

STRAIN NAME : **StingV154M**

REAGENTS AND SOLUTIONS:

- Kapa Mouse Genotyping kit (Sigma)
- NcoI-HF 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
StingV154MF	0,30 μl
StingV154MR	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

- StingV154MF            5' ATAGCAGTGCTGAGAGCAAGC 3'
- StingV154MR        5' GGGATCTAATGCTCTCTTCTGG 3'

EXPECTED AMPLIFICATIONS : all alleles amplifiat 504 bp

ENZYMATIC RESTRICTION REACTION MIX :

MIX	Quantity / well
Water	7,70 μl
10X Cutsmart Buffer	2,00 μl
NcoI	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 504 bp Mutant allele : 164 bp + 340 bp

*Perfection date : 13/01/2017*

*Update :*