PCR NOD beta2m IAbeta

2 PCR reactions

1- PCR mix for beta2m

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl2)
0,25 µl dNTP (20 mM total)
0,20 µl primer beta2m neo (30µM)
0,30 µl primer beta2 sens (30 µM)
0,30 µl primer beta2 antisens (30 µM)
0,20 µl Taq polymerase (Q BIOgene ; 5U/µl)
19,25 µl distilled water

2- PCR mix for IAbeta

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl2)
0,25 µl dNTP (20 mM total)
0,20 µl primer Iabeta neo (30 µM)
0,20 µl primer Iabeta neo NOD (30 µM)
0,20 µl primer Iabeta1 (30 µMl)
0,20 µl primer IAbeta2 (30 µM)
0,20 µl Taq polymerase (Q BIOgene ; 5U/µl)
19,25 µl distilled water

3- PCR reactions :

23,0 µl mix
2,0 µl DNA (200ng/µl)

4- PCR program :

5 min	94°C	
1 min	94°C	
1 min	62°C	35 cycles
2 min	72°C	
5 min	72°C	

5- Primers:

-Primer beta2m neo	5' GCTATTCGGCTATGACTGGG 3'
-Primer beta2 sens	5' CTGAGCTCTGTTTTCGTCT 3'
-Primer beta2 antisens	5' TATCAGTCTCAGTGGGGGTG 3'
-Primer Iabeta neo	5' CAGCGCATCGCCTTCTATCGC 3'
-Primer Iabeta neo NOD	5' TTCGTGCACCAGTTCAAGGG 3'
-Primer Iabeta1	5' TAGTTGTGTCTGCACACCGT 3'
-Primer Iabeta2	5' TTCGTGTACCAGTTCATGGG 3'

6-Amplifications:

PCR beta2m :	wildtype allele :	262 bp	mutant allele : 728 bp
PCR IAbeta :	wildtype allele :	230 bp	mutant allele : 250 bp