Genotyping protocol

Slc17a8

IR00005760 / K5760

(ICS internal reference)

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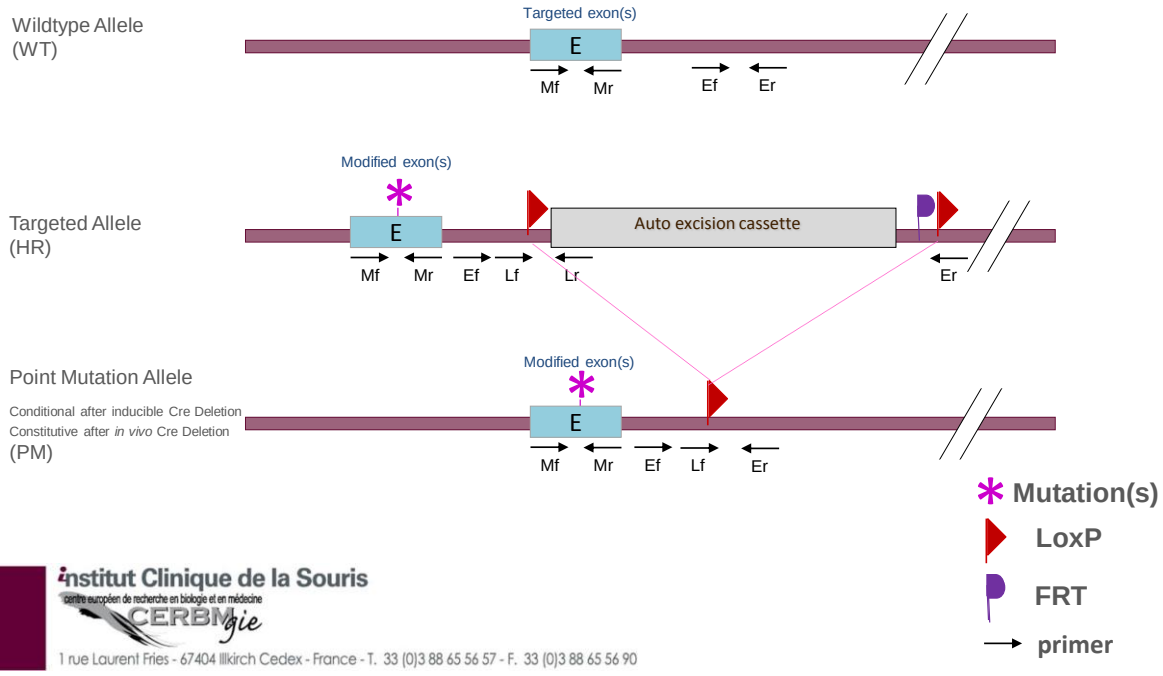
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Slc17a8** 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.

PM pCre Genotyping strategy



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Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	9000	TGTTAGGAATATCACTCACTGCTGGTGCTA
Er	9001	CGGTCTTGGAATTTTCCCACTGCTA
Lf	8998	GTGAACGGCCGCTCTAGTATAACTTCGTA
Lr	8999	CACCAAAGAACGGAGCCGGTT
Mf	8996	GAATTTCAAGTGTTCCTCCAGGGCAA
Mr	8997	TCCCACGGCATTCTTCACTCCTT

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	8996-8997	Mf / Mr	201	201	201
Presence of the distal loxP	8998-8999	Lf / Lr	212	---	---
Excision of the selection marker (with Betaine)	9000-9001 (with 0.5% Betaine)	Ef / Er	4626**	471*	392

*: 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.