

PCR Calpha

1- PCR mix

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl₂)
 0,50 µl dNTP (20 mM total)
 0,60 µl primer alpha1 (200 ng/µl)
 0,40 µl primer alpha2 (200 ng/µl)
 0,20 µl primer Neo55 a (200 ng/µl)
 0,20 µl Taq polymerase (Q BIOgene ; 5U/µl)
 19,60 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	55°C	35 cycles
2 min	72°C	
5 min	72°C	

4- Primers:

- Primer alpha1 5' CGT GGA GAG GGA CTA CTG AA 3'
 - Primer alpha2 5' GCC TGG GAG AAC AGA GAA G 3'
 - Primer Neo 55 a 5' CCT GCC GAG AAA GTA TCC A 3'

5- Amplifications:

wild-type allele: 741 bp
 mutant allele: 1100 bp