nude genotyping

1- PCR mix

2,50 µl 10X buffer(Q BIOgene ; 1,5 mM MgCl2)
0,50 µl dNTP (20 mM total)
0,15 µl primer nu 1 (200 ng/µl)
0,15 µl primer nu2 (200 ng/µl)
0,20 µl Taq polymerase (Q BIOgene ; 5U/µl)
20,50 µl distilled water

2- PCR reaction :

24,0 µl mix
1,0 µl DNA (200ng/µl)

3- PCR program :

30 sec	95°C	
15 sec	94°C	
15 sec	57°C	40 cycles
30 sec	72°C	
5 min	72°C	

4- Primers:

- Primer nu 1 5' GGCCCAGCAGGCAGCCCAAG 3'
- Primer nu 2 5' AGGGATCTCCTCAAAGGCTTC 3'

5- Amplification: 190 bp

6- Restriction mix :

2,00 µl 10X buffer (enzyme *Mwo1*)
0,40 µl enzyme *Mwo1* I (10U/µl)
2,60 µl distilled water

7- Restriction reaction :

5,0 µl mix
15,0 µl PCR product

8- Restriction program : 60°C overnight

9- Restriction migration : 10 µl on 20% polyacrylamide gel

10- Restriction results :

wild type allele : 80 bp
mutant allele : 91 bp