

Genotyping protocol

Q114* PM in *Nxn1*

Nxn1^{tm1.1}

(PHENOMIN-ICS reference IR00004997 / K4997)

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1. Genotyping protocol and data

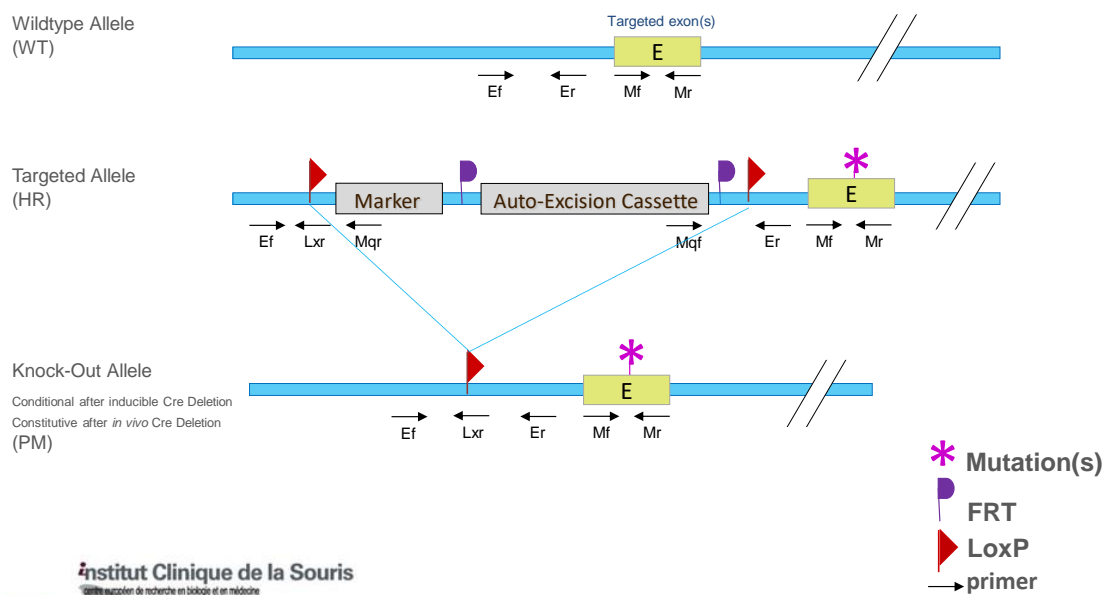
This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the Q114* PM in *Nxn1* mouse line.

1.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.



PM Genotyping strategy



Sequence of primers used for genotyping:

Position	Sequence
Ef	CAAACATCACACCCACGGAGAGG
Ef ²	CAGTTAAGATCTCCCCAGGGTCTTC
Er	GGGCAGCGATTTCAAACAGCGAG
Lxr	GAAGTTATACTAGAGCGGCCGTTTAC
Mf	CCTCCCTCTTCACGTTGACAGAC
Mq1f	AGGGCCAGCTCATTCTCCCACTC
Mq1r	TGCTAAAGCGCATGCTCCAGACTGC
Mr	CAAATCCTCCGTTGCAGGAAGC

²: for a selected position, a second primer was designed

PCR fragments expected size (bp):

Region analyzed	Position on the primer (see the map above)	Targeted allele (HR)	PM allele	WildType allele
WildType / Mutated alleles	Mf / Mr	376	376	376
Excision of the selection marker	Ef / Er	4376*	221	126
5' part of the selection marker	Ef / Mq1r	214	---	---
3' part of the selection marker	Mq1f / Er	374	---	---
LoxP specific PCR	Ef2 / Lxr	218	218	---

*: this PCR product will not be observed using our PCR genotyping conditions (see description below)

---: no Amplicon should be obtained



1.2. PCR protocol

This section describes the composition of the mix and cycling conditions used for genotyping.

Reagents:	Volume:
- FastStart PCR Master (Roche)	7.5µl
- DNA (50ng/µl)	1.5µl
- 5' primer (100 µM)	0.06µl
- 3' primer (100 µM)	0.06µl
- Sterile H ₂ O	up to 15 µl

Cycling conditions:

Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
72°C	1min	
72°C	7min	1
20°C	5min	1

NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

2. Cre and Flp genotyping method

You will find the genotyping protocol in the publication:

[Highly-efficient, fluorescent, locus directed cre and FlpO deleter mice on a pure C57BL/6N genetic background.](#)

Birling MC, Dierich A, Jacquot S, Héroult Y, Pavlovic G.

Genesis. 2012 Jun;50(6):482-9. doi: 10.1002/dvg.20826. Epub 2012 Mar 20.

