Genotyping protocol

Conditional Bcl2l1-Mito

/ K5090

(ICS internal reference)

This report has been prepared by: **David Moulaert**

This report has been validated by: **Sylvie Jacquot, PhD, Head of Genotyping Service**

The first version of this report was generated the: 24 Feb 2016
The final version was corrected by Marie-Christine Birling (PhD), Head of Genetic Engineering, birlingm@igbmc.fr

For any question, please contact:

Institut Clinique de la Souris - ICS - Mouse Clinical Institute
1 rue Laurent Fries, BP 10142
67404 Illkirch Cedex, France
Email: mutagenesis@igbmc.fr
Web site: http://www-mci.u-strasbg.fr/

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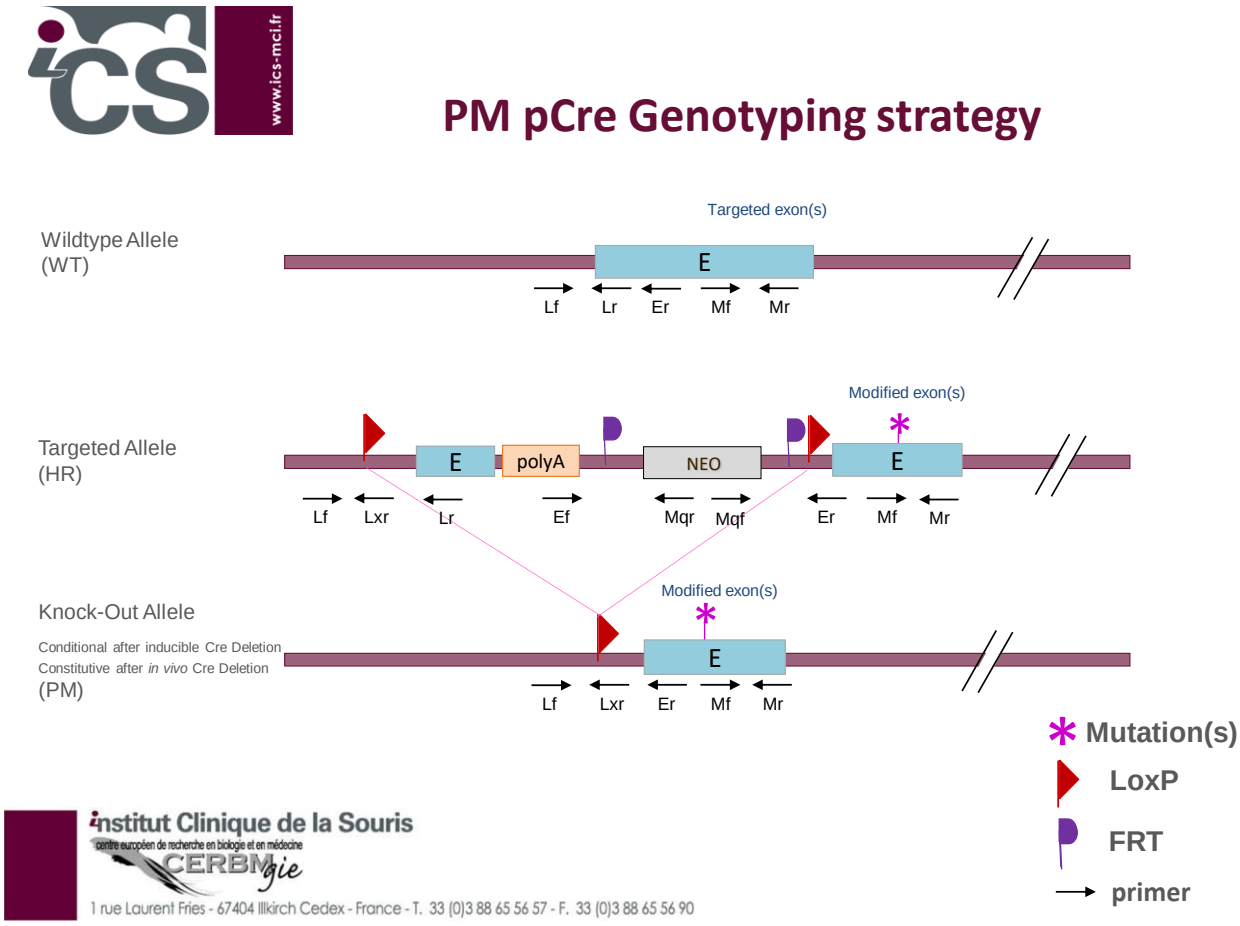
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **BCL2L1-RE cPM** Conditional Point mutation / Knockout (PM-cKO) project.

1.1. Genotyping strategy

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



institut Clinique de la Souris
centre européen de recherche en biologie et en médecine
CERBMgie
1 rue Laurent Fries - 67404 Illkirch Cedex - France - T. 33 (0)3 88 65 56 57 - F. 33 (0)3 88 65 56 90

Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	2687	CTGCATTCTAGTTGTGGTTTGTC
Er	8297	CAGACAGCTTGGAGCGAACACCAG
Er[2]	8298	GGTTAAAGGCTGACCTTCTGAAGCCC
Lf	8299	GAACATCCCAAAACCACTAACTGAGTGC
Lf[2]	8295	GTTTCCCCCACCTACTAGCCAGC
Lr	8296	GACAGCTTGGAGCGAACACCAGC
Lxr	4724	CGAAGTTATCTGCAGGTCGACCTTAAG
Mf	8293	CCCCATTCTGCCCATTTTGCTC
Mr	8294	CCCAACCCTGTGATAGGGCAAG
Mqf	1499	CCCACTTTGTGGTTCTAAGTACTGTG
Mqr	265	TGCTAAAGCGCATGCTCCAGACTGC

[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	KO allele	WildType allele
WildType / Mutated alleles	8293-8294	Mf / Mr	354	354	---	333
Presence of the distal loxP	8299-8296	Lf / Lr	277	277	---	198
Excision of the selection marker	2687-8297	Ef / Er	2105*	251	---	---
5' part of the selection marker	2687-265	Ef / Mqr	160	---	---	---
3' part of the selection marker	1499-8297	Mqf / Er	340	---	---	---
LoxP specific PCR	8295-4724	Lf[2] / Lxr	220	220	220	---
Excision of the floxed exon(s), i.e. knock out	8295-8297	Lf[2] / Er	345*	345*	361**	266*
Excision of the floxed exon(s), i.e. knock out 2	8299-8298	Lf / Er[2]	375	375	391**	296

*: 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.