S421A PM in Htt - Huntingtin (IR00001848 / K449 ICS internal reference) mouse line genotyping protocol

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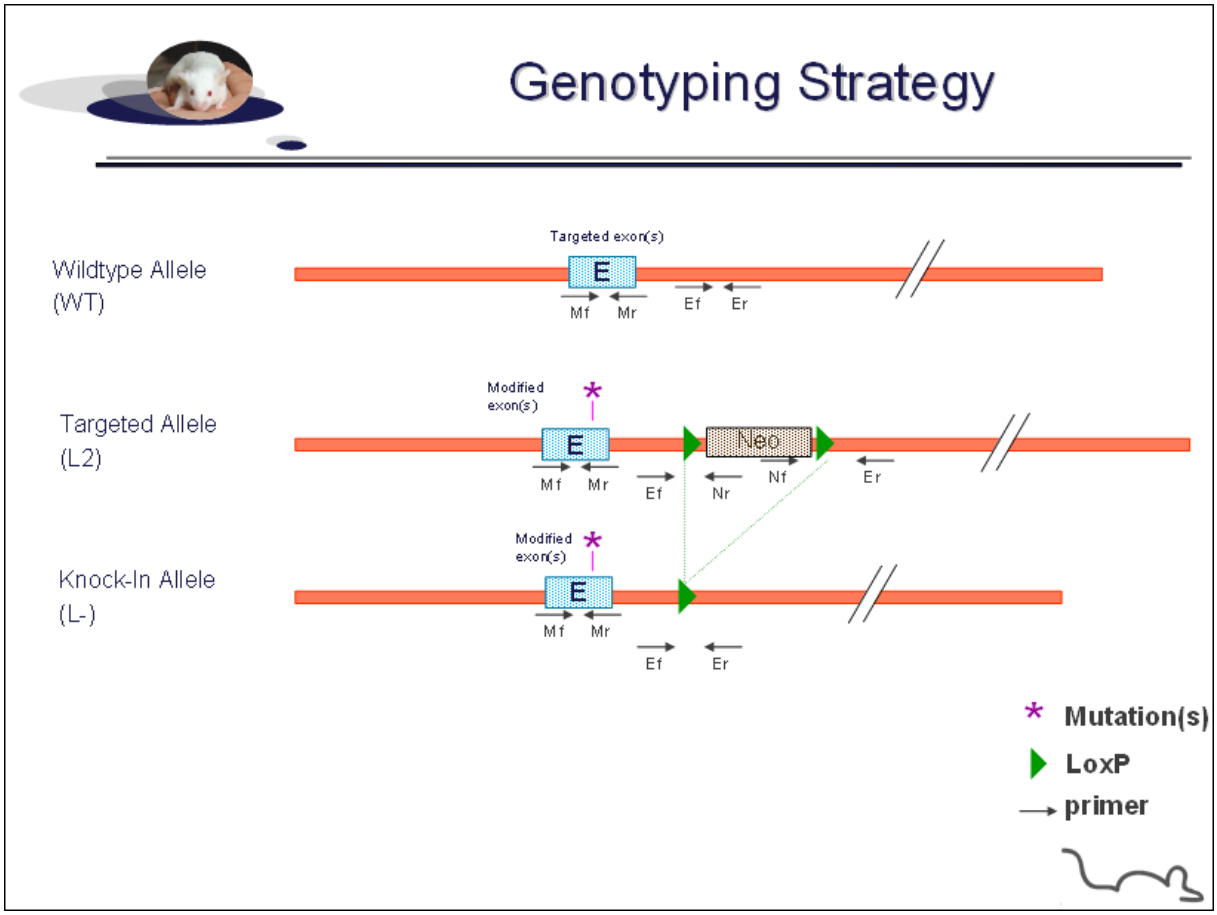
This protocol has been validated by Guillaume Pavlovic.

1. Genotyping protocol and data

This section describes the condition used at the Mouse Clinical Institute (ICS) to genotype the S421A PM in Htt 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	GCTGTACTGCGCTGAGCTACCC
Ef	TTACAATGTAGGCGTCTTGCTG
Er	AGGGTTCTCTAAAGTCAGGATCC
Mf	TCATACTCAGCACCAAGACCAC
Mr	CTCCATGCACAATGGTTAGAAG
Nf	CAGCTCATTCCTCCCACTCATGATC
Nr	TGACTAGGGGAGGAGTAGAAGGTG

PCR fragments expected size (bp):

Region analyzed	Position on the primer (see the map above)	Conditional allele (L2)	Knock-Out allele (L-)	WT allele (WT)
Excision of the selection marker	Ef / Er	2058*	389	302
5' part of the selection marker	Ef / Nr	411	---	---
3' part of the selection marker	Nf / Er	243	---	---
WildType / Mutated alleles	Mf / Mr	225	225	225

* 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:
-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.