

## Genotyping protocol

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

Gt(ROSA)26Sor<sup>tm31(CAG-NONO/TFE3)Ics</sup>/Ics

(PHENOMIN-ICS reference IR00007475 / Kos7475)

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pCAG-lox-STOP-lox-NONO-TFE3-FRT-IRES-eGFP-FRT in Rosa –  
Gt(ROSA)26Sortm31(CAG-NONO/TFE3)Ics/Ics

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

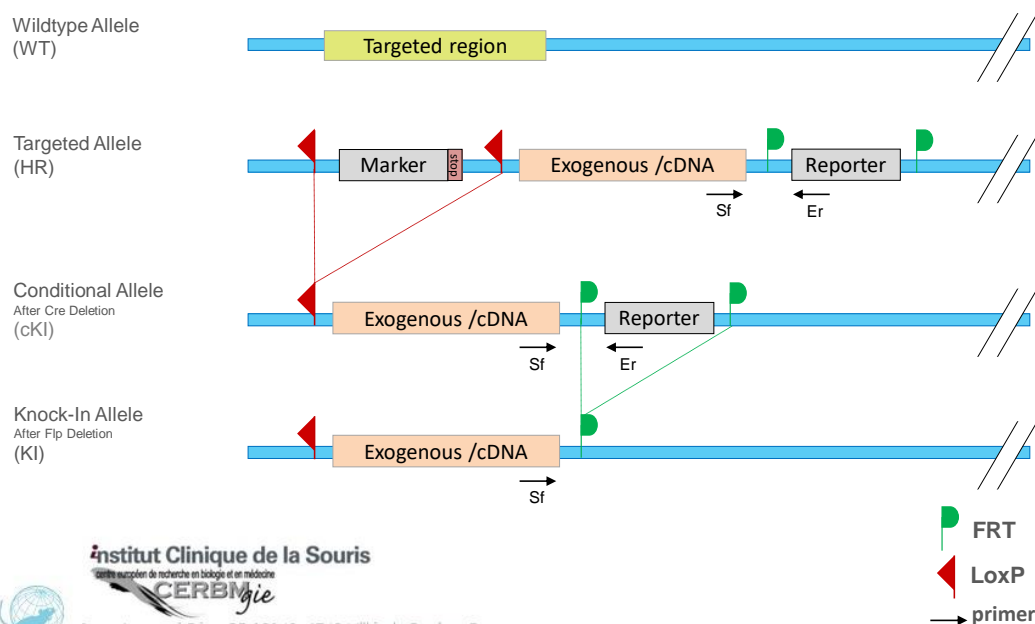
## 1. PCR Genotyping protocol

### 1.1. Genotyping strategy

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



### KI Genotyping strategy



Sequence of primers used for genotyping:

Position	Primers	Sequence
Sf	10384	GCATGGAAGAGGAGTCCTGAGG
Er	5735	CAAGCGGCTTCGCCAGTAACGTTAG

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 3' (with Betaine + DMSO)	10384-5735	Sf / Er	159	159	---	---

---: no Amplicon should be obtained



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## 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 <sub>2</sub> O	up to 15 µl

If mentioned in table “PCR fragments expected size” add 0.5% of Betaine and 5% of DMSO 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.**



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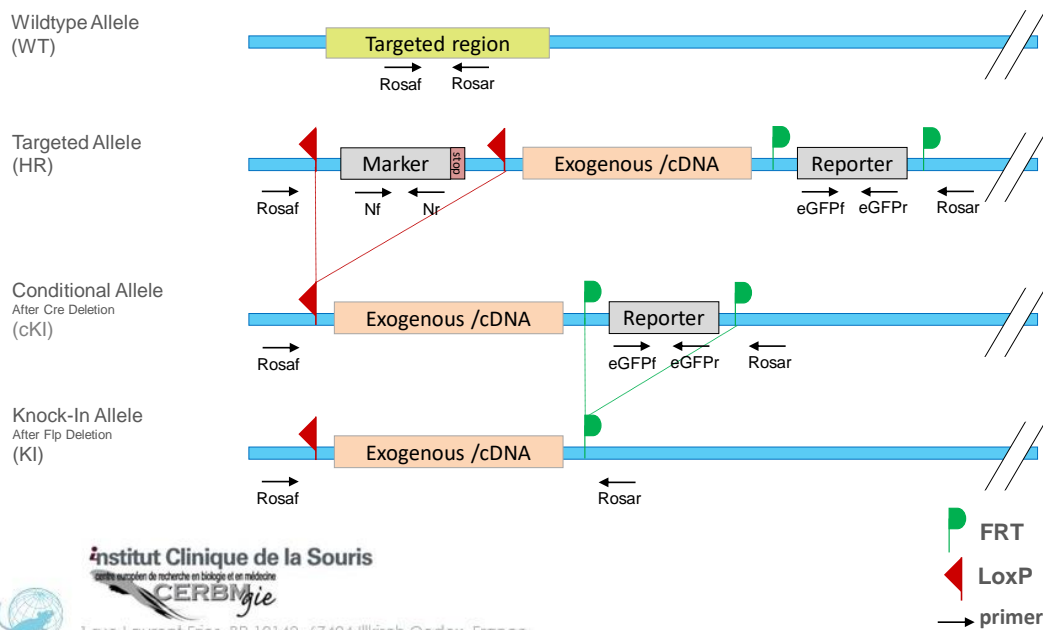
## 2. qPCR Genotyping protocol

### 2.1. Genotyping strategy

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



### KI Genotyping strategy



#### Sequence of primers used for genotyping:

Position	Primers	Sequence
eGFPf	eGFP f1	GACAACCACTACCTGAGCAC
eGFPPr	eGFP r1	CAGGACCATGTGATCGCG
Nf	Neo f1	TGAATGAACTGCAGGACGAG
Nr	Neo r1	TTCCCCTTCAGTGACAAC
Rosaf	Rosa f1	CCCTCTCCCTCGTGATCT
Rosar	Rosa r1	CACACACCAGGTTAGCCTTA

#### 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/ eGFPPr	72	72	---	---
Neomycine qPCR	Neo f1 – Neo r1	Nf / Nr	96	---	---	---
Rosa qPCR	Rosa f1 – Rosa r1	Rosaf / Rosar	8318*	5813*	4420*	88

\*: this amplicon will not be observed using our genotyping conditions (see description below)

---: no Amplicon should be obtained



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## 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	45
60°C	20s	

### 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.**



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### 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, Héroult 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 through this link <http://bit.ly/2sxxWvO>

