Genotyping protocol

Itgb5

IR00002672 / E83

(ICS internal reference)

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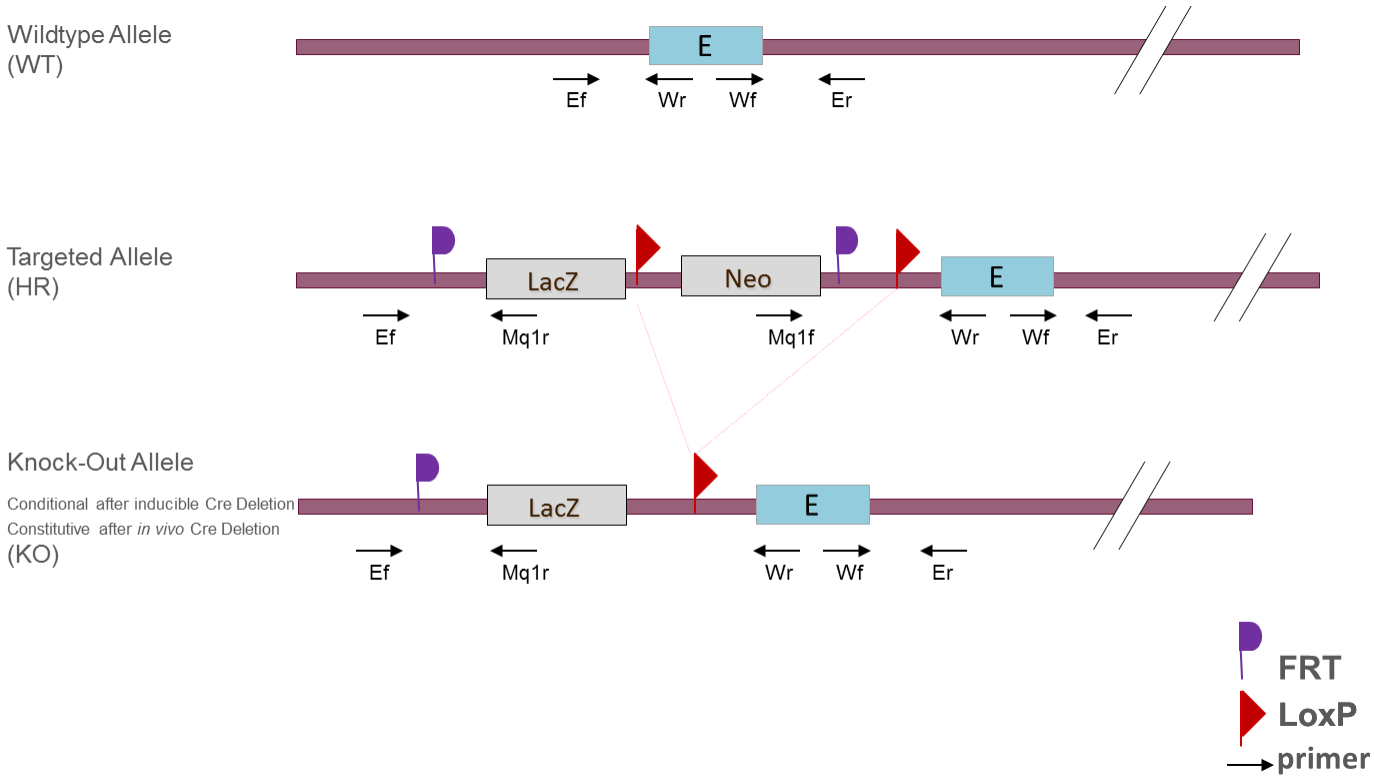
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Itgb5** Conventional or Constitutive Knockout (KO) 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	4976	AGGCCTTGCCCAGAGCAGGTGG
Er	4980	AGTGTGGACGCTCTACACGGAGC
Er[2]	4977	GCAGAGATCAGCTCTCTTCTATC
Mq1f	4981	GGGATCTCATGCTGGAGTTCTTCG
Mq1r	3209	CCAACAGCTTCCCCACAACGG
Wf	4979	TGCGATAGGCTGGTGTCCTTGCC
Wr	4978	GCAGAGATCAGCTCTCTTCTATC

[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)	KO allele	WildType allele
WildType allele specific PCR (5' part of the targeted locus)	4976-4978	Ef / Wr	---	---	172
WildType allele specific PCR (3' part of the targeted locus)	4979-4980	Wf / Er	---	---	466
Excision of the selection marker	4976-4977	Ef / Er[2]	7223*	319**	172*
5' part of the selection marker	4976-3209	Ef / Mq1r	293	---	---
3' part of the selection marker	4981-4977	Mq1f / Er[2]	495	---	---

*: 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.