



STRAIN NAME :

**ERN1**

REAGENTS AND SOLUTIONS:

- Kapa Mouse Genotyping kit (Sigma)
- MfeI restriction enzyme 20u/μl (NEB)
- Primers 30μM (Eurofins)
- Mineral Oil (Fisher Scientific)
- DNA (extracted with Kapa Mouse Genotyping kit)

PCR REACTION MIX :

MIX	Quantity / well
Water	7,40 μl
2X Kapa Buffer	10,00 μl
ERN1F	0,30 μl
ERN1R	0,30 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

- ERN1F                    5' AGCCATGGAGAAGTGCTGC 3'
- ERN1R                    5' AGCTAGACCCACCTCTTGTAGG 3'

EXPECTED AMPLIFICATIONS : all alleles amplifiat 599 bp

ENZYMATIC RESTRICTION REACTION MIX :

MIX	Quantity / well
Water	7,70 μl
10X Buffer	2,00 μl
MfeI	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 599 bp Mutant allele : 189 bp + 410 bp

*Perfection date* : 03/09/2019

*Update* :