

	TRANSGENESE ET ARCHIVAGE D'ANIMAUX MODELES PHENOMIN	BM-DRF-002
	FICHE PROTOCOLE GENOTYPAGE	22/10/2013 Page : 1/2

STRAIN NAME : **Nox4 flox**

REAGENTS AND SOLUTIONS:

- Kapa Mouse Genotyping kit (Sigma)
- Primers 30µM (Eurofins)
- Mineral Oil (Fisher Scientific)
- DNA (extracted with Kapa Mouse Genotyping kit)

REACTION MIX WT and floxed allele :

MIX	Quantity / well
Water	7,40 µl
2X Kapa Buffer	10,00 µl
Nox4WTF	0,30 µl
Nox4R	0,30 µl
DNA	2,00 µl
Final Quantity	20,00 µl

REACTION MIX KO allele :

MIX	Quantity / well
Water	7,40 µl
2X Kapa Buffer	10,00 µl
Nox4KOF	0,30 µl
Nox4R	0,30 µl
DNA	2,00 µl
Final Quantity	20,00 µl

PCR PROGRAM:

3 min	95°C	
15 sec	94°C	
15 sec	60°C	35 cycles
30 sec	72°C	
5 min	72°C	

MIGRATION :

Migration on 1.5% agarose gel.

PRIMERS SEQUENCES

- Nox4WTF 5' AGACATCCAATCATTCCAGTGG 3'
- Nox4KOF 5' TGTCTGTCGGCGCACTCACTA 3'
- Nox4R 5' GTGGATCAAGAAACATGCTGAC 3'

EXPECTED AMPLIFICATIONS

WT allele : 435 bp
Floxed allele : 469 bp
KO allele : 517 bp

STRAIN NAME : **Tie2 cre**

REAGENTS AND SOLUTIONS:

- Kapa Mouse Genotyping kit (Sigma)
- Primers 30µM (Eurofins)

- Mineral Oil (Fisher Scientific)
- DNA (extracted with Kapa Mouse Genotyping kit)

REACTION MIX :

MIX	Quantity / well
Water	7,40 μ l
2X Kapa Buffer	10,00 μ l
Tie2F	0,30 μ l
Tie2R	0,30 μ l
DNA	2,00 μ l
Final Quantity	20,00 μ l

PCR PROGRAM:

3 min	95°C	
15 sec	94°C	
15 sec	60°C	35 cycles
30 sec	72°C	
5 min	72°C	

MIGRATION :

Migration on 1.5% agarose gel.

PRIMERS SEQUENCES

- Tie2F 5' CGCATAACCAAGTGAACAGCATTGC 3'
- Tie2R 5' CCCTGTGCTCAGACAGAAATGAGA 3'

EXPECTED AMPLIFICATIONS

Tg allele : 550 bp