EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Rr5758-DEL

(PHENOMIN-ICS reference None / Rr5758-DEL)

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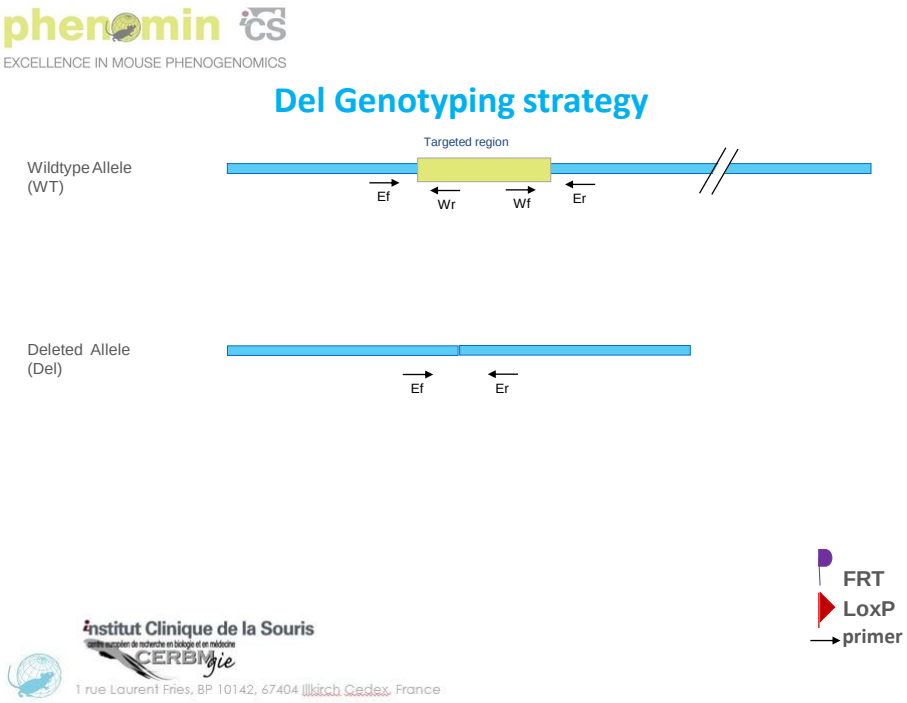
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Rr5758-DEL** Crispr model, deletion of genomic region (DEL) 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	9366	CTTGTAGGCTGTGGGCTGAATG
Er	9370	CAGCTATGATTGGAGCACTTTGTG
Ef[2]	9368	GGCGAGCCTACATGGTTGG
Er[2]	9372	GTTCATCTTGGAACCCGGTG
Wf	9371	GGTCTCATCGTGGCCCATACTC
Wr	9369	GCAGACACCAGCACCCATC

[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)*	Deleted allele	WildType allele
WildType allele specific PCR (5' part of the targeted locus)	9368-9369	Ef[2] / Wr	---	311
WildType allele specific PCR (3' part of the targeted locus)	9371-9372	Wf / Er[2]	---	364
PCR Del	9366-9372	Ef / Er[2]	431	---
PCR_DEL2	9368-9370	Ef[2] / Er	294	---

---: 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.