EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Rr5865-DUP

(PHENOMIN-ICS reference None / Rr5865-DUP)

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The first version of this report was finalized the: 31 Jan 2018
The last update of this report was done the: 31 Jan 2018

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Web site: http://www.ics-mci.fr/

Table of contents

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Rr5865-DUP** Crispr model, tandem duplication of genomic region (DUP) project.

1.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.

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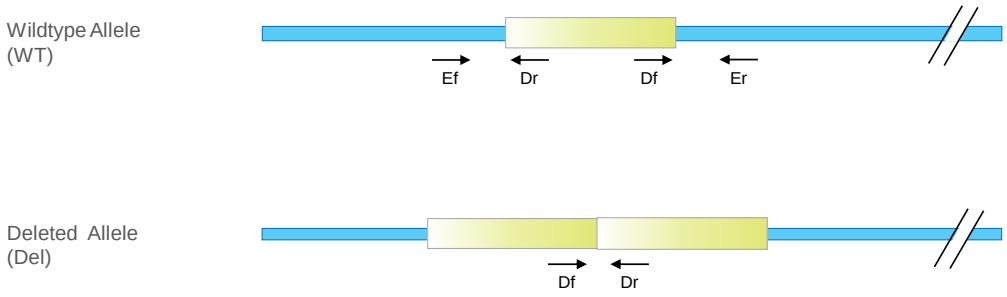
Dup Genotyping strategy



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→primer

Sequence of primers used for genotyping:

Position	Primers	Sequence
Df	9100	AATGCTCTGAACATATCCAGTGACC
Df^{2}	9183	TTACCAGAGTTATCCATGGGACACC
Df^{3}	9104	AAAGCATGACAGACGGGGTAGTAAC
Dr	9105	ACACCTTGAAAGGGGTTTTACAGTG
Dr^{2}	9184	CCTGTCTCAGATTTAGGATGCAGTC
Dr^{3}	9108	ACTGAACCCAGGGTTCTGTATATGC
Ef	9099	CTCGTGGCTGATTGATTCTGTAAAG
Er	9106	CCAATGAACCATTGTCCTGCTATAC

$^{2\,(3)}$: for a selected position, a second (third) primer was designed

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Duplicated allele	WildType allele
WildType allele specific PCR (5' part of the targeted locus)	9099-9105	Ef / Dr	---	320
WildType allele specific PCR (3' part of the targeted locus)	9100-9106	Df / Er	---	390
PCR Dup	9104-9108	Df^{3} / Dr^{3}	373	---
PCR_DUP2	9183-9184	Df^{2} / Dr^{2}	376	---

*: this PCR product will not be observed using our PCR genotyping conditions (see description below)
---: 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.

2. Cre and Flp genotyping method

You will find the genotyping protocol in the publication:

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.