



**Genotyping protocol
C451A PM in Esr1 -Estrogen Receptor α (IR00002665 /
K557 ICS internal reference)**

**C451A PM in Estrogen Receptor α -Esr1 (IR00002665 / K557
ICS internal reference) mouse line genotyping protocol**

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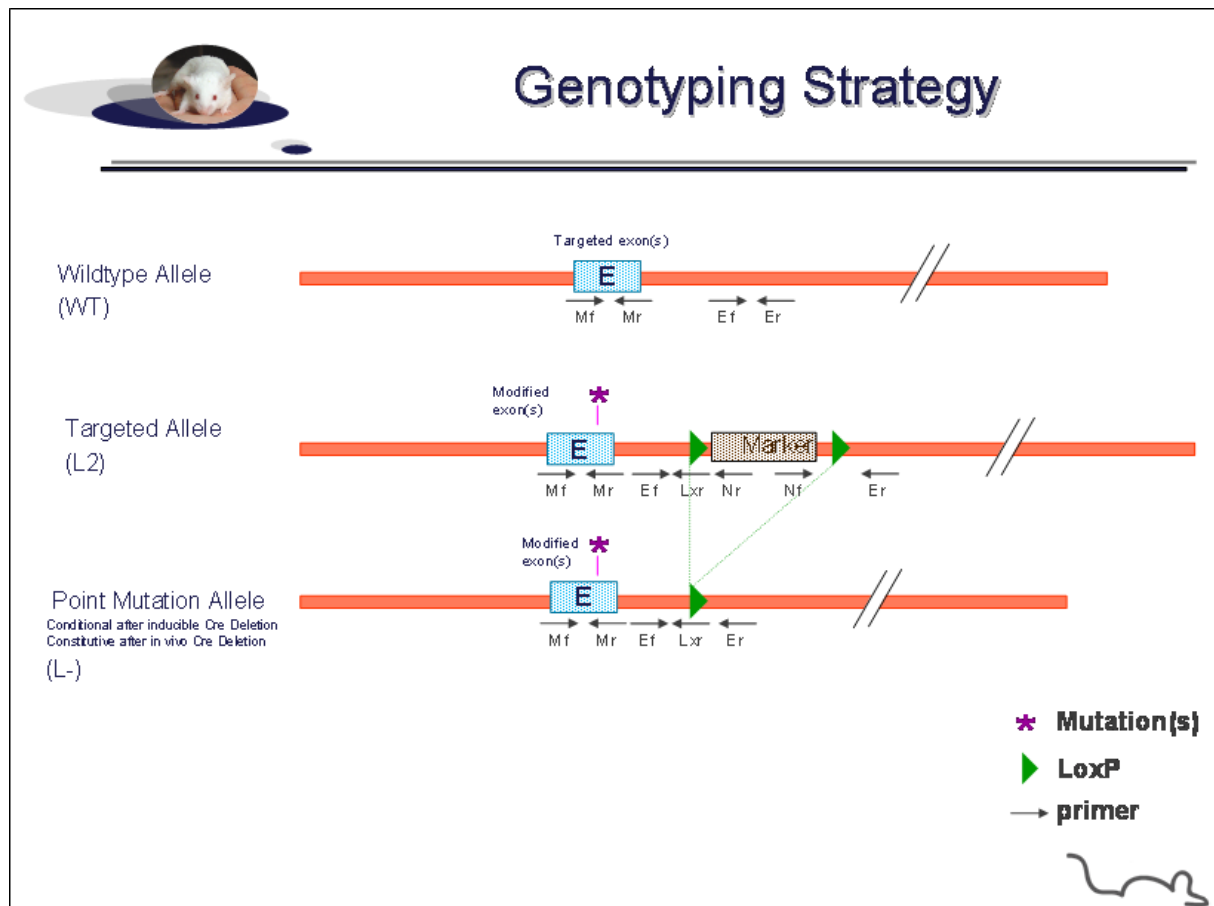
This protocol has been validated by Christelle Roth.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the **C451A 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	GCTTGTGTATCTGTAGATAGCTGC
Ef	GCACATATTTAAAATGGACCTAGACCAGG
Er	GCTTCAGTGGCTCCTAGTCATCTTAAC
Lxr	GAAGTTATACTAGAGCGGCCGTTTAC
Mf	CTGCAAGAAAGTGAGCTGCCAATGG
Mr	GCATTGGCAACTCACCGGAATTAAG
Nf	CAGCTCATTCTCCCACTCATGATC
Nr	GTAGAAGGTGGCGGAAGGGGC



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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	2025*	356**	234
5' part of the selection marker	Ef / Nr	314	---	---
3' part of the selection marker	Nf / Er	315	---	---
WildType / Mutated alleles	Mf / Mr	272	272	272
LoxP specific PCR	Ef / Lxr	106	106	---

* 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:

- 10x Buffer (Roche)
- dNTPs 10mM (Amersham Biosciences)
- Taq DNA Polymerase (Roche)
- DNA (50ng/ μ l)
- 5' primer (100 μ M)
- 3' primer (100 μ M)
- Sterile H₂O

Volume:

- 2.5 μ l
- 0.5 μ l
- 0.2 μ l
- 3 μ l
- 0.125 μ l
- 0.125 μ l
- up to 25 μ l

Cycling conditions:

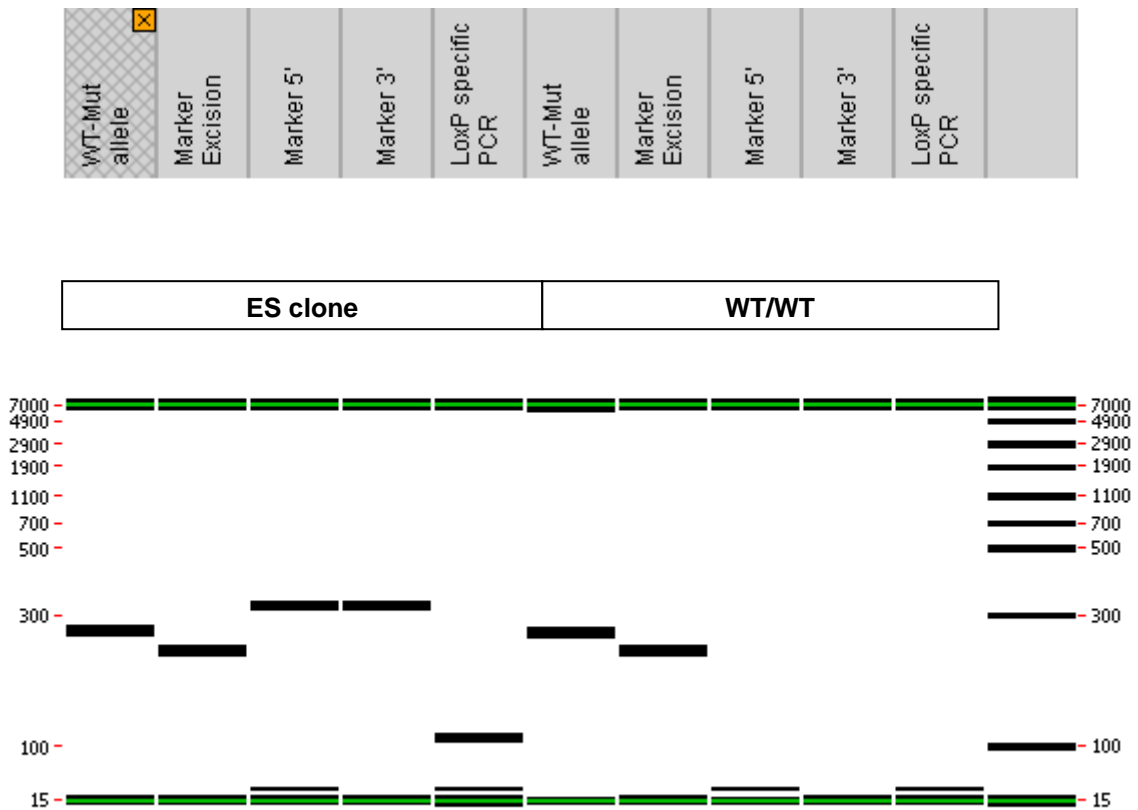
Temp	Time	#Cycles
94°C	3min	1
94°C	1min	2
62°C	1min	
72°C	1min	
94°C	30s	30
62°C	30s	
72°C	30s	
72°C	3min	1
4°C	∞	

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.