Genotyping protocol

DNM2 (Dynamin 2) K562E

IR00003384 / K634

(ICS internal reference)

This report has been prepared by: **Pauline Cayrou**

This report has been validated by: **Sylvie Jacquot, PhD, Head of Genotyping Service**

The first version of this report was finalized the: 16 Apr 2012

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

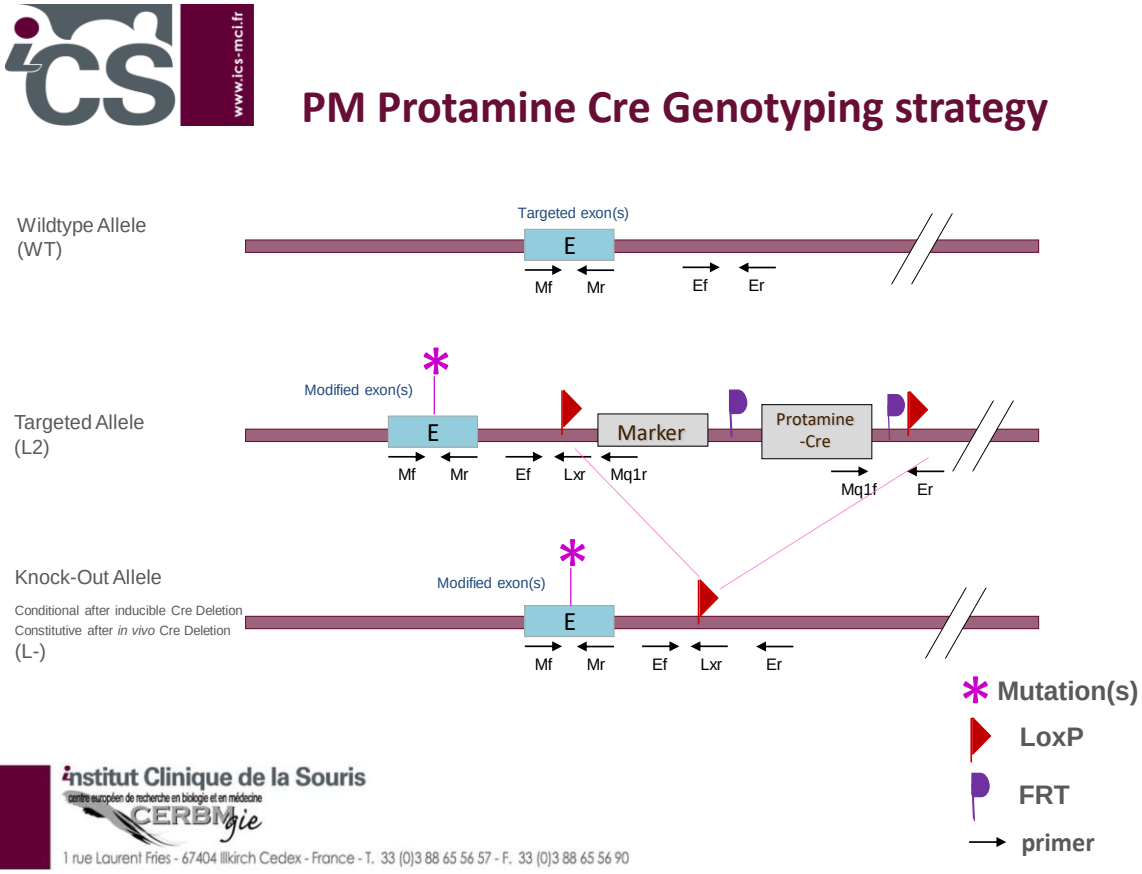
TABLE OF CONTENTS

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype your **DNM2 (Dynamin 2) K562E** 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.



institut Clinique de la Souris
centre européen de recherche en biologie et en médecine
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	6116	GCCATCTTCAACACAGAGCAGAGGTG
Er	6115	TACACTGTCTGCACTGTCGAGCCCTG
Er^2	6114	CATGCAGAGGTCAGAGGTCAGCAC
Lxr	4774	GAAGTTATACTAGAGCGGCCGTTCAC
Mf	6117	AAGGGGACGTTTGAGTAAATGTGGGG
Mq1f	1512	AATGGAAGGATTGGAGCTACGGGG
Mq1r	265	TGCTAAAGCGCATGCTCCAGACTGC
Mr	6118	TGTGCTAGGCAAGACCCTGGCA

PCR fragments expected size (bp):

Region analyzed	Primers used	Position on the primer *(see the map above)*	Targeted allele (L2)	PM allele (L-)	WildType allele (WT)
WildType / Mutated alleles	6117-6118	Mf / Mr	293	293	293
Excision of the selection marker (with DMSO)	6116-6115	Ef / Er	2270*	601	514
5' part of the selection marker (with DMSO)	6116-265	Ef / Mq1r	539	---	---
3' part of the selection marker	1512-6114	Mq1f / Er^2	414	---	---
LoxP specific PCR (with DMSO)	6116-4774	Ef / Lxr	327	327	---

*: 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
- DMSO 5% final	

Cycling conditions:

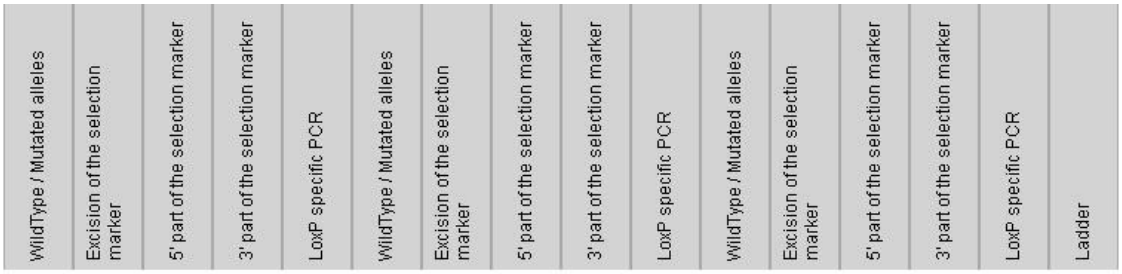
Temp	Time	#Cycles
95°C	4min	1
94°C	30s	
62°C	30s	34
72°C	1min	
72°C	7min	1
20°C	5min	1

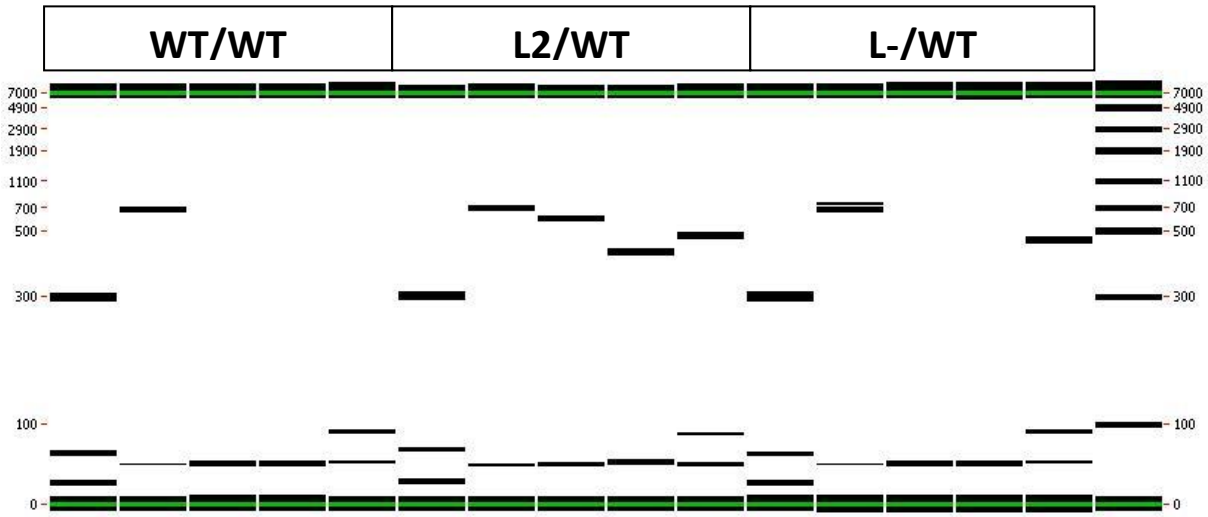
NB: These PCR conditions have been optimized for high-throughput genotyping. Adaptation to small-scale may be required.

1.3. Picture of genotyping with various alleles

Analysis of PCR products pattern was 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.

A migration shift is observed due to DMSO in the reaction mix