



## Genotyping protocol

Ube2b

IR00002024 / E7

(ICS internal reference)

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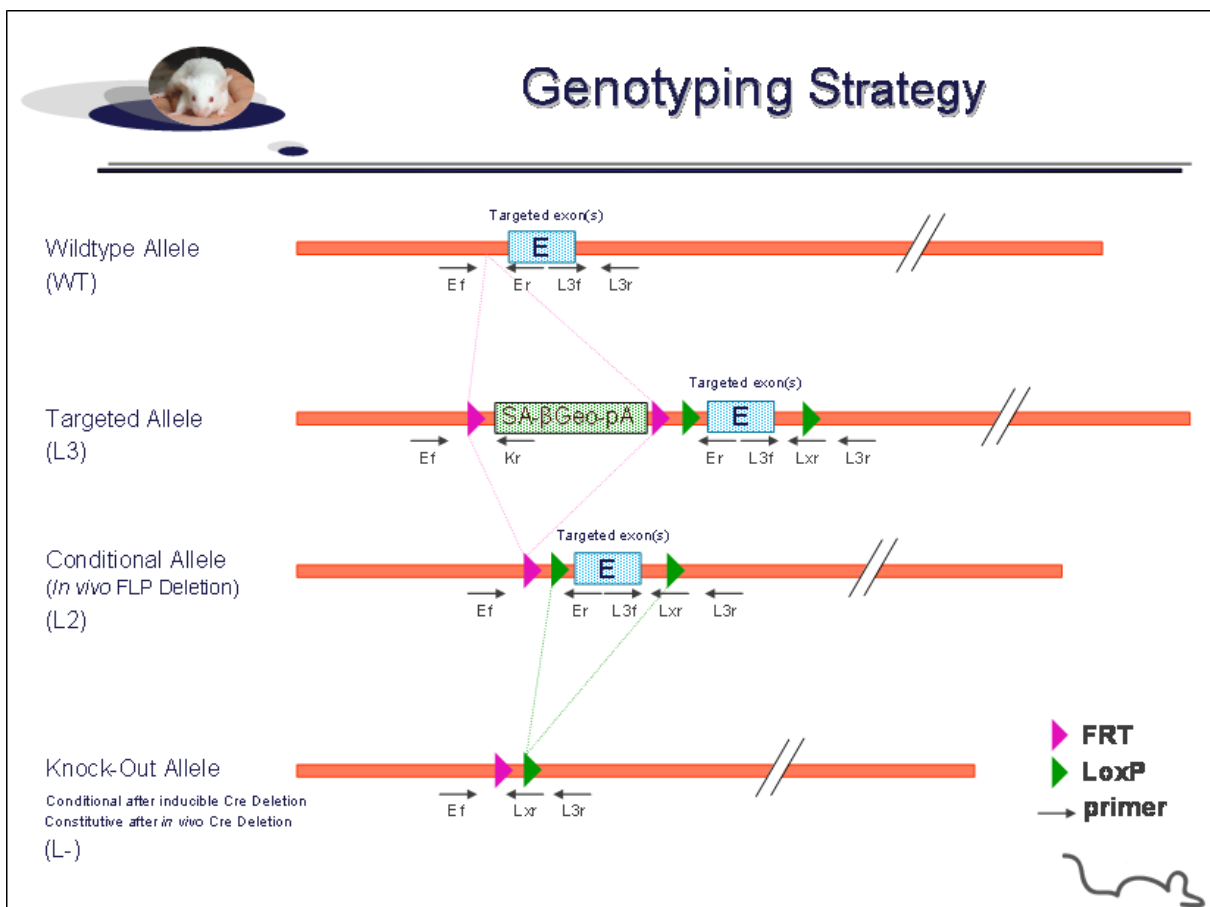
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### 1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Ube2b** Constitutive Knockout / Conditional Knockout (KO-cKO) 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	3476	CCCCTGTGTATGTGTTATAACCATCC
Er	3478	TACAGCTGAGTGTGGCAGCGCACAC
Kr	3277	CTCCTACATAGTTGGCAGTGTGGG
L3f	3479	GCAAATGAAAACCTTAGAGCTCATGG
L3r	3480	TGAATTCAGGACTCAGGGATGCCTG
Lxr	3255	ACTGATGGCGAGCTCAGACCATAAC

### PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer (see the map above)	Targeted allele (KO allele) (L3)	cKO allele (L2)	KO allele (L-)	WildType allele (WT)
5' part of the selection marker	3476-3277	Ef / Kr	224	---	---	---
Presence of the distal loxP	3479-3480	L3f / L3r	340	340	---	332
Distal loxP specific PCR	3479-3255	L3f / Lxr	216	216	---	---
Excision of the selection marker	3476-3478	Ef / Er	5907*	500**	---	359
Excision of the floxed exon(s), i.e. knock out	3476-3480	Ef / L3r	6623*	1216*	350**	1067**

\*: this PCR product will not be observed using our PCR genotyping conditions (see de scription 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 <sub>2</sub> O	up to 15 µl

### Cycling conditions:

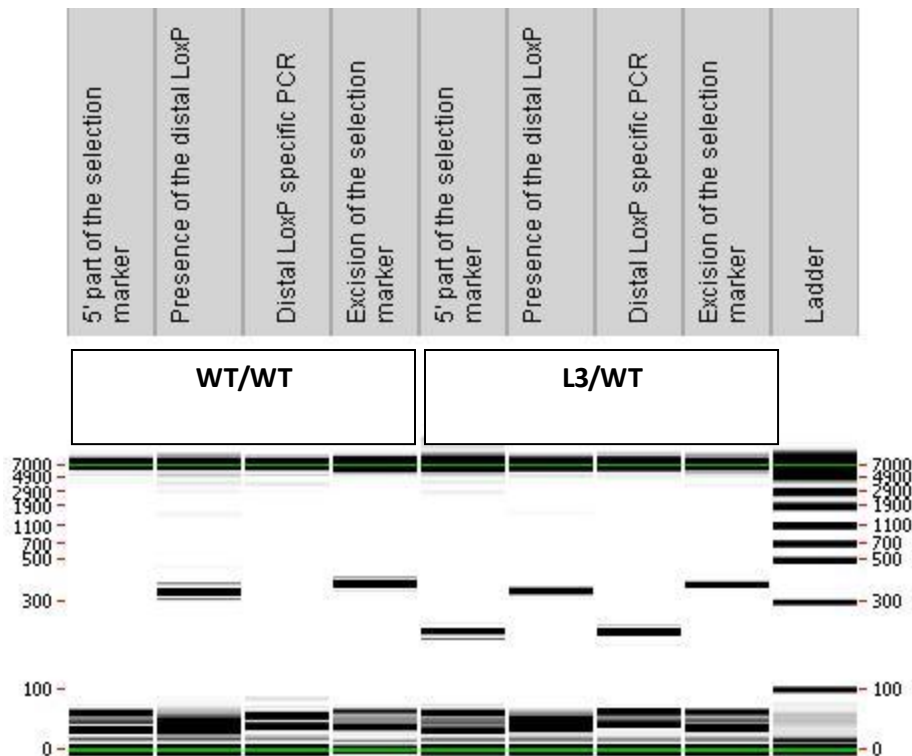
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	34
62°C	30s	
72°C	1min	
72°C	7min	1
20°C	5min	1

**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



Note that as this technology is more sensitive than gel analysis, non specific signals and/or primer dimers may be visible on the picture.

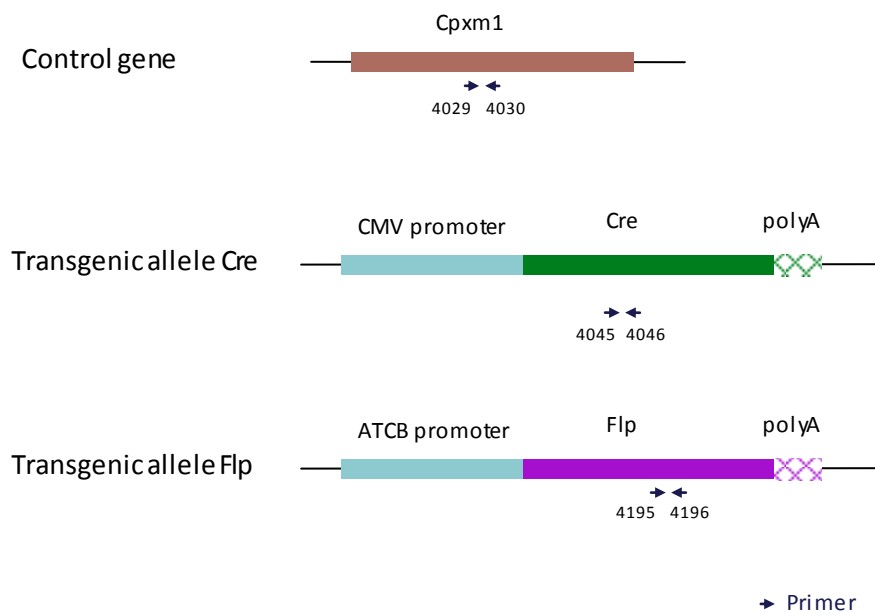
## 2. Cre and Flp genotyping method

The protocol used to segregate the cre and/or flp transgene is indicated below.

Detection of cre transgene and flp transgene is done using a multiplex assay: primer pairs were designed for each gene and for a positive control (Cpxm1 gene).

### 2.1. Cre and Flp genotyping

#### Schematic representation of the genotyping strategy



#### Sequence of primers used for genotyping:

Primers	Sequence
4029	ACTGGGATCTTCGAACTCTTTGGAC
4030	GATGTTGGGGCACTGCTCATTACCC
4045	CCATCTGCCACCAGCCAG
4046	TCGCCATCTTCCAGCAGG
4195	TCTTTAGCGCAAGGGGTAGGATCG
4196	GTCCTGGCCACGGCAGAAGC

#### PCR fragments expected size (bp):

Primer pair	4045-4046	4195-4196	4029-4030
Region analyzed	Middle part of Cre transgene	Middle part of Flp transgene	Cpxm1 control gene
Control gene	/	/	446
Tg allele	281	328	/

## 2.2. 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)	0.05µl
3' primer (100 µM)	0.05µl
Sterile H <sub>2</sub> O	up to 15 µl

Cycling conditions are identical to those described in chapter 1.2