

PCR NrCam

2 PCR reactions

1- PCR mix for wild type allele

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl₂)
 0,50 µl dNTP (20 mM total)
 0,40 µl primer MORE1 (200 ng/µl)
 0,40 µl primer MIMO 71 (200 ng/µl)
 0,20 µl Taq polymerase (Q BIOgene ; 5U/µl)
 19,00 µl distilled water

2- PCR mix for mutant allele

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl₂)
 0,50 µl dNTP (20 mM total)
 0,40 µl primer MORE1 (200 ng/µl)
 0,40 µl primer NeoL1 (200 ng/µl)
 0,20 µl Taq polymerase (Q BIOgene ; 5U/µl)
 19,00 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	60°C	35 cycles
2 min	72°C	
5 min	72°C	

5- Primers:

- Primer MORE1	5' GAATAGATCAGTCGGGTCCTGAGG3'
- Primer MIMO 71	5' CAGGAAGAGAATCAGGGGCACTC3'
- Primer NeoL1	5' CCTGCGTGCAATCCATCTTGTTCATG3'

6- Amplifications:

reaction 1 : wild type allele : 800 bp
 reaction 2 : mutant allele : 1000 bp