EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Snc2a WT first with conditional L1314P PM potential

Scn2a$^{em1.1Ics}$

(PHENOMIN-ICS reference IR00007499 / Kos7499)

For any question, please contact:

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This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype the **Snc2a conditional PM** mouse line.

1. PCR Genotyping protocol

1.1. Genotyping strategy

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

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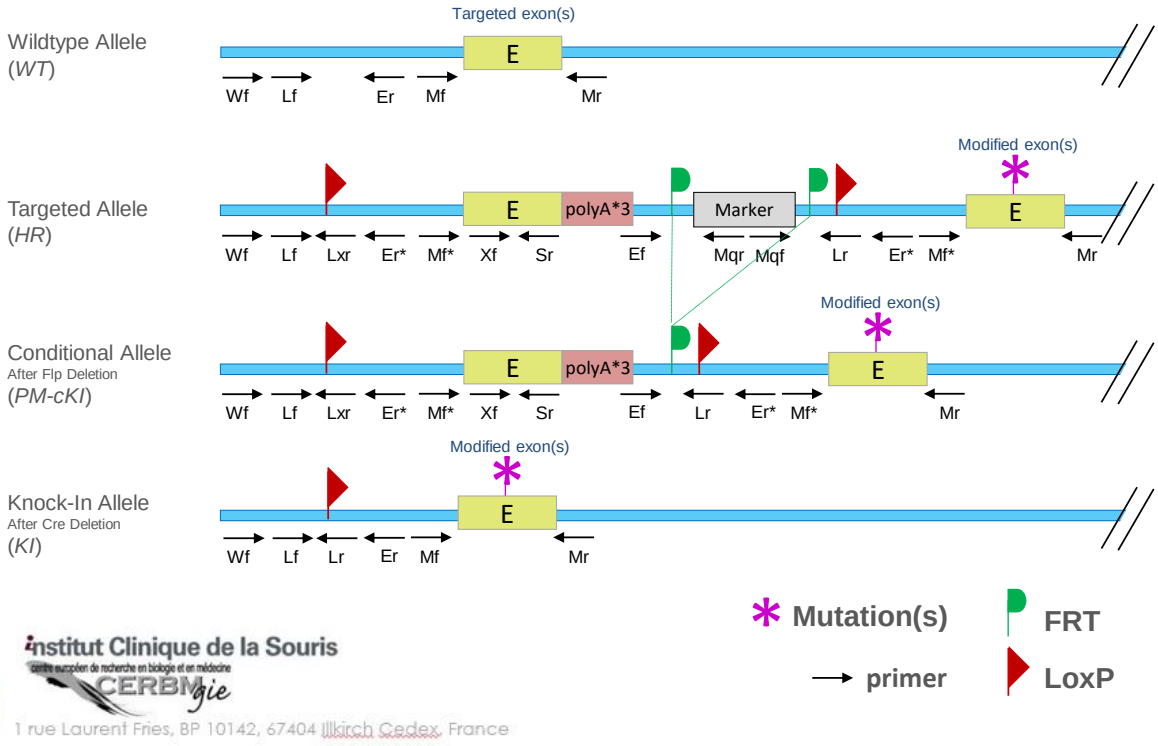
PM-cKI Genotyping strategy



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* Er and Mf primers hybridize at 2 different positions on targeted and conditional alleles

Sequence of primers used for genotyping:

Position	Sequence
Ef	CTTATCATGTCTGCACCGGTGG
Er	CTTGGGTGAGTTTCCCCGTCAC
Lf	CCAAACTGTGCTAGCTCCCG
Lxr	GGTCGATGGATCCATAACTTCGTATAGC
Lr	GGGGGTCGATGGATCCATAACTTC
Mf	GGAACGCTGTGGCATGTAG
Mr	CGCTGGATGCGTATTATTGATTGGTC
Mq1f	GAAGAACGAGATCAGCAGCCTCTGTTCC
Mq1r	TGCTAAAGCGCATGCTCCAGACTGC
Sr	GAGGCCGATGTTAAACAGCGC
Wf	CCGAACTTACTAAACCAAACTGTGCTAG
Xf	ACCAGAGCCAAGAGATGACCAAC

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	Conditional allele (cKI)	PM allele	WildType allele
Mutation PCR	Mf / Mr	220	220	220	220
Presence of the distal loxP	Lf / Er	418	418	432	338
Excision of the selection marker	Ef / Er	2166*	312	---	---
5' part of the selection marker	Ef / Mq1r	120	---	---	---
3' part of the selection marker	Mq1f / Er	387	---	---	---
Exogenous/cDNA5' specific PCR	Xf / Sr	335	335	---	---
LoxP PCR	Lf / Lxr	5430*	3576*	229**	---
LoxP "Ki specific" PCR	Wf / Lr	5447*	3593*	246**	---

*: amplicon will not be observed using our genotyping conditions
**: 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	35
72°C	1min	
72°C	7min	1
14°C	---	---

NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

2. Recommended papers:

2.1. Cre and Flp genotyping method

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.

2.1. Tips and tricks for optimizing your PCR genotyping procedures

Optimizing PCR for mouse genotyping: Recommendations for reliable, rapid, cost effective, robust and adaptable to high-throughput genotyping protocol for any type of mutation.
Jacquot, S, Chartoire, N, Piguet, F, Herault, Y, Pavlovic, G. (2019).
Current Protocols in Mouse Biology, 9, e65. doi: 10.1002/cpmo.65
Free copy of this paper can be accessed online though this link http://bit.ly/2sxxWvO