



16p11.2 DEL and DUP on Long Evans rats by CRISPR Update project

Kur7018 / IM7018

Update done the 24th of june 2019

by Marie-Christine Birling (PhD)

Genotyping protocol

Kur7018-8130-DEL

Sequence with PCR F-R

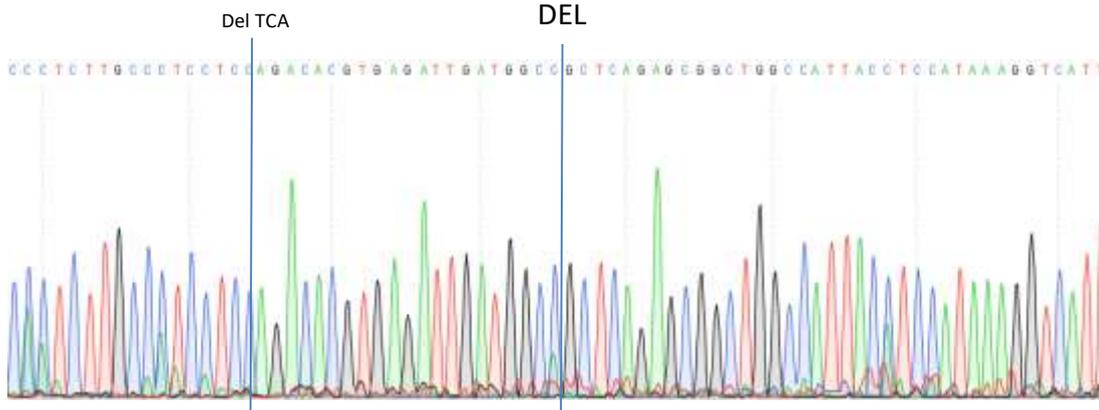
Oligo F1 in orange

Oligo R8 in green



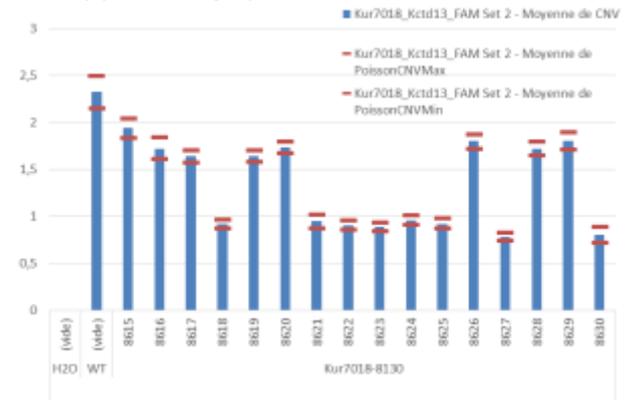
CACCTCCTATGCAGATGGCTATTCCCTCCCCTCAATGTGGCCAGAGGTACGTGGTGGCTTGTAAAGAGCCCAAGAAAAAGTG
 AATCGGGCTGGCAGACTTGAATCTGCCCCAGCCAACCTCATGGGAGAAGGCAACAAAGGAGTTCCTATGATCCAATAAATACTC
 TTCCCTCTCACCATGTCTCCTGGGCCTCACTACGTACAGACCCTCTTGCCCTCCTC --- CAGACACGTGAGATTGATGGCC ----
 -----DEL-----
 GCTCAGAGCGGCTGGCCATTACCTCCATAAAGGTCATTCTCTTGGCAAGCTGCATTGTGTAAAGCACTCTTAGGGTTCTCTAT
 GCTGTCCTAGTTTGGAGAAGAACACTACATGCTTCTGGAAAATTCACATTCAAGCAGCAACATCTTAACATATCTCC

Sanger sequencing

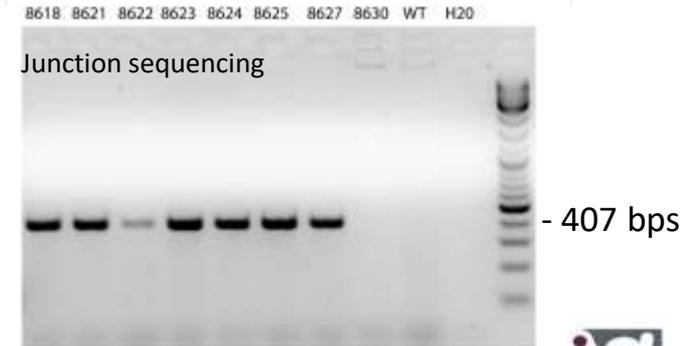


F1 genotyping

Copy counting by ddPCR



FO	Nb F1 born	F1 heterozygote (confirmed by junction sequencing and ddPCR)	
		M	F
Kur7018-8130	16	6	1



Thirteen more pups to genotype soon

A new line with the expected deletion is established.

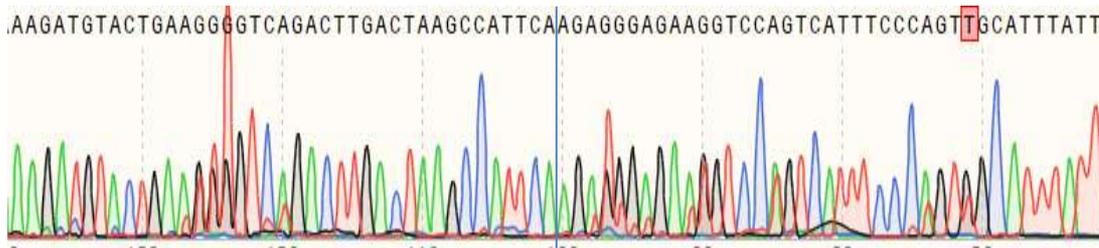
Line Kur7018-8135-DUP



Sequence with PCR F-R
 Oligo F8 in orange
 Oligo R1 in green

AAGCTGAGTCAGAAACATAAACTATATATCAAACGGGTTGGTGGCCACTGACATCGTGACCCTGCTGGGGGAGCTGCCCTCCTAGAGGAAGTAATGGTC
 ACCACTCTTTCCTGCTTGCCCCCTGCTTCTGAGAGAAAGGAAAGATGTACTGAAGGGGTCAGACTTGACTAAGCCATTCA-----DUP-----
 AGAGGGAGAAGGTCCAGTCATTCCCAGTGGCATTATTCTTTCAGTGTAAAATATAGTTTATCTTACTATTTTTTGCATGTTTGTACTTTAGGTTCTTTTAA
 GGTGTTGGGACTGATTGCTTTCCTGACA

Sanger sequencing



F1 genotyping



F0	Nb F1 born	F1 heterozygote	
		M	F
Kur7018-8135	15	1	2

A line (Kur7018-8135-DUP) is now established.

The 2 females can be transferred.

The F0 breeding is continuing in order to obtain more F1 pups



GENOTYPING INSTRUCTIONS



Primer ref.	Sequence	Amplification product size for the deletion	Amplification product size for the duplication
F1	CACCTCCTATGCAGATGGCTATTCC	407 bps	NA
R8	GGAGATATGTTAAGATGTTGCTGCT		
F8	AAGCTGAGTCAGAAACATAAACTAT	NA	315 bps
R1	TGTCAGGAAAGCAATCAGTCCCAAC		

PCR Protocol

This section describes the composition of the mix and the cycling conditions used for genotyping F0.

Reagents:	Volume (per sample):
- Phusion HS (Thermo Scientific) 5X Buffer	4 µl
- 10mM dNTP	0.4 µl
- 5' primer (100 µM)	0.1 µl
- 3' primer (100 µM)	0.1 µl
- DNA (lysate 1/10)	2 µl
- Phusion Hot Start II	0.2 µl
- Sterile H ₂ O	up to 20 µl

Cycling conditions

Temp	Time	#Cycles
96°C	5min	1
96°C	8s	30
62°C	10s	
68°C	45s	
68°C	5min	1
12°C	5min	1