Genotyping protocol

Knock-In of Venus at the ATG of Shank3

IR00004021 / K4021

(ICS internal reference)

This report has been prepared by: **David Moulaert**

This report has been validated by: **Sylvie Jacquot, PhD, Head of Genotyping Service**

The first version of this report was generated the: 18 Nov 2015

For any question, please contact:

Institut Clinique de la Souris - ICS - Mouse Clinical Institute
1 rue Laurent Fries, BP 10142
67404 Illkirch Cedex, France
Email: mutagenesis@igbmc.fr
Web site: http://www-mci.u-strasbg.fr/

TABLE OF CONTENTS

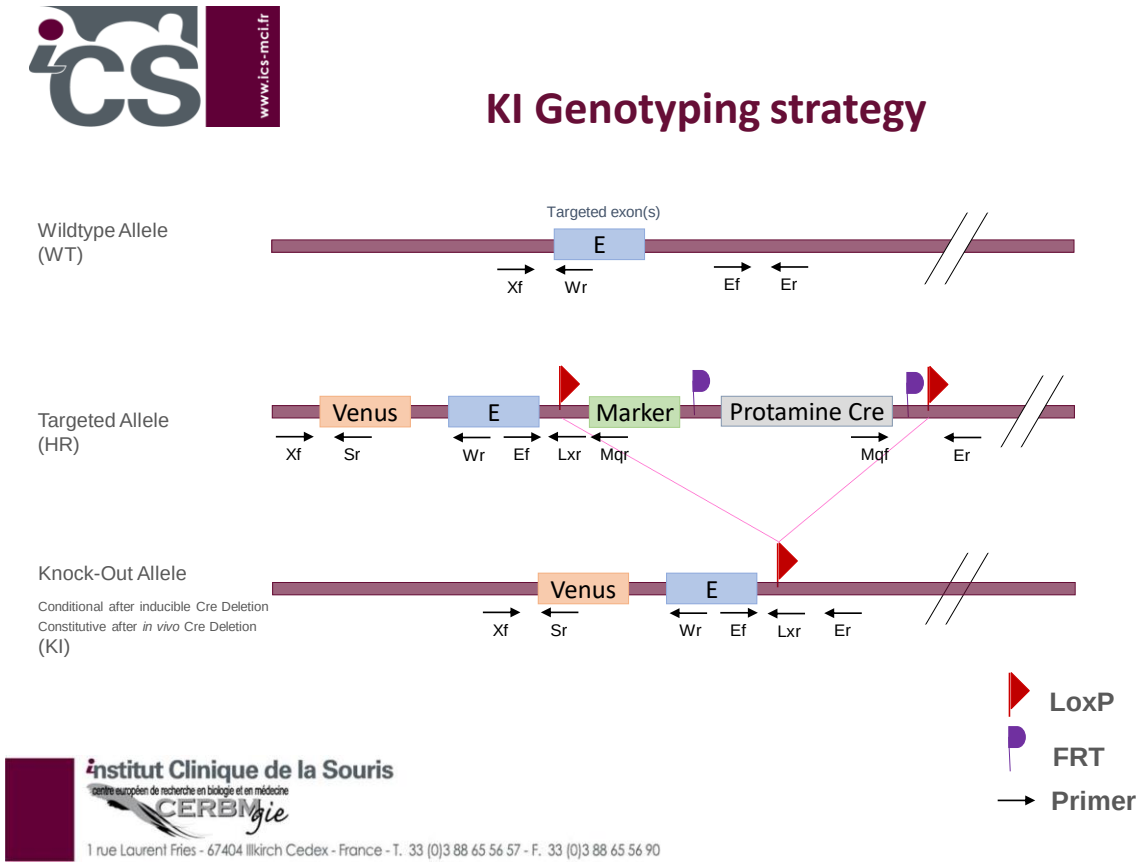
Table of contents **2**
1. Genotyping protocol and data **2**
1.1. Genotyping strategy 2
1.2. PCR protocol 4
2. Cre and Flp genotyping method **5**

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Venus-Shank3** Knockin (KI) project.

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	Primers	Sequence
Ef	7589	GCAGTGTGCTGAGAGACCGAG
Ef[2]	7588	GTGTGATCTTTGAGCTCCGTGGC
Er	7591	CCCTAACGCACTCACTCTTAAACCG
Er[2]	7590	GCATGTGCTCATTGTGTGCTGTCC
Lxr	4559	AGTTATACTAGAGCGGCCGTTCACCG
Mqf	6145	GATAGTGAAACAGGGGCAATGGTGC
Mqr	265	TGCTAAAGCGCATGCTCCAGACTGC
Sr	2244	CCGTCCAGCTCGACCAGGATG
Wr	7585	GGTACGGCGAGATCGCAAAGG
Xf	7586	CTCTCTCCGCCGGGAACAG

[2]: for a selected position, a second primer was designed

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (HR)	KI allele	WildType allele
WildType allele specific PCR (5' part of the targeted locus) (with DMSO)	7586-7585 (with 5% DMSO)	Xf / Wr	862	862	133
Excision of the selection marker	7589-7591	Ef / Er	4582*	336**	241
5' part of the selection marker	7588-265	Ef[2] / Mqr	303	---	---
3' part of the selection marker	6145-7590	Mqf / Er[2]	652	---	---
Exogenous/cDNA specific PCR (with DMSO)	7586-2244 (with 5% DMSO)	Xf / Sr	121	121	---
LoxP specific PCR	7588-4559	Ef[2] / Lxr	209	209	---

*: 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.