Genotyping protocol

D16Ertd472e

/ P5374

(ICS internal reference)

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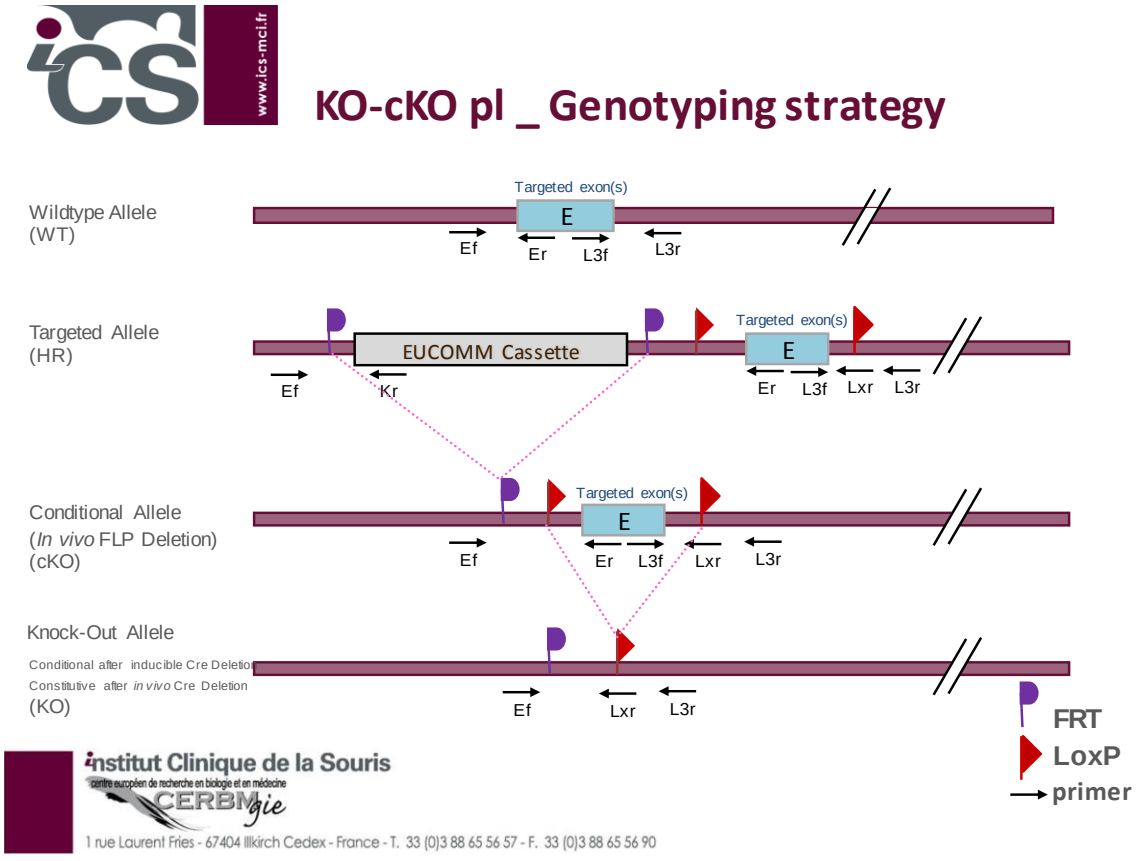
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **D16Ertd472e** 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.



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Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	8361	CAAAGGTTAATGATCGCTGGGAACC
Ef[2]	8362	CCGGGCTAAGACTCCAATTGAATC
Er	8366	CACTAGTATGTTGTATGTGTGGAGGCCC
Kr	3277	CTCCTACATAGTTGGCAGTGTTTGGG
L3f	8364	CCGTGAAGAATGTGAGTAGTGTGTGACG
L3f[2]	8363	CTTTTTGGGGGACGGTTGGGTG
L3r	8365	CCAGTGTATACCGGCCAATAGTTCCAG
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC
Mqf	4981	GGGATCTCATGCTGGAGTTCTTCG

[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)	conditional allele (KO-cKO)	KO allele	WildType allele
5' part of the selection marker	8361-3277	Ef / Kr	297	---	---	---
Presence of the distal loxP	8364-8365	L3f / L3r	453	453	---	407
Distal loxP specific PCR	8363-3255	L3f[2] / Lxr	221	221	---	---
Excision of the selection marker	8362-8366	Ef[2] / Er	5834*	420	---	284
Excision of the floxed exon(s), i.e. knock out 1	8361-8365	Ef / L3r	7081*	---*	475**	1485**

*: 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.

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.