

PCR REACTION Nf1:

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10X PCR buffer	3 λ
MgCL ₂ 25 mM	3 λ
dNTP'S 2.5 mM	3 λ
Primer Nf1_F	5 μ mol per reaction (1,5 λ)
Primer Nf1_R	5 μ mol per reaction (1,5 λ)
Taq	0,6 λ
DNA	1,5 λ
H ₂ O	Up to volume 30 λ (15,9 λ)

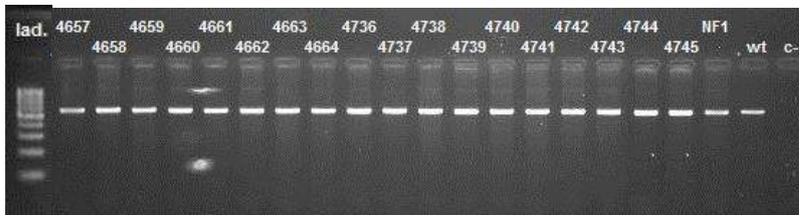
Nf1_F: 5'-GTTTTGGGGCAATCCGTCTT-3'

Nf1_R: 5'-TACATTGCCTTGCACTTGGC-3'

PCR CONDITIONS:

1. 94^oC for 5 min.
2. 94^oC for 45 sec.
3. 58^oC for 30 sec
4. 72^oC for 45 sec.
5. Steps 2-4 for 30 more cycles.
6. 72^oC for 5 min.
7. 4^oC for infinite

PCR product: 608 bp



DIGESTION REACTION OF PCR PRODUCTS

SacI enzyme	0.5 λ
10x 1.1. Buffer	1,5 λ
DNA	5 λ
H ₂ O	Up to volume 15 λ (8 λ)

Incubation at 37^oC for 2h

After digestion:

wt band: 608 bp

mutant bands: 181 bp & 427 bp

