

Genotyping protocol

Project pCAG-HPV16 conditional Flex in Rosa locus

Gt(ROSA)26Sor^{tm26.1(CAG-EGFP,-HPV16E7)}Ics/Ics (PHENOMIN-ICS
reference IR00004760 / I4760)

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pCAG-HPV16 cond in Rosa - Gt(ROSA)26Sor^{tm26.1(CAG-EGFP,-HPV16E7)lcs}/lcs

This protocol describes the condition used at the Mouse Clinical Institute (ICS) to genotype your pCAG-HPV16 cond in Rosa Knockin (KI) project.

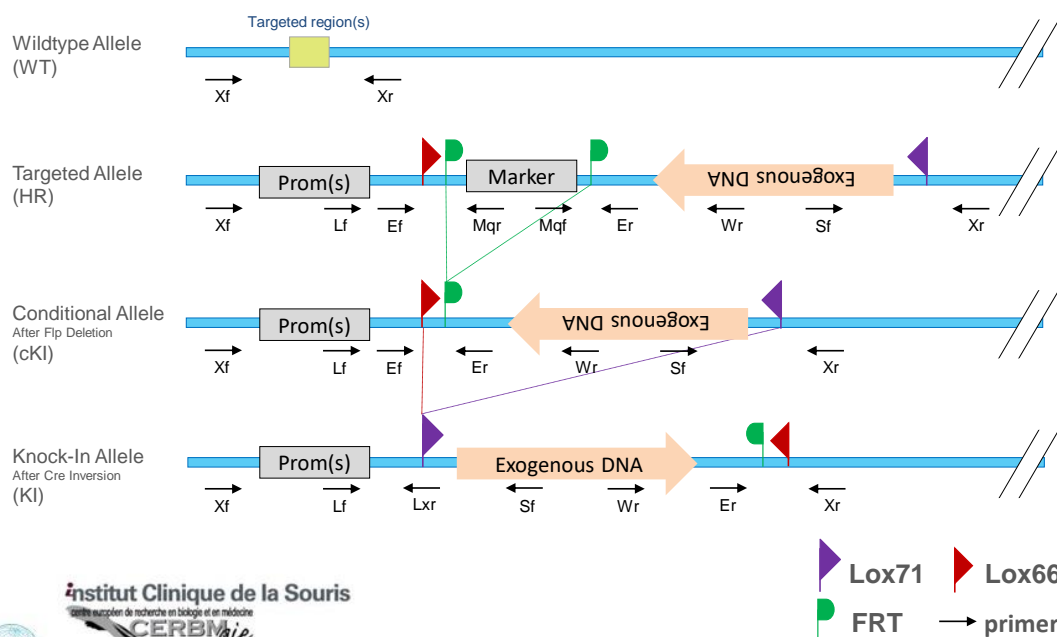
1. PCR Genotyping protocol

1.1. Genotyping strategy

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



KI-FLEX Genotyping strategy



Sequence of primers used for genotyping:

Position	Sequence
Ef	GGCTCTAGAGCCTCTGCTAACCATGT
Ef ²	AGCCTCTGCTAACCATGTTTCATGCCT
Er	GTGGTTTGTCCAAACTCATC
Lf	GCGGAGCCGAAATCTGGGAG
Lxr	TGCTATACGAACGGTAGGCCGG
Mqf	GAAGAACGAGATCAGCAGCCTCTGTTCC
Mqr	TGCTAAAGCGCATGCTCCAGACTGC
Sf	TCCTCGCTGCTGTCGTT
Wr	CACATGGTCTGCTGGAGTTCGT
Xf	AAAGTCGCTCTGAGTTGTTAT
Xr	CCTTTAAGCCTGCCAGAAG

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)	conditional allele (cKI)	KI allele	WildType allele
WildType allele specific PCR (5' part of the targeted locus)	Xf / Xr	5016*	3163*	3163*	239
Excision of the selection marker	Ef / Er	2102*	249	---	---
5' part of the selection marker	Ef ² / Mqr	239	---	---	---
3' part of the selection marker	Mqf / Wr	358	---	---	---
Exogenous DNA3' specific PCR	Sf / Xr	389	389	---	---
Inversion 3' PCR	Wr / Xr	---	---	353	---
Inversion 5' PCR (with DMSO)	Lf / Lxr	---	---	340	---

*: amplicon will not be observed using our genotyping conditions

---: 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 ₂ O	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, Héroult 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, Héroult, 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 through this link <http://bit.ly/2sxxWvO>

