

PCR delta hs 3b 4
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## 1- PCR mix

2,50 µl 10X buffer(Appligene ; 1,5 mM MgCl<sub>2</sub>)  
 0,50 µl dNTP (20 mM total)  
 0,40 µl primer hs4 1 (200 ng/µl)  
 0,40 µl primer hs4 2 (200 ng/µl)  
 0,50 µl primer Neo 204 (20 ng/µl)  
 0,50 µl primer Neo 909 (20 ng/µl)  
 0,20 µl Taq polymerase (Appligene ; 5U/µl)  
 19,00 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 hs4 1                    5' CTGCAGACTCACTGTTCA 3'  
 - Primer hs4 2                    5' AGCTTGGAGTTAGGTGGG 3'  
 - Primer Neo204                5' GCTATTCGGCTATGACTGGG 3'  
 - Primer Neo909                5' GAAGGCGATAGAAGGCGATG 3'

## 5- Amplifications:

wild-type allele:    1400 bp  
 mutant allele:      706 bp