



Genotyping protocol

Bmp10 tm1a, tm1b and tm1c

IR00004990 / P4990

(ICS internal reference)

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

 1.3. Picture of genotyping with various alleles **Erreur ! Signet non défini.**

2. Cre and Flp genotyping method 5

1. Genotyping protocol and data

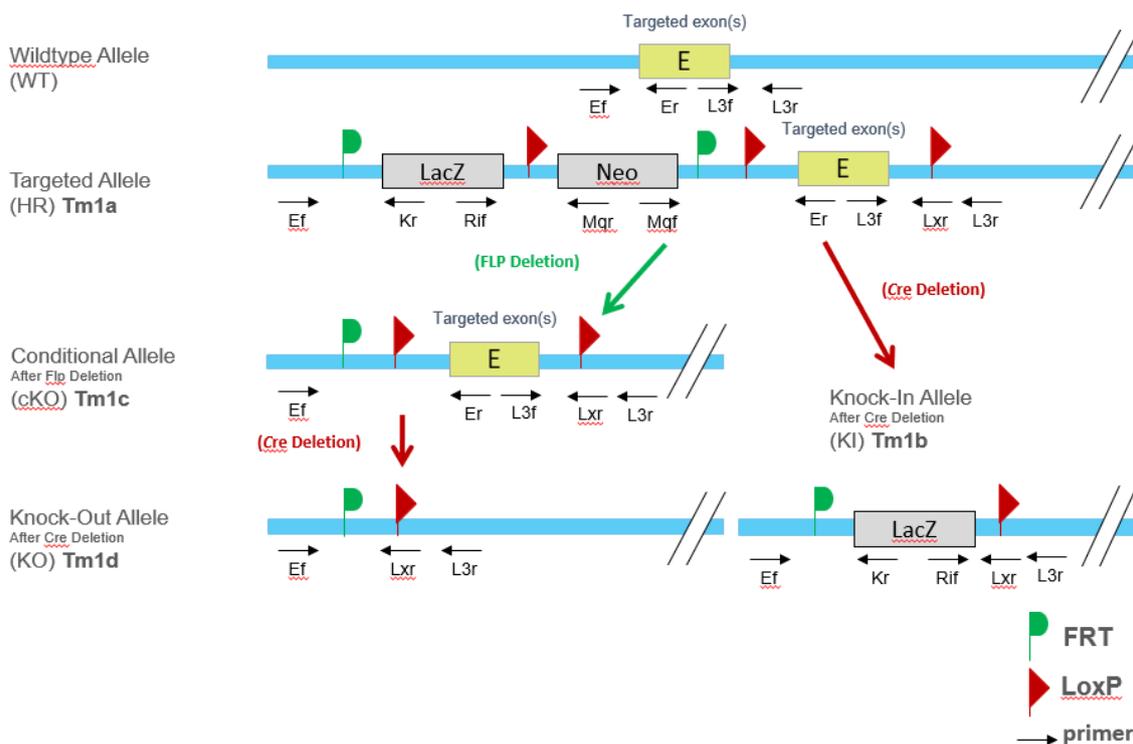
This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the different alleles of the **Bmp10** EUCOMM mouse line.

1.1. Genotyping strategy

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



Genotyping strategy



Sequence of primers used for genotyping:

Position	Sequence
Ef	GGAGAGCACTGCCTTACAAACATAAGC
Er	CCCCTTGATGATAATGGACTGAACC
Kr	CCAACAGCTTCCCCACAACGG
L3f	GGTTGCCACCTTAGGGAACTGTG
L3r	CATTCTGGAGGAAGCCACTCACATG
Lxr	ACTGATGGCGAGCTCAGACCATAAC
Rif	GCACATGGCTGAATATCGACGGT

PCR fragments expected size (bp):

Region analyzed	Position on the primer (see the map above)	Targeted allele (HR)	conditional allele (cKO)	KI allele	WildType allele
5' part of the selection marker	Ef / Kr	356	---	---	---
Presence of the distal loxP	L3f / L3r	355	355	---	284
Distal loxP specific PCR	L3f / Lxr	192	192	---	---
Excision of the selection marker	Ef / Er	7344*	440	---	287
Cre total excision	Rif / Lxr	4057*	---	471	---

*: 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 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.

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.