EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Il-36α reporter (mScarlet)-tagged insertion with conditional potential mouse line

Il36a$^{tm1.1Ics}$

(PHENOMIN-ICS reference IR00007277 / Ros7277)

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Table of contents

1. PCR Genotyping protocol 2
1.1. Genotyping strategy 2
1.2. PCR protocol 4
2. Recommended papers: 5
2.1. Cre and Flp genotyping method 5
2.1. Tips and tricks for optimizing your PCR genotyping procedures 5

This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Il-36α reporter (mScarlet)-tagged insertion with conditional potential** mouse line.

1. PCR Genotyping protocol

1.1. Genotyping strategy

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

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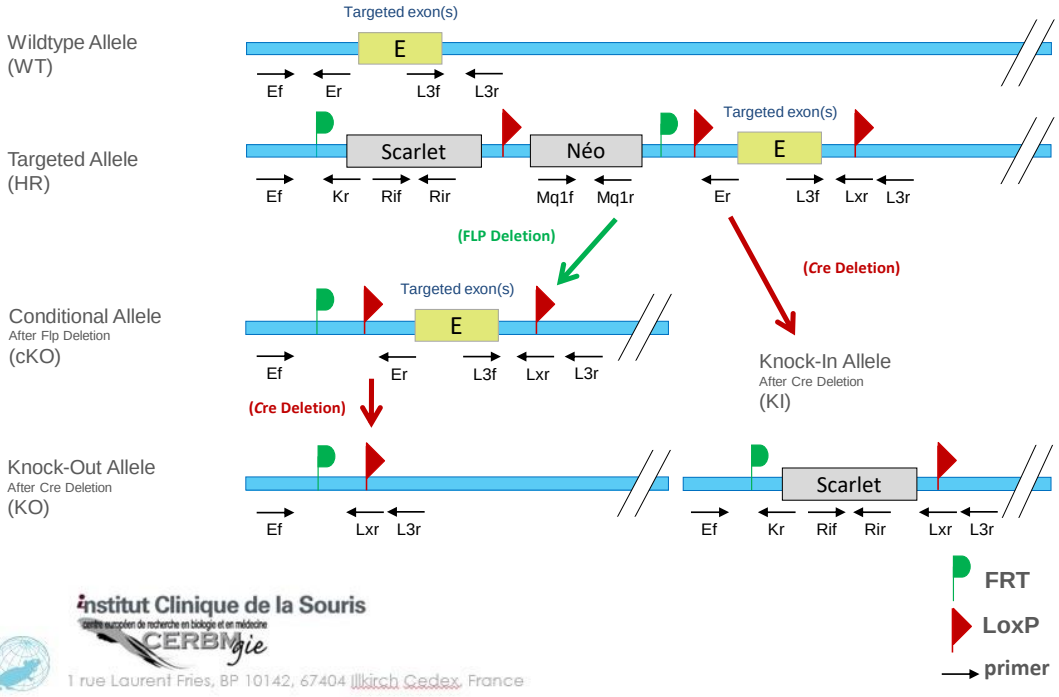
KI-cKO Genotyping strategy



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Sequence of primers used for genotyping:

Position	Sequence
Ef	GTCTTAGACAACCAAGGGATGGGTC
Ef[2]	GGGTCAAGGAAAAAGGAGGTAGG
Er	CTGTCACTGCCACACACCAGTG
Kr	CTCCTACATAGTTGGCAGTGTTTGGG
L3f	GTGAGCACATGTGTGTATAAATAAGTCAG
L3f[2]	GGGGATCCCACGTACATGGG
L3r	GGAGGGGAATCGAGGAAAGAGG
L3r[2]	GGTTTTCGGAGGGGAATCGAGG
Lxr	CCTGCAGGATAACTTCGTATAGCATAC

[2]: for a selected position, a second primer was designed

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	cKO allele	KO allele	KI allele	WildType allele
5' part of the synthetic cassette	Ef / Kr	169	---	---	169	---
Excision of the synthetic cassette	Ef / Er	4124*	417**	---	---	224
Presence of the distal loxP	L3f / L3r	219	219	---	---	156
Distal loxP specific PCR	L3f[2] / Lxr	412	412	---	---	---
Excision of the floxed exon(s), i.e. knock out	Ef[2] / L3r[2]	4815*	1108*	354**	2338*	852
Excision of the floxed exon(s), i.e. knock out 2	Ef / L3r	4828*	1121*	367**	2351*	865

*: this PCR product will not be observed using our PCR genotyping conditions (see description below)
**: this PCR is only verified if mice are generated
---: 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