Genotyping protocol

Project pCAG-Lox-STOP-Lox-Nr3c2 in Rosa

Gt(ROSA)26Sor$^{tm25(CAG-Nr3c2)Ics}$/Ics

(PHENOMIN-ICS reference IR00007177 / Kos7177)

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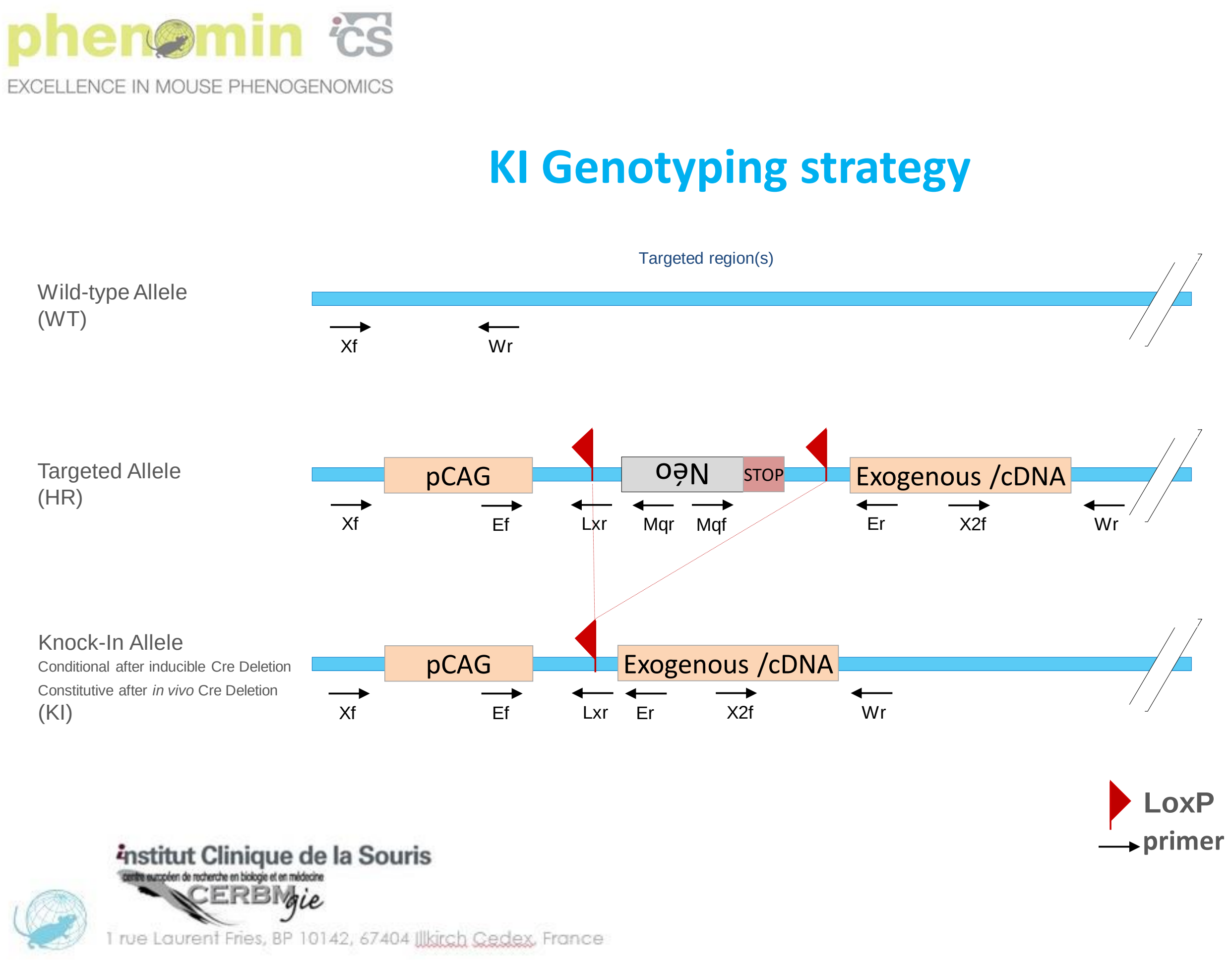
This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **pCAG-Lox-STOP-Lox-Nr3c2 in Rosa** Knockin (KI) project.

1. PCR Genotyping protocol

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	GGCTCTAGAGCCTCTGCTAACC
Ef[2]	CTAGAGCCTCTGCTAACCATGTTC
Er	GCTCCAAGGTCTGAGACACTTGAC
Er[2]	CAAGAGAAGAACGCTCCAAGGTC
Lxr	CGAGGAATTCTTTGCCAAAATGATGAG
Mq1f	CTGCATTCTAGTTGTGGTTTGTC
Mq1r	CCTTCTTGACGAGTTCTTCTGAGGG
Wr	GTTCAATTCCCCTGCAGGACAAC
Xf	GGGGAGGGGAGTGTTGCAATAC
X2f	CTTTCACAGGAAGTGAGGCGCG

[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)	KI allele	Wild-Type allele
Wild-Type allele specific PCR (5' part of the targeted locus)	Xf / Wr	7935*	5430*	203
Excision of the selection marker	Ef / Er	2755*	250**	---
Excision of the selection marker 2	Ef[2] / Er[2]	2763*	258**	---
5' part of the selection marker	Ef / Mq1r	236	---	---
3' part of the selection marker (with Betaine)	Mq1f / Er[2]	219	---	---
Exogenous/cDNA specific PCR	X2f / Wr	464	464	---
LoxP specific PCR	Ef / Lxr	109	109	---

*: 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

If mentioned in table “PCR fragments expected size” add 0.5% of Betaine in the reaction mix

Cycling conditions:

Temp	Time	#Cycles
95°C	4min	1
94°C	30s	35
62°C	30s	
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