EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Tmprss6 EUCOMM alleles

Tmprss6tm1a(EUCOMM)Wtsi

Tmprss6tm1b(EUCOMM)Wtsi

(PHENOMIN-ICS reference IR00005806/ P5806)

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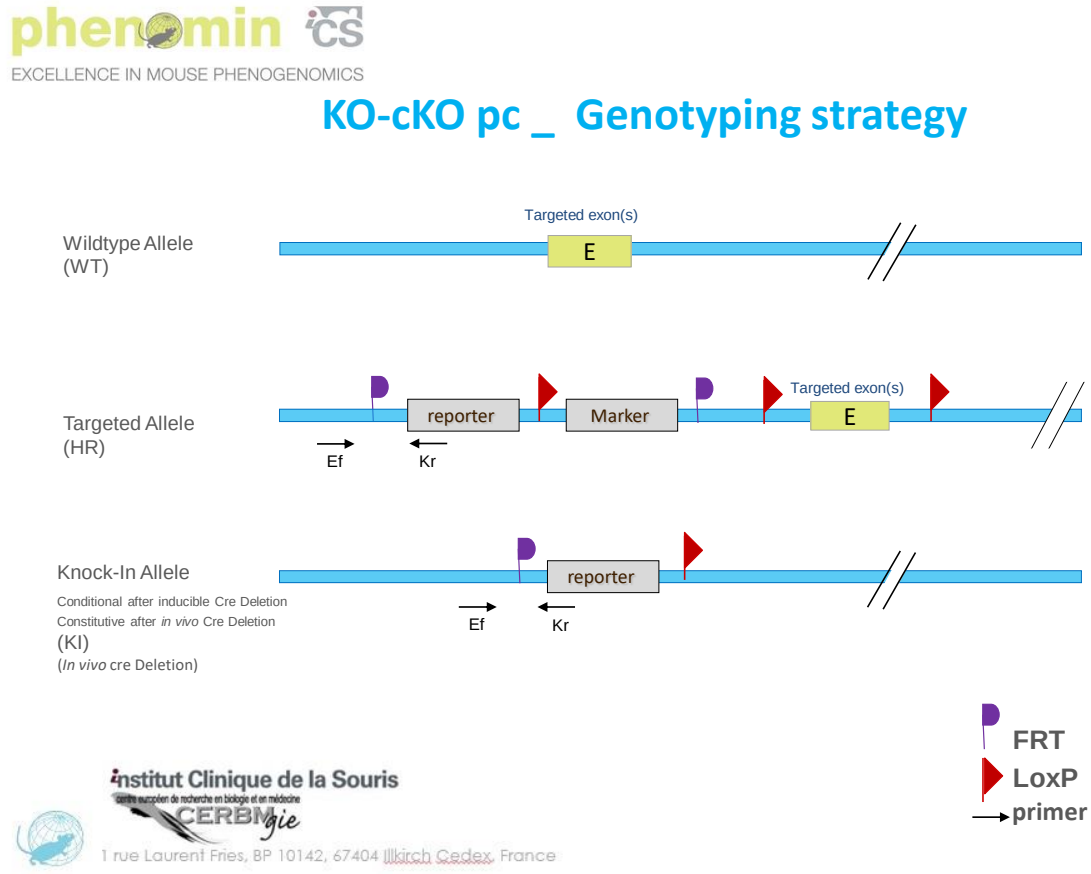
Table of contents

1. PCR Genotyping protocol

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the **Tmprss6** Constitutive Knockout and Conditional Knockout mouse models.

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	Sequence
Ef	CTCAGCTCTCATTCCTTCAGCATC
Kr	CCAACAGCTTCCCCACAACGG

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	302	---	302	---

*: 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_2O	up to 15 µl

Cycling conditions:

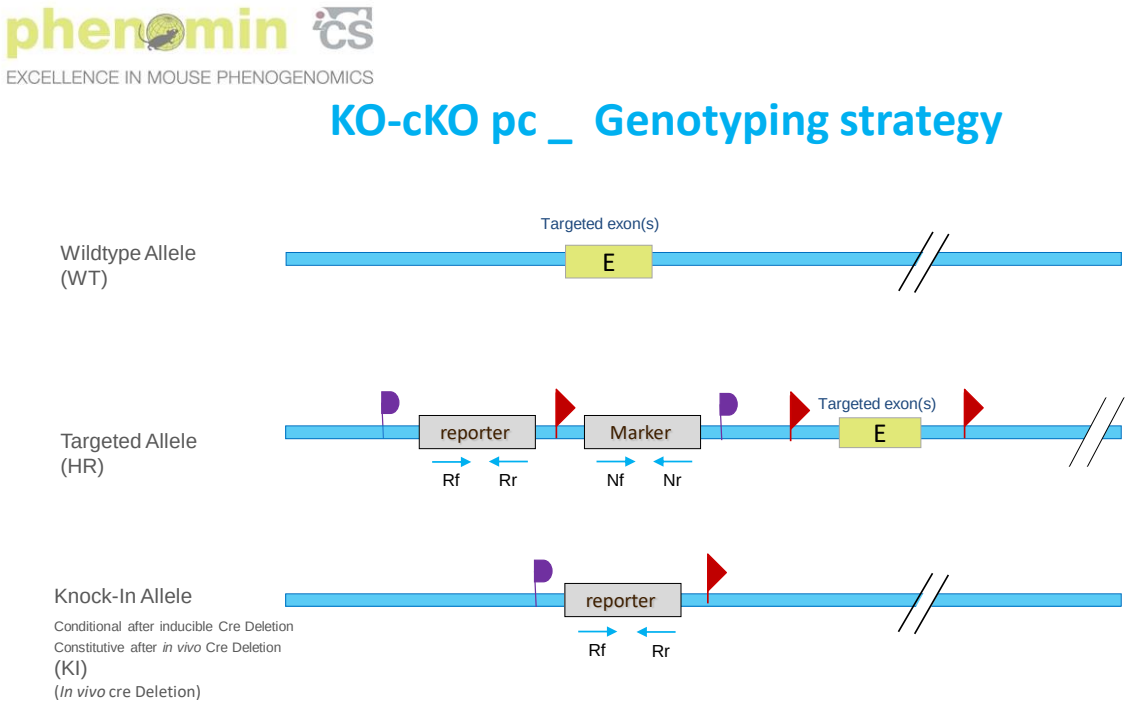
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

2. qPCR Genotyping strategy

2.1. 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	Sequence
Rf	TTATCAGCCGGAAAACCTACC
Rr	CTCGCCACTTCAACATCAAC
Nf	TGAATGAACTGCAGGACGAG
Nr	TTCCCGCTTCAGTGACAAC

Region analyzed	Position on the primer *(see the map above)*	TM +/-1 (°C)	Expected size (bp)
Reporter LacZ	Rf / Rr	80	73
Neomycine Marker	Nf / Nr	88.5	96

---: no Amplicon should be obtained

2.2. qPCR protocol

This section describes the composition of the mix and cycling conditions used for genotyping.

Reagents:	Volume:
- EvaGreen Master (Biorad)	3.5µl
- DNA (10ng/µl)	3µl
- Forward primer (100 µM)	0.06µl
- Reverse primer (100 µM)	0.06µl
- Sterile H2O	up to 7µl

Cycling conditions:

Temp	Time	#Cycles
95°C	10min	1
94°C	5s	
62°C	10s	34
72°C	15min	
Melting Curve analysis		
65°C -> 95°C		

Follow manufacturer's protocol for programming the data acquisition of dsDNA product.

Important note: these qPCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

3. 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.