

## Genotyping protocol

Project R553\* PM in Cftr

Cftr<sup>tm1.1(R553\*)Ics</sup>

(PHENOMIN-ICS reference IR00006344 / Kos6344)

For any question, please contact:

PHENOMIN-ICS

Email: [mutagenesisgbmc.fr](mailto:mutagenesisgbmc.fr)

Web site: <http://www.ics-mci.fr/>

## Table of contents

1. Genotyping protocol and data .....	2
1.1. Genotyping strategy .....	2
1.2. PCR protocol .....	4



## 1. Genotyping protocol and data

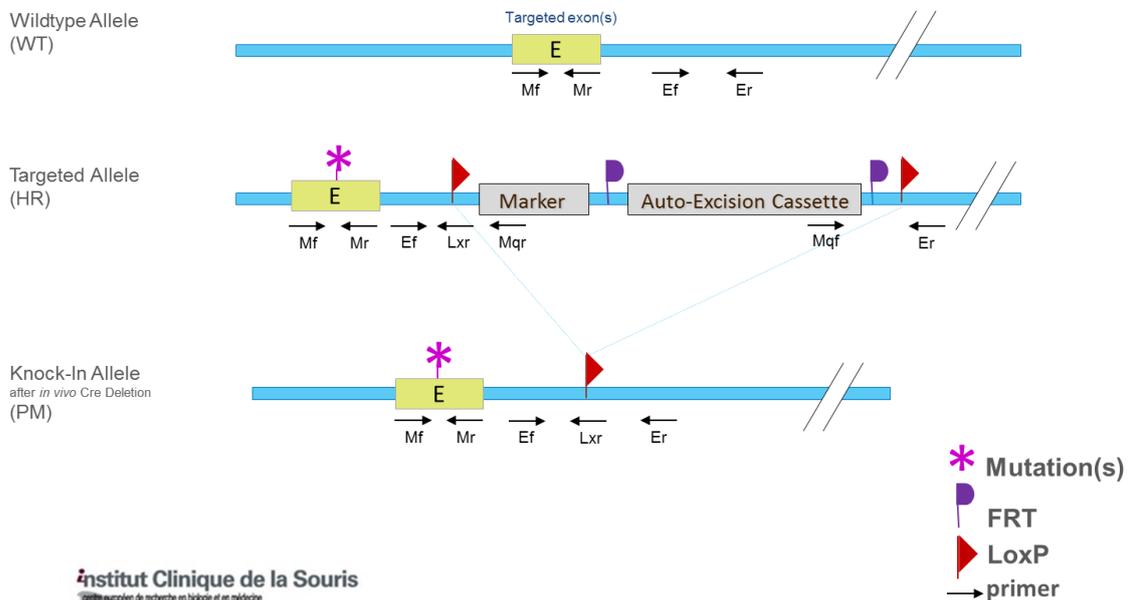
This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the R553\* Point mutation in **Cftr** project.

### 1.1. Genotyping strategy

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



### PM Genotyping strategy



Sequence of primers used for genotyping:

Position	Sequence
Ef	CTTCCTGTTCTGTCTCTGGAATTGATGC
Ef <sup>2</sup>	CTGTGAGCACTTGCTGTAAATACTATGGG
Er	CATCTGGGGACAGGGAAAAAGGG
Er <sup>2</sup>	CAAATCAGAGGCATCTGGGGACAGG
Lxr	AGTTATACTAGAGCGGCCGTTACCG
Mf	GCACTTGAGTTTATATGATGGGCTTATGGG
Mqf	GAAGAACGAGATCAGCAGCCTCTGTTCC
Mqr	TGCTAAAGCGCATGCTCCAGACTGC
Mr	CATCAATTCCAGAGACAGAACAGGAAGC

<sup>2</sup>: for a selected position, a second primer was designed

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 (with Betaine)	Mf / Mr	350	350	350
Excision of the selection marker (with Betaine)	Ef / Er	4491*	341**	257
Excision of the selection marker 2 (with Betaine)	Ef <sup>2</sup> / Er <sup>2</sup>	4563*	413**	329
5' part of the selection marker (with Betaine)	Ef <sup>2</sup> / Mqr	332	---	---
3' part of the selection marker	Mqf / Er <sup>2</sup>	313	---	---
LoxP specific PCR (with Betaine)	Ef / Lxr	178	178	---

\*: 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 <sub>2</sub> 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, Héroult Y, Pavlovic G.

Genesis. 2012 Jun;50(6):482-9. doi: 10.1002/dvg.20826. Epub 2012 Mar 20.

