Genotyping protocol

Atxn7l3

IR00004008 / K719

(ICS internal reference)

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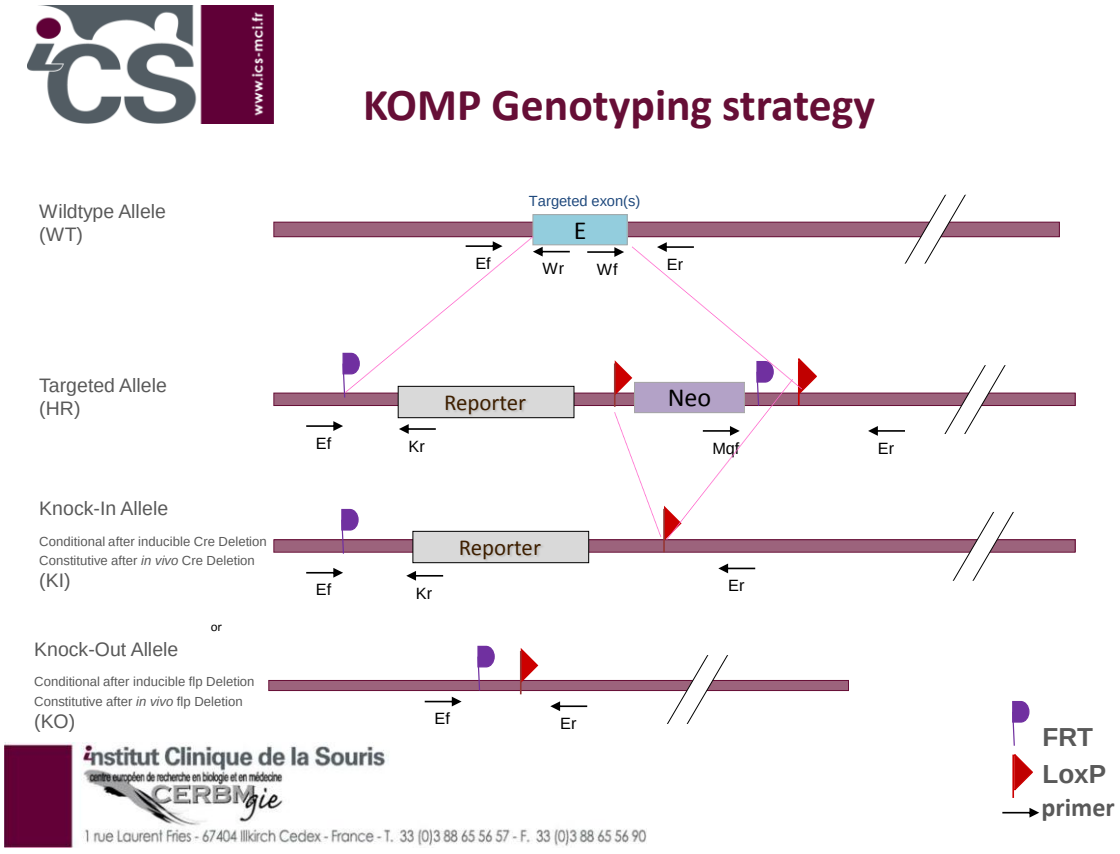
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Atxn7l3** project (KOMP Clone).

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	6581	CAAAGAAAGCAGCATGCTTGGTCAGG
Er	6584	CCTGCAGAGGAAAGAGGCACAGAG
Mqf	2687	CTGCATTCTAGTTGTGGTTTGTC
Kr	3209	CCAACAGCTTCCCCACAACGG
Wf	6583	CGTTCTTCCAGAGGTAGAGAGCTC
Wr	6582	CAGGAAGAAGTAGCCACACTTAACAGC

PCR fragments expected size (bp):

PCR Number	Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	KI allele	KO allele	WildType allele (WT)
1	WildType allele specific PCR (5' part of the targeted locus)	6581-6582	Ef / Wr	---	---	---	215
2	WildType allele specific PCR (3' part of the targeted locus)	6583-6584	Wf / Er	---	---	---	253
3	Excision of the selection marker	6581-6584	Ef / Er	7272*	5361*	368**	2956*
4	5' part of the reporter	6581-3209	Ef / Kr	277	277	---	---
5	3' part of the selection marker	2687-6584	Mqf / Er	318	---	---	---

*: 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:

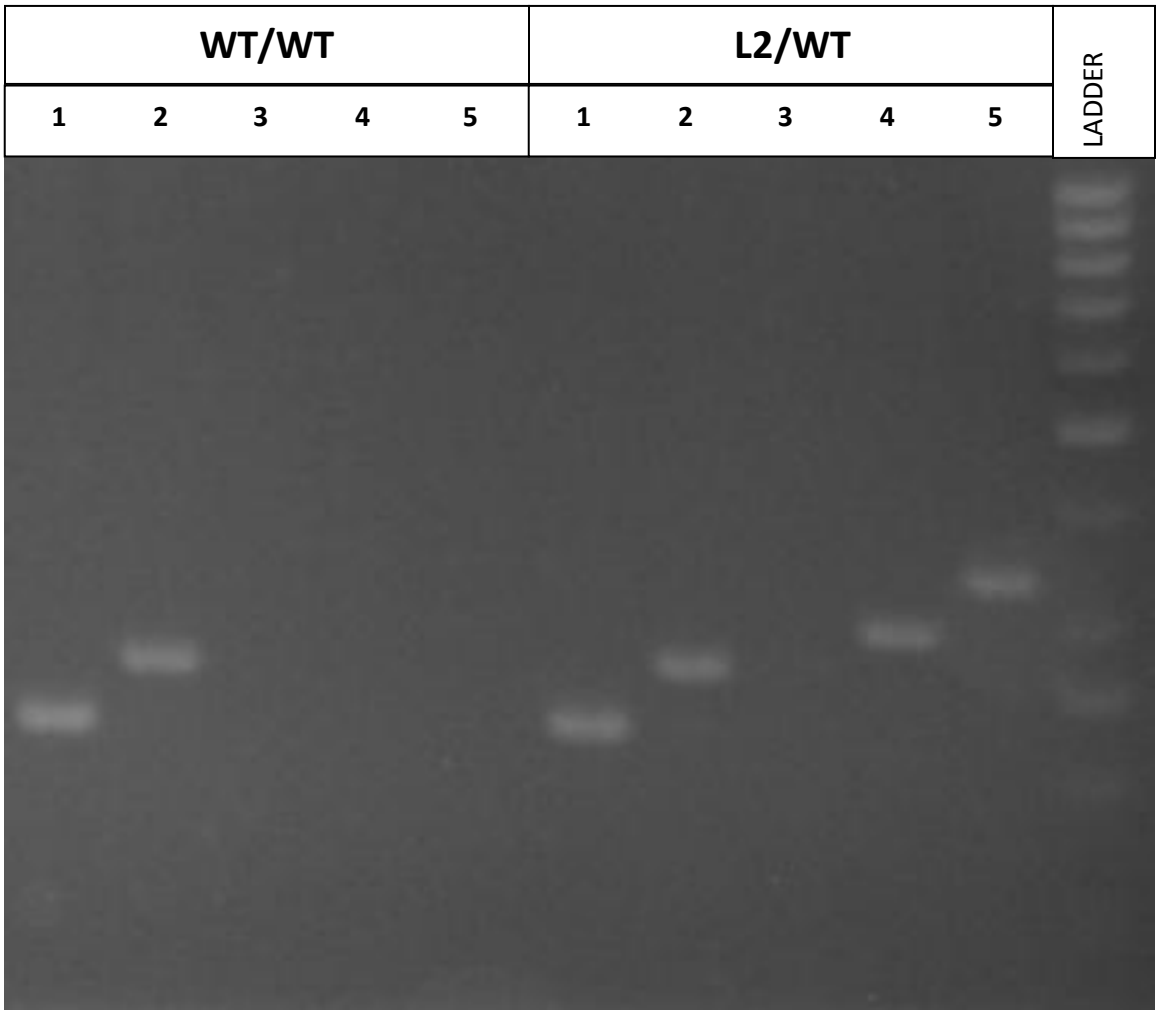
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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.

Representative genotyping picture

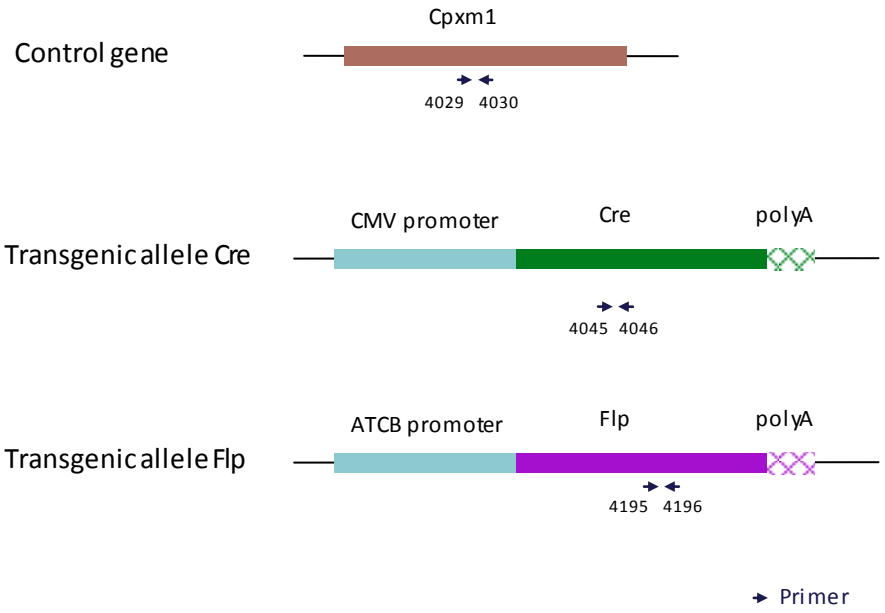


2. Cre and Flp genotyping method

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	GATGTTGGGGCACTGCTCATTCACC
4045	CCATCTGCCACCAGCCAG
4046	TCGCCATCTTCCAGCAGG
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)	7.5µl
DNA (50ng/µl)	1.5µl
5' primer (100 µM)	0.05µl
3' primer (100 µM)	0.05µl
Sterile H_2O	up to 15 µl

Cycling conditions are identical to those described in chapter 1.2