



## **Fam63a (4930504E06Rik) (IR00002284 / E20 ICS internal reference) mouse line genotyping protocol**

### Table of contents

Table of contents .....	1
1. Genotyping protocol and data .....	2
1.1. Genotyping strategy .....	2
1.2. PCR protocol .....	3
1.3. Picture of genotyping with various alleles .....	4
2. Cre and Flp genotyping method .....	5
2.1. Cre genotyping .....	5
2.2. Flp genotyping .....	6

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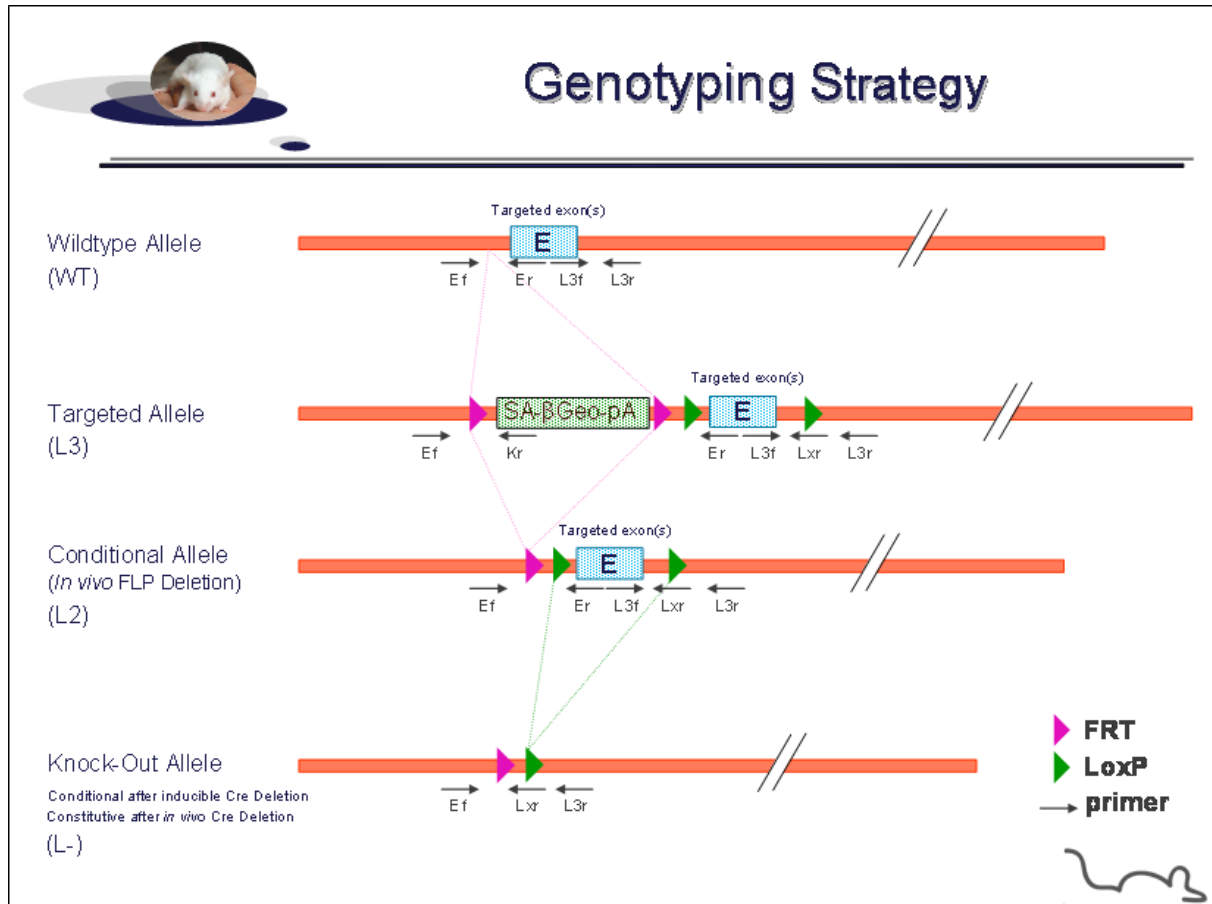
This protocol has been validated by Karim Essabri.

## 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Fam63a (4930504E06Rik)** 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	3941	CTAAGGTTGAACCTCACCACAG
Er	3943	GGCAGGTGGATCTCTA TAGGAGTG
Kr	3277	CTCCTACA TAGTTGGCAGTGTTTGGG
L3f	3944	AACCTCGTGACAGAAGGTAATGG
L3r	3942	AAATCATCCTCAGCTCCAAAGC
Lxr	3254	TTATCATTAA TTGCGTTGCGCCATC



**Genotyping protocol**  
**Fam63a (4930504E06Rik) (IR00002284 / E20 ICS internal reference)**

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	3941-3277	Ef / Kr	192	---	---	---
Presence of the distal loxP	3944-3942	L3f / L3r	381	381	---	394
Distal loxP specific PCR	3944-3254	L3f / Lxr	225	225	---	---
Excision of the selection marker	3941-3943	Ef / Er	5840*	440**	---	285
Excision of the floxed exon(s), i.e. knock out	3941-3942	Ef / L3r	8150*	2750*	300**	2608**

\* 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:

-10x Buffer (Roche)  
 -dNTPs 10mM (Amersham Biosciences)  
 -Taq DNA Polymerase (Roche)  
 -DNA (50ng/μl)  
 -5' primer (100 μM)  
 -3' primer (100 μM)  
 -Sterile H2O

### Volume:

2.5μl  
 0.5μl  
 0.2μl  
 3μl  
 0.125μl  
 0.125μl  
 up to 25 μl

### Cycling conditions:

Temp	Time	#Cycles
94°C	3min	1
94°C	1min	2
62°C	1min	
72°C	1min	
94°C	30s	30
62°C	30s	
72°C	30s	
72°C	3min	1
4°C	∞	

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

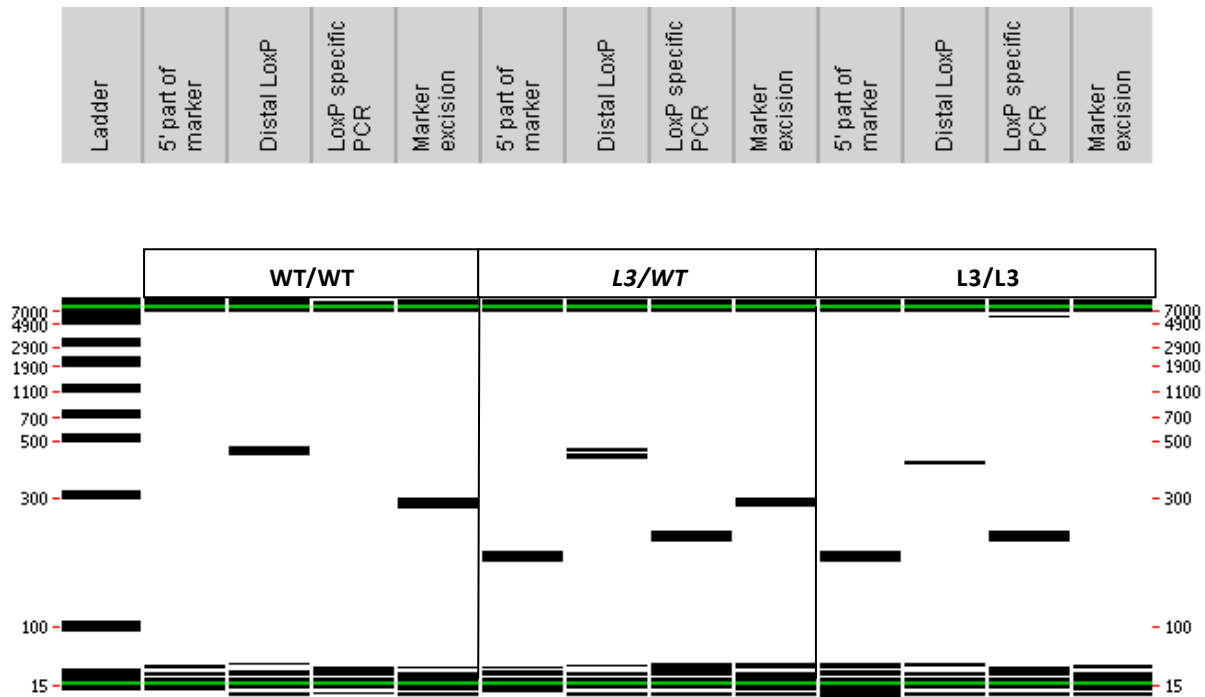


# Genotyping protocol Fam63a (4930504E06Rik) (IR00002284 / E20 ICS internal reference)

### 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



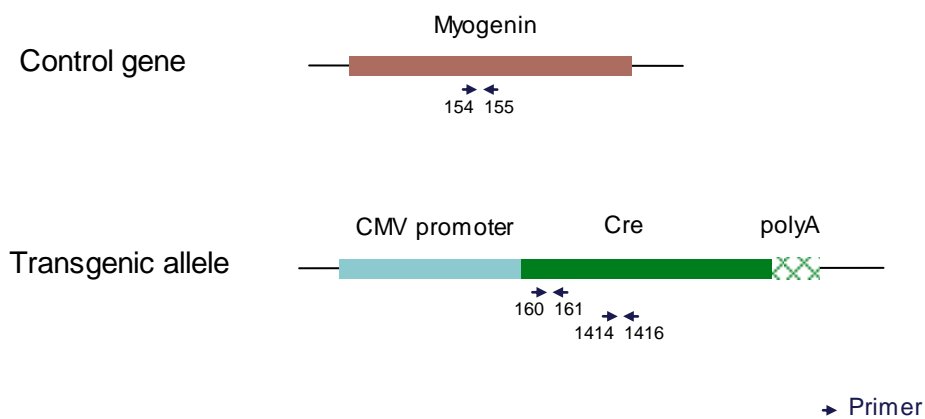
Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

## 2. Cre and Flp genotyping method

The protocol used to segregate the cre and/or flip 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	GAACCTGATGGACATGTTTCAGG
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	/

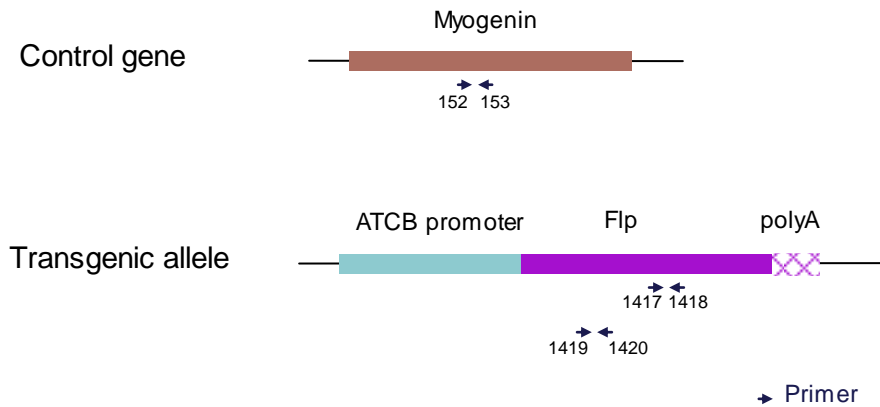
Cycling conditions:

Temp	Time	#Cycles
95°C	3min	1
95°C	10s	35
62°C	20s	
72°C	20s	
95°C	5s	1 (melting curve generation)
62°C	30s	
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	TTACGTCCATCGTGACAGC
153	TGGGCTGGGTGTTAGCCTTA
1417	TTCTTTAGCGCAAGGGGTAG
1418	GCTCCAATTTCCCACAA CAT
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