



Genotyping protocol

Ankrd11

IR00003730 / G16

(ICS internal reference)

This report has been prepared by:

Pauline Cayrou
genotyping@igbmc.fr

This report has been validated by:

Sylvie Jacquot, PhD, Head of Genotyping Service
33 (0)3 88 65 57 44
genotyping@igbmc.fr

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For any question, please contact:

Institut Clinique de la Souris - ICS - Mouse Clinical Institute
1 rue Laurent Fries, BP 10142
67404 Illkirch Cedex, France
Email: genotyping@igbmc.fr
Web site: <http://www-mci.u-strasbg.fr/>

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1. Genotyping protocol and data

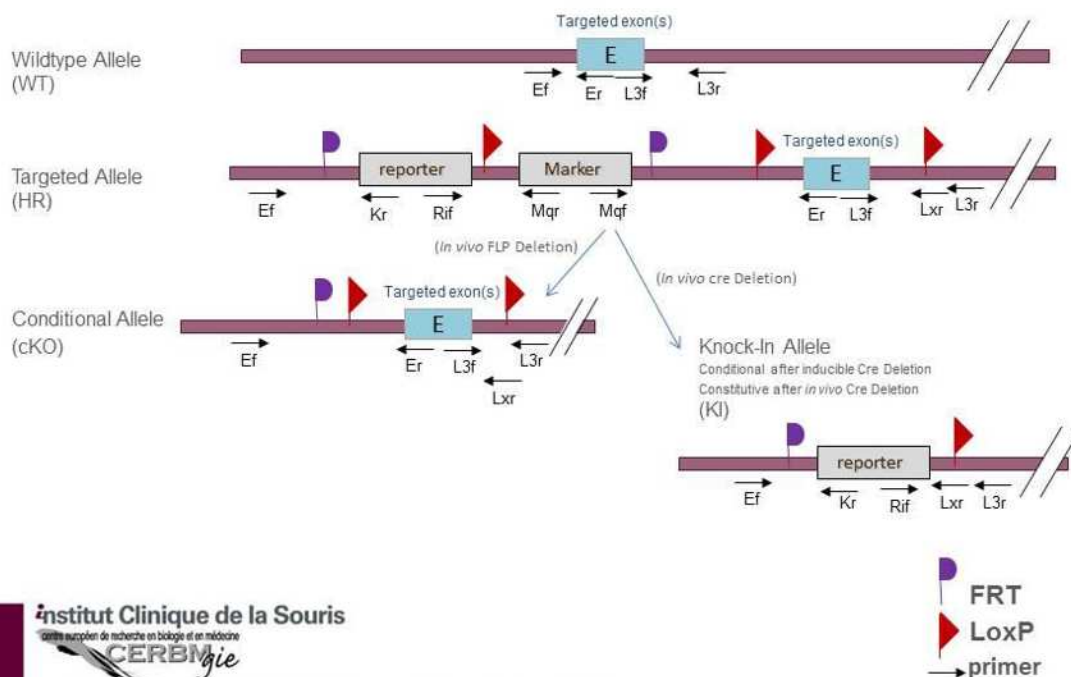
This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Ankrd11** Constitutive Knockout / Conditional Knockout (KO-cKO x Cre) project.

1.1. Genotyping strategy

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



G16 _ Genotyping strategy



Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	6434	CTGTCTCAGAGAGGAGAGTGAGGAGGAC
Er	6438	TACCTTACACCCTGAGACGGCGTC
Kr	3278	GGCAAGAACATAAAGTGACCCTCC
L3f	6435	CAACTGTTAGCCGCTGGTGACG
L3f ²	6436	TAAGGGCCTGGATGATGACACACCTT
L3r	6437	TCATGCAGTCAGAAGAGCCAGCAC
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)	cKO allele (cKO)	KI allele (KI)	WildType allele (WT)
5' part of the selection marker	6434-3278	Ef / Kr	270	---	---	---
Presence of the distal loxP	6435-6437	L3f / L3r	438	438	---	384
Distal loxP specific PCR	6436-3255	L3f ² / Lxr	270	270	---	---
Excision of the selection marker	6434-6438	Ef / Er	7359*	455	---	259
Cre total excision	5966-3255	Ri1f / Lxr	3113*	---	471	---

*: 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 ₂ O	up to 15 µl

Cycling conditions:

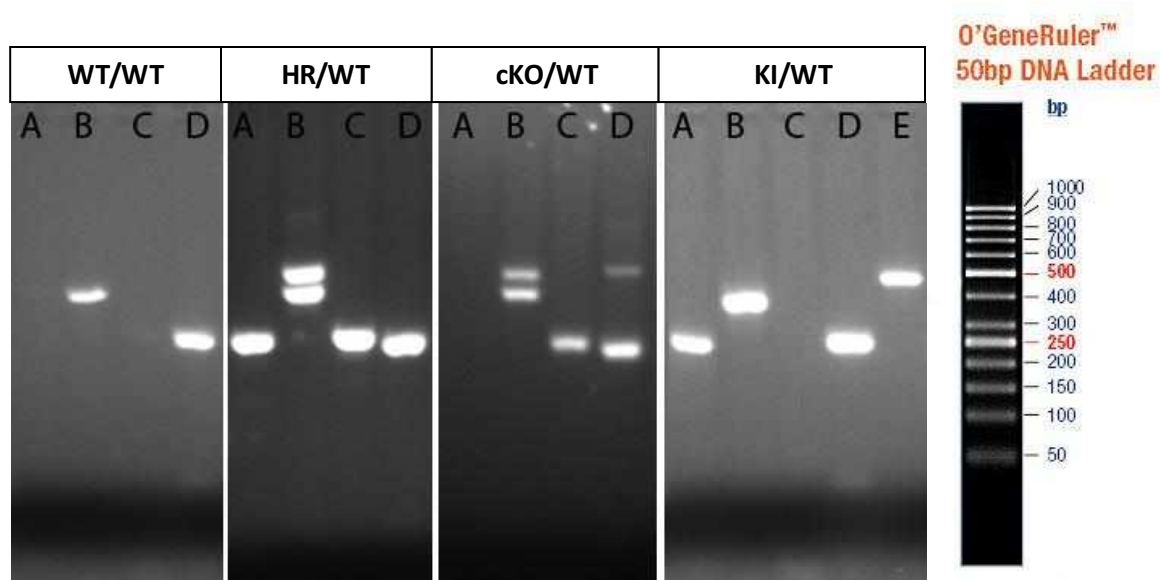
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
72°C	1min	
72°C	7min	1
20°C	5min	1

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

1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was done by gel electrophoresis 2% agarose (SB buffer).

Representative genotyping picture



A: 5' part of the selection marker
 B: Presence of the distal loxP
 C: Distal loxP specific PCR
 D: Excision of the selection marker
 E: Cre total excision

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.