

PCR FOR NEMO flx

PCR REACTION:

10X PCR buffer	2µl
MgCL ₂ 25 mM	1,2µl
dNTP'S 2,5 mM	2µl
oligo A	5 pmol per reaction
oligo B	5 pmol per reaction
Oligo C	5 pmol per reaction
Tag	0,4µl
DNA	1µl
H ₂ O	Up to volume 20µl

Oligo A

Name: NEMO27

Sequence: 5'-GCCTTGGTGCTCCCTAACTCT-3'

Oligo B

Name: NEMO40

Sequence: 5'-TCACATCACATCGTTATCCTT-3'

Oligo C

Name: NEMO61

Sequence: 5'-ATGAACAAGCACCCCTGGAAG-3'

PCR CONDITIONS:

PCR program:

1= 93 °C 3:00

2= 93 °C 0:45

3= 56 °C 0:45

4= 72 °C 0:45

Go to 2, 29 times

6= 72°C 10:00

7= 20 °C 0:30

8= END

Use with oligo A+B for the detection of WT allele (product 431bp) and the floxed allele (product 576bp).

Use with oligo B+C for the detection of the defloxed allele (product 841bp).

Or use all oligos in one reaction to obtain all 3 bands,.