

PCR PRP KO

Substrain: B6;129S7-Prnp<tm1Cwe>/CweOrl
maintained at the EMMA facility at CNRS-CDTA, Orleans (France)

1- PCR mix

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl₂)
0,50 µl dNTP (20 mM total)
0,20 µl primer P3 (200 ng/µl)
0,20 µl primer P10 (200 ng/µl)
0,20 µl primer 3'NC (200 ng/µl)
0,20 µl Taq polymerase (Q BIOgene ; 5U/µl)
20,20 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	60°C	35 cycles
2 min	72°C	
5 min	72°C	

4- Primers:

- Primer P3 5' ATTCGCAGCGCATCGCCTTCTATCGCC 3'
- Primer P10 5' GTACCATAATCAGTGGAAACAAGCCCAGC 3'
- Primer 3'NC 5' ATAACCCCTCCCCCAGCCT 3'

5- Amplifications:

wild type allele: 550 bp
mutant allele : 350 bp