STRAIN NAME : Hsurivong CD4 EGFP (EM:06321)

REAGENTS AND SOLUTIONS:

- Kapa Mouse Genotyping kit (Clinisciences)
- Primers 30µM (Eurogentec)
- DNA (extracted with Kapa Mouse Genotyping kit)

REACTION MIX :

mCD4

MIX	Quantity / well	
Water	6,00	µl
2X Kapa Buffer	10,00	µl
mCD4 sens	1,00	µl
mCD4 as	0,80	µl
LTAP3	0,20	µl
DNA	2,00	µl
Final Quantity	20,00	µl

Beta2m

MIX	Quantity / well	
Water	6,00	µl
2X Kapa Buffer	10,00	µl
b2M0	1,00	µl
b2M5	0,50	µl
Neo55a	0,50	µl
DNA	2,00	µl
Final Quantity	20,00	µl

IAbeta

MIX	Quantity / well	
Water	7,20	µl
2X Kapa Buffer	10,00	µl
AbetaR	0,40	µl
AbetaWTF	0,20	µl
AbetaKOF	0,20	µl
DNA	2,00	µl
Final Quantity	20,00	µl

CD4-EGFP

MIX	Quantity / well	
Water	7,00	µl
2X Kapa Buffer	10,00	µl
Dbx1GFP for	0,50	µl
Dbx1GFP rev	0,50	µl
DNA	2,00	µl
Final Quantity	20,00	µl

Dp alpha

MIX	Quantity / well	
Water	7,40	µl
2X Kapa Buffer	10,00	µl
Dpa sens	0,30	µl
Dpa as	0,30	µl
DNA	2,00	µl
Final Quantity	20,00	µl

Dp beta

MIX	Quantity / well	
Water	7,40	µl
2X Kapa Buffer	10,00	µl
Dpb sens	0,30	µl
Dpb as	0,30	µl
DNA	2,00	µl
Final Quantity	20,00	µl

hCD4

MIX	Quantity / well	
Water	7,00	µl
2X Kapa Buffer	10,00	µl
hCD4 sens	0,50	µl
hCD4 as	0,50	µl
DNA	2,00	µl
Final Quantity	20,00	µl

HLAA2 / HLADRA1

MIX	Quantity / well	
Water	6,00	µl
2X Kapa Buffer	10,00	µl
HLAA2 F	0,50	µl
HLAA2 R	0,50	µl
HLADRA1 F	0,50	µl
HLADRA1 R	0,50	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

- mCD4 sens 5' GGAGTTGTGGGTGTTCAAAGTG 3'
- mCD4 as 5' AGAGTTGCTATCCAAGGTCAGGG 3'
- LTAP3 5' 'ACCGCTTCCTCGTGCTTTACGG 3'

- b2M0 5' CTGAGCTCTGTTTTCGTCTG 3'
- b2M5 5' TATCAGTCTCAGTGGGGGTG 3'
- Neo55a 5' CCTGCCGAGAAAGTATCCA 3'

- AbetaR 5' AGGGAGGTGTGGGTCTCC 3'
- AbetaWTF 5' GTACCAGTTCATGGGCGAGT 3'
- AbetaKOF 5' GTGTTGGGTCGTTTGTTCG 3'

- Dpa sens 5' TAATACAAAGTCTGCAGCTGGC 3'
- Dpa as 5' AGCAATGTTAGCCAGCC 3'

- Dpb sens 5' GGGATTGGAAAGAGGCTC 3'
- Dpb as 5' GCACTGCCCGCTTCTCC 3'

- hCD4 sens 5' TCAGTGCAATGTAGGAGTCCAAG 3'
- hCD4 as 5' CACGATGTCTATTTTGAACTCCAC 3'

- HLAA2 F 5' CATTGAGACAGAGCGCTTGGCACAGAAGCAG 3'
- HLAA2 R 5' GGATGACGTGAGTAAACCTGAATCTTTGGAGTACGC 3'
- HLADRA1 F 5' CTCCAAGCCCTCTCCCAGAG 3'
- HLADRA1 R 5' ATGTGCCTTACAGAGGCCCC 3'

Dbx1GFP for 5' GACCCTGAAGTTCATCTGC 3'
- Dbx1GFP rev 5' TGTCGGCCATGATATAGACG 3'

EXPECTED AMPLIFICATIONS

mCD4 :
WT allele : 375 bp
Modified allele: 200 bp

Beta2m :
WT allele : 262 bp
Modified allele: 768 bp

IAbeta :
WT allele : 252 bp
Modified allele: 170 bp

CD4-EGFP

Dp alpha :
Transgenic allele: 445 bp

Dp beta :
Transgenic allele: 815 bp

hCD4 :
Transgenic allele: 146 bp

HLAA2 / HLADRA1 :
Transgenic allele: 400 bp
Transgenic allele: 153 bp

Transgenic allele : 350 bp

Perfection date : 30/06/2011
Update :