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Genotyping protocol

Project pCAG-lox-STOP-lox-TFE3-FRT-IRES-eGFP-FRT in Rosa

Gt(ROSA)26Sor$^{tm29(CAG-TFE3,-EGFP)Ics}$/Ics

(PHENOMIN-ICS reference IR00007474 / Kos7474)

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This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **pCAG-lox-STOP-lox-TFE3-FRT-IRES-eGFP-FRT** 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.

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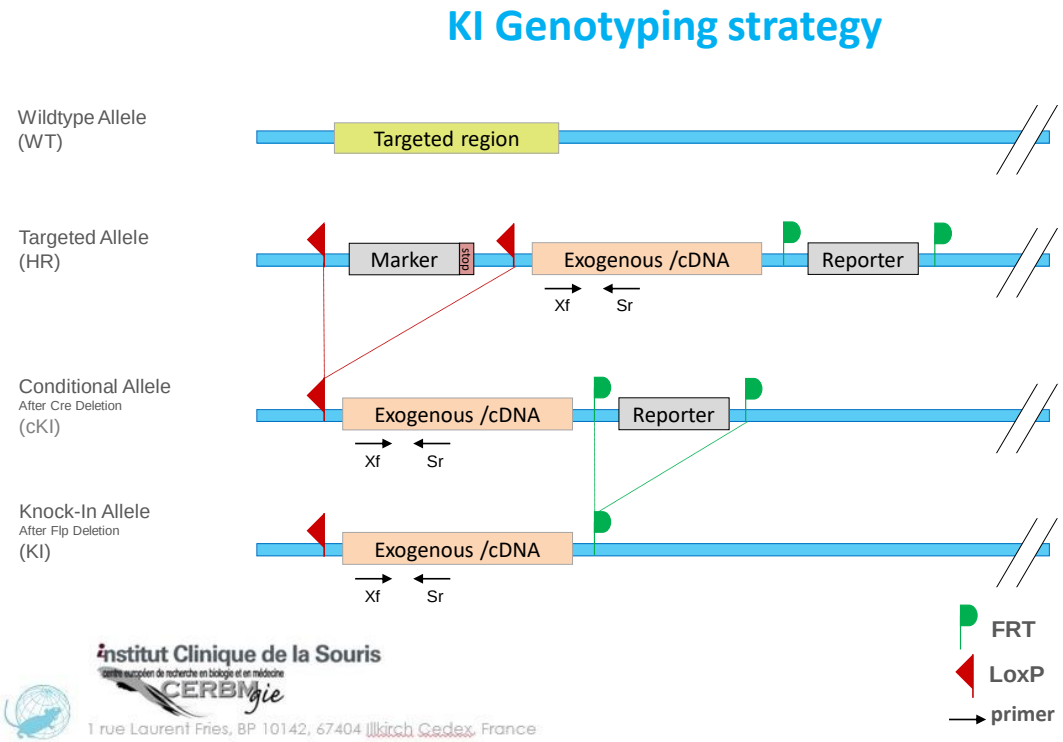

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Sequence of primers used for genotyping:

Position	Primers	Sequence
Xf	10346	CTCCAGGGCTGCTTTCCTTGG
Sr	10347	CATGGAACGTTGCTGCGCC

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	cKI allele	KI allele	WildType allele
Exogenous/cDNA specific PCR	10346-10347	Xf / Sr	105	105	105	---

---: 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. qPCR Genotyping protocol

2.1. Genotyping strategy

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

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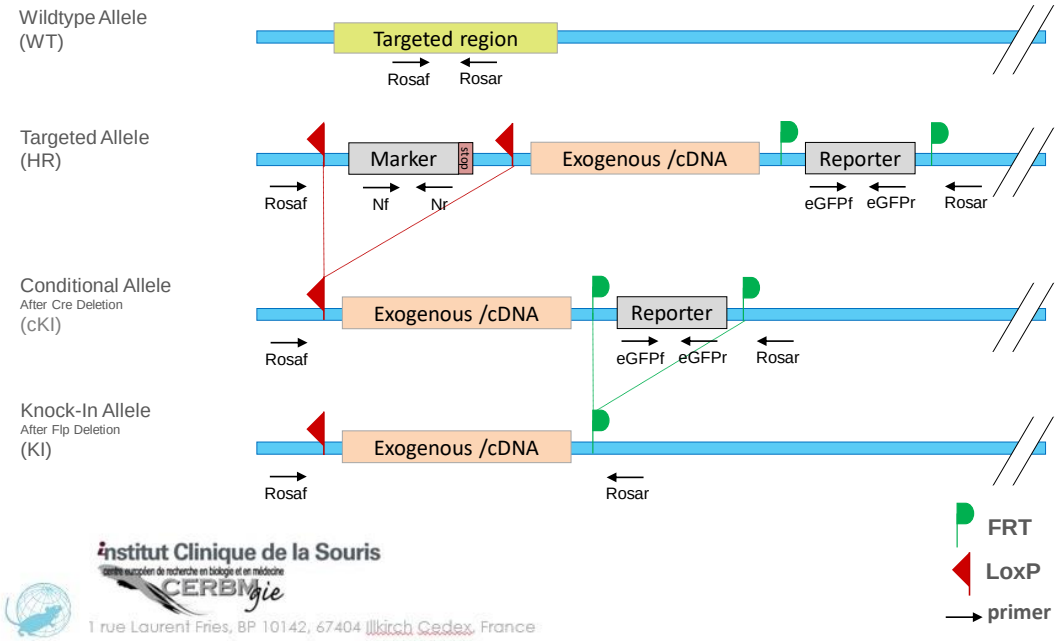
KI Genotyping strategy



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Sequence of primers used for genotyping:

Position	Primers	Sequence
eGFPf	eGFP f1	GACAACCACTACCTGAGCAC
eGFPr	eGFP r1	CAGGACCATGTGATCGCG
Nf	Neo f1	TGAATGAACTGCAGGACGAG
Nr	Neo r1	TTCCCGCTTCAGTGACAAC
Rosaf	Rosa f1	CCCTCTTCCCTCGTGATCT
Rosar	Rosa r1	CACACACCAGGTTAGCCTTTA

qPCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	cKI allele	KI allele	WildType allele
eGFP qPCR	eGFP f1- eGFP r1	eGFPf/ eGFPr	72	72	---	---
Neomycine qPCR	Neo f1 – Neo r1	Nf / Nr	96	---	---	---
Rosa qPCR	Rosa f1 – Rosa r1	Rosaf / Rosar	8079*	5574*	4181*	88

*: this amplicon will not be observed using our genotyping conditions (see description below)
---: no Amplicon should be obtained

2.2. qPCR protocol

Reagents:	Volume:
- EvaGreen (biorad)	3,5µl
- DNA (10ng/µl)	3µl
- Forward primer (100µM)	0,06µl
- Reverse primer (100µM)	0,06µl
- Sterile H2O	up to 7µl

Cycling conditions:

Temp	Time	#Cycles
98°C	2min	1
98°C	5s	
60°C	20s	45
Melting curve analysis 65°c -> 95°c		

Follow manufacturer's protocol for programming the data acquisition of dsDNA product.

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

3. Recommended papers:

3.1. 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.

3.1. 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