B9d1 (IR00002232 / E17 ICS internal reference) mouse line genotyping protocol

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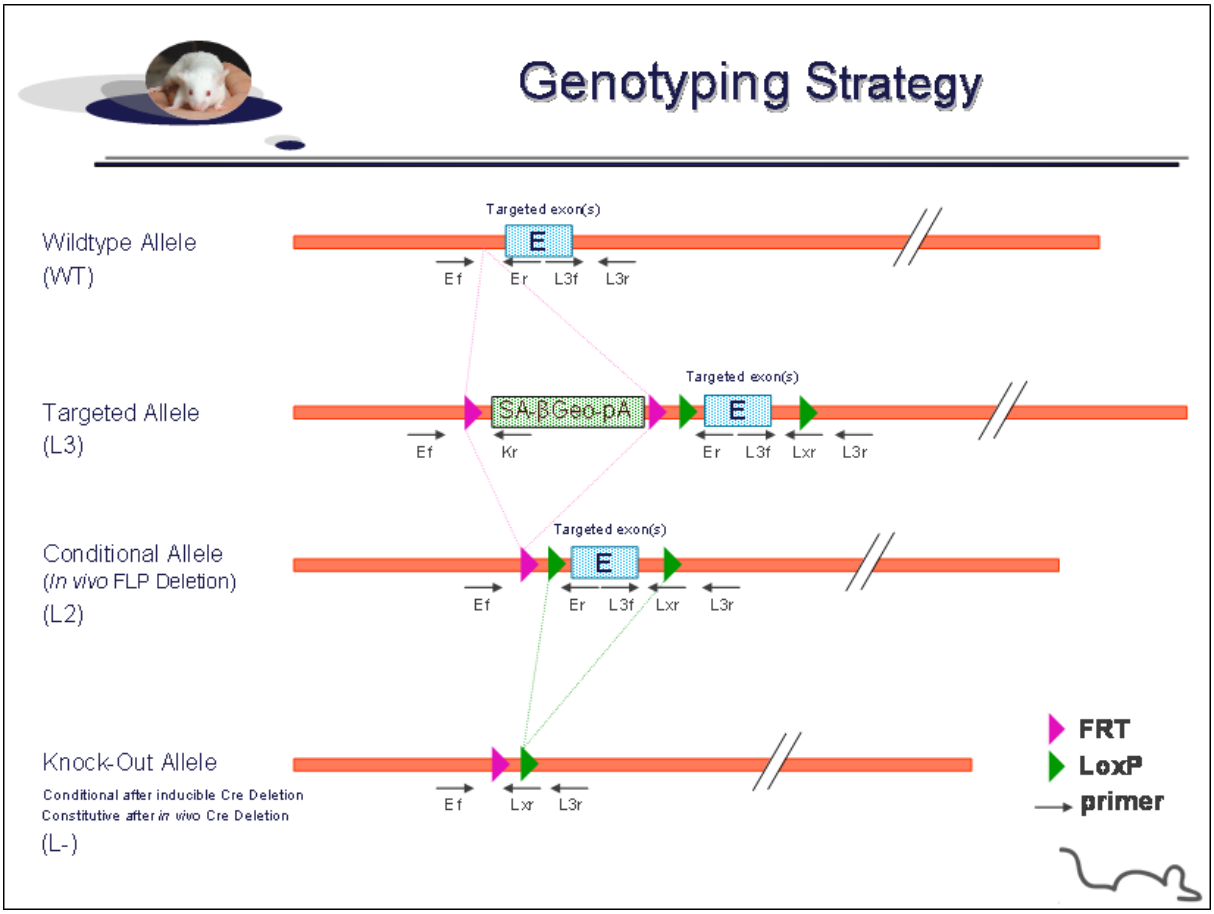
This protocol has been validated by Karim Essabri.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **B9d1** 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	3859	TGTTGTAAGCTGCCTTTGGGGCTGG
Er	3860	TCCCTCTTCATTCCACCCATCTTTGGC
Kr	3278	GGGCAAGAACATAAAGTGACCCTCC
L3f	3862	ACGGCAAGCACTGGTGTGGAAC
L3f[2]	3863	CTGTGGGCCATGCTGGAAGGGG
L3r	3861	GCTTGCTTTGCAAGGACGCAGA
Lxr	3864	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	3859-3278	Ef / Kr	392	---	---	---
Presence of the distal loxP	3862-3861	L3f / L3r	365	365	---	412
Distal loxP specific PCR	3863-3864	L3f[2] / Lxr	223	223	---	---
Excision of the selection marker	3859-3860	Ef / Er	6457*	445**	---	257
Excision of the floxed exon(s), i.e. knock out	3859-3861	Ef / L3r	7064*	1052*	394**	911**

* 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:

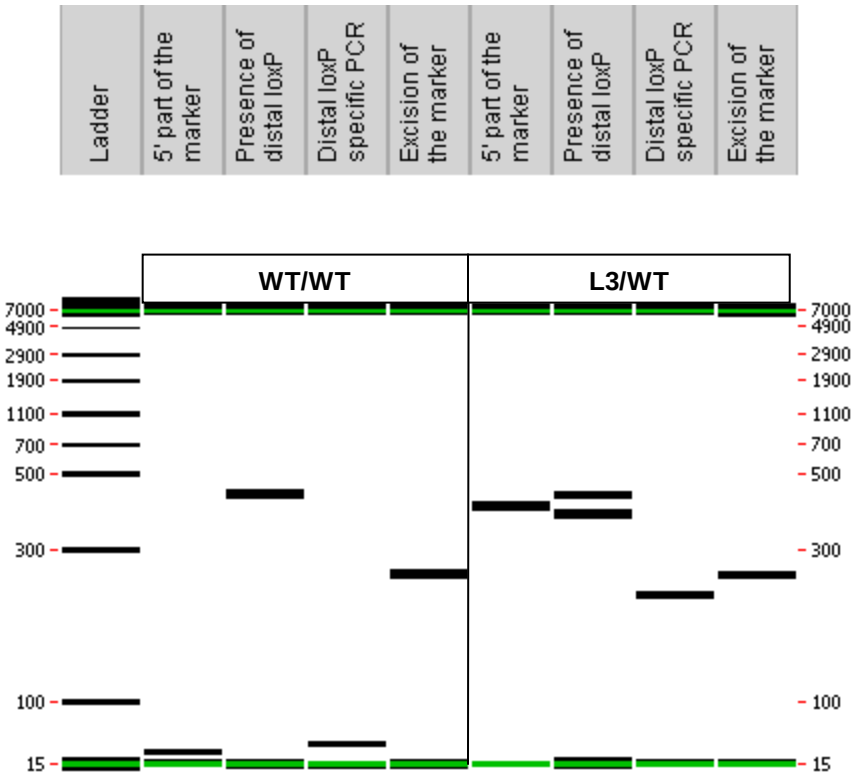
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5 min	1

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 not done by gel electrophoresis but using LabChip® 90 microfluidic apparatus. PCR products were run on the HT DNA 5K LabChip® 90 Assay Kit.

Representative genotyping picture



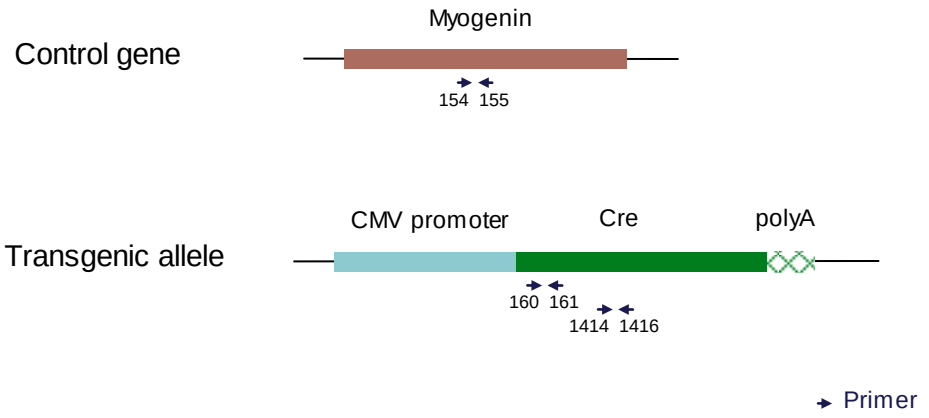
Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

2. Cre and Flp genotyping method

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

2.1. Cre genotyping

Schematic representation of the genotyping strategy



Sequence of primers used for genotyping

Primers	Sequence
154	ACTCCCTTACGTCCATCGTG
155	ACCCAGCCTGACAGACAATC
160	GAACCTGATGGACATGTTCAGG
161	AGTGCGTTCGAACGCTAGAGCCTGT
1414	CGTACTGACGGTGGGAGAAT
1416	CCCGGCAAAACAGGTAGTTA

PCR fragments expected size (bp):

Primer pair	160-161	1414-1416	154-155
Region analyzed	5' part of Cre transgene	Middle of Cre transgene	Myogenin control gene
Control gene	/	/	99
Tg allele	345	165	/

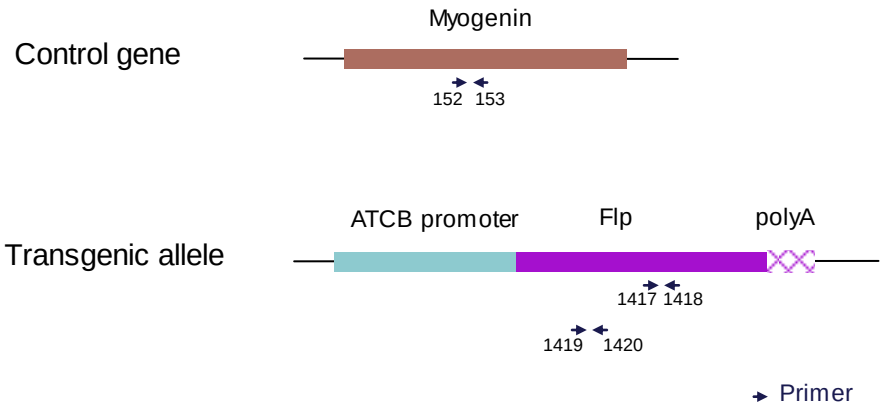
Cycling conditions:

Temp	Time	#Cycles
95°C	3min	1
95°C	10s	
62°C	20s	35
72°C	20s	
95°C	5s	
62°C	30s	1 (melting curve generation)
72°C	72s	
37°C	30s	1
4°C	∞	

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

2.2. Flp genotyping

Schematic representation of the genotyping strategy



Sequence of primers used for genotyping

Primers	Sequence
152	TTACGTCCATCGTGGACAGC
153	TGGGCTGGGTGTTAGCCTTA
1417	TTCTTTAGCGCAAGGGGTAG
1418	GCTCCAATTTCCCACAACAT
1419	TGGGAAATTGGAGCGATAAG
1420	CTGCCACTCCTCAATTGGAT

PCR fragments expected size (bp):

Primer pair	1417-1418	1419-1420	152-153
Region analyzed	Middle part of Flp transgene	5' of Flp transgene	Myogenin control gene
Control gene	/	/	245
Tg allele	299	175	/

PCR protocol and cycling conditions are identical to those described in chapter 1.2