Genotyping protocol

H132Qfs*21 PM in Zbtb24

Zbtb24$^{tm1.1Ics}$

IR00004994 / K4994

(ICS internal reference)

For any question, please contact:

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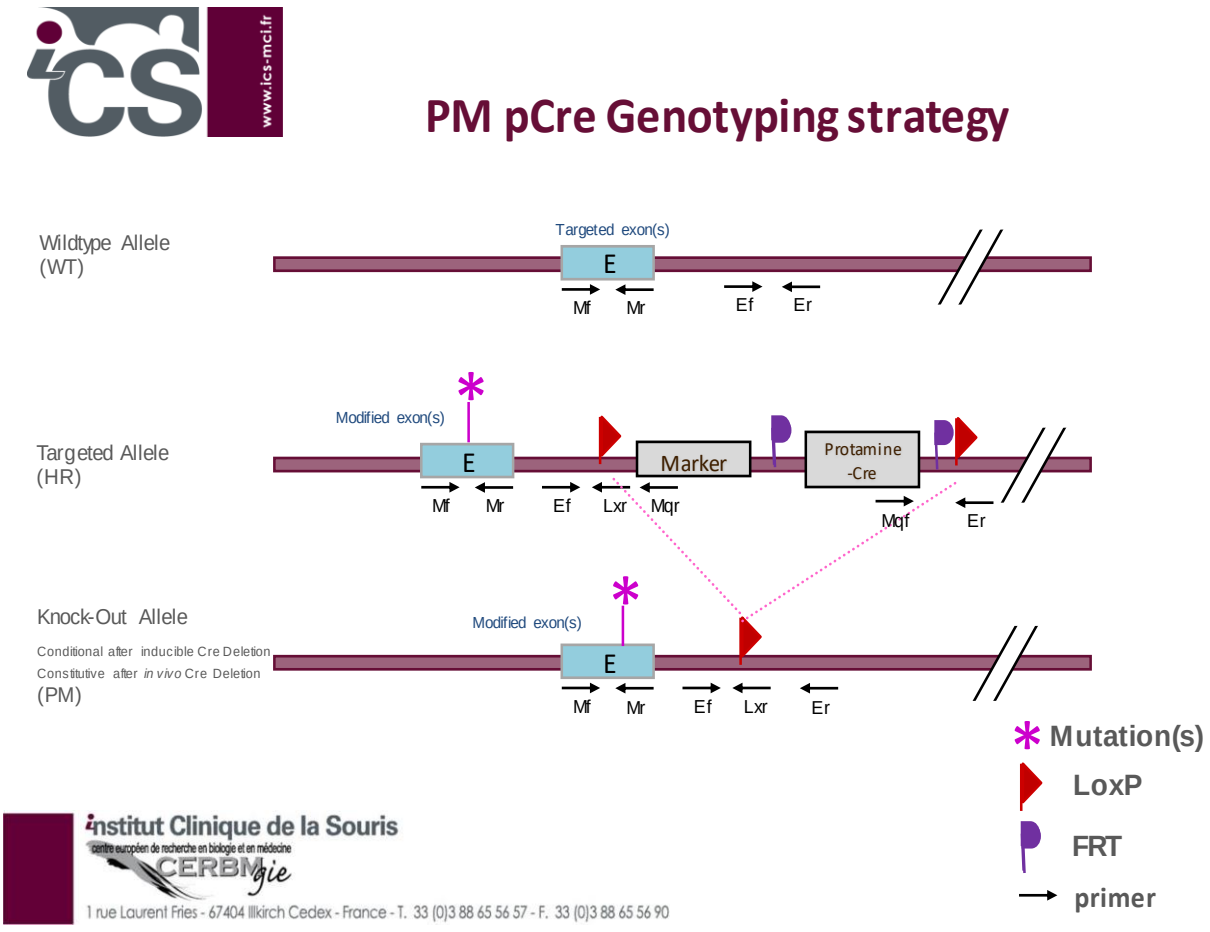
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the **Zbtb24$^{tm1.1Ics}$** mouse line.

1.1. Genotyping strategy

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



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Sequence of primers used for genotyping:

Position	Sequence
Ef	GTACTATCTCCAGTGTGCAGATGAGG
Er	GGCCAATGCCAAGTCAGAGACAC
Lxr	AGTTATACTAGAGCGGCCGTTCACCG
Mf	GGAAGGCATGGTTGCAGACACCTTTG
Mqf	CAGCTCATTCCTCCCACTCATGATC
Mqr	TGCTAAAGCGCATGCTCCAGACTGC
Mr	GCAAACCATTGGCTTTTCTTGGTCTTCC

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	276	276	278
Excision of the selection marker	Ef / Er	4447*	292	213
5' part of the selection marker	Ef / Mqr	271	---	---
3' part of the selection marker	Mqf / Er	383	---	---
LoxP specific PCR	Ef / Lxr	177	177	---

*: 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:

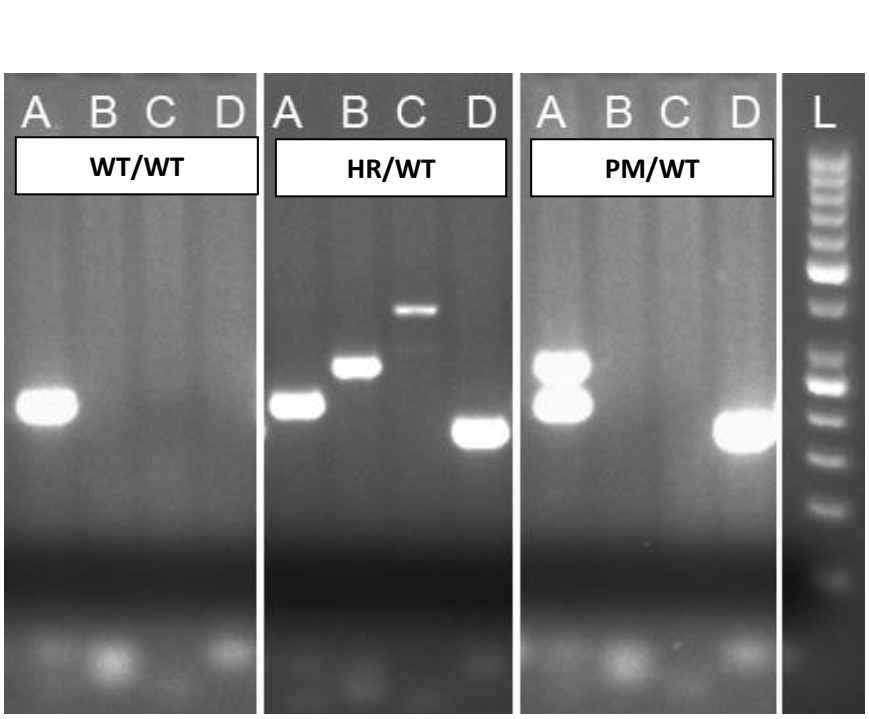
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

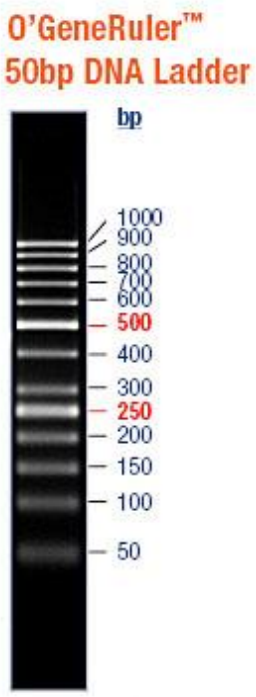
NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

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
L: 0'GeneRuler 50bp DAN Ladder

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.