

STRAIN NAME : **P14Rag2 Ly5.1 (EM:11491)**

P14

REAGENTS AND SOLUTIONS:

- Kapa Mouse Genotyping kit (Sigma)
- Primers 30µM (Eurofins)
- Mineral Oil (Fisher Scientific)
- DNA (extracted with Kapa Mouse Genotyping kit)

REACTION MIX :

MIX	Quantity / well
Water	6,40 µl
2X Kapa Buffer	10,00 µl
P14a	0,30 µl
P14b	0,30 µl
D9mit10F	0,50 µl
D9mit10R	0,50 µl
DNA	2,00 µl
Final Quantity	20,00 µl

PCR PROGRAM:

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

MIGRATION :

Migration on 1.5% agarose gel.

PRIMERS SEQUENCES

- P14a 5' GGCTGCAGTCACCCAAAGTCCAAG 3'
- P14b 5' CCGATAAGTCGGGTGCCCGCACC 3'
- D9mit10F 5' TAACCAACCCTTCAAGGCAC 3'
- D9mit10R 5' AATCCTTGGCTGAAGGGAAT 3'

EXPECTED AMPLIFICATIONS

Transgene : 331 bp
Internal control : 150 bp



RAG2

REAGENTS AND SOLUTIONS:

- Kapa Mouse Genotyping kit (Clinisciences)
- Primers 30 μ M (Eurogentec)
- Mineral Oil (Fisher Scientific)
- DNA (extracted with Kapa Mouse Genotyping kit)

REACTION MIX :

MIX	Quantity / well
Water	6,80 μ l
2X Kapa Buffer	10,00 μ l
RAG2F	0,60 μ l
RAG2R	0,30 μ l
RAG2neoR	0,30 μ l
DNA	2,00 μ l
Final Quantity	20,00 μ l

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

- RAG2F 5' GGGAGGACTCACTTGCCAGTA 3'
- RAG2R 5' AGTCAGGAGTCTCCATCTCACTGA 3'
- RAG2neoR 5' CGCCGGAGAACCTGCGTGCAA 3'

EXPECTED AMPLIFICATIONS

WT allele : 263 bp
Modified allele: 350 bp

Ly5

REAGENTS AND SOLUTIONS:

- Kapa Mouse Genotyping kit (Clinisciences)
- XhoI restriction enzyme 20u/ μ l (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	
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

- 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)