Genotyping protocol

Msln- CreERT2_EGFP

IR00004725b / E4725b

(ICS internal reference)

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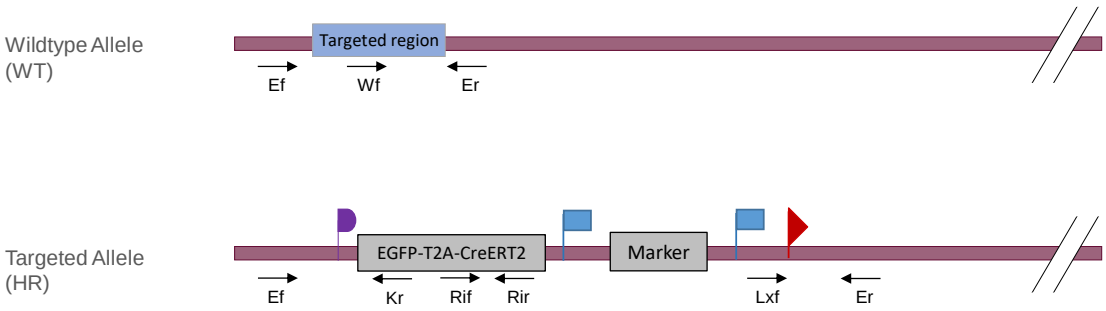
1. PCR Genotyping protocol

This protocol describes the condition used at the Institut Clinique de la Souris (ICS) to genotype your **Msln** Eucommtools Knockin (KI E-Tool markerless) project.

1.1. Genotyping strategy

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

KI Eucommtools Genotyping strategy With Marker



Rox LoxP FRT primer

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Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	7457	CTTAGCTCTCTCTACACCCAGTGGGC
Er	7458	CTGACAAAAGGCTCTTCGTCCCAC
Kr	3278	GGGCAAGAACATAAAGTGACCCTCC
Lxf	6956	TTAGGCGCGCCATAACTTC
Rif	2344	CGACCACTACCAGCAGAACACC
Rir	5626	GGTTCTTGCGAACCTCATCACTCGT
Wf	7459	AGTCATGAGTGGGACTGTGTTGTGCTA

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	WildType allele
Internal reporter	2344-5626	Rif / Rir	317	---
WildType allele specific PCR (3' part of the targeted locus)	7459-7458	Wf / Er	---	282
5' Exogenous/cDNA specific PCR	7457-3278	Ef / Kr	329	---
Rox/LoxP specific PCR	6956-7458	Lxf / Er	182	---

---: 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