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Genotyping protocol

S122A point mutation in Esr1 (Estrogen receptor α)

IR00003880 / K712

(ICS internal reference)

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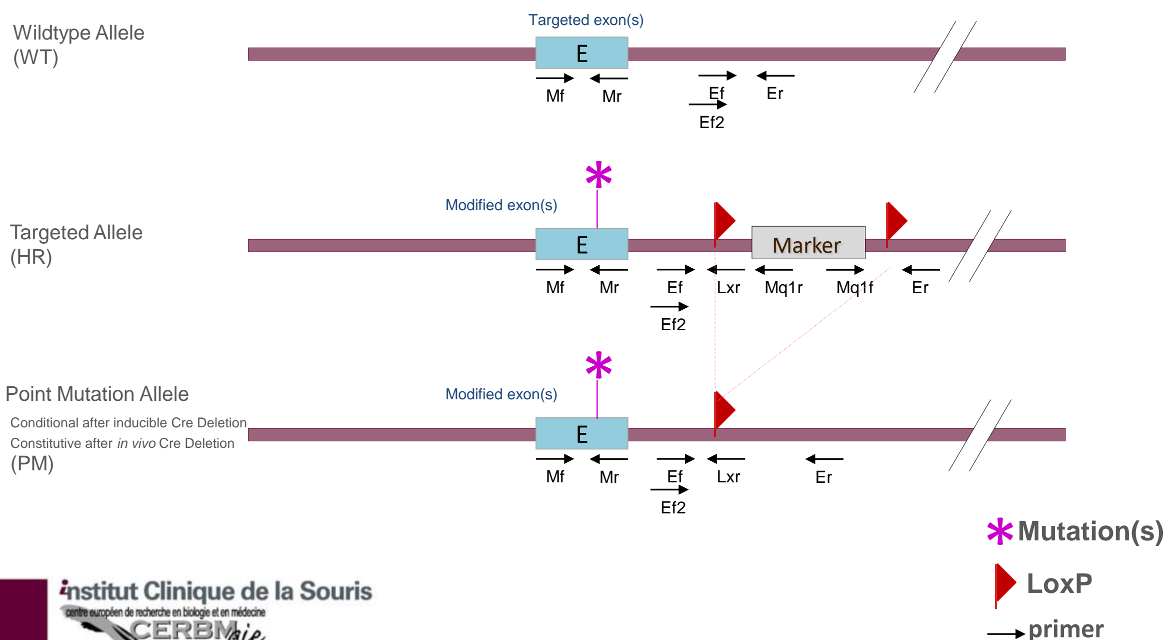
1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **Estrogen receptor Alpha** Point mutation or few bp modification Knockin (PM) project.

1.1. Genotyping strategy

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

PM Genotyping strategy



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Sequence of primers used for genotyping:

Position	Sequence
Ef	GGGAAGTCAGAAAGGATTCTTTGGCAG
Ef2	CTGCAGGTCTCCCCATCCCAG
Er	GGTTCCTTGCTAGCACAGGCCAC
Lxr	GAAGTTATACTAGAGCGGCCGTTCAC
Mf	CTTTCCCCCAGCTCAACAGCGTG
Mq1f	GAAGGGTGAGAACAGAGTACCTAC
Mq1r	TGCTAAAGCGCATGCTCCAGACTGC
Mr	CTGGGCTCGTTCTCCAGGTAGTAG

PCR fragments expected size (bp):

	Region analyzed	Position on the primer *(see the map above)*	Targeted allele (HR)	PM allele (PM)	WildType allele (WT)
A	WildType / Mutated alleles (with DMSO)	Mf / Mr	124	124	124
B	Excision of the selection marker	Ef / Er	2003*	334	247
C	5' part of the selection marker	Ef / Mq1r	246	---	---
D	3' part of the selection marker	Mq1f / Er	398	---	---
E	LoxP specific PCR	Ef2 / Lxr	181	181	---

*: this PCR product will not be observed using our PCR genotyping conditions (see description below)
---: 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:

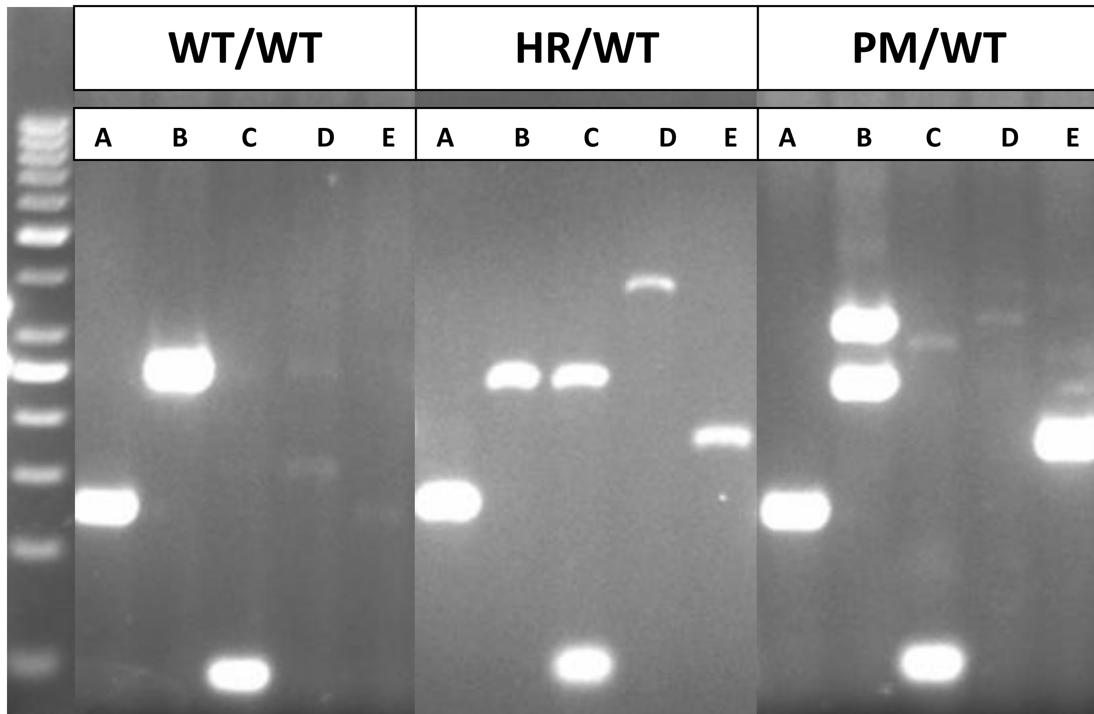
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

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 done by agarose gel electrophoresis 3%.

<u>Representative genotyping picture</u>



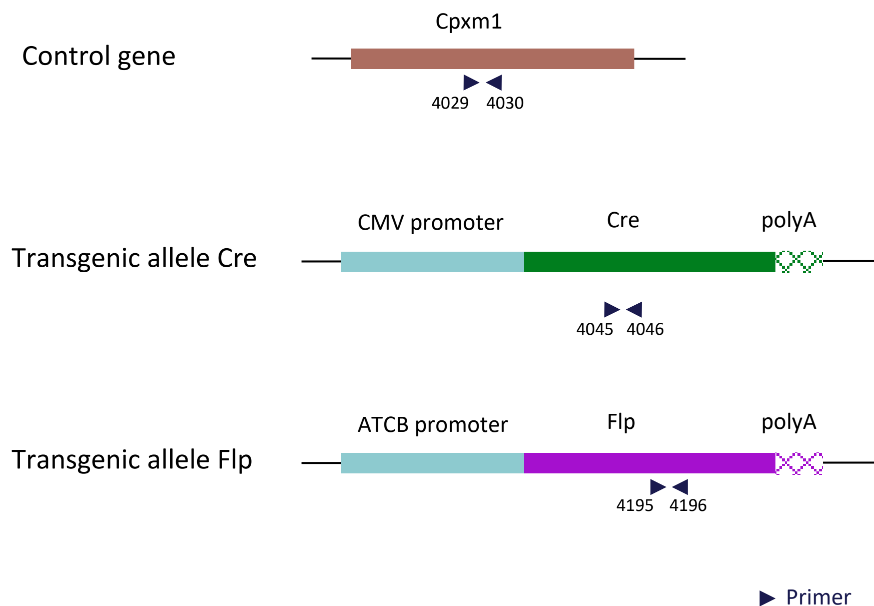
Note that primer dimers may be visible on the picture.

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	GATGTTGGGGCACTGCTCATTCACC
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	/	/	397
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_2O	up to 15 µl

Cycling conditions are identical to those described in chapter 1.2

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