Genotyping protocol

Kctd13 KO

/ T5619

(ICS internal reference)

This report has been prepared by: **Valérie Rousseau**
genotyping@igbmc.fr

This report has been validated by: **Sylvie Jacquot, PhD, Head of Genotyping Service**
33 (0)3 88 65 57 44
genotyping @igbmc.fr

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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: genotyping@igbmc.fr
Web site: http://www-mci.u-strasbg.fr/

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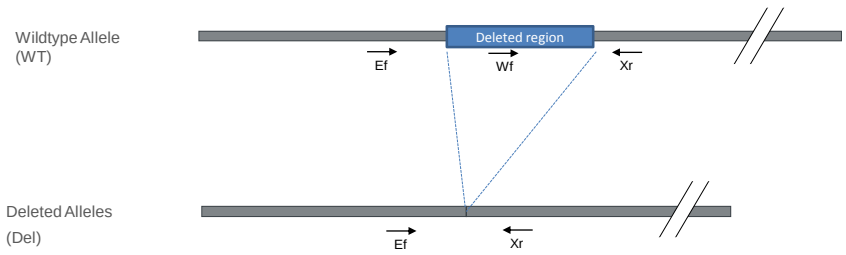
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Kctd13 KO** Transgenic model, deletion of genomic region (DEL) project.

1.1. Genotyping strategy

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

Del Genotyping strategy



→ primer

Institut Clinique de la Souris
CERBMgie
1 rue Laurent Fries - 67404 Illkirch Cedex - France - T. 33 (0)3 88 65 56 57 - F. 33 (0)3 88 65 56 90

Sequence of primers used for genotyping:

Position	Primers	Sequence
Ef	8754	ACCTCTTAGCTGGGCATGCTAAATT
Xr	8753	AGCCTATGCTAACTATTATCACAGG
Wf	8751	CTCATCCCCACAGTGACATCACCCC
Xr[2]	8752	GAGCTCTGCCAAACCTAGGATTCTG

[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)*	Deleted alleles	WildType alleles
WildType allele specific PCR (3' part of the targeted locus)	8751-8752	Wf / Xr	---	295
PCR Del	8754-8753	Ef / Xr[2]	239/288*	668

* The expected size of deleted allele depends on the mouse line
---: 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.