

PCR Des lacz KO
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## 1- PCR mix

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl<sub>2</sub>)  
 0,25 µl dNTP (20 mM total)  
 0,20 µl primer Des1F (200 ng/µl)  
 0,20 µl primer Des1R (200 ng/µl)  
 0,20 µl primer LacZ 430 R (200 ng/µl)  
 0,20 µl Taq polymerase (Q BIOgene ; 5U/µl)  
 19,45 µl distilled water

## 2- PCR reaction :

23,0 µl mix  
 2,0 µl DNA (100ng/µl)

## 3- PCR program :

5 min	94°C	
1 min	94°C	35 cycles
1 min	62°C	
2 min	72°C	
5 min	72°C	

## 4- Primers:

- Primer DES1 F                    5' TTGGGGTCGCTGCGGTCTAGCC 3'  
 - Primer DES1 R                    5' GGTCGTCTATCAGGTTGTCACG 3'  
 - Primer LacZ 430 R                5' GATCGATCTCGCCATACAGCGC 3'

## 5- Amplifications:

wild-type allele:    around 300 bp  
 mutant allele:        around 400 bp