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EXCELLENCE IN MOUSE PHENOGENOMICS

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

Kur7018 / IM7018

Update done the 24th of june 2019

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Instituts thématiques Inserm Institut national de la santé et de la recherche médicale

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INVESTISSEMENTS D'AVENIR

Genotyping protocol

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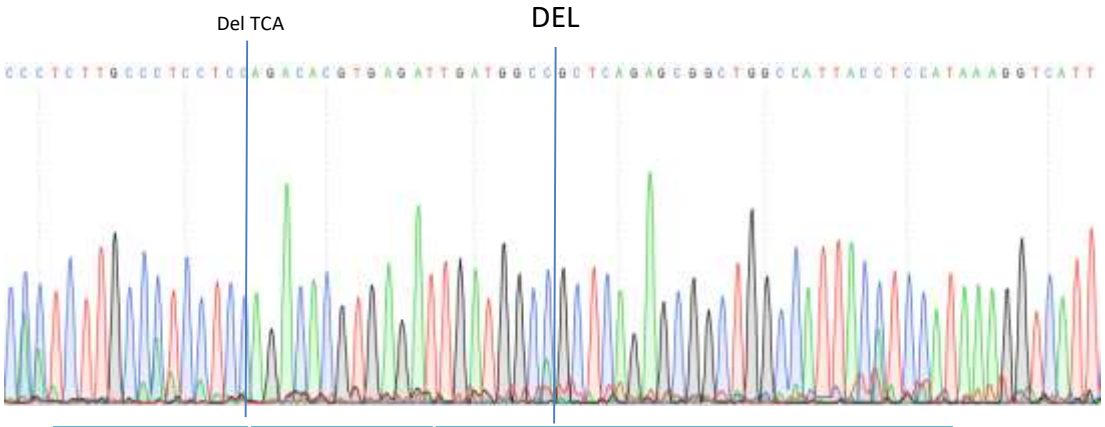
Kur7018-8130-DEL

Sequence with PCR F-R
Oligo F1 in orange
Oligo R8 in green

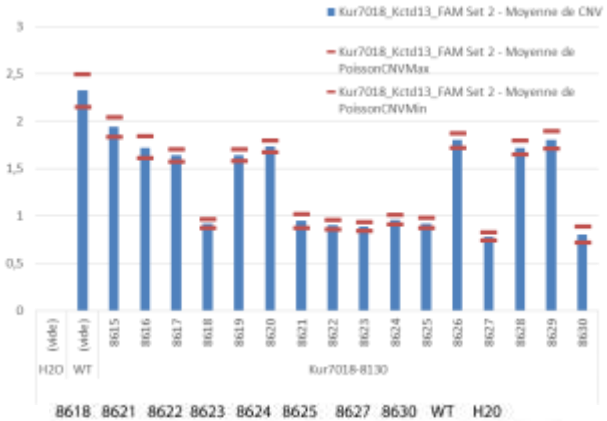
CACCTCCTATGCAGATGGCTATTCCCCTCCCCTCAATGTGGCCCAGAGGTACGTGGTGGCTTGTAAGGAGCCCAAGAAAAAGTG
AATCGGGCTGGCAGACTTGAATCTGCCCCAGCCAACCTCATGGGAGAAGGCAACAAAGGAGTTCCTATGATCCAATAAATACTC
TTCCCTCTCACCATGTCTCCTGGGCCTCACTACGTCAGACCCTCTTGCCCTCCTC---CAGACACGTGAGATTGATGGCC----
----------DEL----------
GCTCAGAGCGGCTGGCCATTACCTCCATAAAGGTCATTCTCTTGGCAAGCTGCATTTGTGTAAGGCACTCTTAGGGTTCTCTAT
GCTGTCCTAGTTTGGAGAAGAACACTACATGCTTCTGGAAAATTCACATTCAAGCAGCAACATCTTAACATATCTCC

Sanger sequencing



F1 genotyping

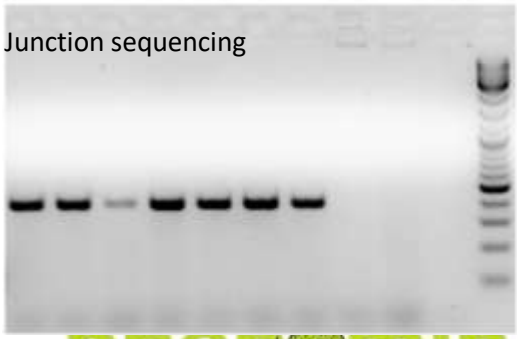
Copy counting by ddPCR



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

Junction sequencing



A new line with the expected deletion is established.

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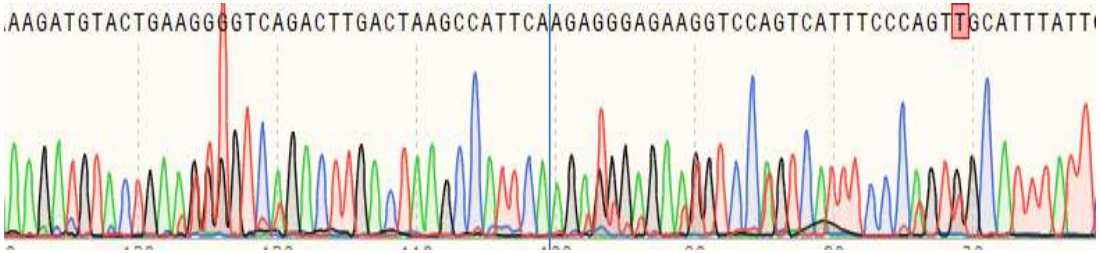
Line Kur7018-8135-DUP

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

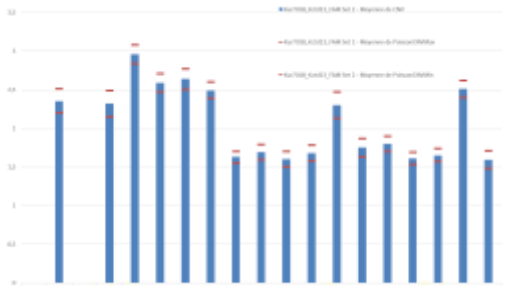
AAGCTGAGTCAGAAACATAAACTATATATCAAACGGGTTGGTGGCCACTGACATCGTGACCCTGCTGGGGGAGCTGCCCTCCTAGAGGAAGTAATGGTC
ACCACTCTTTCCCTGCTTGCCCCCTGCTTCCTGGAGAGAAAGGAAAGATGTACTGAAGGGGTCAGACTTGACTAAGCCATTCA----------DUP----------
AGAGGGAGAAGGTCCAGTCATTTCCCAGTGGCATTTATTCTTTCAGTGTAAAATATAGTTTATCTTACTATTTTTTGCATGTTTGTACTTTAGGTTCTTTTTA
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

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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 H2O	up to 20 µl

Cycling conditions

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

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