Genotyping protocol

Nxnl2 KI (LacZ)/KO

IR00003942 / K3942

(ICS internal reference)

For any question, please contact:

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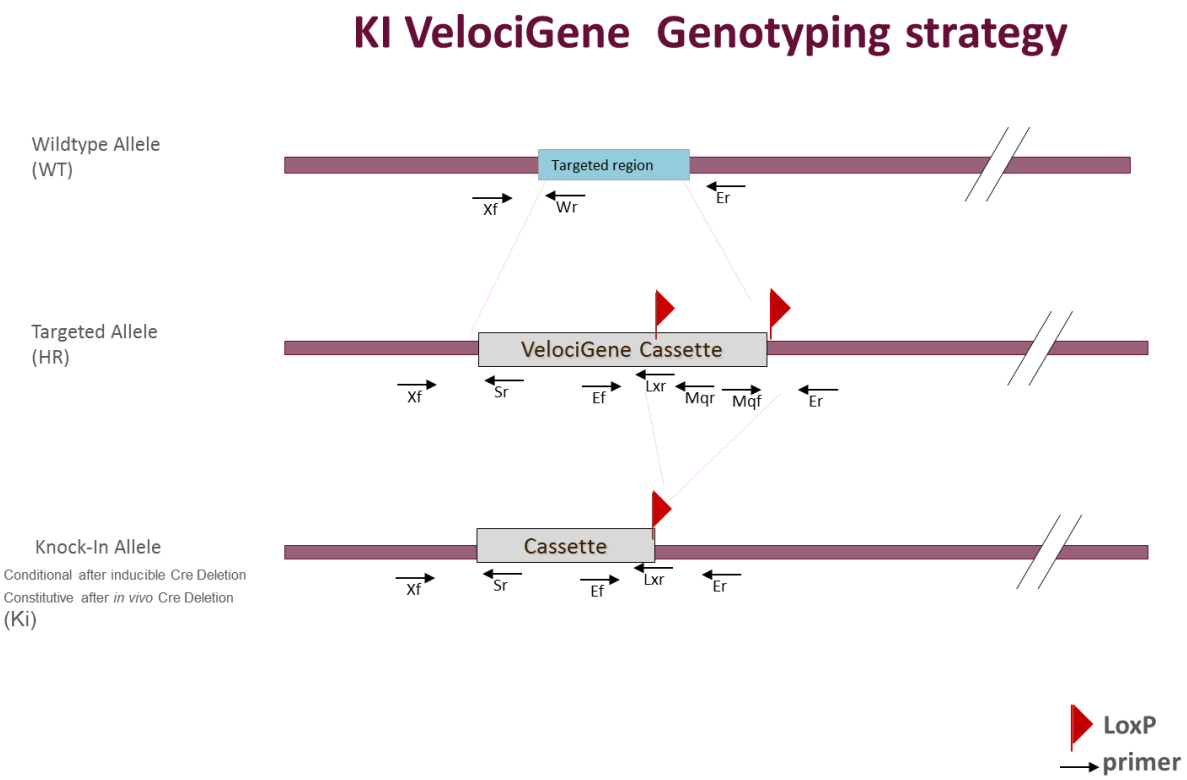
TABLE OF CONTENTS

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the **Nxnl2** Knockin (KI/KO) 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	GTGGTTTGTCCAAACTCATCA
Er	TGCTCAAAGTGTGAGTGTTGG
Lxr	GAAGTTATCTCGAGTCGCTACCTTAG
Mq1f	GAAGAACGAGATCAGCAGCCTCTGTTCC
Mq1r	GTTCTAAGGCCGAGTCTTATGAGCAG
Sr	CTAGTCTGTTCAGCTGTGTCACACC
Wr	GGTAGCATCGCAACTTGGATAAC
Xf	CCGCTTCTAGGACAGATGTCAG
Xf2	TGGTGGGTAGAGAGGGTTTG

PCR fragments expected size (bp):

N° PCR	Region analyzed	Position on the primer *(see the map above)*	Targeted allele (L2)	KI allele (L-)	WildType allele (WT)
1	WildType allele specific PCR (5' part of the targeted locus)	Xf / Wr	---	---	80
2	Excision of the selection marker	Ef / Er	3169*	555**	---
3	5' part of the selection marker	Ef / Mq1r	316	---	---
3	3' part of the selection marker	Mq1f / Er	530	---	---
4	Exogenous/cDNA specific PCR	Xf2 / Sr	375	375	---
4	LoxP specific PCR	Ef / Lxr	130	130	---

*: 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.

1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was not done by gel electrophoresis but using LabChip® 90 microfluidic apparatus. PCR products were run on the HT DNA 5K LabChip® 90 Assay Kit.

Representative genotyping picture