

## Genotyping protocol

Project Tmprss6 cKO

Tmprss6<sup>tm1c(EUCOMM)Wtsi</sup>

(PHENOMIN-ICS reference IR00005806/ P5806)

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## Table of contents

<b>1. PCR Genotyping protocol.....</b>	<b>3</b>
1.1. Genotyping strategy .....	3
1.2. PCR protocol .....	4
<b>3. Cre and Flp genotyping method .....</b>	<b>5</b>



## 1. PCR Genotyping protocol

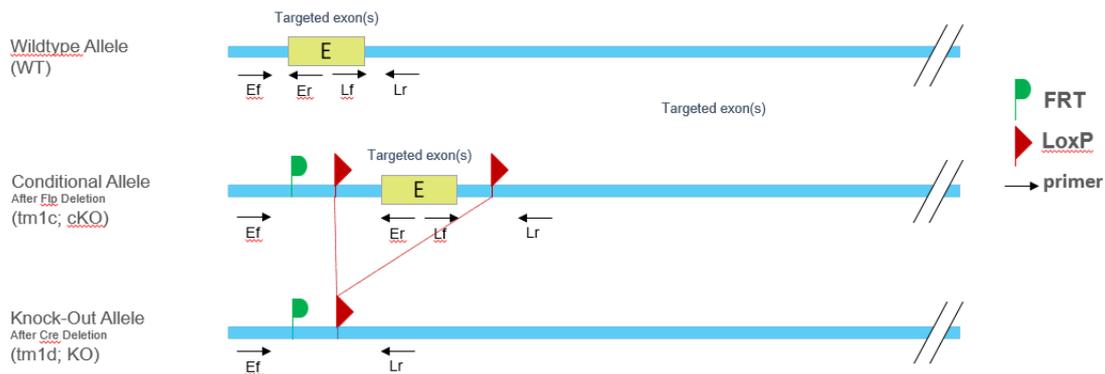
This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Tmprss6** Conditional Knockout allele.

### 1.1. Genotyping strategy

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



### cKO Genotyping strategy



#### Sequence of primers used for genotyping:

Position	Sequence
Ef	CTCAGCTCTCATTTCCTTCAGCATC
Er	GGAACGAGAGATTGTAGGGACTGGC
Lf	GTCCTGGGTACCATGGGAAAGGAC
Lr	ATGCCTACAGCATTGCTTCTAGCAGC

#### PCR fragments expected size (bp):

Region analyzed	Position on the primer (see the map above)	conditional allele (cKO; tm1c)	KO allele (KO; tm1d)	WildType allele
5' LoxP	Ef / Er	438	---	243
3' LoxP	Lf / Lr	272	---	251
KO	EF/Lr	---	368	1246*

\*: this PCR product will not be observed using our PCR genotyping conditions (see description below)  
 ---: 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, Hérault Y, Pavlovic G.

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

