

PCR Tga20

2 PCR reactions

1- PCR mix for PrP KO

2,50 µl 10X buffer (KALYS)
 2,00 µl MgCl₂ (50 mM)
 0,25 µl dNTP (20 mM total)
 0,20 µl primer BF3 (30 µM)
 0,50 µl primer BF7 (30 µM)
 0,20 µl primer BF9 (30 µM)
 0,20 µl Taq polymerase (KALYS ; 5U/µl)
 18,15 µl distilled water

2- PCR mix for Tga20 transgene

2,50 µl 10X buffer (KALYS)
 2,00 µl MgCl₂ (50 mM)
 0,25 µl dNTP (20 mM total)
 0,25 µl primer BF7 (30 µM)
 0,25 µl primer BF8 (30 µM)
 0,20 µl Taq polymerase (KALYS ; 5U/µl)
 18,55 µl distilled water

3- PCR reactions :

24,0 µl mix
 1,0 µl DNA (200ng/µl)

4- PCR program :

3 min	96°C	
40 sec	94°C	
30 sec	63°C	35 cycles
1 min	72°C	
9 min	72°C	

5- Primers:

-Primer BF3	5' TCATGCCCATTTCCAAATTCCACTACA 3'
-Primer BF7	5' GGGTAGCGGTACATGTTTTACGGTAG 3'
-Primer BF8	5' ACTCTTCATTTTAGGCCCCACGTA 3'
-Primer BF9	5' ACGCGTCACCTTAATATGCGAAGTGGA 3'

6-Amplifications:

PCR PrP :	wildtype allele : 779 bp	mutant allele : 566 bp
PCR Tg :	wildtype allele : 1046 bp	