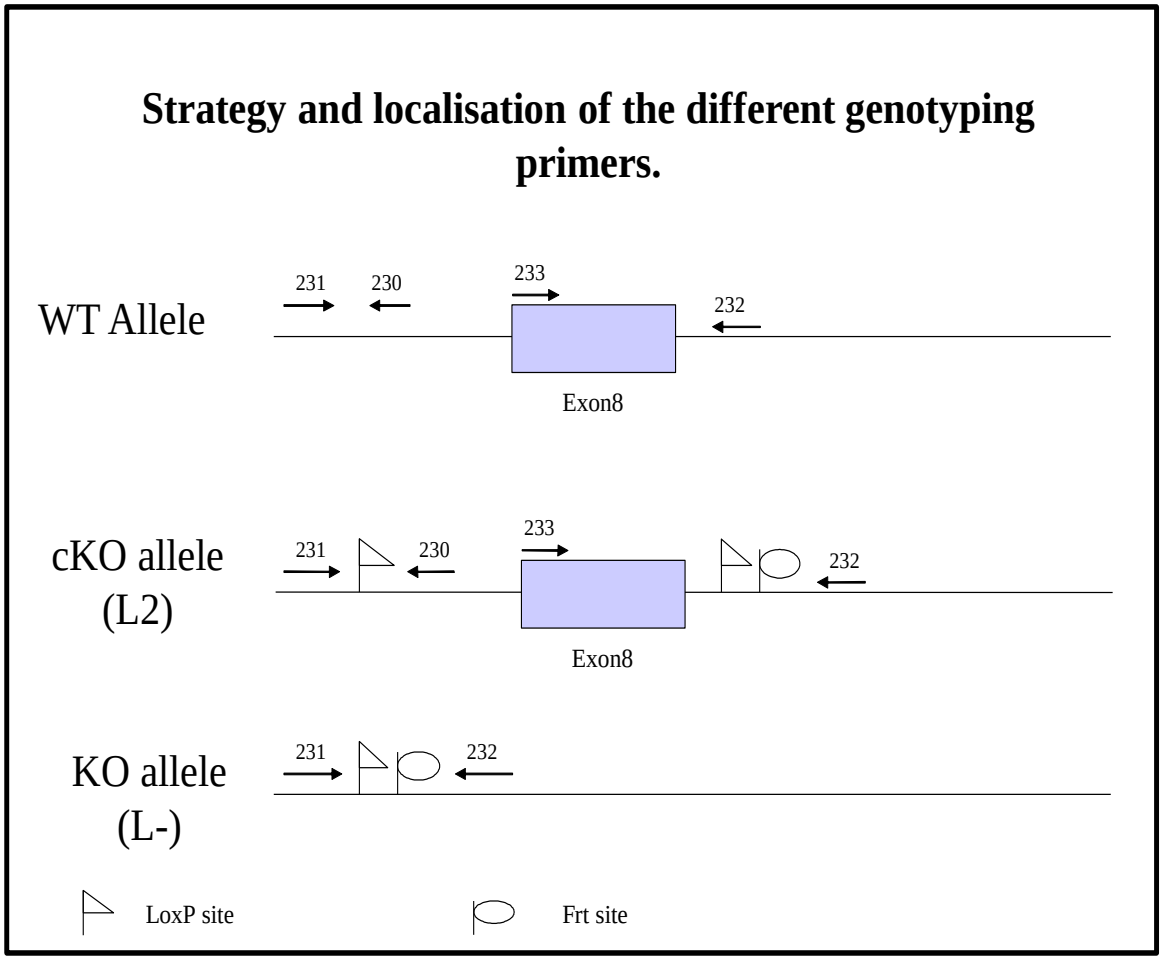
Strasbourg, le

Slit2 cKO (IR11/ICS internal reference: K48) mouse line genotyping protocol

This protocol describes the strategy and the PCR conditions used for Slit2 conditional knock out line genotyping.



Primers sequence

Oligo 230 : 5'- TGGTTCACAAACACGAGTCAATTCC-3'

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Oligo 231 : 5'- ATGCCTCTTTATGTCCACAGCTCTG–3'

Oligo 232 : 5'- TTCCCATGGGATGAAAATGGGCATG–3'

Oligo 233 : 5'- TCGAACAACTTGTACTGCGACTGCC–3'

PCR to be done: (please see the scheme above)

- Characterisation of L2 animals: PCR 231/230, PCR 233/232.
- Characterisation of L2/L- animals (after cross with a Cre mouse line)*****: PCR 231/230, PCR 233/232, PCR 231/232.
- Characterisation of L- animals: PCR 231/230, PCR 233/232, PCR 231/232.

Expected size (bp):

	PCR 231/230	**PCR** 233/232	**PCR** 231/232
WT	259	372	783
L2 allele (cKO)	317	490	959
L- allele (KO)	N/A	N/A	472

PCR genotyping conditions:

Reagents:	**Volume:**
-10x Thermopol Reaction Buffer (Biolabs)	*2.5µl*
-dNTPs 10mM (Amersham Biosciences)	*0.5µl*
-Taq DNA Polymerase (Sigma can be used)	*0.2µl*
-DNA (50ng/µl)	*2µl*
-5' primer (100 µM)	*0.25µl*
-3' primer (100 µM)	*0.25µl*
-Sterile H2O	*up to 25 µl*
Total volume	*25 µl*

Cycling conditions:

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

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