



Genotyping protocol

Spns1

IR00002984 / E172

(ICS internal reference)

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1. Genotyping protocol and data

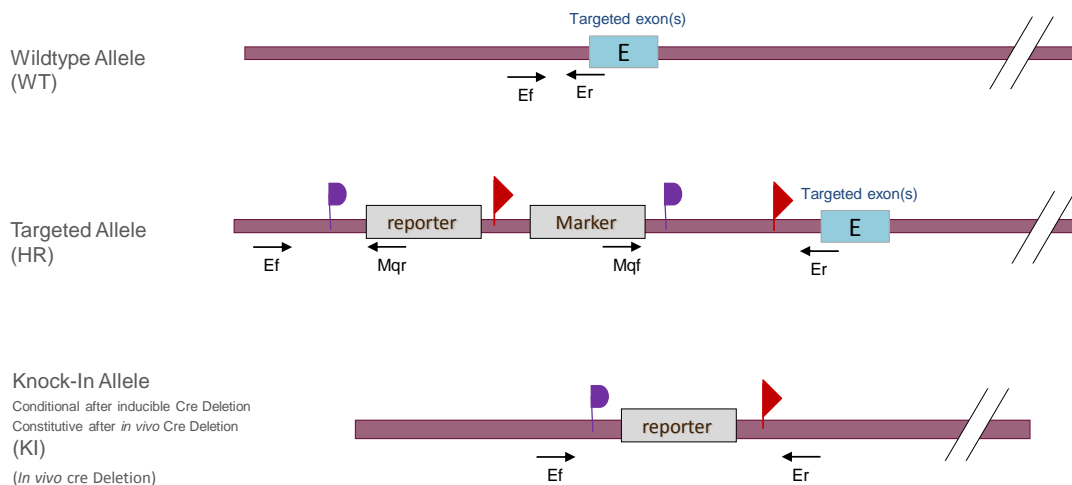
This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Spns1** Conventional or Constitutive Knockout (KO) project.

1.1. Genotyping strategy

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



KO-cKO pc _ Genotyping strategy



Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	5400	GGTAGAGCCAGGTGTGTTGGC
Ef ²	5401	GAGATGCAGACCTGGCCCAC
Er	5402	GATCTAACCCACCTCCTCCTTTCC
Er ²	5403	CCACACACATTAGGGTTCAGCACC
Mq1f	4981	GGGATTCATGCTGGAGTTCTTCG
Mq1r	3278	GGCAAGAACATAAAGTGACCCTCC

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)	KO allele	WildType allele
WildType allele specific PCR	5400-5402	Ef / Er	---	---	321
WildType allele specific PCR	5401-5403	Ef ² / Er ²	---	---	367
Excision of the selection marker	5401-5402	Ef ² / Er	7516*	612**	425*
5' part of the selection marker	5400-3278	Ef / Mq1r	415	---	---
3' part of the selection marker	4981-5403	Mq1f / Er ²	528	---	---

*: 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.

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.