



Genotyping protocol
Conditional BCL2L1-RE
/ K5088
(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: 19 Jan 2016
Corrected the 8 Jan 2025 by Marie-christine Birling (PhD) Head of Genetic Engineering and Genome Editing

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/>

TABLE OF CONTENTS

Table of contents 2

1. Genotyping protocol and data 2

 1.1. Genotyping strategy 2

 1.2. PCR protocol 4

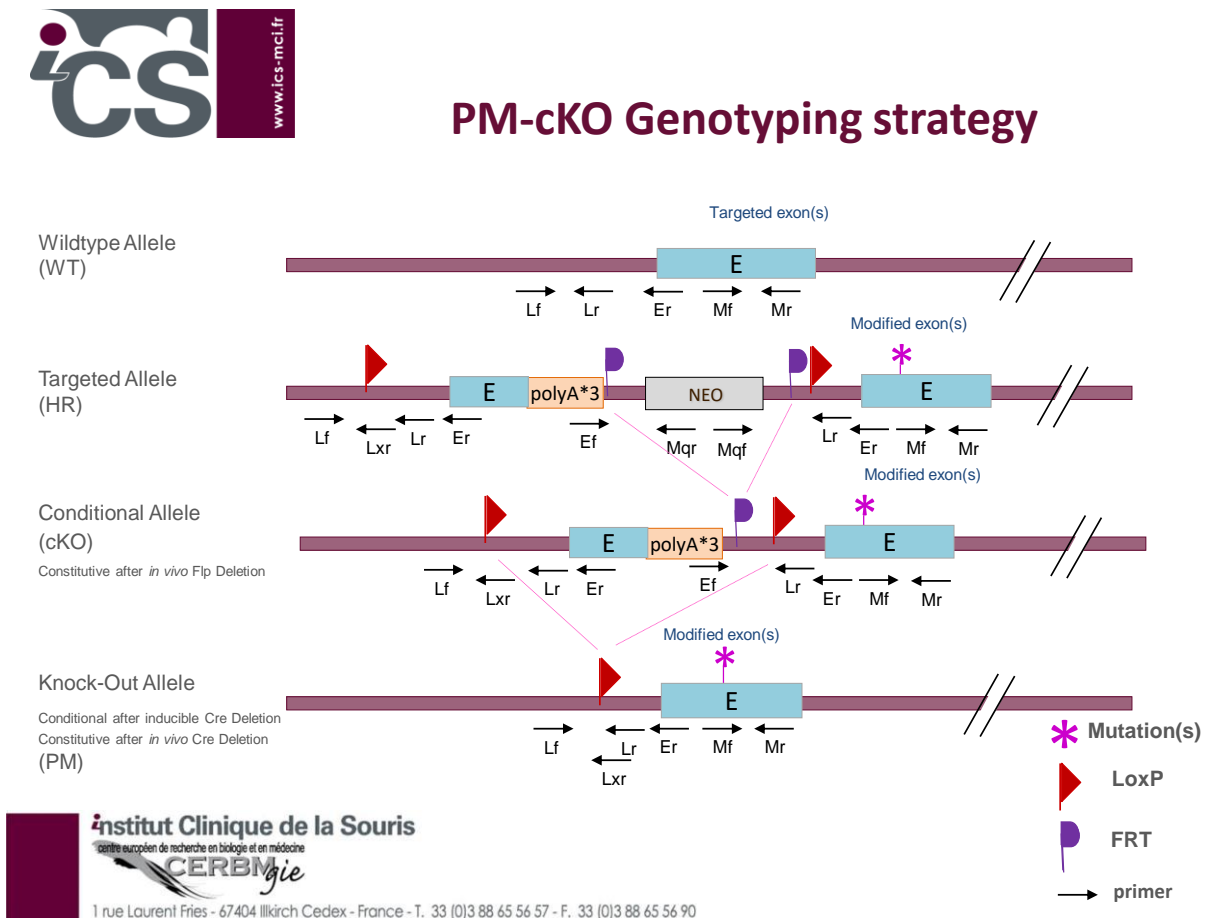
2. Cre and Flp genotyping method Erreur ! Signet non défini.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **BCL2L1-MITO 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.



Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	2687	CTGCATTCTAGTTGTGGTTTGTC
Er	8297	CAGACAGCTTGGAGCGAACACCAG
Er ²	8298	GGTTAAAGGCTGACCTTCTGAAGCCC
Lf	8299	GAACATCCCAAACCCTAAGTACTGAGTGC
Lf ²	8295	GTTTCCCCCACCTACTAGCCAGC
Lr	8296	GACAGCTTGGAGCGAACACCAGC
Lxr	4724	CGAAGTTATCTGCAGGTCGACCTTAAG
Mf	8293	CCCCATTCTGCCATTTTGCTC
Mr	8294	CCCAACCCTGTGATAGGGCAAG
Mqf	1499	CCCACTTTGTGGTTCTAAGTACTGTG
Mqr	238	TGACTAGGGGAGGAGTAGAAGGTG

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	381	381	---	333
Presence of the distal loxP	8299-8296	Lf / Lr	277	277	---	198
Excision of the selection marker	2687-8297	Ef / Er	2105*	251 (505-759***)	---	---
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 1	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

***: size of the others products

---: 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 ₂ O	up to 15 µl

Cycling conditions:

Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
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.