Genotyping protocol

A385E point mutation in Fgfr3

IR00004697 / K4697

(ICS internal reference)

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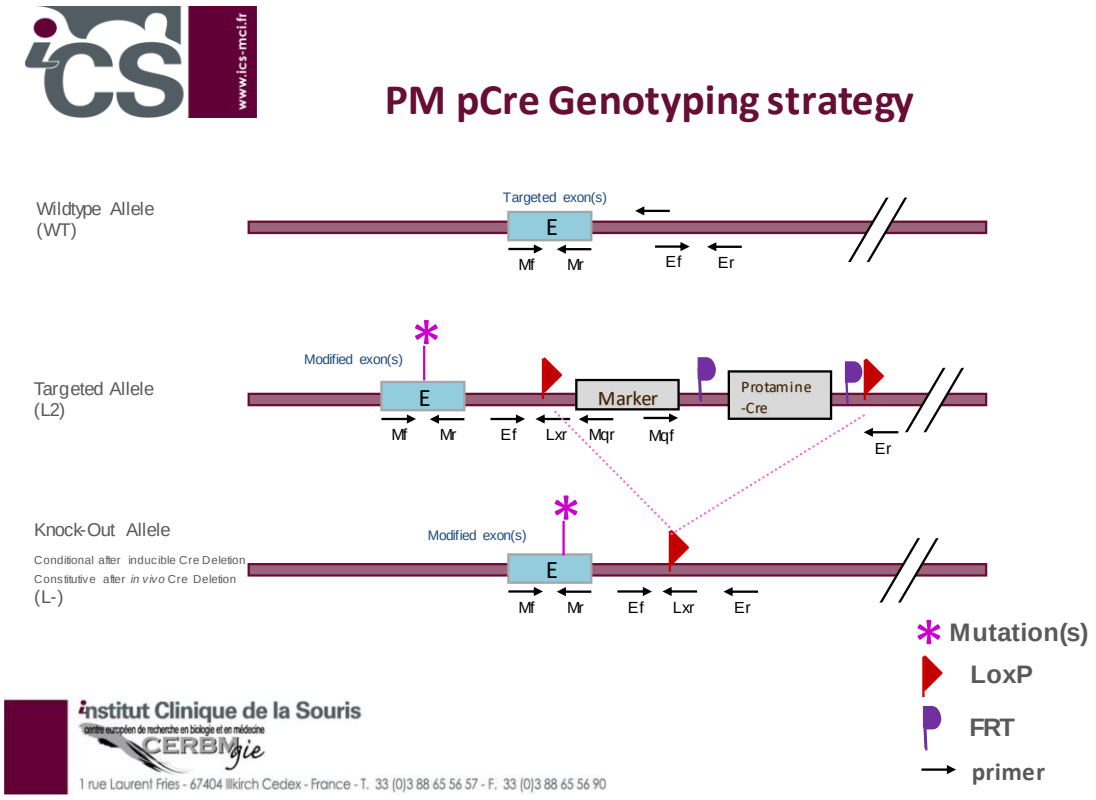
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the A385E Point mutation in **Fgfr3** 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	Sequence
Ef	GCTTCCCGCTTAAGCGACAGGTAAC
Er	AAAGGGCTAGCTGCTCGGACTTCTATA
Lxr	GAAGTTATACTAGAGCGGCCGTTCAC
Mf	CACTGGGGCCTGTGGTTCCG
Mq1f	GAAGAACGAGATCAGCAGCCTCTGTTCC
Mq1r	TGCTAAAGCGCATGCTCCAGACTGC
Mr	CTCTAGGAGACACGAGGCAGGACAG

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	329	329	329
Excision of the selection marker	Ef / Er	4492*	337	237
5' part of the selection marker	Ef / Mq1r	247	---	---
3' part of the selection marker	Mq1f / Er	327	---	---
LoxP specific PCR	Ef / Lxr	155	155	---

*: 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:

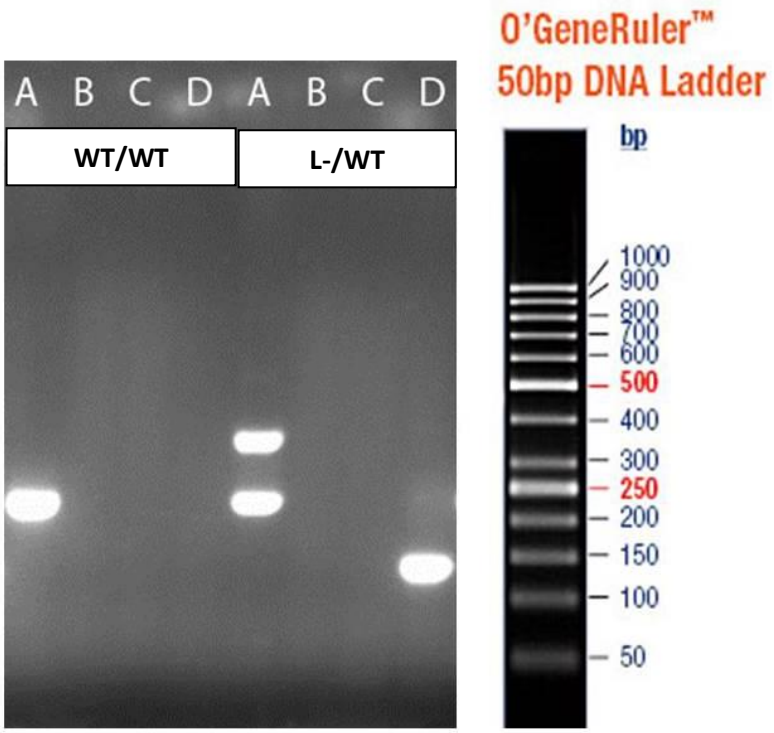
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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

1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was done by gel electrophoresis 2% agarose (SB buffer).

Representative genotyping picture



A: Excision of the selection marker
B: 5' part of the selection marker
C: 3' part of the selection marker
D: LoxP specific PCR

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.