



Genotyping protocol

Pak3/ P5884

(ICS internal reference)

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1. Genotyping protocol and data

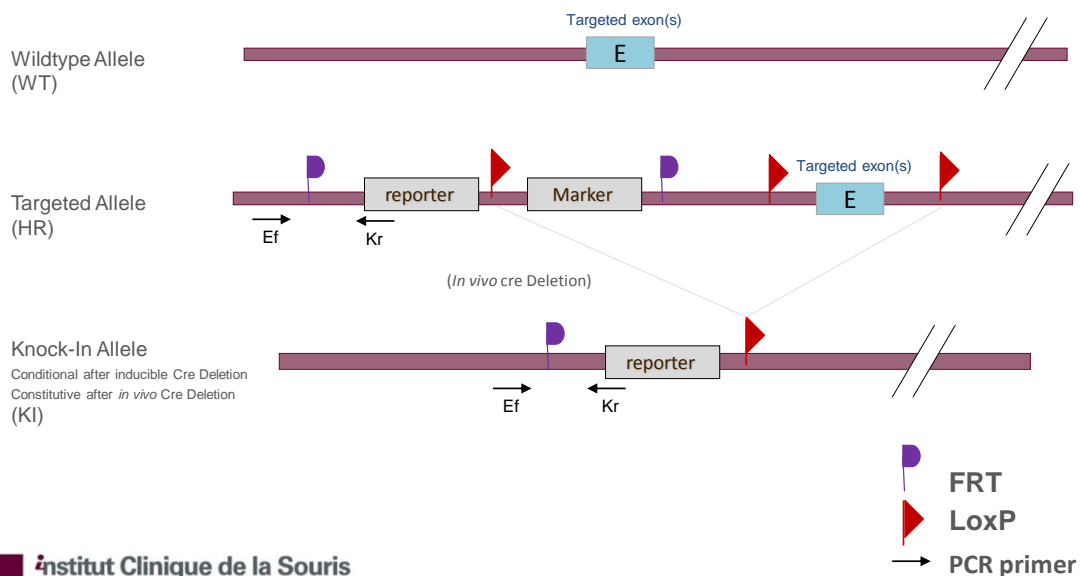
This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Pak3** Constitutive Knockout / Conditional Knockout (KO first) project.

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	8857	CTGTGTCATAACAATGTAGGAGAG
Kr	3209	CCAACAGCTTCCCCACAACGG

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer (see the map above)	Targeted allele (HR)	conditional allele (KO-cKO)	KI allele	WildType allele
5' part of the selection marker	8857-3209	Ef / Kr	253	---	253	---

*: 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. qPCR Genotyping protocol and data

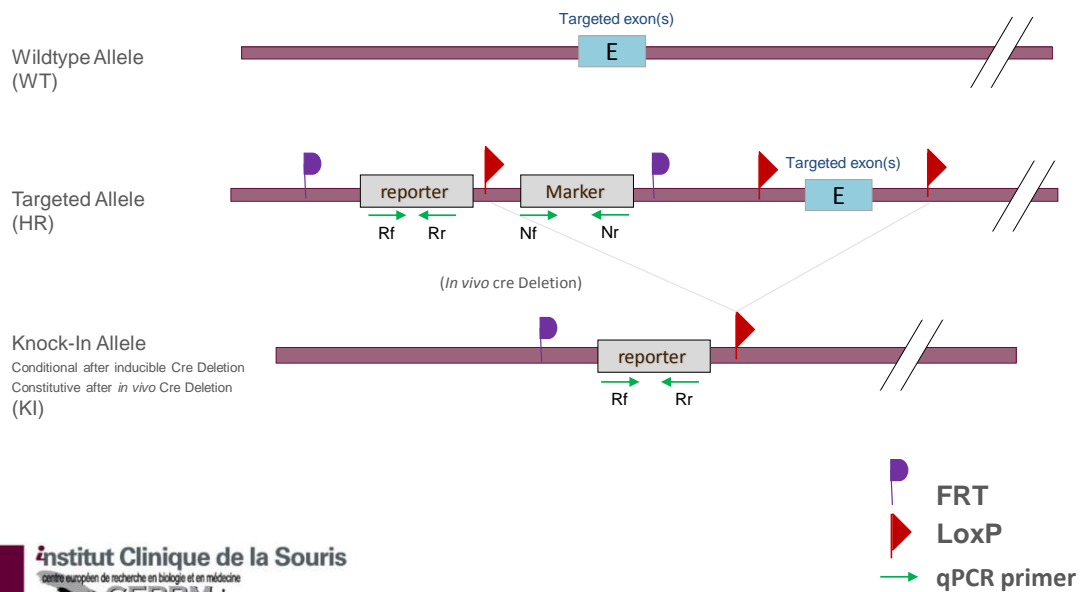
This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Bscl2** Constitutive Knockout / Conditional Knockout (KO first) project.

2.1. 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
Rf	7443	CTCGCCACTTCAACATCAAC
Rr	7445	TTATCAGCCGGAAAACCTACC
Nf	Neo f1	TGAATGAACTGCAGGACGAG
Nr	Neo r1	TTCCCGCTTCAGTGACAAC

2.2. qPCR protocol

Reagents:	Volume:
- EvaGreen (biorad)	3,5µl
- DNA (10ng/µl)	3µl
- Forward primer (100µM)	0,06µl
- Reverse primer (100µM)	0,06µl
- Sterile H2O	up to 7µl

Cycling conditions:

Temp	Time	#Cycles
95°C	10min	1
95°C	5s	
62°C	10s	34
95°C	15min	
Melting curve analysis		
65°C -> 95°C		

Follow manufacturer's protocol for programming the data acquisition of dsDNA product.

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

3. 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.