EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project Smad4 I499V conditional PM

Smad4$^{tm1.1Ics}$

(PHENOMIN-ICS reference IR00005805 / K5805)

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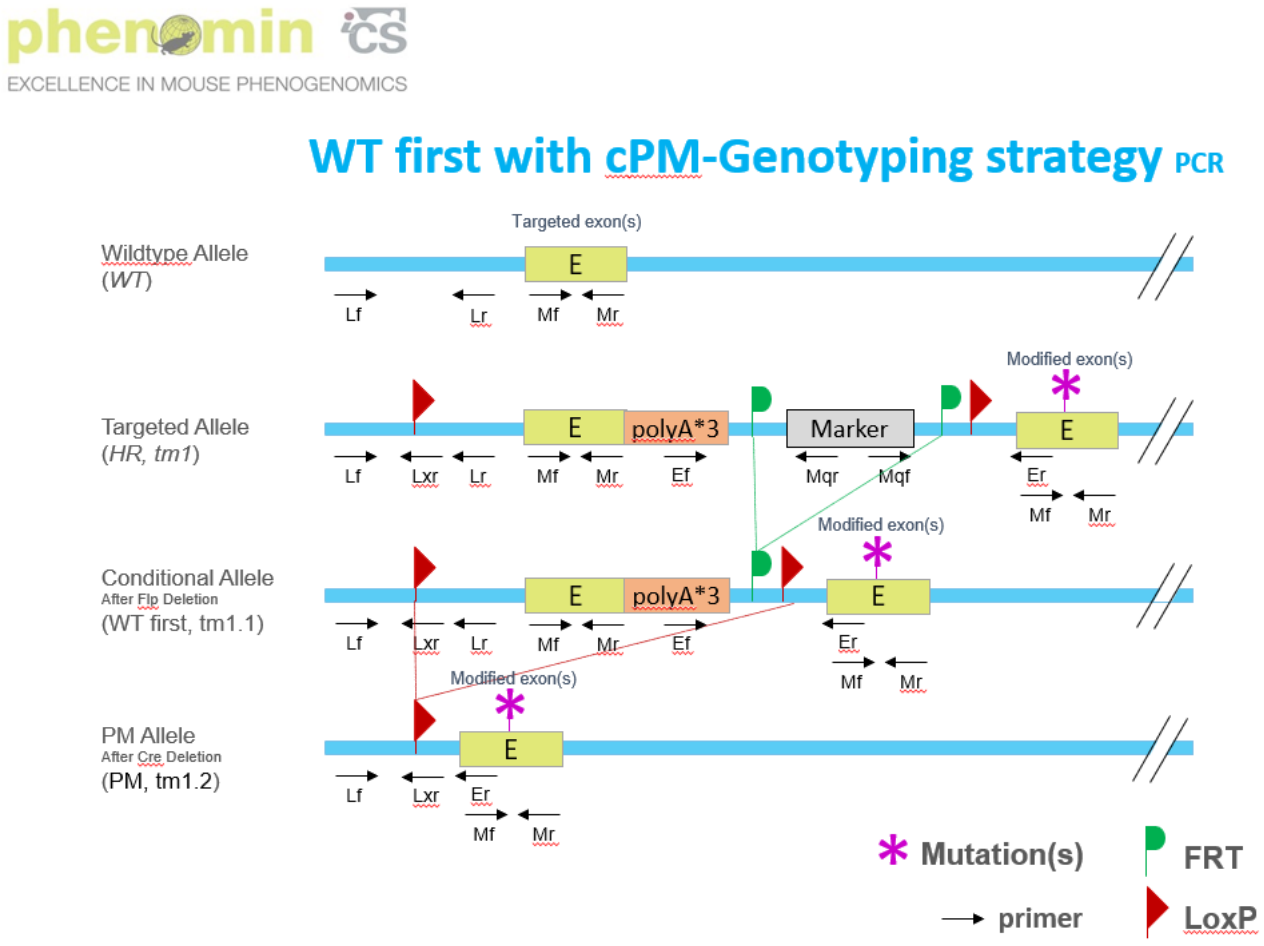
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the **Smad4** Conditional Point mutation 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	CTGCATTCTAGTTGTGGTTTGTC
Er	CGGTTGTAAGAAGCCCAGCCTCTAC
Er[2]	CAGGGGGTCGATGGATCCATAAC
Er[3]	GGTCGATGGATCCATAACTTCGTATAGC
Lf	GATTTTTATCCATGCTTTTCCCCCTCC
Lf[2]	CAGTGGCCCTGCAGGGTCTCAT
Lr	CCAACTTCTCTGTAAGAGGCAGGGC
Lxr	CGAAGTTATCTGCAGGTCGACCTTAAG
Mf	CCCGTGATAAGGTTTAGATCTGCGG
Mq1f	GAAGAACGAGATCAGCAGCCTCTGTTCC
Mq1r	TGCTAAAGCGCATGCTCCAGACTGC
Mr	GCATGGTGTGCAGGACTTCATCC

[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	KO allele	WildType allele
WildType / Mutated alleles	Mf / Mr	336	336	336	336
Presence of the distal loxP (with Betaine 0,5%)	Lf / Lr	252	252	266	192
Excision of the selection marker (with Betaine 0,5%)	Ef / Er	2085*	231	---	---
5' part of the selection marker	Ef / Mq1r	160	---	---	---
3' part of the selection marker	Mq1f / Lr	298	---	---	---
LoxP specific PCR	Lf[2] / Lxr	341	341	341	---
Excision of the floxed exon(s), i.e. knock out	Lf[2] / Er[2]	3690*	1836*	384**	---
Excision of the floxed exon(s), i.e. knock out 2	Lf / Er[3]	3458*	1604*	152**	---

*: 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.