EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Rr5758-802

(PHENOMIN-ICS reference None / Rr5758-DUP)

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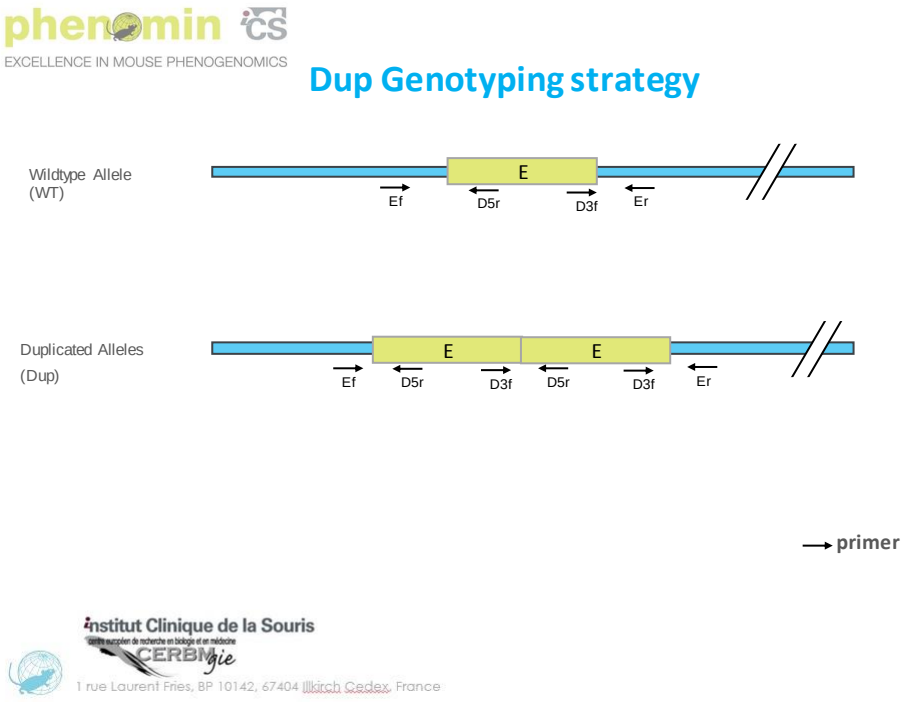
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Rr5758-** 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.



Sequence of primers used for genotyping:

Position	Primers	Sequence
D3f	9371	GGTCTCATCGTGGCCCATACTC
D5r	9369	GCAGACACCAGCACCCATC
D5r[2]	9366	CTTGTAGGCTGTGGGCTGAATG
D5r[3]	9373	CAAGCACACAAGTCGTTGCTGAG
Ef	9368	GGCGAGCCTACATGGTTGG
Er	9372	GTTCATCTTGGAACCCGGTG

[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)*	Duplicated allele	WildType allele
WildType allele specific PCR (5' part of the targeted locus)	9368-9369	Ef / D5r	311	311
WildType allele specific PCR (3' part of the targeted locus)	9371-9372	D3f / Er	364	364
PCR Dup	9371-9373	D3f / D5r[3]	126	---
PCR_DUP2	9371-9366	D3f / D5r[2]	351	---

---: 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.