# V128M KI in Adat3

# Adat3$^{\mathrm{em1Ics}}$

# Genotyping protocol

**Kus6768 / IM6768**

By Marie-Christine Birling (PhD)

birlingm@igbmc.fr

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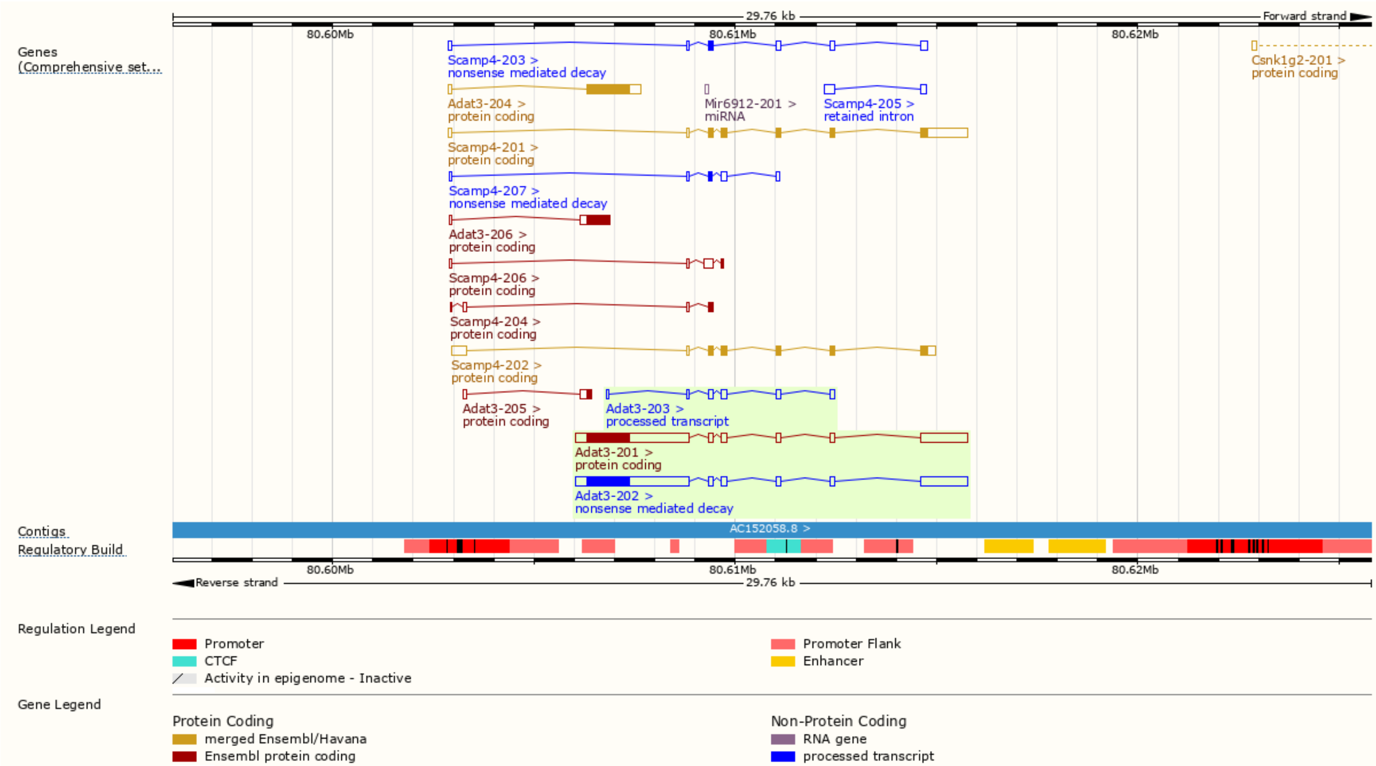
# Strategiy undertaken

24/07/2018

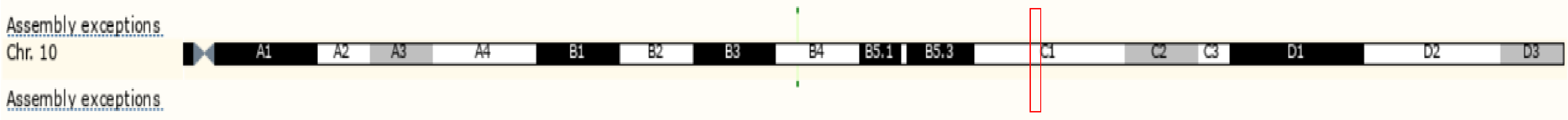
# Adat3 Mouse Genomic locus

**Gene: Adat3 ENSMUSG00000035370**

**Gene: Adat3 ENSMUSG00000113640**



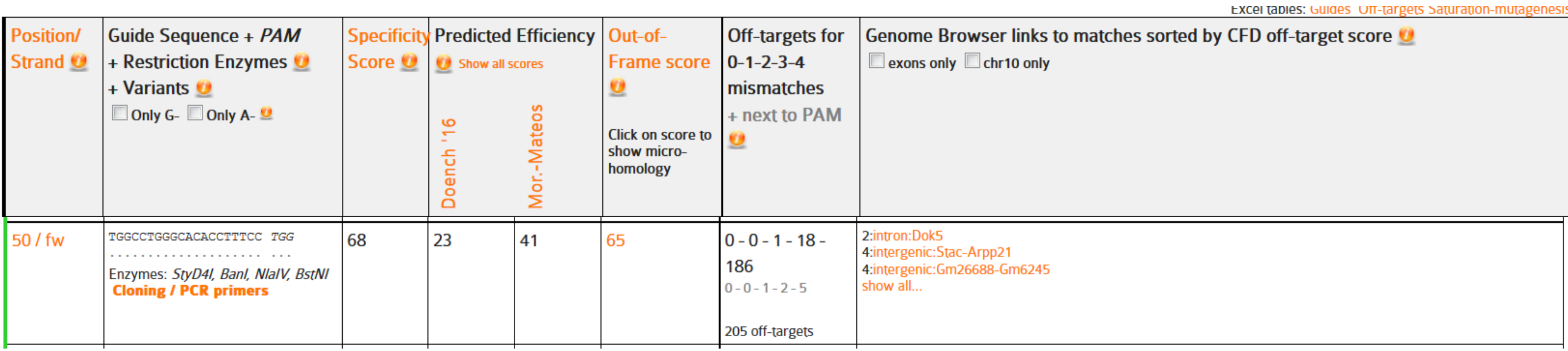
**Chromosome 10: 80,606,021-80,615,784**



# Adat3 mRNAs and proteins

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | RefSeq |
|---|---|---|---|---|---|---|---|
| Adat3-201 | ENSMUST00000178231.1 | 4516 | 349aa | Protein coding | CCDS48637 | Q6PAT0 | |
| Adat3-202 | ENSMUST00000218067.1 | 4517 | 349aa | Nonsense mediated decay | CCDS48637 | - | |
| Adat3-203 | ENSMUST00000219462.1 | 612 | No protein | Processed transcript | - | - | |
| Adat3-204 | ENSMUST00000038411.4 | 1411 | 349aa | Protein coding | CCDS48637 | Q6PAT0 | NM_001100606<br>NP_001094076 |
| Adat3-205 | ENSMUST00000221670.1 | 359 | 35aa | Protein coding | - | - | - |
| Adat3-206 | ENSMUST00000221960.1 | 795 | 183aa | Protein coding | - | - | - |

# sgRNA search



Excel tables: Guides Off-targets Saturation-mutagenesis

| Position/ Strand | Guide Sequence + *PAM* + Restriction Enzymes + Variants<br>☐ Only G- ☐ Only A- | Specificity Score | Predicted Efficiency<br>Show all scores | | Out-of-Frame score<br>Click on score to show micro-homology | Off-targets for 0-1-2-3-4 mismatches<br>+ next to PAM | Genome Browser links to matches sorted by CFD off-target score<br>☐ exons only ☐ chr10 only |
|---|---|---|---|---|---|---|---|
| | | | Doench '16 | Mor.-Mateos | | | |
| 50 / fw | `TGGCCTGGGCACACCTTTCC` *TGG*<br>.................... ...<br>Enzymes: *StyD4I, BanI, NlaIV, BstNI*<br>**Cloning / PCR primers** | 68 | 23 | 41 | 65 | 0 - 0 - 1 - 18 - 186<br>0 - 0 - 1 - 2 - 5<br>205 off-targets | 2:intron:Dok5<br>4:intergenic:Stac-Arpp21<br>4:intergenic:Gm26688-Gm6245<br>show all... |

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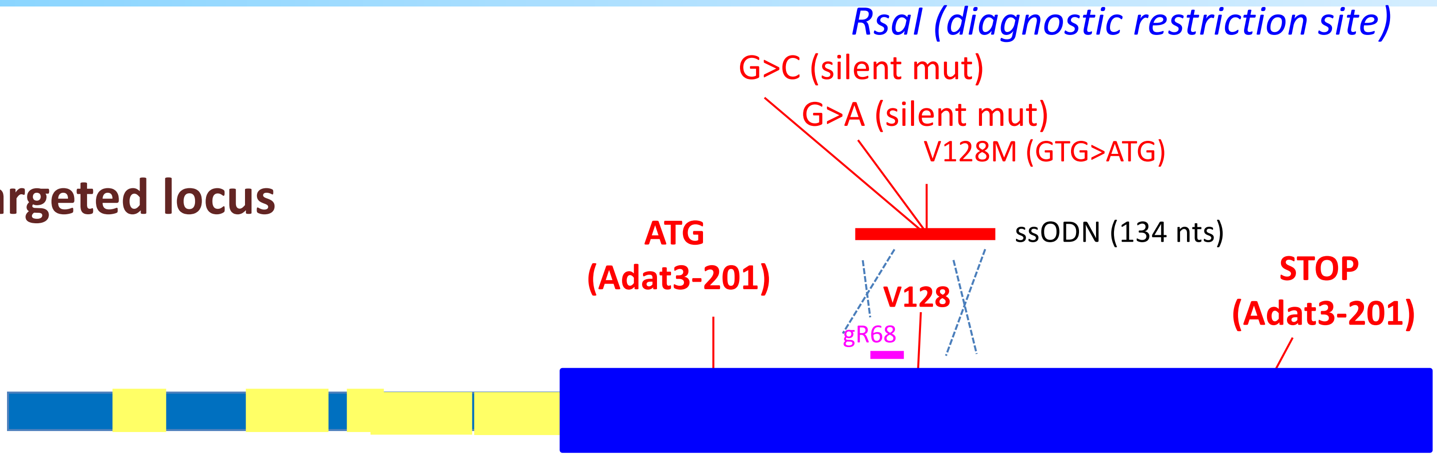
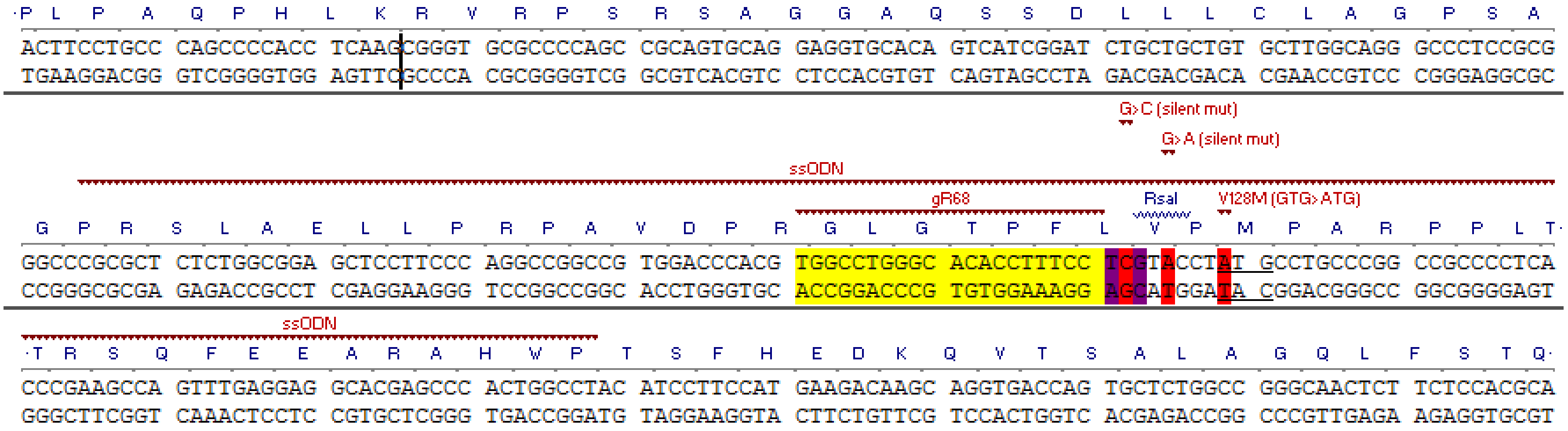
# Strategy proposed: insertion of the V128M mutation

## Targeted locus



## Sequence expected after homologous directed repair (HDR)



```
·P L  P   A   Q  P  H   L   K  R   V   R  P   S   R  S  A   G   G  A   Q   S  S   D   L  L  L   C   L  A   G   P  S  A
ACTTCCTGCC CAGCCCCACC TCAAGCGGGT GCGCCCCAGC CGCAGTGCAG GAGGTGCACA GTCATCGGAT CTGCTGCTGT GCTTGGCAGG GCCCTCCGCG
TGAAGGACGG GTCGGGGTGG AGTTCGCCCA CGCGGGGTCG GCGTCACGTC CTCCACGTGT CAGTAGCCTA GACGACGACA CGAACCGTCC CGGGAGGCGC

                                                                        G>C (silent mut)
                                                                           G>A (silent mut)
                                                  ssODN
                                                      gR68                   RsaI    V128M (GTG>ATG)
 G  P  R   S   L  A   E   L  L   P   R  P   A   V   D  P   R  G   L  G   T  P  F   L   V  P   M   P  A   R   P  P  L T·
GGCCCGCGCT CTCTGGCGGA GCTCCTTCCC AGGCCGGCCG TGGACCCACG TGGCCTGGGC ACACCTTTCC TCGTACCTAT GCCTGCCCGG CCGCCCCTCA
CCGGGCGCGA GAGACCGCCT CGAGGAAGGG TCCGGCCGGC ACCTGGGTGC ACCGGACCCG TGTGGAAAGG AGCATGGATA CGGACGGGCC GGCGGGGAGT

                    ssODN
·T R  S   Q  F  E   E   A  R  A   H   W  P   T   S  F   H   E  D  K   Q   V  T   S   A  L   A   G  Q   L   F  S   T  Q·
CCCGAAGCCA GTTTGAGGAG GCACGAGCCC ACTGGCCTAC ATCCTTCCAT GAAGACAAGC AGGTGACCAG TGCTCTGGCC GGGCAACTCT TCTCCACGCA
GGGCTTCGGT CAAACTCCTC CGTGCTCGGG TGACCGGATG TAGGAAGGTA CTTCTGTTCG TCCACTGGTC ACGAGACCGG CCCGTTGAGA AGAGGTGCGT
```

# Sequence of the mRNA and protein after HDR

```
 ex1
                                                                                       ATG
 GCGGTGGCAGGCGTGGCGGCCCGGTTGCTAGGACTTGGATAAGCGTGGCGTTCGCTCGGGTGTCCCTCAGTCGAGCCCCGTTGACCATGGAGCCCACCTCAGGCTTTGCAGAGCAACCTGGGCCTGTGAAGGCTGAAAGTGAGGA
                                                                                      ▸ M  E  P  T  S  G  F  A  E  Q  P  G  P  V  K  A  E  S  E  E
 GCAAGAGCCGGCGCAATGGCAAGCCCTCCCTGTCCTGTCGGAGCAGCAGTCGGGGGCCGTGGAGCTGATACTGGCCTATGCTGCACCTGTCCTGGACAAGCGCCAGACGTCCCGCCTCCTCCGGGAGGTGTCTGCTGTCTATCCA
▸  Q  E  P  A  Q  W  Q  A  L  P  V  L  S  E  Q  Q  S  G  A  V  E  L  I  L  A  Y  A  A  P  V  L  D  K  R  Q  T  S  R  L  L  R  E  V  S  A  V  Y  P
 CTTCCTGCCCAGCCCCACCTCAAGCGGGTGCGCCCCAGCCGCAGTGCAGGAGGTGCACAGTCATCGGATCTGCTGCTGTGCTTGGCAGGGCCCTCCGCGGGCCCGCGCTCTCTGGCGGAGCTCCTTCCCAGGCCGGCCGTGGACC
▸ L  P  A  Q  P  H  L  K  R  V  R  P  S  R  S  A  G  G  A  Q  S  S  D  L  L  L  C  L  A  G  P  S  A  G  P  R  S  L  A  E  L  L  P  R  P  A  V  D
                             G>A (silent mut)
                           RsaI  V128M (GTG>ATG)
                          G>C (silent mut)
 CACGTGGCCTGGGCACACCTTTCCTCGTACCTATGCCTGCCCGGCCGCCCCTCACCCGAAGCCAGTTTGAGGAGGCACGAGCCCACTGGCCTACATCCTTCCATGAAGACAAGCAGGTGACCAGTGCTCTGGCCGGGCAACTCTT
▸P  R  G  L  G  T  P  F  L  V  P  M  P  A  R  P  P  L  T  R  S  Q  F  E  E  A  R  A  H  W  P  T  S  F  H  E  D  K  Q  V  T  S  A  L  A  G  Q  L  F
 CTCCACGCAGGAGCGAGCTGCCATGCAAACCCACATGGAACGGGCGGTATGTGCGGCCCAGAGGGCAGCGGCTCAGGGGCTGCGGGCAGTGGGCGCCGTCGTGGTGGACCCAGCCTCGGACCGCGTGTTGGCCACAGGCCATGAC
▸  S  T  Q  E  R  A  A  M  Q  T  H  M  E  R  A  V  C  A  A  Q  R  A  A  A  Q  G  L  R  A  V  G  A  V  V  V  D  P  A  S  D  R  V  L  A  T  G  H  D
 TGCAGCAGTGTAGCCAGCCCCCTGCTGCATGCCGTCATGGTGTGCATCGACCTGGTGGCCCAGGGGCAGGGCCGCGGTTCCTGTGACCTCAGAAGCCACCCGGCTTGCTCCTTTACACAGGCCACTGCCACTCAAGGTGCCCGAG
▸ C  S  S  V  A  S  P  L  L  H  A  V  M  V  C  I  D  L  V  A  Q  G  Q  G  R  G  S  C  D  L  R  S  H  P  A  C  S  F  T  Q  A  T  A  T  Q  G  A  R
                                                               RsaI
 CCGGTAGCGTGCGCAAGCTGGATGAGGACAGCCTGCCCTATGTGTGCACTGGCTATGACCTGTACGTCACCCGAGAGCCCTGTGTCATGTGTGCTATGGCCCTAGTCCATGCCCGCATCCAGCGAGTCTTCTATGGGGCTCCCTC
▸A  G  S  V  R  K  L  D  E  D  S  L  P  Y  V  C  T  G  Y  D  L  Y  V  T  R  E  P  C  V  M  C  A  M  A  L  V  H  A  R  I  Q  R  V  F  Y  G  A  P  S
                                                                                                                        STOP
 CCCTGATGGTGCCTTGGGCACACTCTTCCGTGTCCATGCTCGGCCAGACCTCAACCACCGCTTCCAGGTGTTCCGCGGTATTCTCGAGGACCAGTGCCGCCAGCTGGACCCCGACCCATAGTTCTGGTGACCTGCTTCTAGACCA
▸  P  D  G  A  L  G  T  L  F  R  V  H  A  R  P  D  L  N  H  R  F  Q  V  F  R  G  I  L  E  D  Q  C  R  Q  L  D  P  D  P
 TTCTCTGCTCCTGGCCAGCCCGTGTCCCCTGCGGCTCCATGGCCTGGGGGCCTCACCCTCTGTGATGATACTGATCACCTGTGCCAGACTGTGGGCCTCTCAGTTTCCAGACTGCAGCTTCTCTGCTGGCTGCGACGACAGTGGG
 GCTCAGCTCTGCTTACAAGCCCAGTGGCTGTCTTACAGTCCCCAGGTCCTCCAGGCTCGGGGCAGGGAGCAGCCATCGAGTGTGAAAATAAAACACCTCAAACCAA
```

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# Data

Genotyping protocol

# Line Kus6768-17-PM (internal ICS code)

## F1 genotype

Sequence with PCR F1-R2

Oligo F in orange

Oligo R in purple

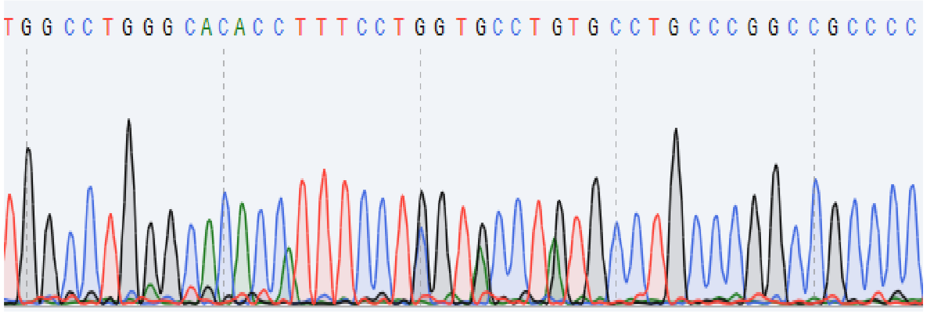
in red silent mutations

in green asked mutation

*Rsal site in italic and underscored*

ACCTGTCCTGGACAAGCGCCAGACGTCCCGCCTCCTCCGGGAGGTGTCTGCTGTCTATCCACTTCCTGCCCAGCCCCACCTCAA
GCGGGTGCGCCCCAGCCGCAGTGCAGGAGGTGCACAGTCATCGGATCTGCTGCTGTGCTTGGCAGGGCCCTCCGCGGGCCCG
CGCTCTCTGGCGGAGCTCCTTCCCAGGCCGGCCGTGGACCCACGTGGCCTGGGCACACCTTTCCTC*GTAC*CTA**TGCCTGCCCG**
**GCCGCCCCTCACCCGAAGCCAGTTTGAGGAGGCACGAGCCCACTGGCCTACATCCTTCCATGAAGACAAGCAGG**TGACCAGT
GCTCTGGCCGGGCAACTCTTCTCCACGCAGGAGCGAGCTGCCATGCAAACCCACATGGAACGGGCGGTATGTGCGGCCCAGA
GGGCAGCGGCTCAGGGGCTGCGGGCAGTGGGCGCCGTCGTGGTGGACCCAGCCTCGGACCGCGTGTTGGCCACAGGCCAT
GACTGCAGCAGTGTAGCCAGCCCCCTGCTGCATGCCGTCATGGTGTGCATCGACCTGGTGGCCCAGGGGCAGGGCCGCGGTT
CCTGTGACCT

### Sanger sequencing

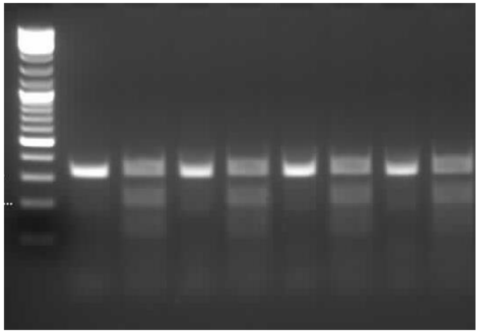


TGGCCTGGGCACACCTTTCCTCGTACCTATGCCTGCCCGGCCGCCCC

G L G T P F L V P M P A R P P

### F1 genotyping

F3-R1



WT 333 bps

**211 bps**

**122 bps**

**After RsaI digest (diagnostic RE)**

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# GENOTYPING INSTRUCTIONS

## PCR genotyping strategy for the PM

| Primer ref. | Sequence | Amplification product size WT | After RsaI digest (if PM and diagnostic restriction site present |
|---|---|---|---|
| F1 | ACCTGTCCTGGACAAGCGCCAGACG | 585 bps | 351 bps + 234 bps |
| R2 | AGGTCACAGGAACCGCGGCCCTGCC | | |
| F3 | GGTGCACAGTCATCGGATCTGCTGC | 333 bps | 211 bps + 122 bps |
| R2 | AGGTCACAGGAACCGCGGCCCTGCC | | |

# GENOTYPING INSTRUCTIONS

## PCR Protocol

This section describes the composition of the mix and the cycling conditions used for genotyping animals.

The Red Hot polymerase is no longer sold, so it is better to optimize conditions with another Taq polymerase.

| Mix PCR | Initial conc | Final conc | Volume (µl)/reaction |
|---|---|---|---|
| H20 | / | / | 6,23 |
| Buffer IV | 10X | 1X | 2 |
| MgCl2 | 25mM | 1,5mM | 1,2 |
| dNTP | 10mM | 0,2mM | 0,4 |
| Red Hot | 5U/µl | 0,0375U | 0,15 |
| PWO | 5U/µl | 0,005U | 0,02 |
| ADN | | | 6 |
| Oligo (F+R) | | 0,125µM/oligo | 4 |

### Cycling conditions

| Temp | Time | #Cycles |
|---|---|---|
| 96°C | 5min | 1 |
| | | |
| 96°C | 8s | |
| 62°C | 10s | 30 |
| 68°C | 45sec | |
| | | |
| 68°C | 10min | 1 |
| | | |
| 15°C | ∞ | |

| **Digestion protocol** | **Volume / sample** |
|---|---|
| PCR product | 10 µl |
| Buffer 10X | 2 µl |
| Restriction enzyme | 0,2 µl |
| H20 | 7,8 µl |

This reaction is incubated 15 mins at 37°C then loaded on a 3% agarose. The 10 µl left over PCR reaction serves as negative control