EXCELLENCE IN MOUSE PHENOGENOMICS

Genotyping protocol

Project pCAG-HPV18 conditional Flex in Rosa locus

Gt(ROSA)26Sortm$^{27.1(CAG-EGFP,-HPV18E7)Ics}$/Ics

(PHENOMIN-ICS reference IR00004761 / I4761)

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This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **pCAG-HPV18cond in Rosa** Knockin (KI) project.

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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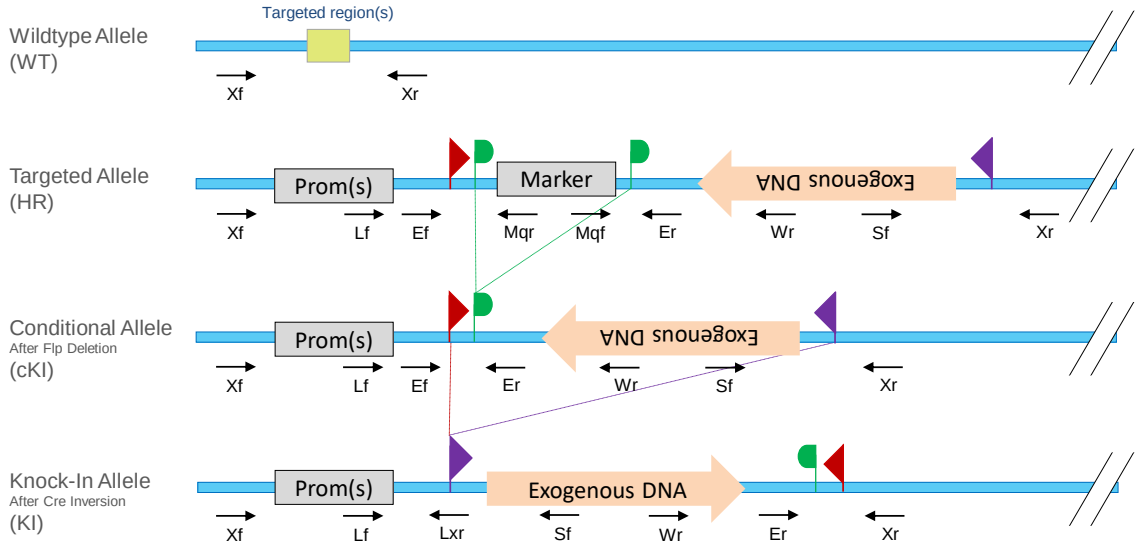
KI-FLEX Genotyping strategy



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Sequence of primers used for genotyping:

Position	Sequence
Ef	GGCTCTAGAGCCTCTGCTAACCATGT
Er	GTGGTTTGTCCAAACTCATC
Lf	GCGGAGCCGAAATCTGGGAG
Lxr	TGCTATACGAACGGTAGGCCGG
Mqf	GAAGAACGAGATCAGCAGCCTCTGTTCC
Mqr	TGCTAAAGCGCATGCTCCAGACTGC
Sf	TTCTCTTCCTCGCTGTCGCT
Wr	CACATGGTCCTGCTGGAGTTCGT
Xf	AAAGTCGCTCTGAGTTGTTAT
Xr	CCTTTAAGCCTGCCCAGAAG

PCR fragments expected size (bp):

Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	conditional allele (cKI)	KI allele	WildType allele
WildType allele specific PCR (5' part of the targeted locus)	Xf / Xr	5044*	3191*	3191*	239
Excision of the selection marker	Ef / Er	2202*	250	---	---
5' part of the selection marker	Lf / Mqr	464	---	---	---
3' part of the selection marker	Mqf / Wr	359	---	---	---
Exogenous DNA3' specific PCR	Sf / Xr	404	404	---	---
Inversion 3' PCR	Wr / Xr	---	---	354**	---
Inversion 5' PCR (**with DMSO**)	Lf / Lxr	---	---	340**	---

*: amplicon will not be observed using our genotyping conditions
**: this PCR is only verified if mice are generated
---: no Amplicon should be obtained

1.1. 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

If mentioned in table "PCR fragments expected size" add 5% of DMSO in the reaction mix

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