EXCELLENCE IN MOUSE PHENOGENOMICS

# Genotyping protocol

Project Myc-NRIP-FRT- IRES-eGFP-FRT

Gt(ROSA)26Sor$^{tm28(CAG-NRIP1,-EGFP)Ics}$/Ics

(PHENOMIN-ICS reference IR00007391 / Kos7391)

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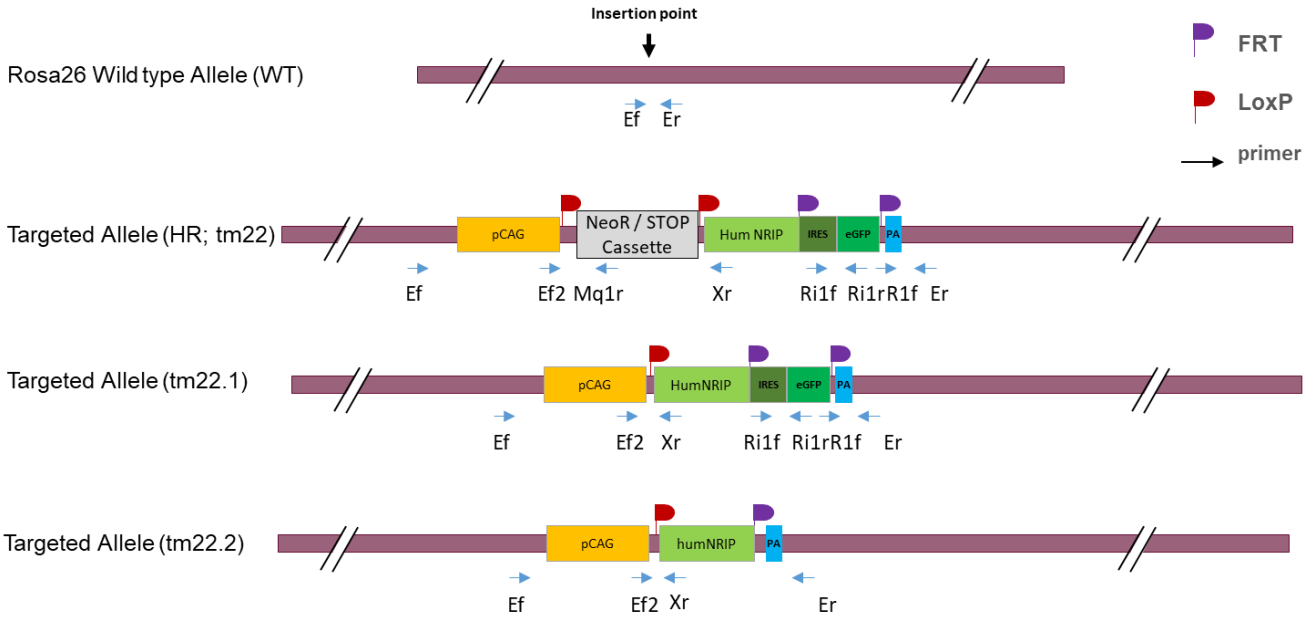
# Table of contents

This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Myc-NRIP-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.




Sequence of primers used for genotyping:

| Position | Sequence |
|---|---|
| Xr | CTGGACCATTACTTTGACAGGTGGG |
| Ef | AAAGTCGCTCTGAGTTGTTAT |
| Er | GCCTTTAAGCCTGCCCAGAAGA |
| Ef2 | GCGGCTCTAGAGCCTCTGCTAACCAT |
| Mq1r | ATTCGCAGCGCATCGCCTTC |
| R1f | CTCGGCATGGACGAGCTGTACAAG |
| Ri1f | GCTGAAGGATGCCCAGAAGGTAC |
| Ri1r | GTGAACAGCTCCTCGCCCTTG |

PCR fragments expected size (bp):

| Region analyzed | Position on the primer *(see the map above)* | Tm28 (HR) | Tm28.1 KI allele GFP+ | Tm28.2KI allele GFP- | WildType allele (WT) |
|---|---|---|---|---|---|
| WildType allele specific PCR (5' part of the targeted locus) | Ef / Er | 10004* | 7465* | 6120* | 239 |
| Excision of the selection marker | Ef2 / Xr | 2895* | 390** | 390** | --- |
| 5' part of the selection marker | Ef2 / Mq1r | 263 | --- | --- | --- |
| 3' reporter | R1f / Er | 512 | 512 | --- | --- |
| Internal reporter | Ri1f / Ri1r | 193 | 193 | --- | --- |

*: 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:

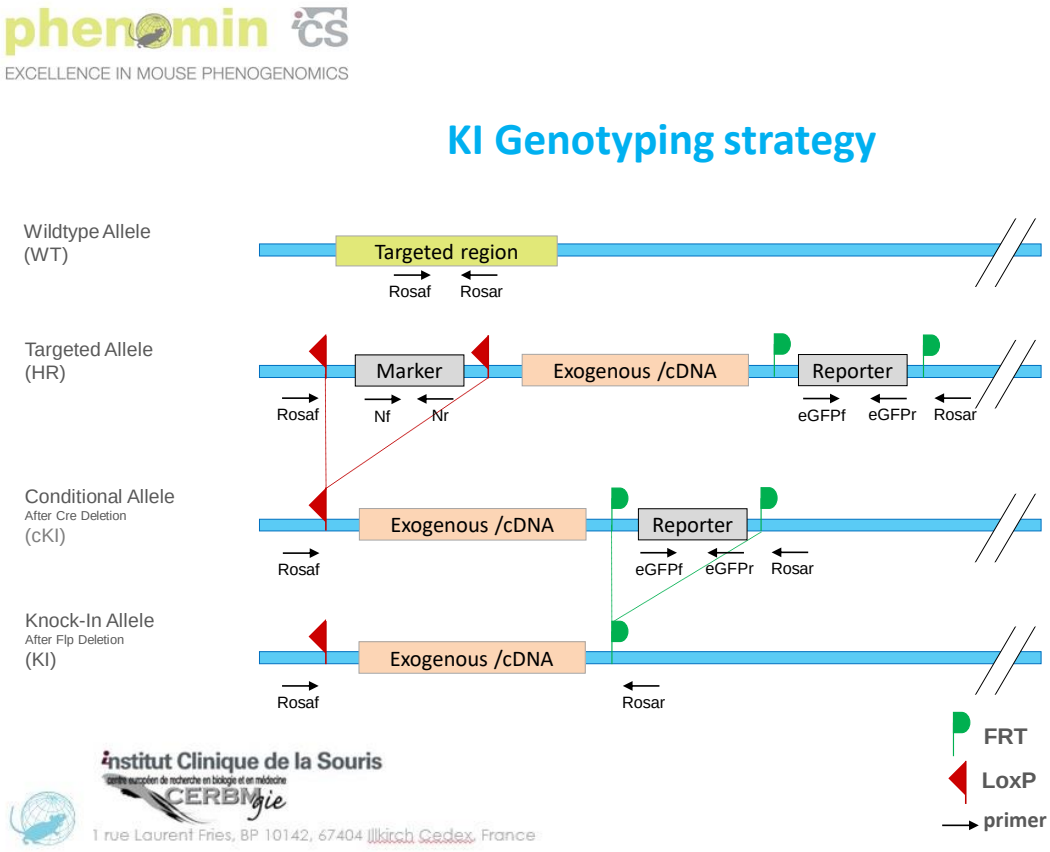
| Temp | Time | #Cycles |
|---|---|---|
| 95°C | 4min | 1 |
| 94°C | 30s | 35 |
| 62°C | 30s | |
| 72°C | 1min | |
| 72°C | 7min | 1 |
| 14°C | --- | --- |

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

# 2. qPCR Genotyping protocol

## 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 |
|---|---|---|
| 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 | 9852* | 7347* | 5954* | 88 |

*: this PCR product will not be observed using our PCR 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