EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project pCAG-FRT-STOP-FRT_LoxP-dTomato-LoxP-SyneGFP in Rosa26

(PHENOMIN-ICS reference IR00005942 / K5942)

This report has been **prepared** by: David MOULAERT

This report has been **validated** by: Sylvie Jacquot, PhD
Head of Genotyping Service

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For any question, please contact:

PHENOMIN-ICS
Email: mutagenesis@igbmc.fr
Web site: http://www.ics-mci.fr/

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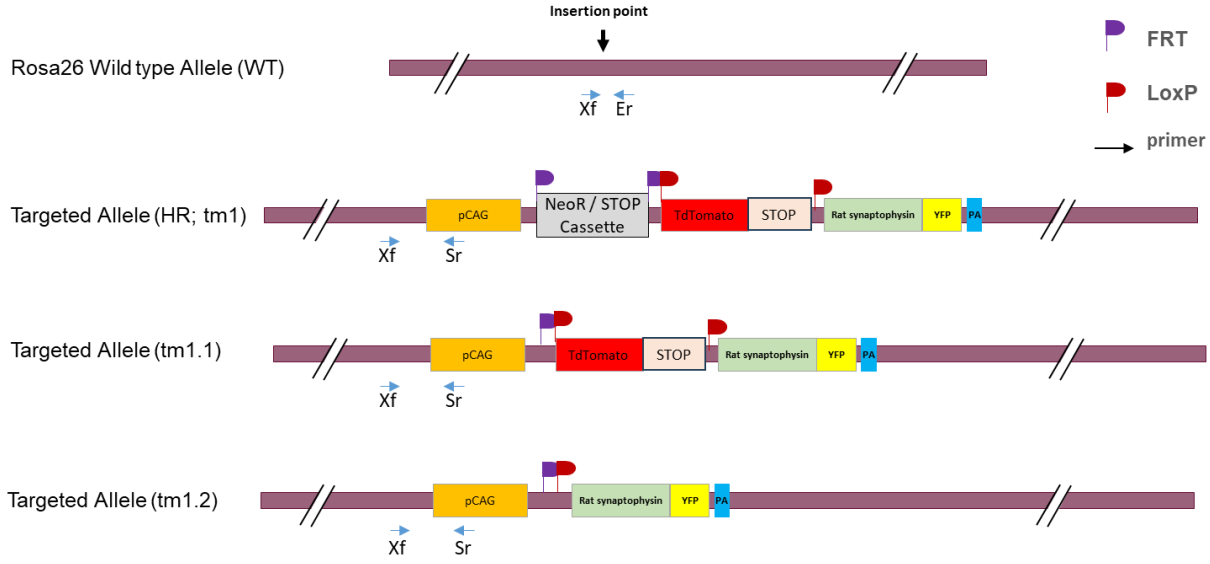
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the project**: pCAG-FRT-STOP-FRT_LoxP-dTomato-LoxP-SyneGFP in rosa**.

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
Sr	CGTAAGTTATGTAACGCGGAACTCCAT
Xf	AAAGTCGCTCTGAGTTGTTAT
Er	CACACACCAGGTTAGCCTTTAAGCCT

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	Tm1.1 allele	Tm1.2 allele	WildType allele
WT allele specific PCR	Xf / Er	9721*	7052*	4246*	386
KI specific PCR	Xf / Sr	319	319	319	---

*: this PCR product will not be observed using our PCR genotyping conditions (see description below)
---: 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:

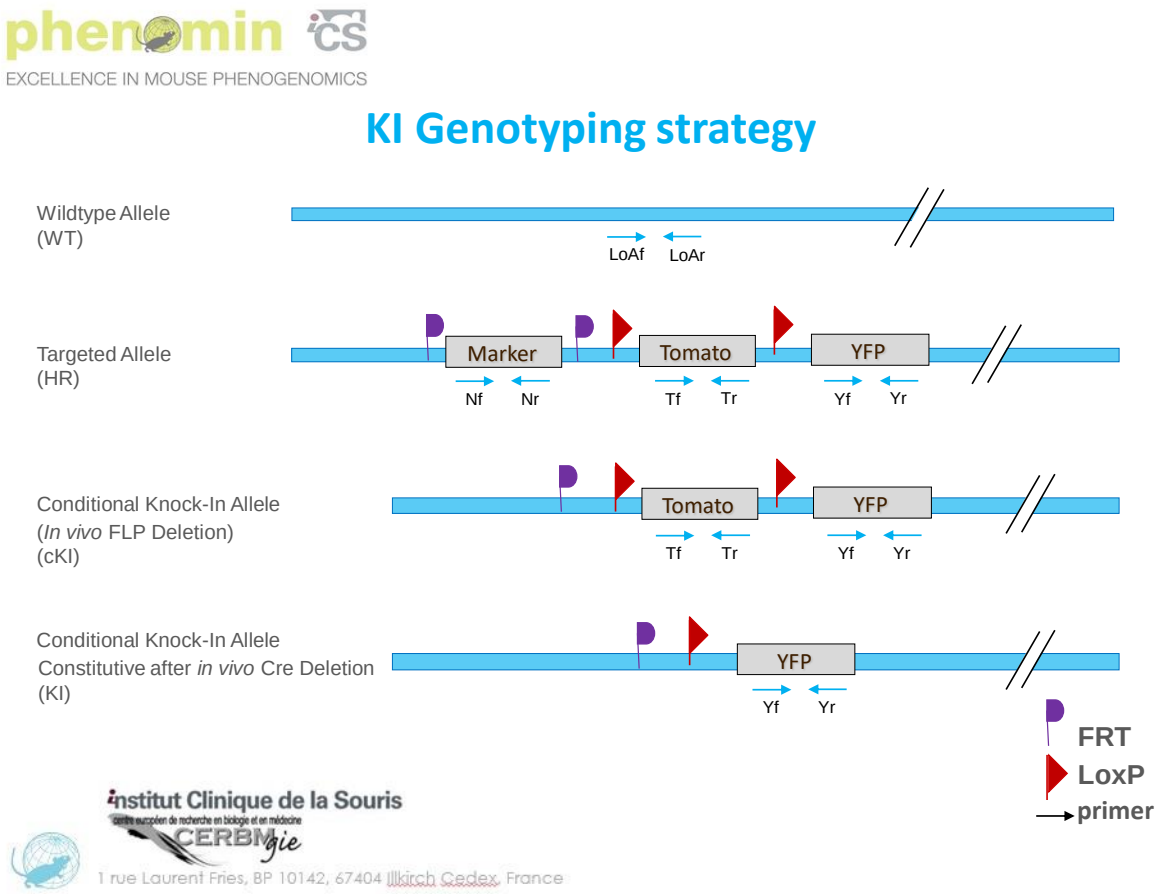
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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

2. qPCR Genotyping protocol and data

2.1. 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	Primers	Sequence
LoAf	LOAf1	CCCTCTTCCCTCGTGATCT
LoAr	LOAr1	CACACACCAGGTTAGCCTTTA
Nf	Neof1	TGAATGAACTGCAGGACGAG
Nr	Neor1	TTCCCGCTTCAGTGACAAC
Tf	Tomf1	CACTACCTGGTGGAGTTCAAG
Tr	Tomr1	GATGGTGTAGTCCTCGTTGTG
Yf	YFPf1	AACCACTACCTGAGCTACCA
Yr	YFPr1	TCGTCCATGCCGAGAGT

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	cKI	KI allele	WildType allele
Loss of allele Rosa qPCR	LoAf1 – LoAr1	LoAf / LoAr	---	---	---	88
Neomycine qPCR	Neof1 - Neor1	Nf / Nr	96	---	---	---
Tomato	Tomf1 – Tomr1	Tf / Tr	117	117	---	---
YFP	YFPf1 – YFPr1	Yf/Yr	113	113	113	---

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
95°C	10min	1
95°C	5s	
62°C	10s	34
95°C	15min	
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. Cre and Flp genotyping method

You will find the genotyping protocol in the publication:

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.