Genotyping protocol

Pth<tm2(EGFP/Cre/ERT2)Wtsi>/Ics

ICS code: IR5326 / E5326

(ICS internal reference)

For any question, please contact:

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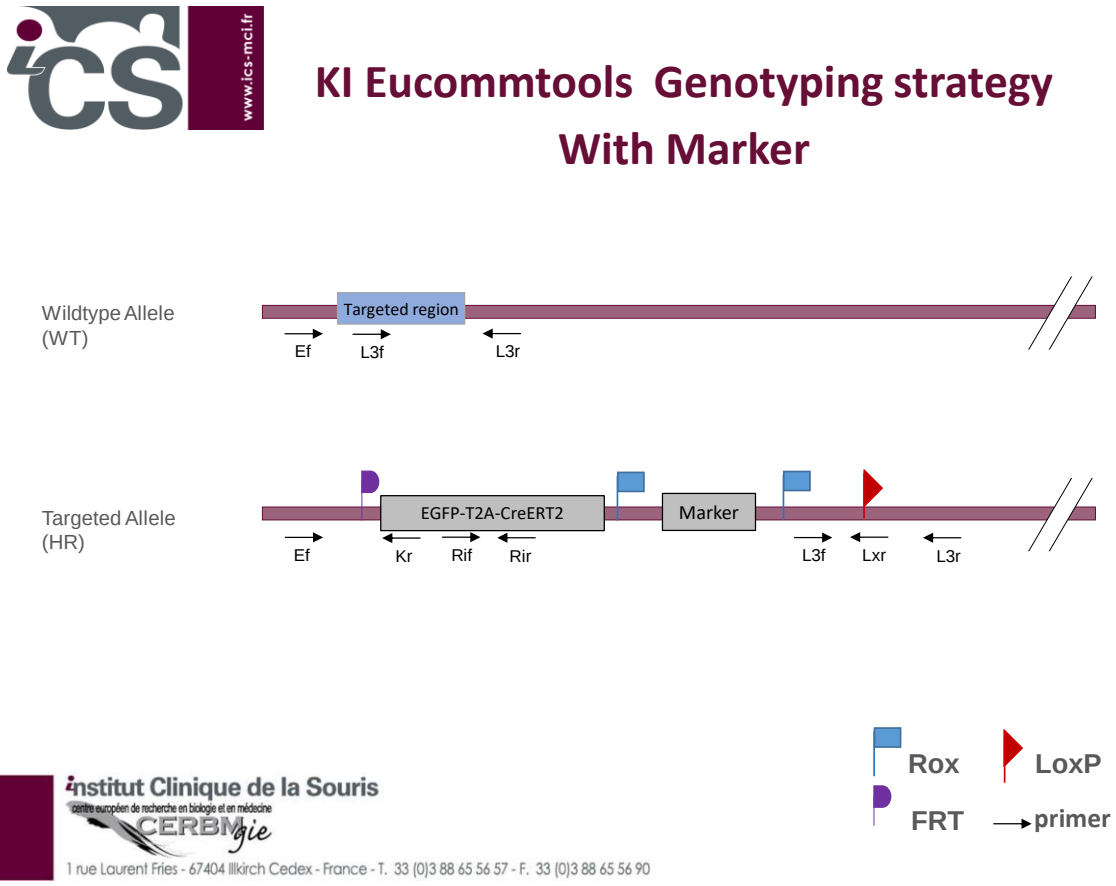
1. PCR Genotyping protocol

This section describes the condition used at the Institut Clinique de la Souris (ICS) to genotype your **Pth** Knockin (KI E-Tool marker) project.

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	GCTAGCATCTGAAAACAAGCTTAGCCAT
Kr	GGGCAAGAACATAAAGTGACCCTCC
L3f	GTGAGGGAGACAAAGCTGATGTGGAT
L3r	CCAACCCTCCAACTGAGACAAAGTTTTA
Lxr	ACTGATGGCGAGCTCAGACCATAAC
Rif	CGACCACTACCAGCAGAACACC
Rir	GGTTCTTGCGAACCTCATCACTCGT

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	WildType allele
Internal reporter	Rif / Rir	317	---
5' Exogenous/cDNA specific PCR	Ef / Kr	413	---
Presence of the distal loxP	L3f / L3r	420	340
Distal loxP specific PCR	L3f / Lxr	227	---

---: 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	35
72°C	1min	
72°C	7min	1
14°C	---	---

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

2. Recommended papers:

2.1. Cre and Flp genotyping method

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.

2.1. Tips and tricks for optimizing your PCR genotyping procedures

Optimizing PCR for mouse genotyping: Recommendations for reliable, rapid, cost effective, robust and adaptable to high-throughput genotyping protocol for any type of mutation.
Jacquot, S, Chartoire, N, Piguet, F, Herault, Y, Pavlovic, G. (2019).
Current Protocols in Mouse Biology, 9, e65. doi: 10.1002/cpmo.65
Free copy of this paper can be accessed online though this link http://bit.ly/2sxxWvO