

STRAIN NAME :

**Lrp4 R1170Q**

REAGENTS AND SOLUTIONS:

- Kapa Mouse Genotyping kit (Sigma)
- XmaI restriction enzyme 5u/μl (NEB)
- Primers 30μM (Eurofins)
- Mineral Oil (Fisher Scientific)
- DNA (extracted with Kapa Mouse Genotyping kit)

PCR REACTION MIX :

MIX	Quantity / well
Water	6,80 μl
2X Kapa Buffer	10,00 μl
Lrp4R1170QF	0,60 μl
Lrp4R1170QR	0,60 μl
DNA	2,00 μl
Final Quantity	20,00 μl

PCR PROGRAM:

3 min	95°C	
15 sec	94°C	
15 sec	60 °C	35 cycles
30 sec	72°C	
5 min	72°C	

MIGRATION : Migration on 1.5% agarose gel.

PRIMERS SEQUENCES

- Lrp4R1170QF            5' AGACGGGCTTGCAGTGGATG 3'
- Lrp4R1170QR           5' CTCCGGGCTCCTCTATTTTC 3'

EXPECTED AMPLIFICATIONS : all alleles amplifiat 541 bp

ENZYMATIC RESTRICTION REACTION MIX :

MIX	Quantity / well
Water	7,70 μl
Cutsmart Buffer 10X	2,00 μl
XmaI	0,30 μl
PCR	10,00 μl
Final Quantity	20,00 μl

RESTRICTION PROGRAM: at least 3 hours at 37°C

EXPECTED FRAGMENTS :

WT allele 409 + 132 bp bp  
 Mutant allele : 541 bp

*Perfection date : 28/05/2018*

*Update :*