PCR MHC°

Double KO Abeta° + beta2M

Abeta°

1- PCR mix

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl2)
0,50 µl dNTP (20 mM total)
0,65 µl primer Abeta3 (200 ng/µl)
0,50 µl primer Abeta2 (200 ng/µl)
0,15 µl primer Neo55 a (200 ng/µl)
0,20 µl Taq polymerase (Q BIOgene ; 5U/µl)
19,50 µl distilled water

2- PCR reaction :

24,0 µl mix
1,0 µl DNA (200ng/µl)

3- PCR program :

5 min	94°C	
1 min	94°C	
1 min	57°C	35 cycles
2 min	72°C	
5 min	72°C	

4- Primers:

- Primer Abeta3 5' TTC GTG TAC CAG TTC ATG GG 3'
- Primer Abeta2 5' TAG TTG TGT CTG CAC ACC GT 3'
- Primer Neo55 a 5' CCT GCC GAG AAA GTA TCC A 3'

5- Amplifications:

wild-type allele: 230 bp
mutant allele: 730 bp

beta2M

1- PCR mix

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl2)
0,50 µl dNTP (20 mM total)
0,70 µl primer beta2M0 (200 ng/µl)
0,50 µl primer beta2M4 (200 ng/µl)
0,20 µl primer Neo55 a (200 ng/µl)
0,20 µl Taq polymerase (Q BIOgene ; 5U/µl)
19,40 µl distilled water

2- PCR reaction :

24,0 µl mix
1,0 µl DNA (200ng/µl)

3- PCR program :

5 min	94°C	
1 min	94°C	
1 min	57°C	35 cycles
2 min	72°C	
5 min	72°C	

4- Primers:

- Primer beta2M0 5' CTG AGC TCT GTT TTC GTC TG 3'
- Primer beta2M4 5' CTT AAC TCT GCA GGC GTA TG 3'
- Primer Neo 55 a 5' CCT GCC GAG AAA GTA TCC A 3'

5- Amplifications:

wild-type allele: 270 bp
mutant allele: 600 bp