



Genotyping protocol

Stradb KOMP alleles

Stradbtm1a(KOMP)Wtsi

IR00005001 / P5001

(ICS internal reference)

For any question, please contact:

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1. Genotyping protocol and data

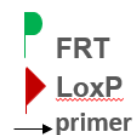
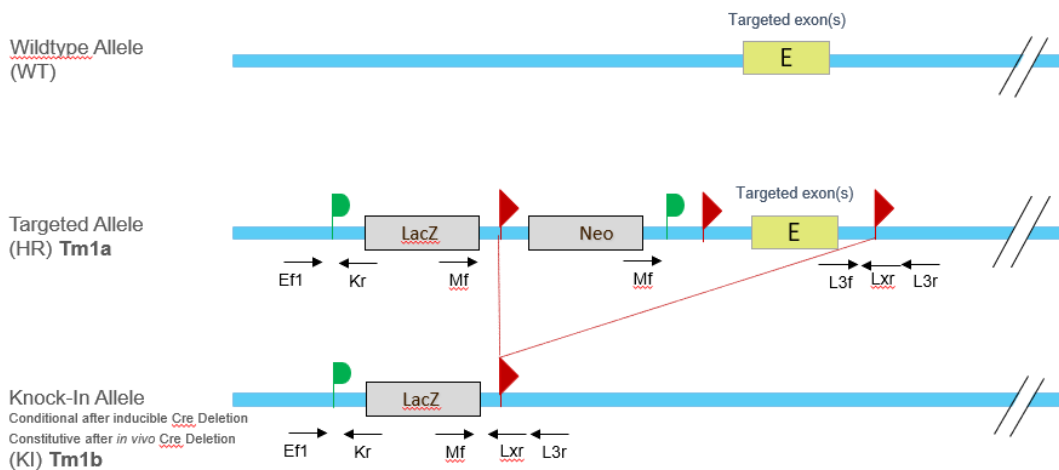
This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Rspo1** Constitutive Knockout / Conditional Knockout (KO-cKO x Cre) project.

1.1. Genotyping strategy

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



IMPC - KO-cKO Genotyping strategy PCR



Sequence of primers used for genotyping:

Position	Sequence
Ef	TTCCCAGCACCCACATGGCAT
Kr	GGGCAAGAACATAAAGTGACCCTCC
L3f	CTAAGTCATGCAGTTACCAGATTGGGAA
L3r	CATCTCCTCACCCGCATAGAAAATAGTTT
Mf	GCATTCTAGTTGTGGTTTGTCCAAAC
Lxr	ACTGATGGCGAGCTCAGACCATAAC

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

PCR fragments expected size (bp):

Region analyzed	Position on the primer (see the map above)	Targeted allele (HR, tm1a)	KI/KO allele (tm1b)	WildType allele
5' part of the LacZ	Ef / Kr	336	336	---
Presence of the 3' distal loxP	L3f / L3r	375	---	350
Distal loxP specific PCR	L3f / Lxr	177	---	---
Excision of NeoR K7 and critical exon (total Cre excision)	Mf / L3r	1222* 3066*	311	---

*: 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 ₂ O	up to 15 µl

Cycling conditions:

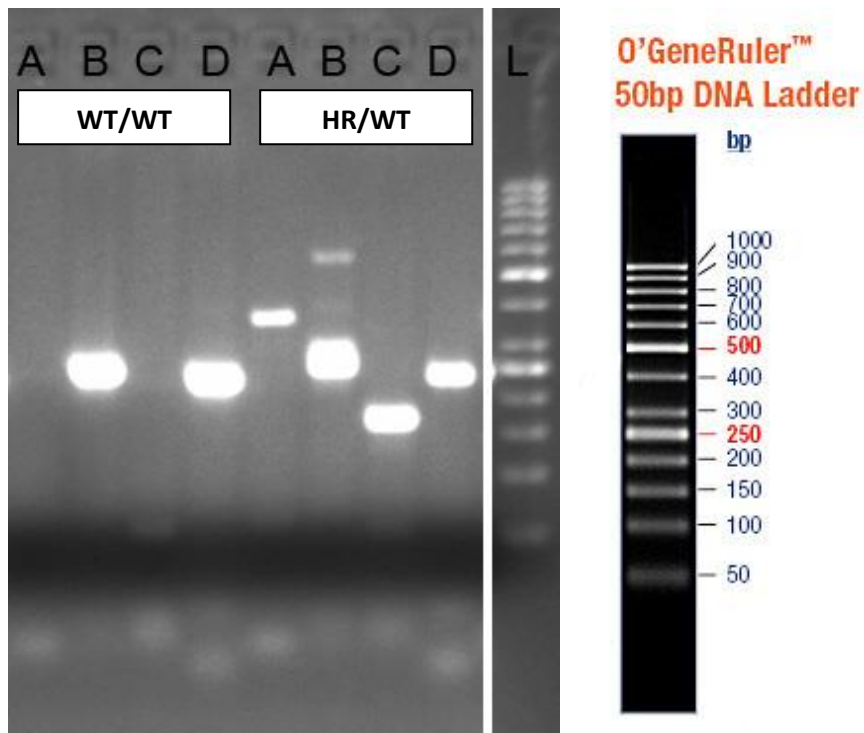
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
72°C	1min	
72°C	7min	1
20°C	5min	1

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
- L: O'GeneRuler 50bp DNA Ladder

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.