PCR Xtp Pasteur (pmn)

1- PCR mix

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl2)
0,25 µl dNTP (20 mM total)
0,20 µl primer pmn1 (200 ng/µl)
0,20 µl primer pmn2 (200 ng/µl)
0,20 µl Taq polymerase (Q BIOgene ; 5U/µl)
19,65 µl distilled water

2- PCR reaction :

23,0 µl mix
2,0 µl DNA (200ng/µl)

3- PCR program :

5 min	94°C	
1 min	94°C	
1 min	60°C	40 cycles
2 min	72°C	
5 min	72°C	

4- Primers:

- Primer pmn1 5' GTCTTACTGCTCCCTACTTG 3'
- Primer pmn2 5' GTGAAAACAGAAAGGGCAGAG 3'

5- Amplifications:

Wild-type and mutant allele : 290 bp

6- Enzymatic rectriction :

2,30 µl distilled water
2,00 µl Mnl110X buffer (Biolabs R0163S)
0,20 µ BSA 100X
0,50 µl Mnl1 (5u/µl; Biolabs)
15,0 µl PCR

37°C for 2 hours

7- Restriction patterns

Wild-type : 100 bp + 190 bp
pmn/+ : 100 bp + 190 bp + 110 bp + 80 bp
pmn/pmn : 100 bp + 110 bp + 80 bp