R264A PM in Esr1 – Estrogen receptor α (IR00002392 / K505 ICS internal reference) mouse line genotyping protocol

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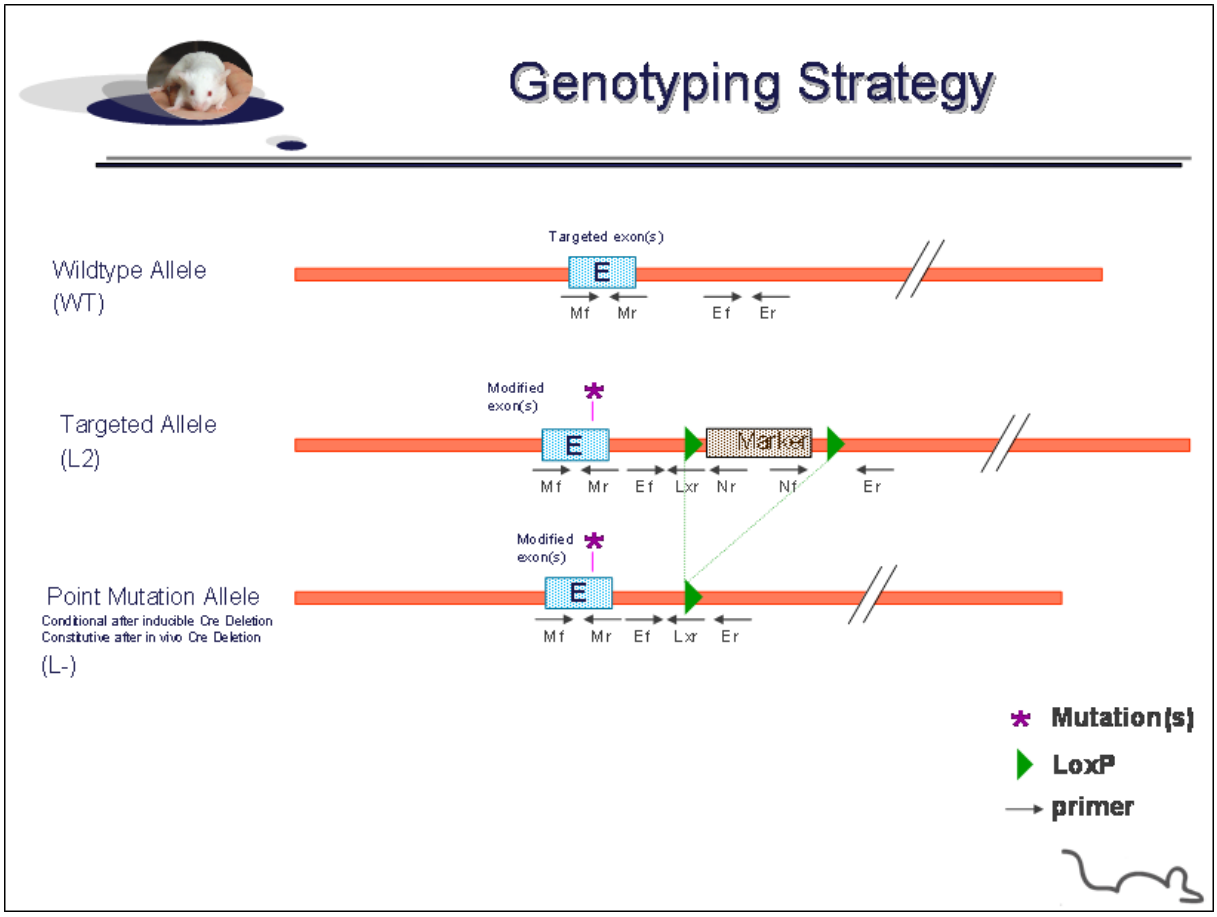
This protocol has been validated by Karim Essabri.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the R264A PM in Esr1 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	TAATGCTCAGGGATCTATGG
Er	ATCCATAAGCCAGAAAACAG
Er	TATGAAACCCATTGGTTGAG
Nf	AATGCCTGCTCTTTACTGAAGGCTC
Mf	AGTCTGCATGTGCTTTCTGT
Mr	AGGCTTCACTGAAGGGTCTA
Nr	TGCTAAAGCGCATGCTCCAGACTGC

PCR fragments expected size (bp):

Region analyzed	Position on the primer (see the map above)	Targeted allele (L2)	PM allele (L-)	WildType allele (WT)
Excision of the selection marker	Ef / Er	2142*	473	385
5' part of the selection marker	Ef / Nr	312	---	---
3' part of the selection marker	Nf / Er	449	---	---
Wild Type / Mutated alleles	Mf / Mr	390	390	390

* 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:
-10x Buffer (Roche)	2.5µl
-dNTPs 10mM (Amersham Biosciences)	0.5µl
-Taq DNA Polymerase (Roche)	0.2µl
-DNA (50ng/µl)	3µl
-5' primer (100 µM)	0.125µl
-3' primer (100 µM)	0.125µl
-Sterile H2O	up to 25 µl

Cycling conditions:

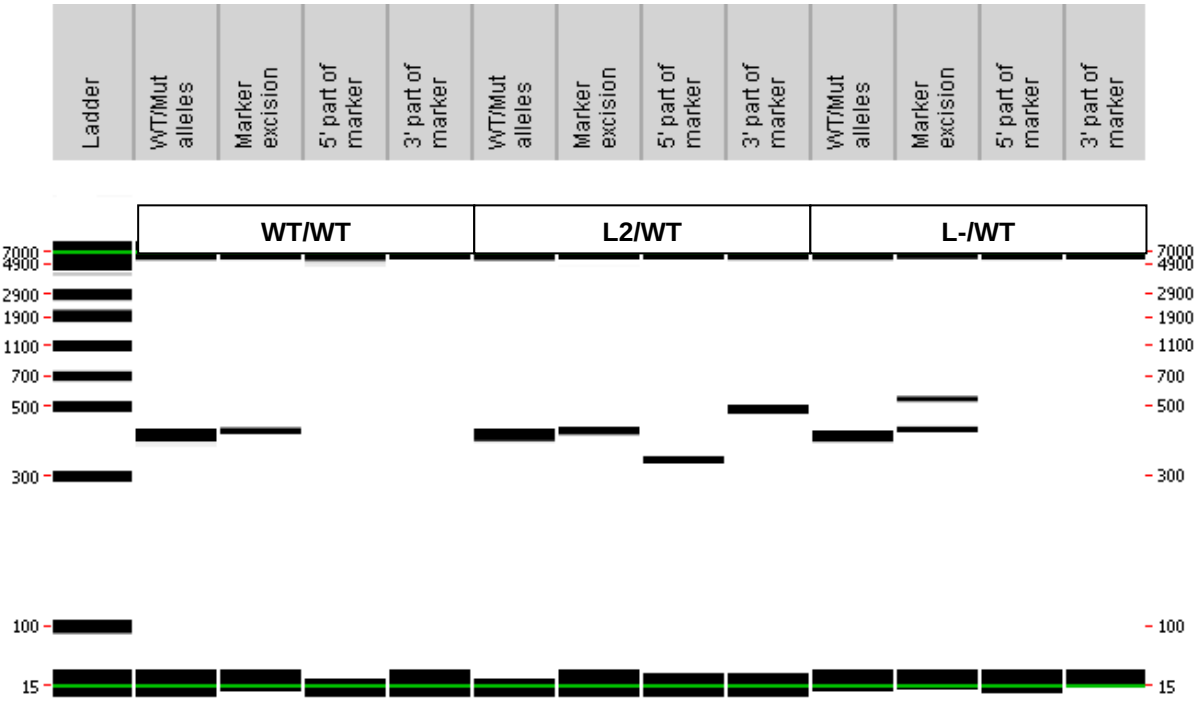
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	
62°C	1min	2
72°C	1min	
94°C	30s	
62°C	30s	30
72°C	30s	
72°C	3min	1
4°C	∞	

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.