

PCR SRIF KO

1- PCR mix

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl₂)
 0,25 µl dNTP (20 mM total)
 0,30 µl primer SRIF1 (200 ng/µl)
 0,15 µl primer SRIF2 (200 ng/µl)
 0,15 µl primer SRIF3 (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 (200ng/µl)

3- PCR program :

5 min	94°C	
30 sec	94°C	
30 sec	68°C	35 cycles
1 min	72°C	
5 min	72°C	

4- Primers:

- Primer SRIF1 5' GTCTCCAGTGCGCCCTGGCT 3'
 - Primer SRIF2 5' GGGACTTTCCACACCCTAACTGA 3'
 - Primer SRIF3 5' GGGAGCTTAGGGGCTGAGCG 3'

5- Amplifications:

wild-type allele: 250 bp
 mutant allele: 170 bp