



STRAIN NAME :

CD3 epsilon

REAGENTS AND SOLUTIONS:

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

REACTION MIX :

| MIX | Quantity / well |
|----------------|-----------------|
| Water | 3,40 µl |
| 2X Kapa Buffer | 10,00 µl |
| CD3EF | 2,00 µl |
| CD3ER | 2,00 µl |
| 3neo500 | 0,30 µl |
| 5neo500 | 0,30 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

- CD3EF 5' GAGGCATGTGTAGGGATTAC 3'
- CD3ER 5' GCTACTACGTCTGCTACAC 3'
- 3neo500 5' CGGGAGCGGCGATACCGTAAAGC 3'
- 5neo500 5' GAAGCGGGAAGGGACTGGCTGCTA 3'

EXPECTED AMPLIFICATIONS

WT allele : 643 bp
Modified allele: 500 bp

Perfection date : 21/02/2017

Update :

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 | |
| 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 :