



Genotyping protocol

Hcn4

IR00003017 / E191

(ICS internal reference)

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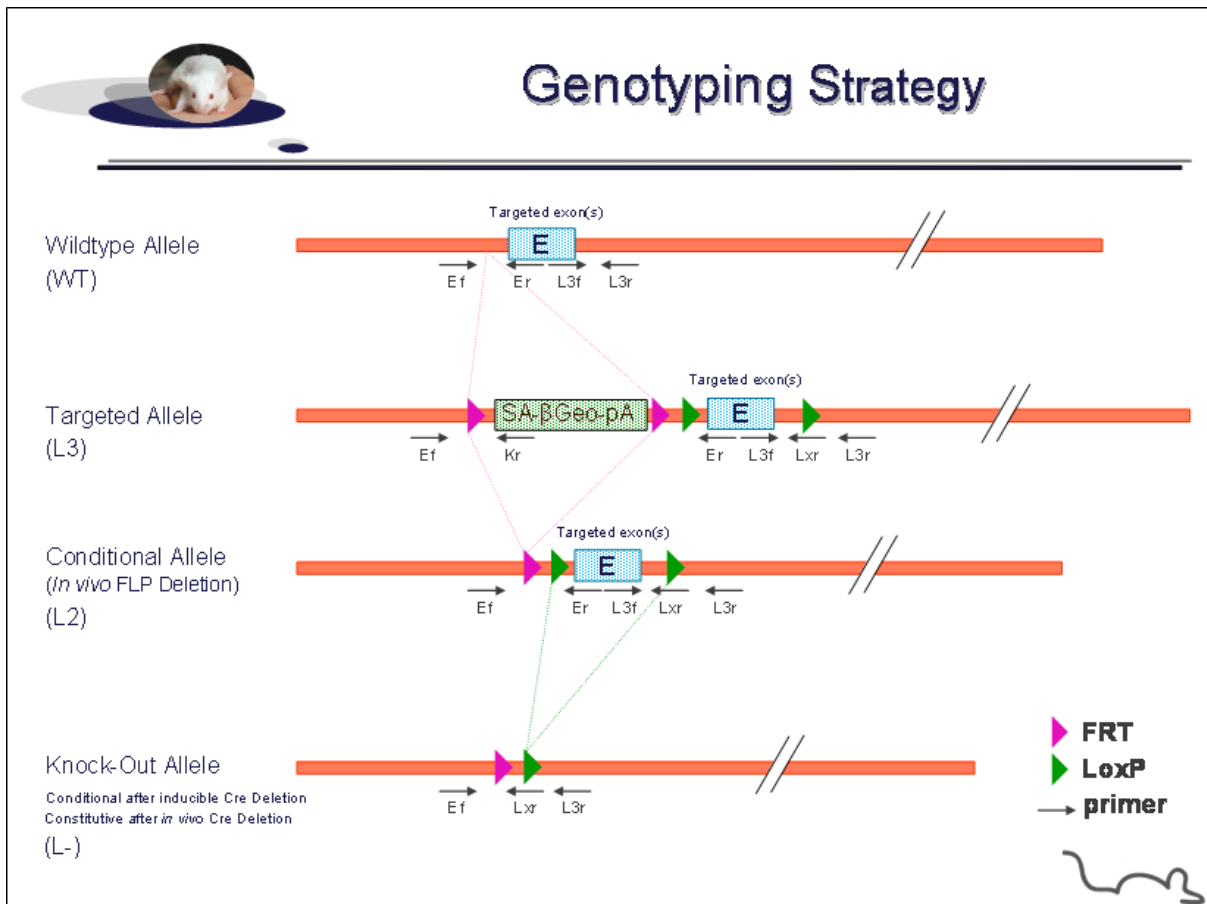
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1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Hcn4** 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	5427	GAGCCAGTGTGAGACATGCTAGGTCA
Er3	5432	AACTCACATCACCGTCCCCAGG
Kr	3209	CCAACAGCTTCCCCACAACGG
L3f	5428	TCCCCGCCTCATTGATACATTCA
L3f ²	5430	ATCAGTGGGAAGAGGTGAGTGGCTG
L3r	5429	CAAATCTGTATGCCGCCCCATA
L3r ²	5431	TGTGAAAGTCCCCATACCAGAGAACA
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC

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 (KO allele) (L3)	cKO allele (L2)	KO allele (L-)	WildType allele (WT)
5' part of the selection marker	5427-3209	Ef / Kr	360	---	---	---
Presence of the distal loxP	5428-5429	L3f / L3r	401	401	---	363
Distal loxP specific PCR	5430-3255	L3f ² / Lxr	297	297	---	---
Excision of the selection marker	5427-5432	Ef / Er	7381*	477	---	283
Excision of the floxed exon(s), i.e. knock out	5427-5431	Ef / L3r ²	8528*	1624*	455**	1392**

*: 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.

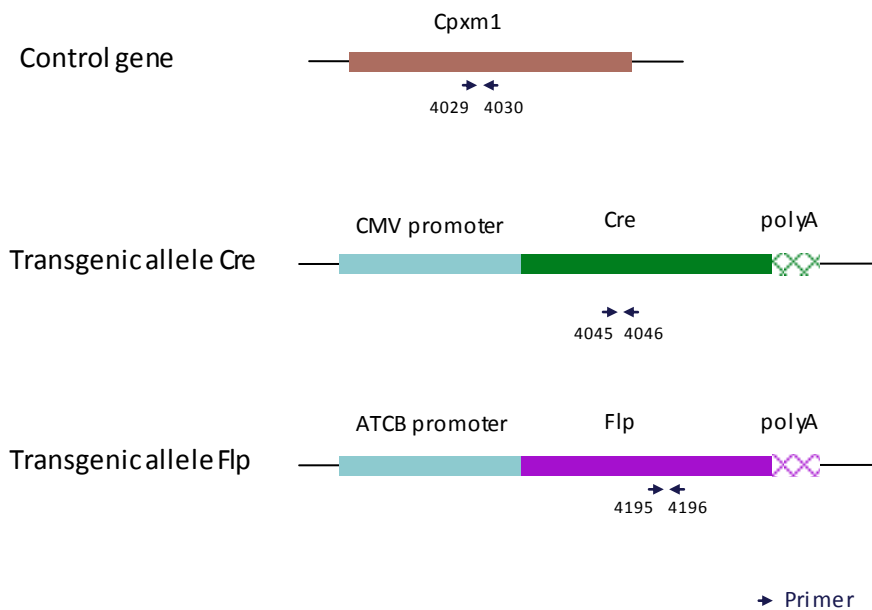
2. Cre and Flp genotyping method

The protocol used to segregate the cre and/or flp transgene is indicated below.

Detection of cre transgene and flp transgene is done using a multiplex assay: primer pairs were designed for each gene and for a positive control (Cpxm1 gene).

2.1. Cre and Flp genotyping

Schematic representation of the genotyping strategy



Sequence of primers used for genotyping:

Primers	Sequence
4029	ACTGGGATCTTCGAACTCTTTGGAC
4030	GATGTTGGGGCACTGCTCATTACC
4045	CCATCTGCCACCAGCCAG
4046	TCGCCATCTCCAGCAGG
4195	TCTTTAGCGCAAGGGGTAGGATCG
4196	GTCCTGGCCACGGCAGAAGC

PCR fragments expected size (bp):

Primer pair	4045-4046	4195-4196	4029-4030
Region analyzed	Middle part of Cre transgene	Middle part of Flp transgene	Cpxm1 control gene
Control gene	/	/	397
Tg allele	281	328	/

2.2. PCR Protocol

This section describes the composition of the mix and cycling conditions used for genotyping.

Reagents	Volume
FastStart PCR Master (Roche)	7.5 μ l
DNA (50ng/ μ l)	1.5 μ l
5' primer (100 μ M)	0.05 μ l
3' primer (100 μ M)	0.05 μ l
Sterile H ₂ O	up to 15 μ l

Cycling conditions are identical to those described in chapter 1.2