EXCELLENCE IN MOUSE PHENOGENOMICS

# Genotyping protocol

Project Zc4h2

(PHENOMIN-ICS reference IR00004650 / G4650)

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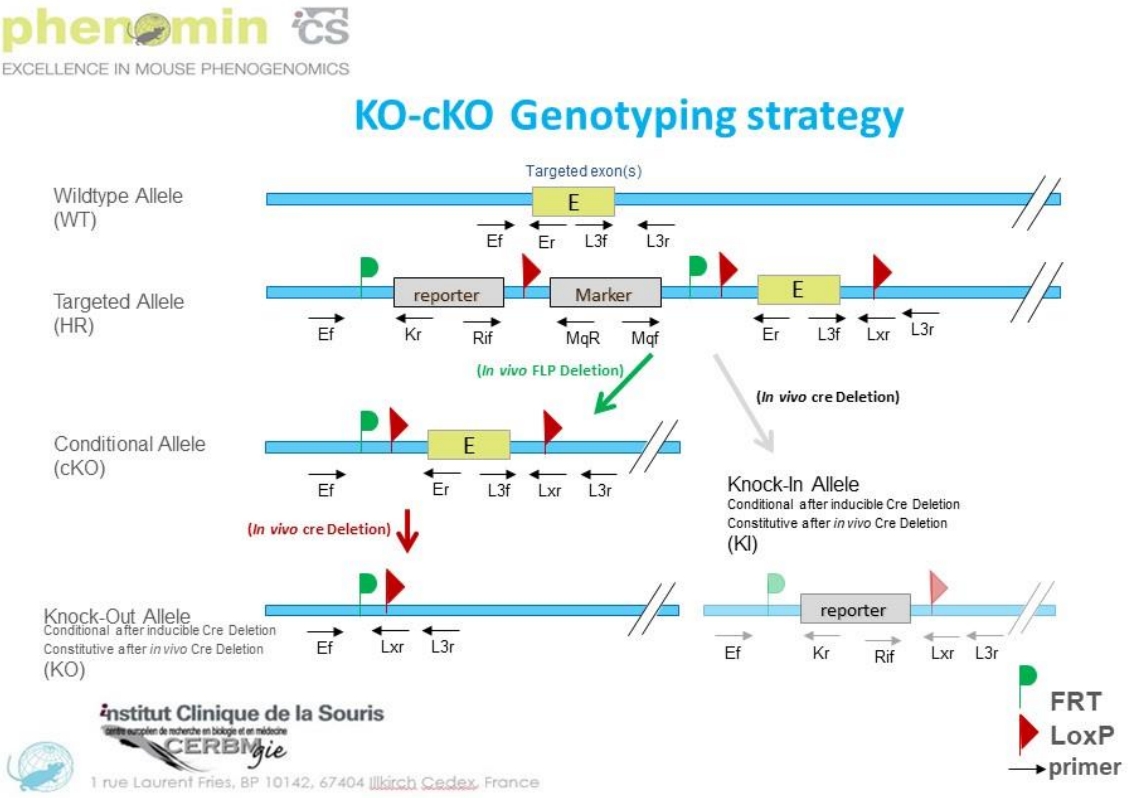
# Table of contents

This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Zc4h2** Constitutive Knockout / Conditional Knockout (KO-cKO x Cre) 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 | Primers | Sequence |
|---|---|---|
| Ef | 7715 | GGTTCTTGACTCAACACTCACTCC |
| Er | 7718 | GCATATGTAGAATCCTTGATGTAGC |
| Kr | 3209 | CCAACAGCTTCCCCACAACGG |
| L3f | 7716 | CCTGAGTTCAAATCCCAGCAACCTTG |
| L3r | 7717 | CACAGTAACTTTAGAACGTCTCCCCTG |
| Lxr | 3255 | ACTGATGGCGAGCTCAGACCATAAC |
| Ri1f | 5966 | GCACATGGCTGAATATCGACGGT |

[2]: for a selected position, a second primer was designed

PCR fragments expected size (bp):

| Region analyzed | Primers used | Position on the primer *(see the map above)* | Targeted allele (HR) | conditional allele (cKO) | KO allele | WildType allele |
|---|---|---|---|---|---|---|
| 5' part of the selection marker | 7715-3209 | Ef / Kr | 396 | --- | --- | --- |
| Presence of the distal loxP | 7716-7717 | L3f / L3r | 268 | 268 | --- | 215 |
| Distal loxP specific PCR | 7716-3255 | L3f / Lxr | 186 | 186 | --- | --- |
| Excision of the selection marker | 7715-7718 | Ef / Er | 7349* | 445 | --- | 264 |
| Cre total excision | 5966-3255 | Ri1f / Lxr | 3305* | --- | --- | --- |
| Excision of the floxed exon(s), i.e. knock out | 7715-7717 | Ef / L3r | ---* | ---* | 433** | 1122** |

*: 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 |

If mentioned in table "PCR fragments expected size" add 0.5% of Betaine (5% of DMSO) in the reaction mix

Cycling conditions:

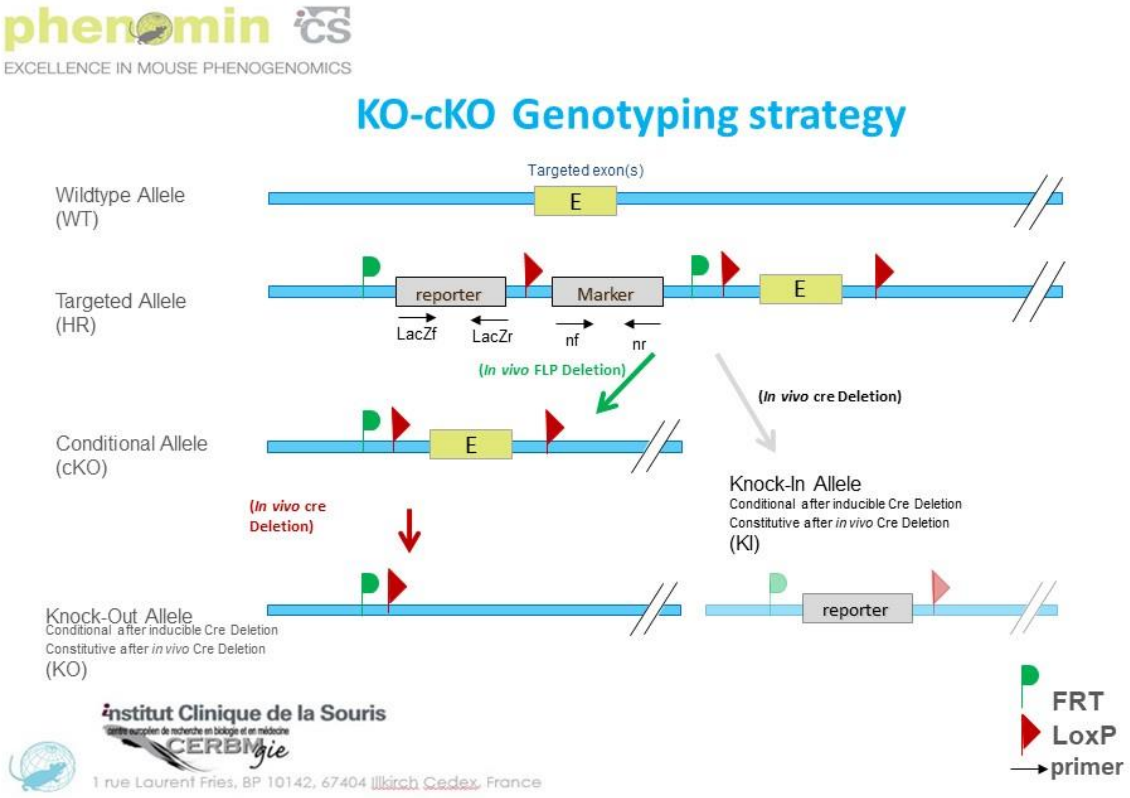
| Temp | Time | #Cycles |
|---|---|---|
| 95°C | 4min | 1 |
| 94°C | 30s | |
| 62°C | 30s | 35 |
| 72°C | 1min | |
| 72°C | 7min | 1 |
| 14°C | --- | --- |

**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.


phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS
KO-cKO Genotyping strategy
Targeted exon(s)
Wildtype Allele
(WT)
E
Targeted Allele
(HR)
reporter
Marker
E
LacZf
LacZr
nf
nr
(In vivo FLP Deletion)
(In vivo cre Deletion)
Conditional Allele
(cKO)
E
Knock-In Allele
Conditional after inducible Cre Deletion
Constitutive after in vivo Cre Deletion
(KI)
(In vivo cre
Deletion)
Knock-Out Allele
Conditional after inducible Cre Deletion
Constitutive after in vivo Cre Deletion
(KO)
reporter
FRT
LoxP
primer
Institut Clinique de la Souris
CERBMgie
1 rue Laurent Fries, BP 10142, 67404 Illkirch Cedex, France

Sequence of primers used for genotyping:

| Position | Primers | Sequence |
|---|---|---|
| LacZf | 7443 | CTCGCCACTTCAACATCAAC |
| LacZr | 7445 | TTATCAGCCGGAAAACCTACC |
| Nf | Neo f1 | TGAATGAACTGCAGGACGAG |
| Nr | Neo r1 | TTCCCGCTTCAGTGACAAC |

qPCR fragments expected size (bp):

| Region analyzed | Primers used | Position on the primer *(see the map above)* | Targeted allele (HR) | Conditional allele (cKO) | Knock out allele (KO) |
|---|---|---|---|---|---|
| LacZ qPCR | 7443 - 7445 | LacZf / LacZr | 73 | --- | --- |
| Neomycine qPCR | Neof1 - Neor1 | Nf / Nr | 96 | --- | --- |

---: 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**