phenomin

EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

P1003Q PM in Pik3r4 (corresponding to R998Q in human)

IR00005809 / K5809

(ICS internal reference)

For any question, please contact:

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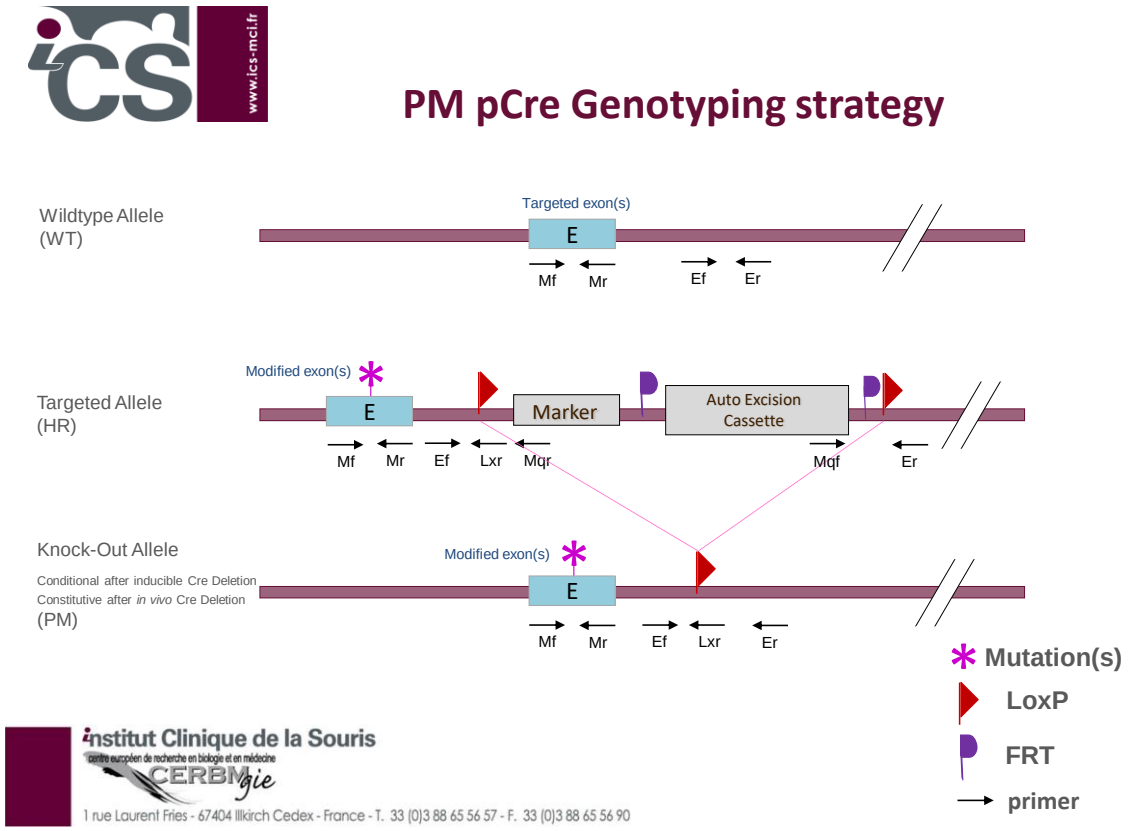
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Pik3r4** Point mutation or few bp modification Knockin (PM) project.

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	Primers	Sequence
Ef	9054	GAGCCACACAGGAATGTTGAATGTTCTG
Ef^2	9055	GGGCTGGAGTGTCTATAAGACACTGCC
Er	9056	CCACATTTACCCTGTGTCAACTCAGCC
Er^2	9057	CCAATCCCTGCAATCACAGTGACG
Lxr	4774	GAAGTTATACTAGAGCGGCCGTTCAC
Mf	9052	GTCATGGTCCAGTAGACCCCTCTGG
Mqf	6	GAAGAACGAGATCAGCAGCCTCTGTTCC
Mqr	265	TGCTAAAGCGCATGCTCCAGACTGC
Mr	9053	GCACTGCTGACGTAGCTGGATTGG

^{2}: for a selected position, a second primer was designed

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	PM allele	WildType allele
WildType / Mutated alleles	9052-9053	Mf / Mr	366	366	366
Excision of the selection marker (with DMSO)	9054-9056 (with 5% DMSO)	Ef / Er	4496*	341	262
Excision of the selection marker 2 (with DMSO)	9055-9057 (with 5% DMSO)	Ef^2 / Er^2	4617*	462	383
5' part of the selection marker (with DMSO)	9054-265 (with 5% DMSO)	Ef / Mq1r	227	---	---
3' part of the selection marker	6-9057	Mq1f / Er^2	398	---	---
LoxP specific PCR (with DMSO)	9055-4774 (with 5% DMSO)	Ef2 / Lxr	209	209	---

*: this PCR product will not be observed using our PCR genotyping conditions (see description below)
**: this PCR is only verified if mice are generated
---: 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.