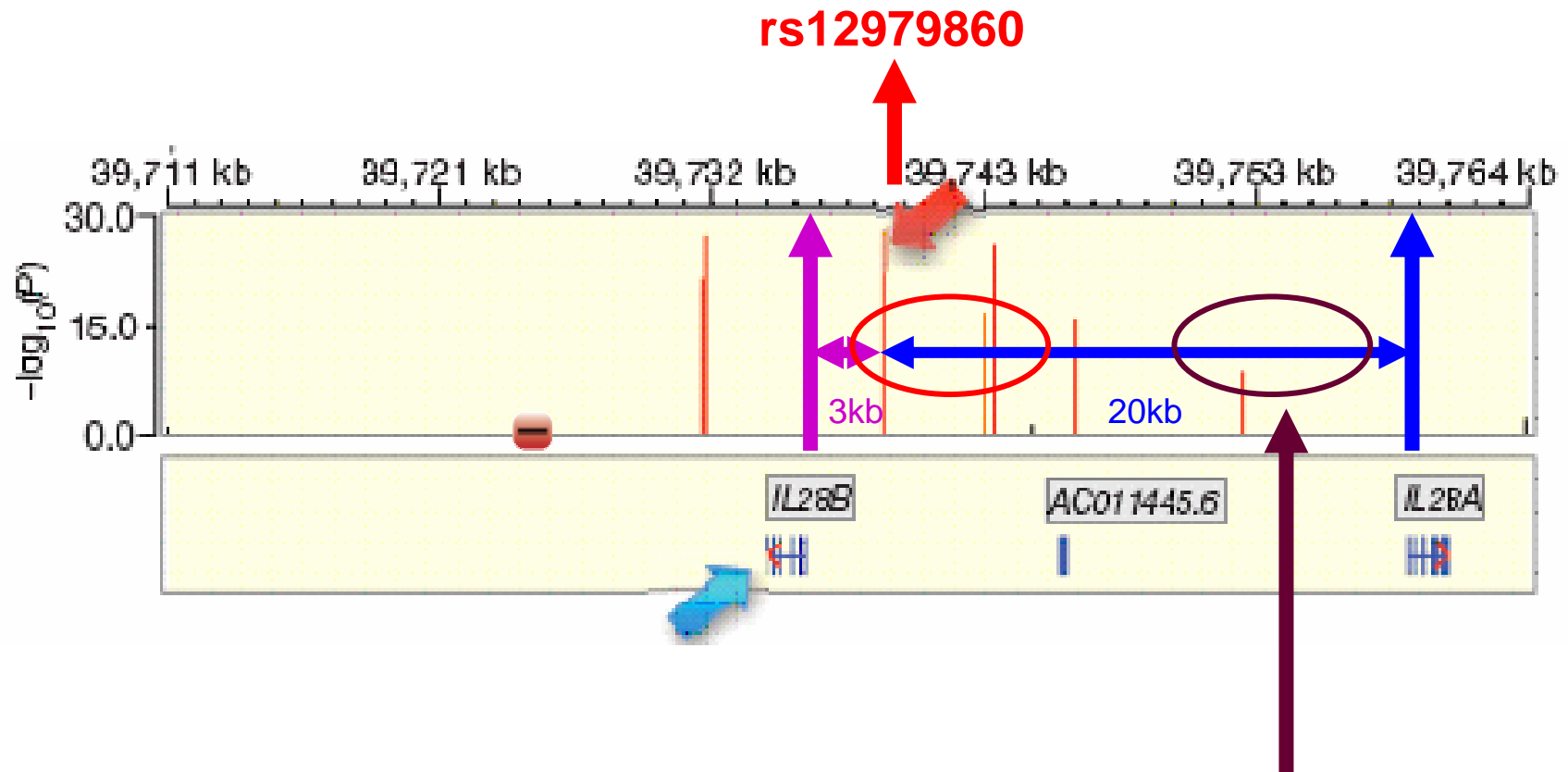


1 IL28B 3mM55C C2022



2 UKTS 8878 60cycasall





Second primer binding sites in inverted repeat !

Inverted repeat 98% homologous with mutation at site of interest explains why all samples tested had 2 peaks

Site of interest



```
TC C C C G A A G G C G C GA A C C A G G G T T G A A T T G C A C T C C G C G C T C C C C C A G C  
T G C A G C A G G C G A G A G A C G T C A G G G A A G C C A A A G A G A G G G T C C A G C G C G T
```

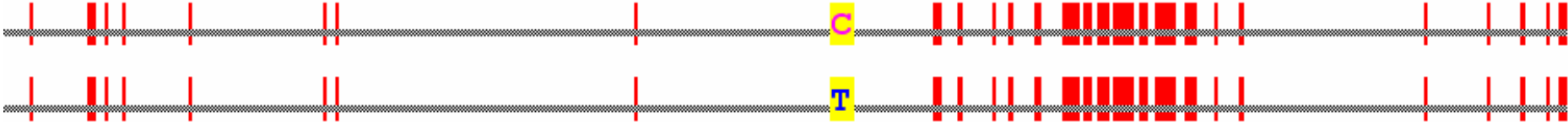
```
TC C C C G A A G G C G T G A A C C A G G G T T G A A T T G C A T C C C G C T C T C C C C C G G C
```



Inverted repeat
(inverted complement)

Partial 3' region of IL28B

rs12979860 C/T



Partial „inverted repeat“ as
inverse complement

Final version with selective primers

