EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Elk (Net) cKO

(PHENOMIN-ICS reference IR00000351 / K123)

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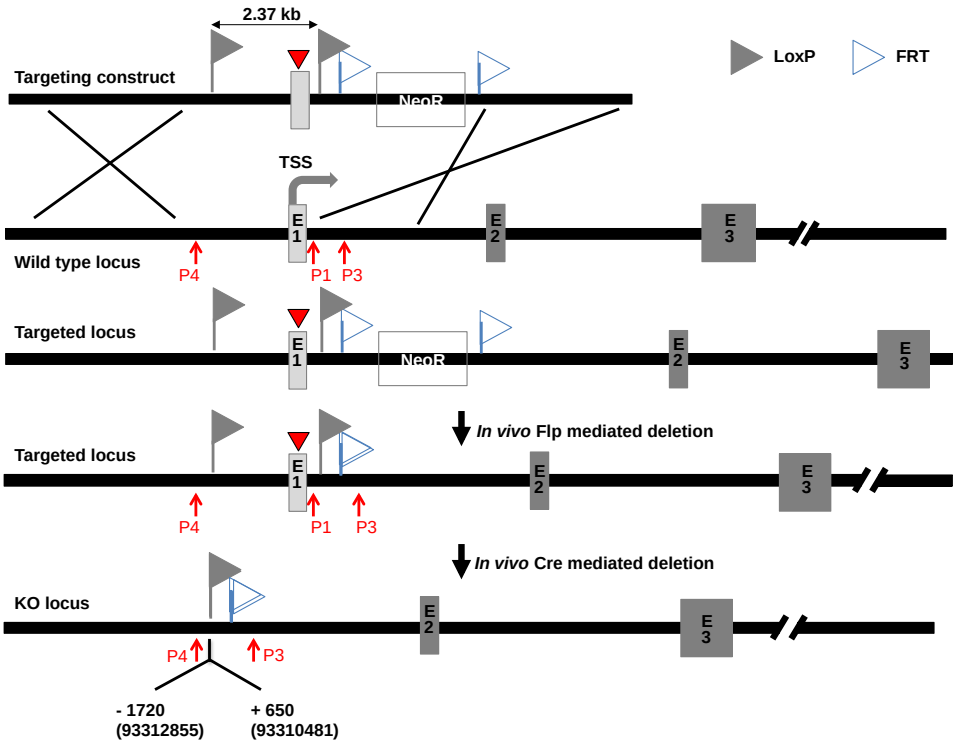
This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype the **Elk cKO mouse line**

1. PCR Genotyping protocol

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	Sequence
P1	GGTTCCTCCTAGAAATCTCCCCAAG
P2	ATGATCCCACCTCTCCCAGAAAAGG
P3	TTTGCACTCAGGGTGTCTCCTCC
P4	CACAGTTCACCTGATGGCTCACTC

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	conditional allele (KO-cKO)	KO allele	WildType allele
Presence of the distal loxP	P4 / P2	250	---	200
Distal loxP specific PCR	P1 / P3	498	---	364
Exon excision	P4 / P3	---	239	---

*: 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

If mentioned in table "PCR fragments expected size" add 0.5% of Betaine (5% of DMSO) in the reaction mix

Cycling conditions:

Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	35
72°C	1min	
72°C	7min	1
14°C	---	---

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