



STRAIN COMMON NAME : Simone (EM :02419)

Marilyn [Tg(TcraH-Y,TcrbH-Y)1Pas]

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	6,80 µl
2X Kapa Buffer	10,00 µl
Ia-2b4	0,40 µl
Samaup	0,40 µl
Fabpi200F	0,20 µl
FabpiR	0,20 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

- Ia-2b4 5' GCAGAGGAACCTGGGAGCTGT 3'
- Samaup 5' TGCTGTCTGTACCACCAGAAATAC 3'
- Fabpi200F 5' TGGACAGGACTGGACCTCTGCTTTCCTAGA 3'
- FabpiR 5' TAGAGCTTTGCCACATCACAGGTCATTTCAG 3'

EXPECTED AMPLIFICATIONS

Transgenic allele: 350 bp
Internal control : 200 bp

Perfection date : 21/04/1998

Update :



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' GGGAGGACACTCACTTGCCAGTA 3'
- RAG2R 5' AGTCAGGAGTCTCCATCTCACTGA 3'
- RAG2neoR 5' CGGCCGGAGAACCTGCGTGCAA 3'

EXPECTED AMPLIFICATIONS

WT allele : 263 bp
Modified allele: 350 bp

Perfection date : 20/04/2012

Update :



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	
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)

Perfection date : 06/07/2012

Update :



FoxP3-EGFP

(Modification on chromosome X)

REAGENTS AND SOLUTIONS:

- Kapa Mouse Genotyping kit (Sigma)
- Primers 30 μ M (Eurofins)
- 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
FoxP3a	0,20 μ l
FoxP3b	0,60 μ l
EGFP3	0,40 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	55°C	35 cycles
30 sec	72°C	
5 min	72°C	

MIGRATION :

Migration on 1.5% agarose gel.

PRIMERS SEQUENCES

- FoxP3a 5' GCCAATCCCAGCCTAGCCCCTAGT 3'
- FoxP3b 5' CAGCTGACCACAGTTTATTGGATG 3'
- EGFP3 5' CAAGTCCGCCATGCCCGAAGGCTACG 3'

EXPECTED AMPLIFICATIONS

WT allele : 650 bp
KI allele : 1000 bp

Perfection date : 01/03/2007

Update :