EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Conditional R897Q point mutation in Nedd4l

FleX2 approach

Nedd4l$^{tm1.1Ics}$

(PHENOMIN-ICS reference IR00005810 / K5810)

For any question, please contact:

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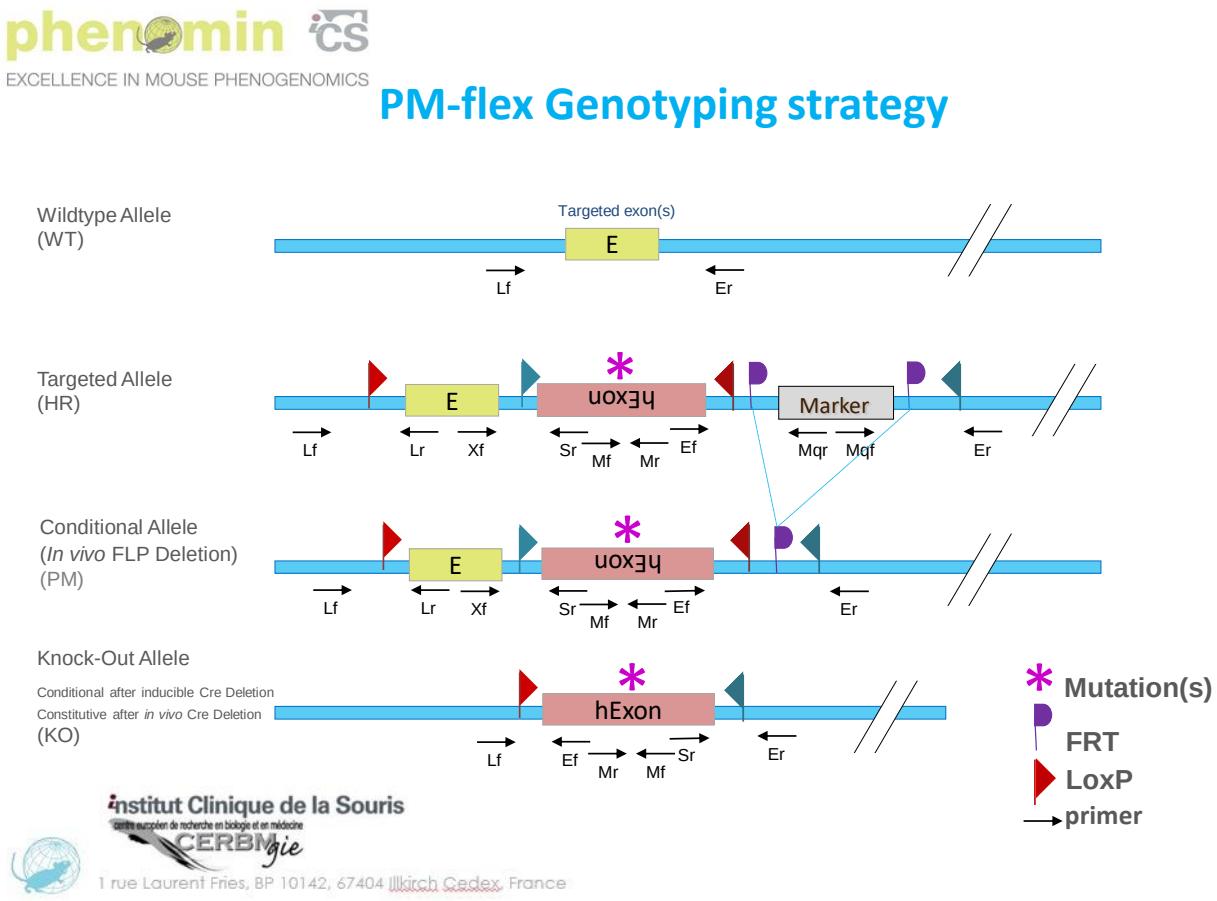
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the **Nedd4l** conditional point mutation (FleX2 approach) mouse line.

1.1. Genotyping strategy

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



Sequence of primers used for genotyping:

Position	Sequence
Lf	GTCATGTGCTGATGAGATACAGAGGGGAA
Lr	GGGGTCAAGCTTAGGTTTCTGCGTT
Xf	ACGGAGATGCAGAACAGAGGGCTT
Sr	CAGTGTGCCATGCATAGGTTCCAT
Mf	ATTCCCAGGAGGACAACTGAATCCAA
Mr	AATAAGATTAAGTTCTGCCTGCCTGCAT
Ef	ACTGAGTGATCACAATGTCCGTCTCTGCTA
Er	GGGACTTAGGGAAAGGGAGAAGGAA
Mqr	TGCTAAAGCGCATGCTCCAGACTGC
Mqf	GAAGAACGAGATCAGCAGCCTCTGTTCC

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	cKO deleted allele	KI allele	WildType allele
Mutated alleles	Mf / Mr	227	227	227	---
Presence of the distal loxP	Lf / Lr	369	369	---	319
Presence of the loxP after inversion	Lf / Ef	---	---	352	---
Excision of the selection marker	Ef / Er	2189*	336	---	---
5' part of the selection marker	Ef / Mqr	196	---	---	---
3' part of the selection marker	Mqf / Er	334	---	---	---
Exogenous/cDNA specific PCR	Xf / Sr	376	376	---	---
Presence of the lox511 after inversion	Sr / Er	---	---	310	---

*: 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_2O	up to 15 µl

Cycling conditions:

Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
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, Hérault Y, Pavlovic G.
Genesis. 2012 Jun;50(6):482-9. doi: 10.1002/dvg.20826. Epub 2012 Mar 20.