Genotyping protocol

Satb1

IR00004167 / P4167

(ICS internal reference)

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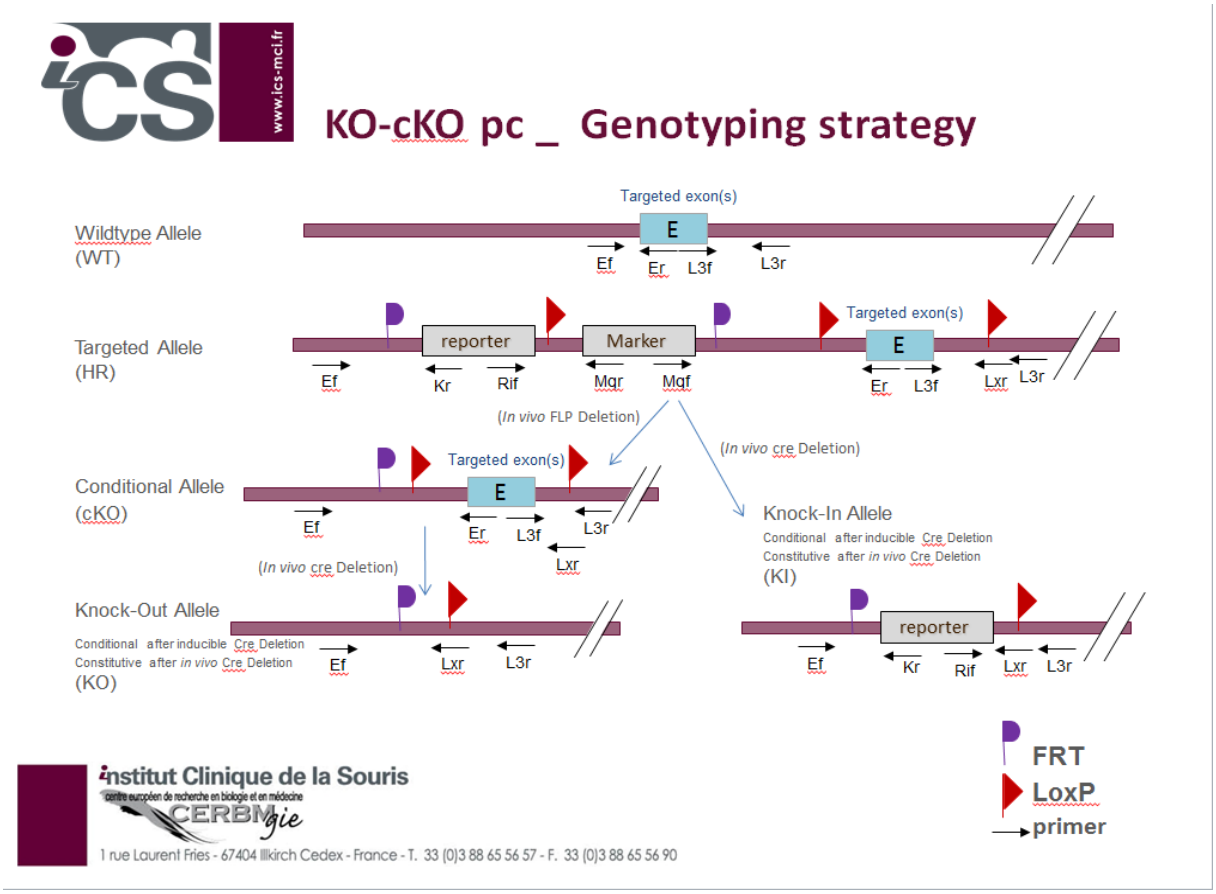
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Satb1** Constitutive Knockout / Conditional Knockout (KO-cKO) 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	6677	TTTGCTCATGTGGAATGTCGAGGTA
Ef[2]	6674	AATAATCTGCTCCACTGAGGACCCAC
Er[2]	1936	GTGGATGTGGAATGTGTGCGAGG
Er[3]	6678	CCCTATTGCAGTGGGAATCAGCAT
Kr	3278	GGGCAAGAACATAAAGTGACCCTCC
L3f	6675	TTACACAGGTGAGTCCAGGCAGGGA
L3r	6676	CGTGGCAAAAGCGAATAAGGCA
Lxf[2]	6013	TCATGTCTGGATCCGGAATAACTTCGTA
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	KI allele	(WT)
Lox interne K7 Eur (with DMSO)	6013-1936 (with 5% DMSO)	Lxf[2] / Er[2]	199	---	---	---
5' part of the selection marker	6677-3278	Ef / Kr	352	---	352	---
Presence of the distal loxP	6675-6676	L3f / L3r	340	340	381	381
Distal loxP specific PCR	6675-3255	L3f / Lxr	246	246	---	---
Excision of the selection marker	6674-6678	Ef[2] / Er[3]	7258*	302	176	176
Cre total excision	5966-3255	Ri1f / Lxr	3229*	---	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

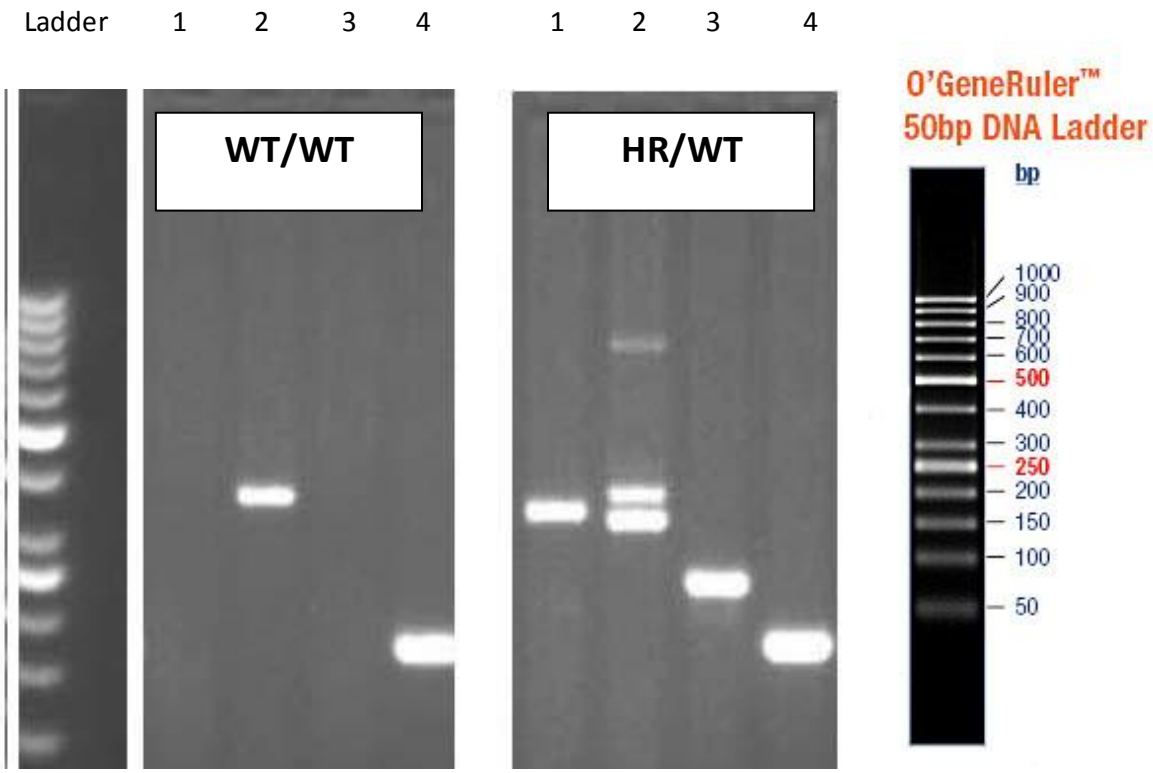
Cycling conditions:

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.

1.3. Picture of genotyping with various alleles

Representative genotyping picture



PCR number:

1. 5' part of the selection marker
2. Presence of the distal loxP
3. Distal loxP specific PCR
4. Excision of the selection marker

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.