



Ala353Val point mutation in *Lrpprc* *Lrpprc*^{em1.lcs}

Genotyping protocol

Kus6776 / IM00006776

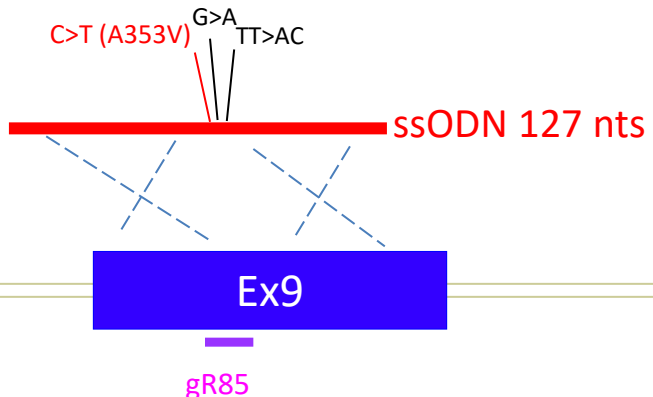
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Strategy proposed: C>T (A353V) point mutation



CRISPR approach (HDR)



Sequence of the mutated allele (after homology directed repair)

Variation_2002

ssODN

Ex9

A M

CCGTGCTGCT GGCCCTATTT TTTCTTTAGT GTTGATTACA ATTTAACAGA TTGCTCAGTT TAGTAACAAC AGATATCTTT GTTTCTCTCG CAGATGCAAT
 GGCACGACGA CCGGGATAAA AAAGAAATCA CAACTAATGT TAAATGTCT AACGATCAA ATCATTGTTG TCTATAGAAA CAAAAGAAGC GTCACGTTA

C>T (Ala353Val) T>C

gR85

A353V G>A T>A

ssODN

Ex9

Scal
Tat
RsaI

M N L I L F L A T E K L E D T V F Q V L L A L P L S K D E S S D N F

GAACCTCATT TTGTTTTAG CCACTGAGAA GTTAGAAGAC ACTGTTTCC AGTACTATT GGCATTACCC CTGTCCAAGG ACGAGAGCTC CGATAACTTT
 CTTGGAGTAA AACAAAATC GGTGACTCTT CAATCTTCTG TGACCAAAGG TCCATGATAA CCGTAATGGG GACAGGTTC TGTCTCGAG GCTATTGAA

ssODN

Ex9

Variation_2000

G S F F L R H C V T L D L

GCAGTTTTCT TTTTGCAGCA CTGTGTGACT CTGGATTTCG TATTCTGCCT GTTCCACTT TGTAGATTGT GTTTAGTGCT GAAGTGAGAC TCACAGGAGA
 CCGTCAAAGA AAACGCCGT GACACACTGA GACCTAAACC ATAAGACGGA CAAGGTGAA ACATCTAACA CAAATCACGA CTTCACTCTG AGTGTCTCT

Introduction of diagnostic restriction site (Scal, 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)



ATG

CTCTGCAGGAGCGTGCATCCTATCCTGCCTTGGGTACGCTGAGCC ATG GCA GCC CTG CTG AGA CCC GCG CGT TGG CTG CTC GGG GCC GCG GCG GCC CCG CGC
 ▶ M A A L L R P A R W L L G A A A A P R

ex2

CTC CCG CTG TCC CTG CGC CTC CCT GCG GGC GTC CCG GGC GCG CTG TCC TCC GTC GTC CCG GTC GCG GCT GTT GGT AGC CCG CCG GCT GCA GGA
 ▶ L P L S L R L P A G V P G R L S S V V R V A A V G S R P A A G
 GAG CGT CTG AGC CAA GCC AGA TTG TAT GCC ATC GTT GCT GAG AAA AGG GAT CTT CAA GAG GAG CCT GCT CCT GTG AGA AAG AAC AGC AGT CAA
 ▶ E R L S Q A R L Y A I V A E K R D L Q E E P A P V R K N S S Q
 TTT GAC TGG GCT CTG ATG AGA CTG GAT AAT TCT GTC CCG AGA ACA GGC CGC ATC ACA AAG GGG CTT CTG CAG AGA GTC TTT GAG AGC ACG TGT
 ▶ F D W A L M R L D N S V R R T G R I T K G L L Q R V F E S T C
 AGC TCA GGT AGC CCA GGG AGC AAT CAA GCT CTG CTT CTG CTG CGC AGC TGT GGC TCG CTC CTG CCC GAA CTG AGT CTC GCC GAG AGG ACA GAG
 ▶ S S G S P G S N Q A L L L L R S C G S L L P E L S L A E R T E

ex4

TTT GCT CAC AAG ATC TGG GAC AAA CTT CAG CAG TTA GGT GTC GTA TAT GAT GTC AGT CAT TAC AAT GCT TTA CTT AAA GTA TAT CTT CAA AAT
 ▶ F A H K I W D K L Q Q L G V Y D V S H Y N A L L K V Y L Q N
 GAA TAC AAA TTT TCA CCT ACT GAC TTC CTG GCA AAG ATG GAG GGA GCA AAC ATC CAA CCA AAT CGA GTA ACA TAC CAG AGG CTG ATA GCT GCC
 ▶ E Y K F S P T D F L A K M E G A N I Q P N R V T Y Q R L I A A

ex6

TAC TGT AAT GTT GGG GAC ATT GAA GGT GCC AGC AAG ATC CTT GGA TTT ATG AAA ACG AAA GAC CTT CCG ATC ACA GAG GCC GTG TTC AGT GCT
 ▶ Y C N V G D I E G A S K I L G F M K T K D L P I T E A V F S A
 CTC GTC ACA GGG CAT GCG AGA GCT GGG GAT ATG GAA AAT GCA GAA AAT ATT CTC ACA GTG ATG AAA CAG GCC GGC ATT GAG CCT GGC CCA GAC
 ▶ L V T G H A R A G D M E N A E N I L T V M K Q A G I E P G P D

ex8

ACG TAT CTG GCC TTG TTG AAT GCA CAT GCT GAG AGG GGT GAC ATT GGC CAG GTT AGG CAG ATT CTG GAG AAA GTG GAG AAG TCA GAC CAT TAC
 ▶ T Y L A L L N A H A E R G D I G Q V R Q I L E K V E K S D H Y
 TTC ATG GAC CGC GAC TTC TTG CAG GTT ATT TTT AGC TTC AGT AAG GCT GGC TAC CCT CAG TAT GTC TCA GAA ATA CTG GAG AAG ATT ACC TAT
 ▶ F M D R D F L Q V I F S F S K A G Y P Q Y V S E I L E K I T Y

C>T (A353V) G>A TT>AC

GAG AGA CCG TCT ATT CCA GAT GCA ATG AAC CTC ATT TTG TTT TTA GCC ACT GAG AAG TTA GAA GAC ACT GTG TTC CAA GTA CTA TTG GCA TTA
 ▶ E R R S I P D A M N L I L F L A T E K L E D T V F Q V L L A L

ex10

CCC CTG TCC AAG GAC GAG AGC TCC GAT AAC TTT GGC AGT TTC TTT TTG CCG CAC TGT GTG ACT CTG GAT TTG CCC CCT GAG AAG CTG ATA GAC
 ▶ P L S K D E S S D N F G S F F L R H C V T L D L P P E K L I D
 TAC TGT CCG AGG CTG AGG GAC GCC AAG CTG CAC AGC TCC TCA CTG CAG TTC ACG CTG CAC TGT GCT CTT CAA GCC AAT AGG ACA GCT TTG GCA
 ▶ Y C R R L R D A K L H S S S L Q F T L H C A L Q A N R T A L A
 AAA GCA GTG ATG GAG GCT TTG AGG GAA GAA GGG TTT CCT ATC CGA CCG CAC TAT TTC TGG CCG TTG CTT GCT GGG CAT CAG AAA ACA AAA AAT
 ▶ K A V M E A L R E E G F P I R P H Y F W P L L A G H Q K T K N

ex12

GTT CAA GGA ATA ATA GAT ATC CTC AAA ATA ATG AAC AAA GTG GGA GTG GAT CCT GAT CAG GAA ACA TAT ATA AAC TAT GTG TTT CCG TGC TTT
 ▶ V Q G I I D I L K I M N K V G V D P D Q E T Y I N Y V F P C F
 GAT AGT GCA CAG TCA GTT CGA GCT GCT TTG CAG GAA AAT GAA TGT CTC CTC GCA AGT AGT ACC TTT GCT CAA GCT GAA GTG AAG AAT GAA GCA
 ▶ D S A Q S V R A A L Q E N E C L L A S S T F A Q A E V K N E A

ex14

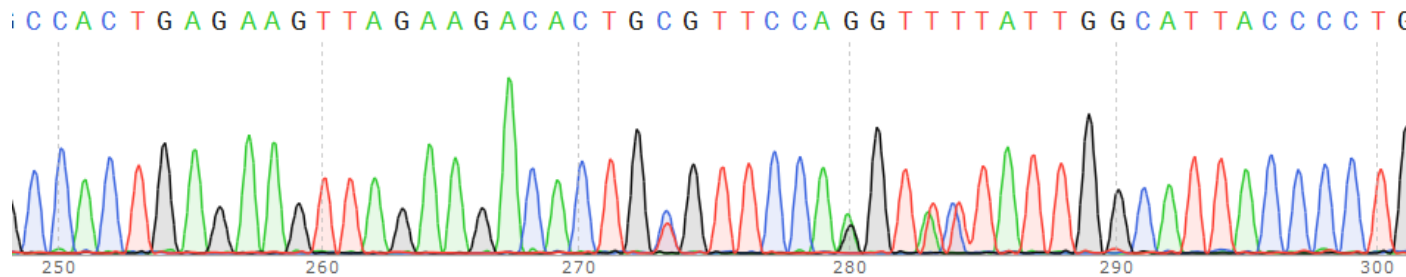
ATA AAT GGG AAC TTA CAG AAC ATT TTG TCA TTT TTG GAA TCG AAT ACA TTG CCT TTC TCG TTT AGT TCT TTG AGA AAC AGC CTA ATC CTA GGC
 ▶ I N G N L Q N I L S F L E S N T L P F S F S S L R N S L I L G

Genotyping protocol

Genotyping of F1 Line Kus6776-19-PM



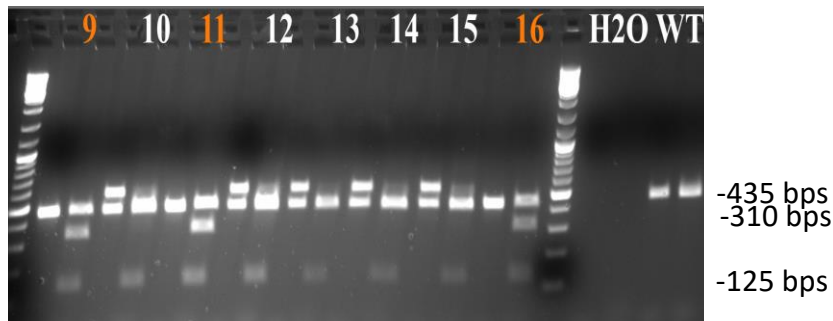
Chromatogram on one F1 animal



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

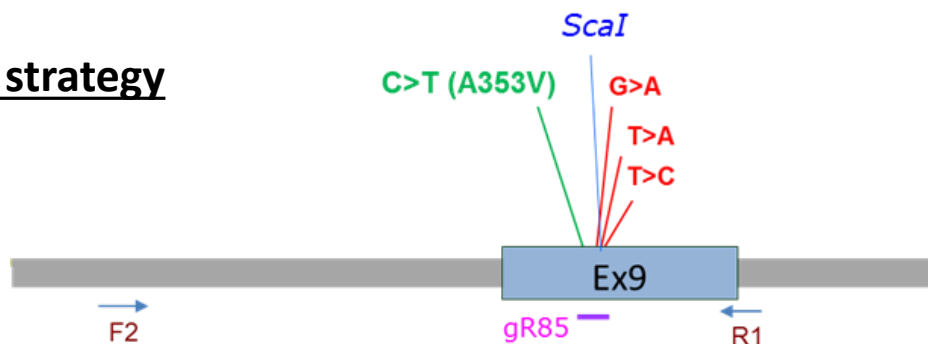
Expected sequence

Example of F1 genotyping



Animal #1, #11 and #16 are positive

PCR genotyping strategy



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

Primers in orange

ScaI in italic

Silent mut in red

Expected mut in green

Sequence of the PCR product (with mutations introduced)

CCTGGGTGGTGCAAGCAGGAATGGGTTACTCTTAGAGATTTAGATTTTTGTGGGGTTTGCAGTTCTTAAGTTTTTTCTTTAA
AAGTATAAACATTTTTGTTTGTGTGTTGGGTGTGCATATACCACAGGTCATGTGTAGTCTTTCTCTGCTGAACCGTGCTGCTG
GCCCTATTTTTCTTTAGTGTTGATTACAATTTAACAGATTGCTCAGTTTAGTAACAACAGATATCTTTGTTTTCTTCGCAGATGC
AATGAACCTCATTTTTGTTTTAGCCACTGAGAAGTTAGAAGACACTGTGTTCCAAGTACTATTGGCATTACCCCTGTCCAAGG
ACGAGAGCTCCGATAACTTTGGCAGTTTCTTTTTGCGGCACTGTGTGACTCTGGATTTGGTATTCTGCCTGTTTCACTTTGTA
GATTGTGTTTAGTGC

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 μ l
- 10mM dNTP	0,4 μ l
- 5' primer (100 μ M)	0.1 μ l
- 3' primer (100 μ M)	0.1 μ l
- DNA (lysate 1/10)	2 μ l
- Phusion Hot Start II	0,2 μ l
- Sterile H2O	up to 20 μ l

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 μ l
Buffer 10X	2 μ l
Restriction enzyme	0,2 μ l
H2O	7,8 μ l

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