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EXCELLENCE IN MOUSE PHENOGENOMICS

Ala353Val point mutation in *Lrpprc*

Lrpprc$^{em1.Ics}$

Genotyping protocol

Kus6776 / IM00006776

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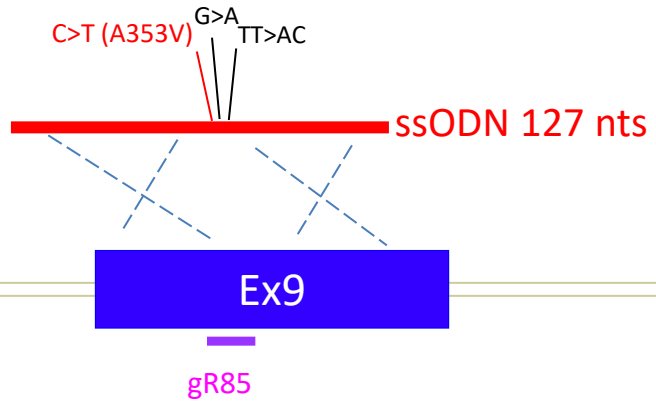
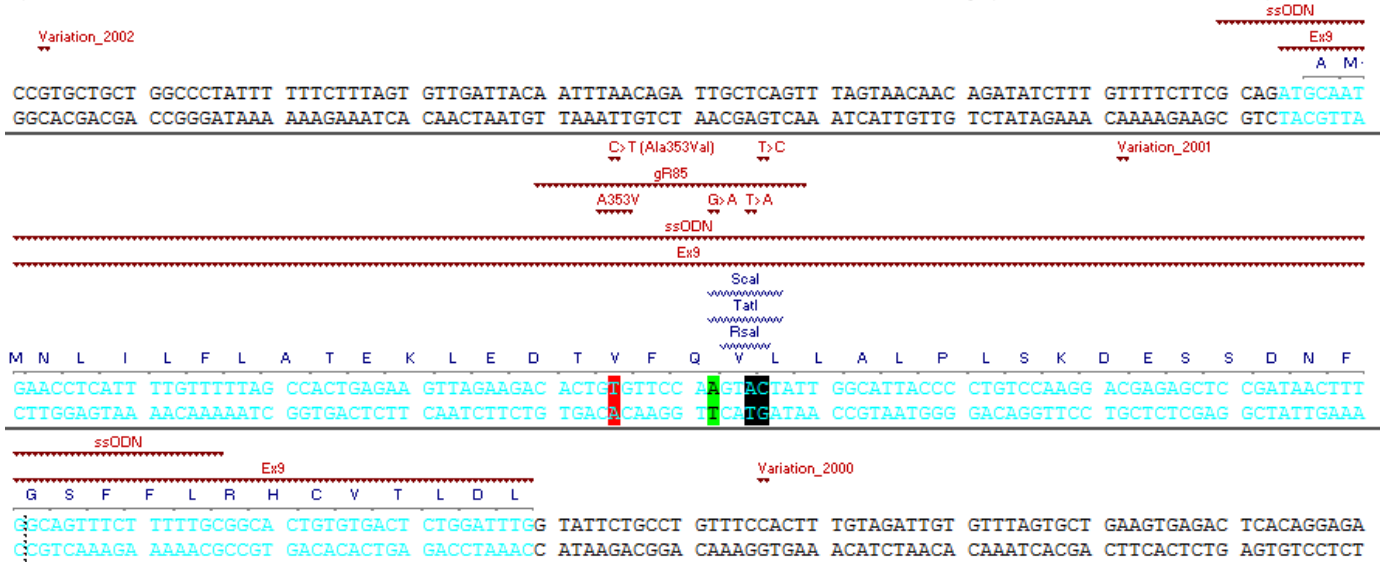
Strategy proposed: C>T (A353V) point mutation

CRISPR approach (HDR)

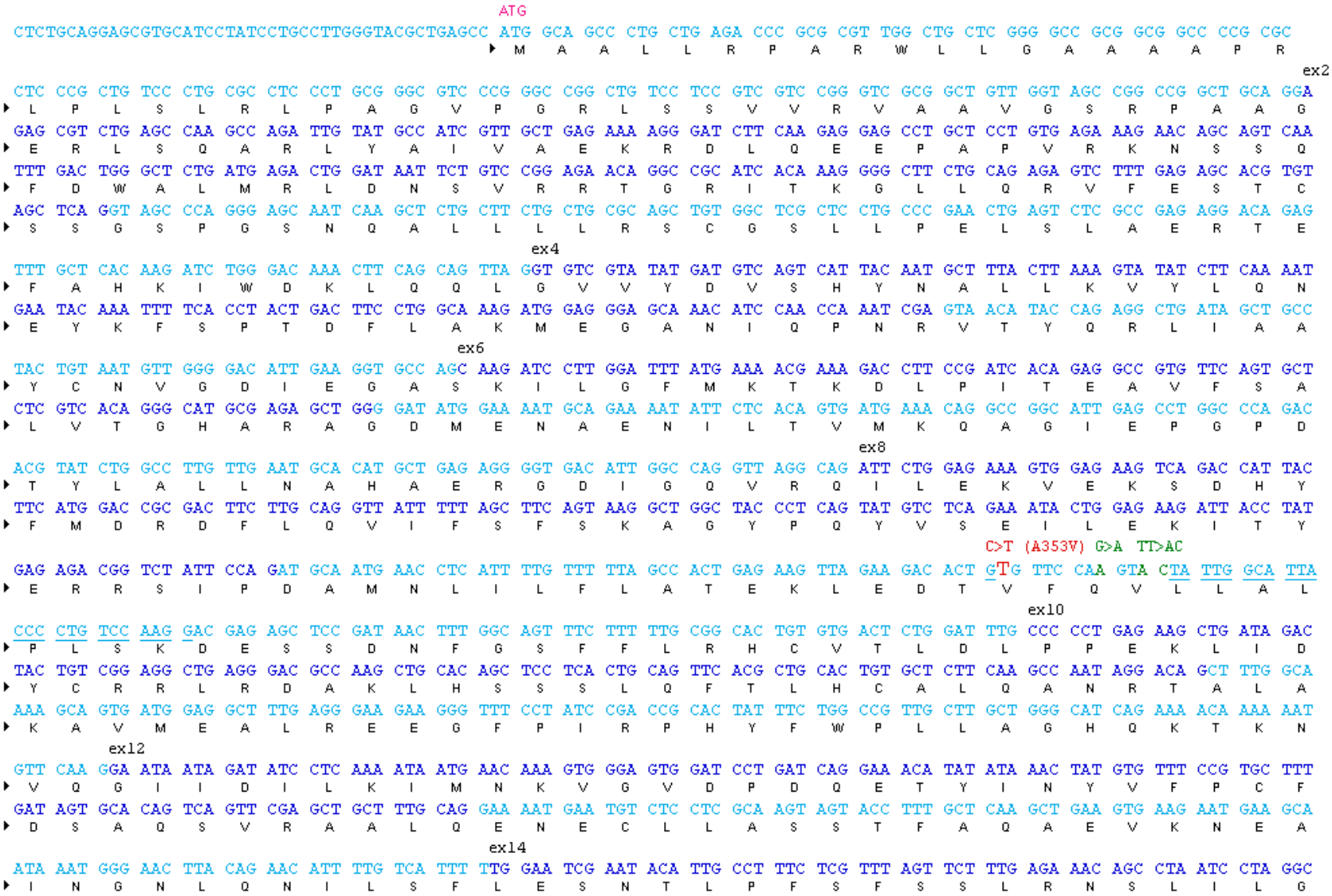


Sequence of the mutated allele (after homology directed repair)



Introduction of diagnostic restriction site (ScaI, RsaI) : will ease the genotyping and will avoid re-cutting after HDR as the 3 silent mutations (plus the asked mutation) are introduced in the 20 bps recognition site of the guide (gR85).

Sequence of the mutated cDNA (partial)



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INVESTISSEMENTS D'AVENIR

Genotyping protocol

cnrs

centre européen de recherche en biologie et en médecine CERBMgie

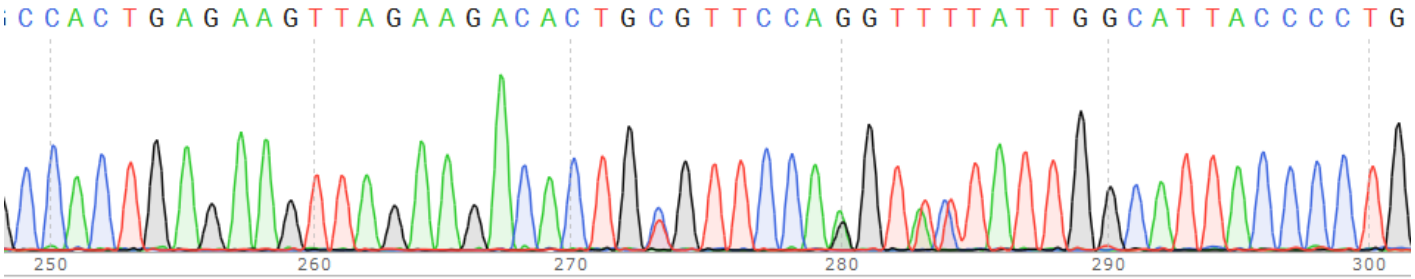
Instituts thématiques Inserm institut national de la santé et de la recherche médicale

UNIVERSITÉ DE STRASBOURG

Aix*Marseille université

Genotyping of F1
Line Kus6776-19-PM

Chromatogram on one F1 animal

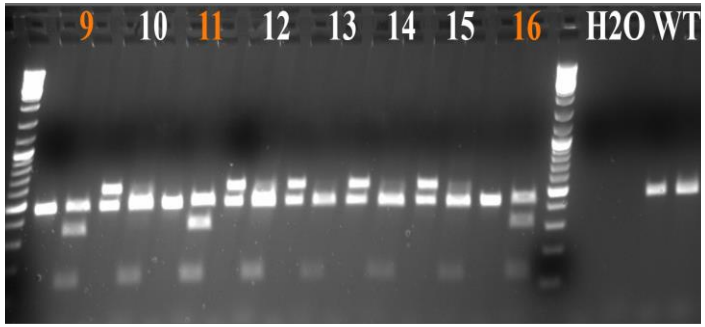


CCACTGAGAAGTTAGAAGACACTGTGTTCCAAGTACTATTGGCATTACCCCTG

T E K L E D T V F Q V L L A L P L

Expected sequence

Example of F1 genotyping



-435 bps
-310 bps

-125 bps

Animal #1, #11 and #16 are positive

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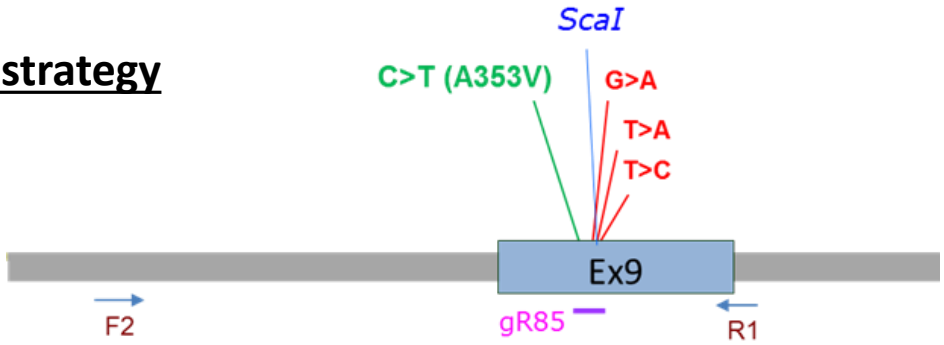
GENOTYPING INSTRUCTIONS

PCR genotyping strategy



Primer ref.	Sequence	Amplification product size WT
F2	CCTGGGTGGTGCAAGCAGGAATGGG	435 bps If ScaI: 310 bps + 125 bps
R1	GCACTAAACACAATCTACAAAGTGG	

Primers in orange
ScaI in italic
Silent mut in red
Expected mut in green

Sequence of the PCR product (with mutations introduced)

CCTGGGTGGTGCAAGCAGGAATGGGGTTACTCTTAGAGATTTAGATTTTTGTGGGGTTTGCAGTTCTTAAGTTTTTTCTTTAA
AAGTATAAACATTTTTGTTTGTGTGTTTGGGTGTGCATATACCACAGGTCATGTGTAGTCTTTCTCTGCTGAACCGTGCTGCTG
GCCCTATTTTTTCTTTAGTGTTGATTACAATTTAACAGATTGCTCAGTTTAGTAACAACAGATATCTTTGTTTTCTTCGCAGATGC
AATGAACCTCATTTTGTTTTTAGCCACTGAGAAGTTAGAAGACACTGTGTTCCA*AGTACT*ATTGGCATTACCCCTGTCCAAGG
ACGAGAGCTCCGATAACTTTGGCAGTTTCTTTTTGCGGCACTGTGTGACTCTGGATTTGGTATTCTGCCTGTTTCCACTTTGTA
GATTGTGTTTAGTGC

GENOTYPING INSTRUCTIONS

PCR Protocol

This section describes the composition of the mix and the cycling conditions used for genotyping F0.

Reagents:	Volume (per sample):
- Phusion HS (Thermo Scientific) 5X Buffer	4 µl
- 10mM dNTP	0,4µl
- 5' primer (100 µM)	0.1µl
- 3' primer (100 µM)	0.1µl
- DNA (lysate 1/10)	2µl
- Phusion Hot Start II	0,2µl
- Sterile H2O	up to 20 µl

Cycling conditions

Temp	Time	#Cycles
96°C	5min	1
96°C	8s	
62°C	10s	30
68°C	45s	
68°C	5min	1
12°C	5min	1

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 leaded on a 3% agarose. The 10 µl left over PCR reaction serves as negative control.