EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Tmem176a and Tmem176b double cKO

(PHENOMIN-ICS reference IR00005004 / K5004)

(PHENOMIN-ICS reference IR00005212 / K5212)

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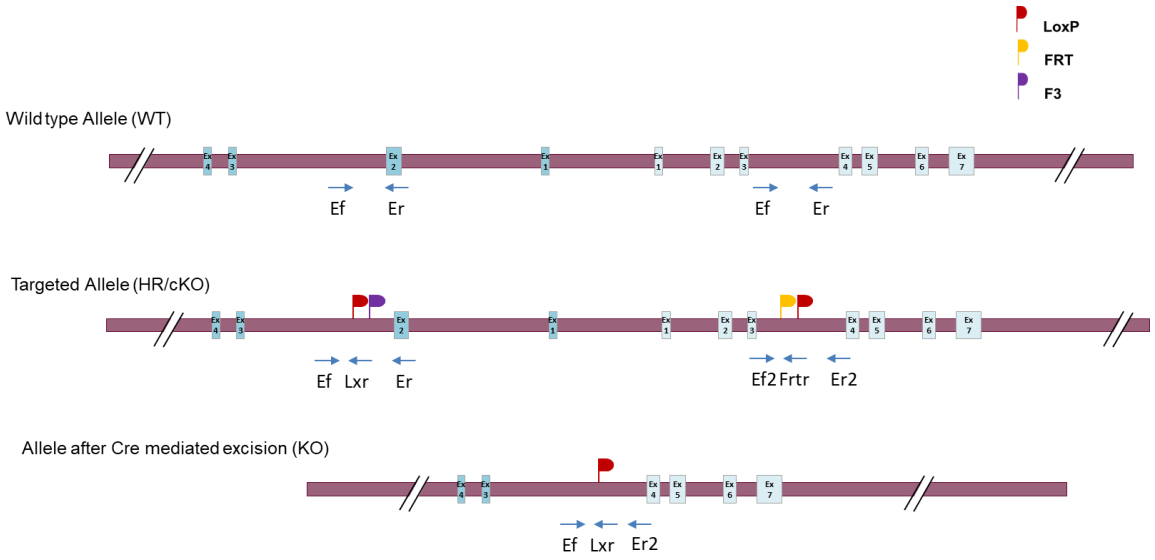
1. Genotyping protocol and data

1.1. Genotyping strategy

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Tmem176a and Tmem176b double cKO** Conventional or Constitutive Knockout (KO) project.

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



Sequence of primers used for genotyping:

Position	Sequence
Ef2	ATGGCGGTCCCTTTGCTTCC
Er2	TGCACTCCGCTTTCCCTGTCTT
Frtr	GAGAATAGGAACTTCCACCGGTG
Ef	CTGTTCCTGGCACTCCTTTACCTTGG
Er	GCTGCAGATGCAAGACAACACTAACCA
Lxr	GTATAGCATACATTATACGAAGTTATCTGCAG

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	cKO allele	WildType allele	KO allele
Excision of the selection marker	Ef2 / Er2	402	280	---
FRT specific PCR	Ef2 / Frtr	257	---	---
Excision of the selection marker	Ef / Er	385	238	---
LoxP specific PCR	Ef / Lxr	274	---	---

KO	Ef / Er2	---	---	366*

---: no Amplicon should be obtained

* Not tested

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.