# Genotyping protocol

## Cd276

## IR00005544 / P5544

(ICS internal reference)


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The first version of this report was generated the: 14 Mar 2016

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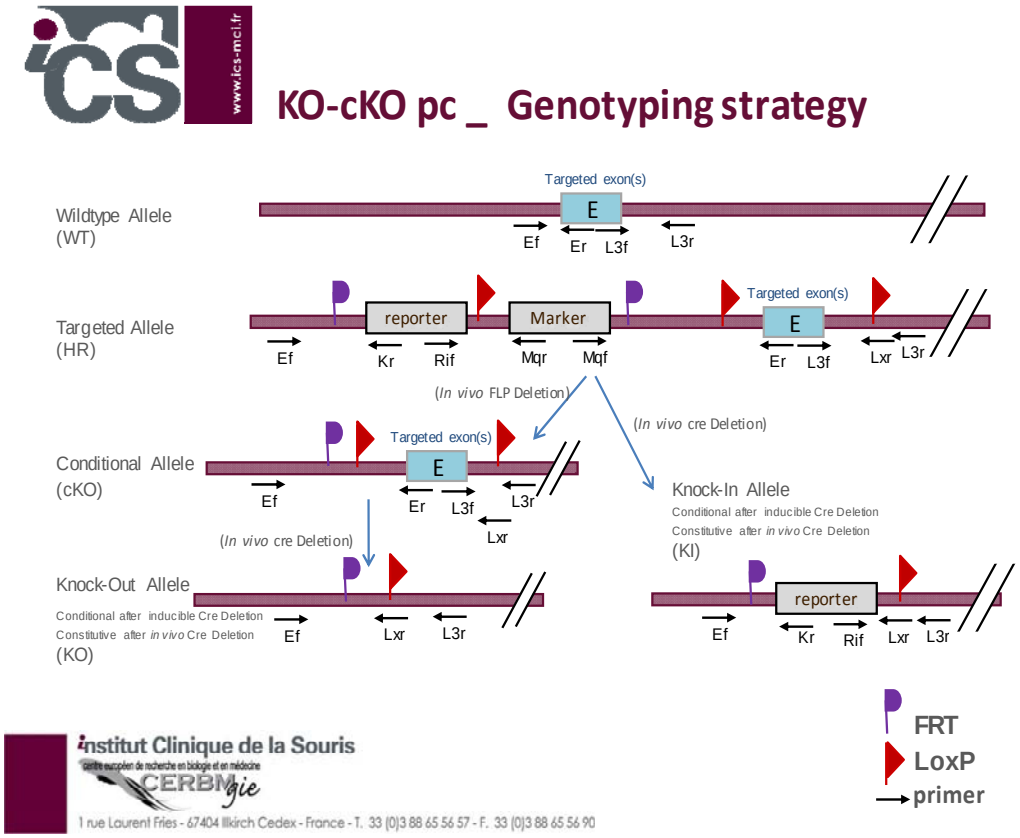
# 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Cd276** Constitutive Knockout / Conditional Knockout (KO-cKO x Cre) project.

## 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 | 8962 | GCTGCAGAGGTCCTATTCCTCCTTAATG |
| Ef[2] | 8963 | GGTCTCTGTGATTCTGACTCAGGAGGC |
| Er | 8966 | CTGGGATAGGCCTGCCCTGGAC |
| Kr | 3209 | CCAACAGCTTCCCCACAACGG |
| L3f | 8964 | CCTCTCCTCTAGGCCCTAACTGGAATATC |
| L3r | 8965 | GAGATGCACCTTCAAGGCTCCTGG |
| 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) | KI allele | WildType allele |
|---|---|---|---|---|---|---|
| 5' part of the selection marker (with DMSO) | 8962-3209 (with 5% DMSO) | Ef / Kr | 325 | --- | --- | --- |
| Presence of the distal loxP (with Betaine) | 8964-8965 (with 0.5% Betaine) | L3f / L3r | 321 | 321 | --- | 342 |
| Distal loxP specific PCR | 8964-3255 | L3f / Lxr | 214 | 214 | --- | --- |
| Excision of the selection marker (with DMSO) | 8963-8966 (with 5% DMSO) | Ef[2] / Er | 7288* | 384 | --- | 232 |
| Cre total excision | 5966-3255 | Ri1f / Lxr | 3401* | --- | 471** | --- |

*: 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 | |
| 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. 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.