

STRAIN NAME : **Ly5**

REAGENTS AND SOLUTIONS:

- Kapa Mouse Genotyping kit (Clinisciences)
- XhoI restriction enzyme 20u/μl (NEB)
- BSA 100X 10mg/ml (NEB)
- Primers 30μM (Eurogentec)
- 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
Ly5for	0,30	μl
Ly5rev	0,30	μl
DNA	2,00	μl
Final Quantity	20,00	μl

PCR PROGRAM:

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

MIGRATION : Migration on 1.5% agarose gel.

PRIMERS SEQUENCES

- Ly5for 5' ACAAGTGCGCAGAATACTGG 3'
- Ly5rev 5' GTGCAACGTTTCAGCTTCTGA 3'

EXPECTED AMPLIFICATIONS : all alleles amplify at 122 bp

ENZYMATIC RESTRICTION REACTION MIX :

MIX	Quantity / well	
Water	7,30	μl
10X Buffer	2,00	μl
BSA 100X	0,20	μl
XhoI	0,50	μl
PCR	10,00	μl
Final Quantity	20,00	μl

RESTRICTION PROGRAM: at least 3 hours at 37°C

EXPECTED FRAGMENTS : Ly5a 83 bp + 39 pb Ly5b : 122 bp (no cut)

Perfection date : 06/07/2012

Update :