Genotyping protocol

D563ENLYFQS PM in Htt

$Htt^{tm3.1Ics}$

K5000

(ICS internal reference)

For any question, please contact:

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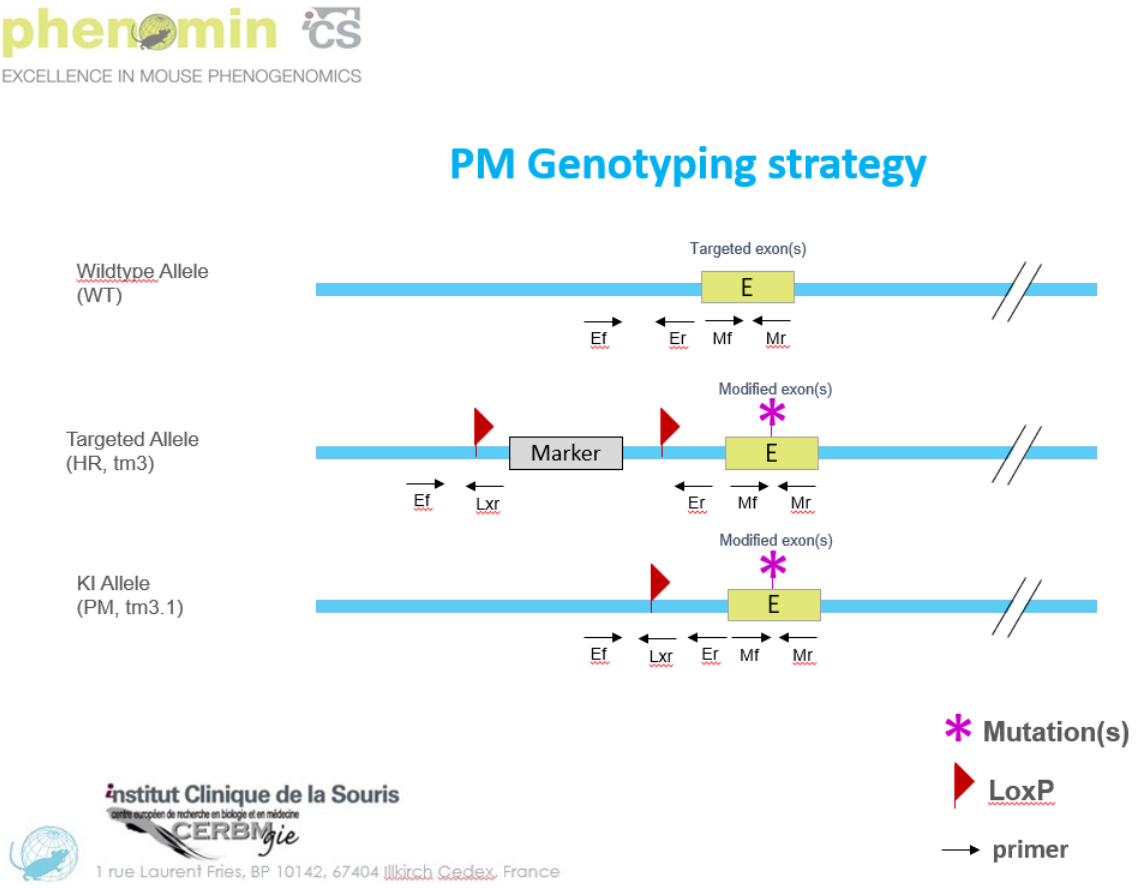
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the D563ENLYFQS PM in Htt mouse line.

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	Sequence
Ef	CACCAGGGCATGCGCAACTTATAAAC
Ef^2	CCTTAGGAAGGAGCAGGGTTCCAC
Er	CCTAGTCAGTGGCCACAGGTAAATG
Er^2	GAAAGCCCTTCAACATGAAGGAAAAGC
Lxr	AGTTATACTAGAGCGGCCGTTCACCG
Mf	GGTGGCTTTTCCTTCATGTTGAAGG
Mr	CACCTGCAGCTCCCTCCTCATC

^{2}: for a selected position, a second primer was designed

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	PM allele	WildType allele
WildType / Mutated alleles	Mf / Mr	159	159	141
Excision of the selection marker 1	Ef / Er	4498*	343	248
Excision of the selection marker 2	Ef^2 / Er^2	4521*	366	271
LoxP specific PCR	Ef / Lxr	193	193	---

*: 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	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.