Genotyping protocol

Arx knock-in

IR00001549 / K420

(ICS internal reference)

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The first version of this report was finalized the: 07 nov 2013

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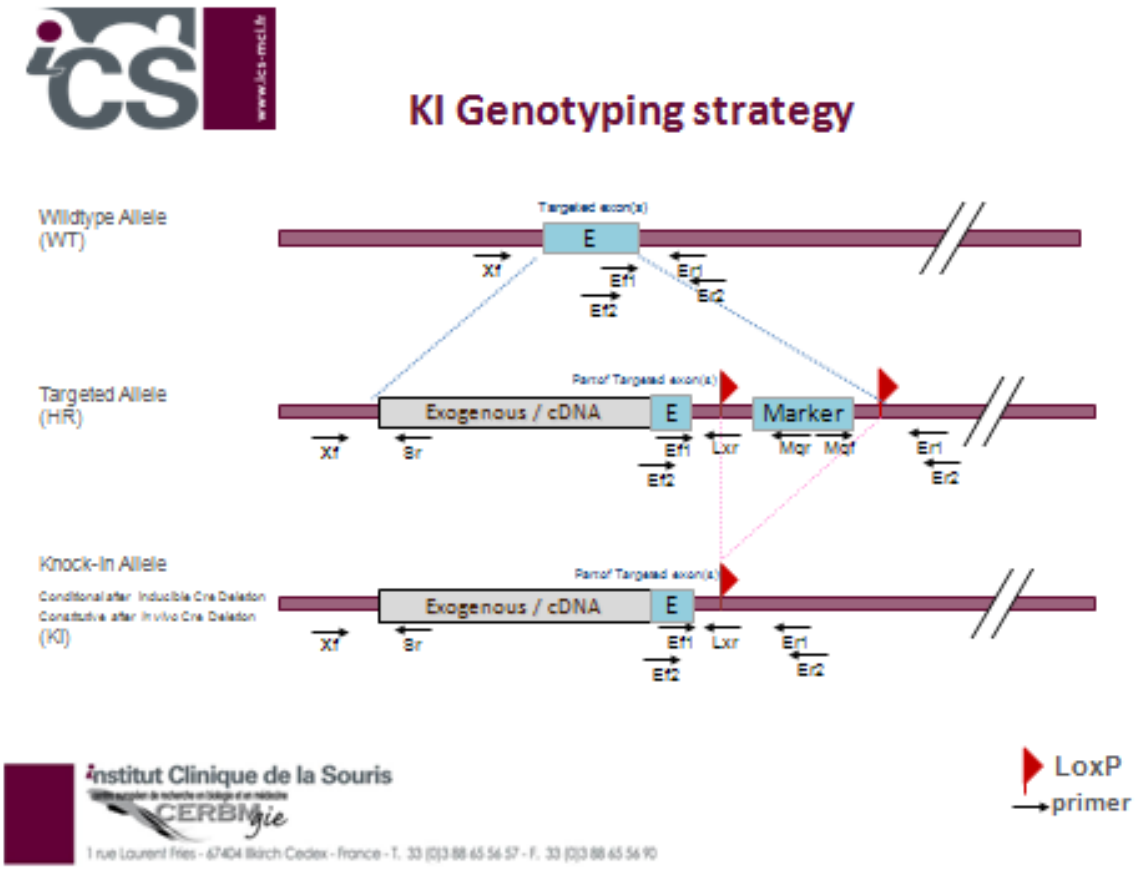
TABLE OF CONTENTS

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **ARX** Knock-In 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
Xf	4322	ACGGTCGGGCTAATGCGCTG
Ef1	4324	TGGCTCCCCAGTACTGCCCC
Ef2	5911	GAAGAGCTGTTGCTGCAC
Er1	4325	CGTGTGTGAGCGGAGAGGCG
Er2	4326	GTTTTCCTGAGAAGAGCGTA
Sr	4327	GCTGCCGCACCCTGAAGGAG
Lxr	4623	CGAAGTTATACTAGAGCGGCCGTTCAC
Mqf	1219	CAGCTCATTCCTCCCACTCATGATC
Mqr	265	TGCTAAAGCGCATGCTCCAGACTGC

PCR fragments expected size (bp):

PCR	Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	KI allele	WildType allele (WT)
A	Excision of the selection marker – PCR1	4324-4325	Ef1 / Er1	1931*	262	159
B	Excision of the selection marker – PCR2	4324-4326	Ef1 / Er2	2064*	395	292
C	5' part of the selection marker	4324-265	Ef1 / Mqr	278	---	---
D	3' part of the selection marker	1219-4326	Mqf / Er2	252	---	---
E	Exogenous/cDNA specific PCR	4322-4327	Xf / Sr	150	150	---
F	LoxP Specific PCR	5911-4623	Ef2 / Lxr	506	506	---

*: 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:

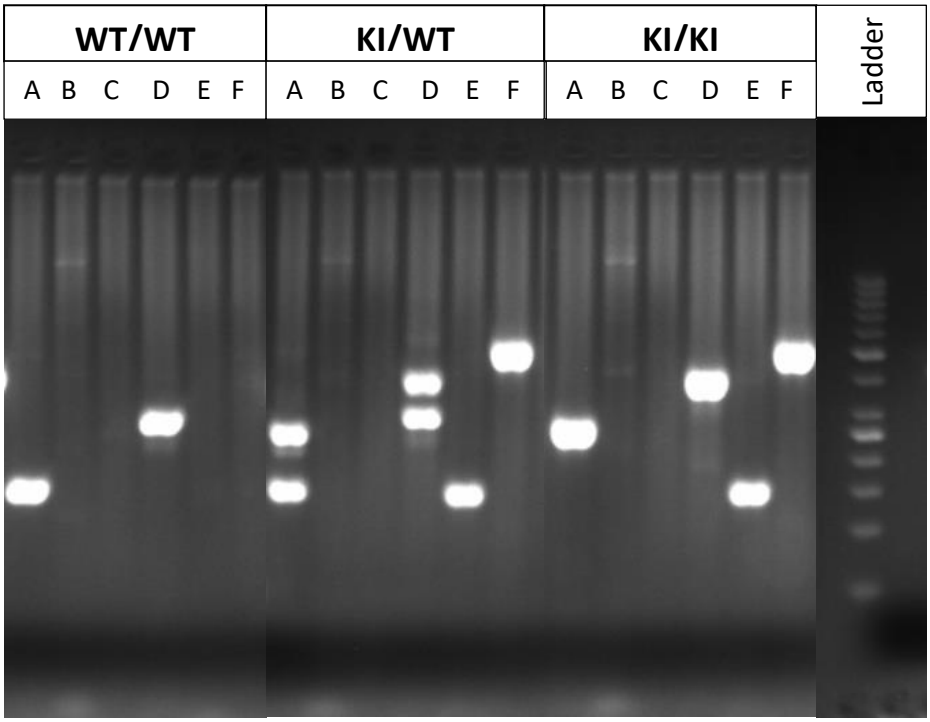
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

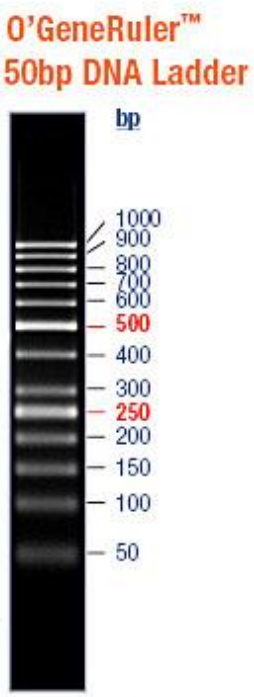
NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was done by gel electrophoresis 2% agarose (SB buffer).

Representative genotyping picture





2. Relevant publications

Cre and Flp genotyping method

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.

Tips and tricks for optimizing your PCR genotyping procedures

Optimizing PCR for mouse genotyping: Recommendations for reliable, rapid, cost effective, robust and adaptable to high-throughput genotyping protocol for any type of mutation.
Jacquot, S, Chartoire, N, Piguet, F, Herault, Y, Pavlovic, G. (2019).
Current Protocols in Mouse Biology, 9, e65. doi: 10.1002/cpmo.65
Free copy of this paper can be accessed online though this link http://bit.ly/2sxxWvO