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EXCELLENCE IN MOUSE PHENOGENOMICS

Kur8011 KO of Clcn4
Internal line name Kur8011-536-DEL

Kur8011 / IM 8011

Report done by Marie-Christine Birling (PhD)
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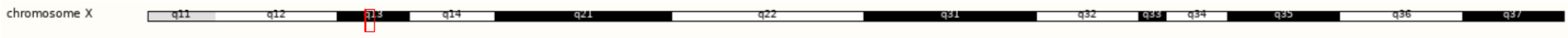
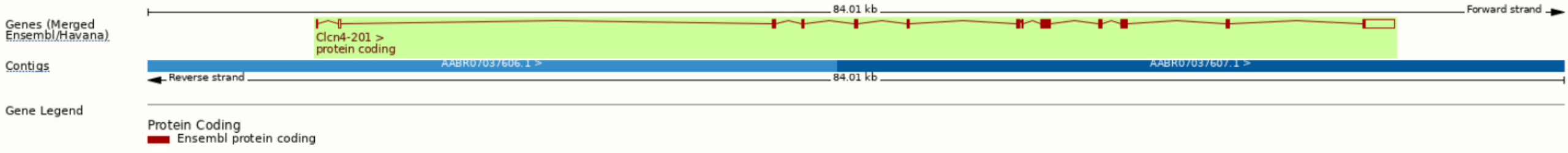
6/10/22

Rat Genomic locus

Location: Chromosome X: 25,016,401-25,080,410



Gene: Clcn4 ENSRNOG00000003533



mRNA and protein

In rat

Name	Transcript ID	bp	Protein	Biotype	UniProt Match	Flags
Clcn4-201	ENSRNOT00000059270.2	4332	754aa	Protein coding	Q56A19	APPRIS P1

In human

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	RefSeq Match	Flags
CLCN4-201	ENST00000380829.5	2418	729aa	Protein coding	-	G3XAG5	-	GENCODE basicTSL:5
CLCN4-202	ENST00000380833.9	6759	760aa	Protein coding	CCDS14137	P51793-1	NM_001830.4	MANE Select v0.93Ensembl CanonicalGENCODE basicAPPRIS P1TSL:1
CLCN4-203	ENST00000421085.7	3581	768aa	Protein coding	-	P51793-2	-	GENCODE basicTSL:5
CLCN4-204	ENST00000454850.1	487	152aa	Protein coding	-	E9PFB5	-	TSL:3CDS 3' incomplete
CLCN4-205	ENST00000674669.1	2227	666aa	Protein coding	CCDS59159	-	-	GENCODE basic
CLCN4-206	ENST00000674959.1	543	No protein	Retained intron	-	-	-	-
CLCN4-207	ENST00000675144.1	3485	103aa	Nonsense mediated decay	-	A0A6Q8PF24	-	-
CLCN4-208	ENST00000675769.1	3777	735aa	Nonsense mediated decay	-	A0A6Q8PG54	-	-

KO and cKO strategy: Selection of the best sgRNAs

5’ sgRNAs

http://crispor.tefor.net/crispor.py?batchId=XCwHOtPv6yaXRedKMMU4

Guide Sequence + *PAM* + Restriction Enzymes ☐ Only G- ☐ Only GG- ☐ Only A-	MIT Specificity Score	CFD Spec. score	Predicted Efficiency Show all scores: Doench '16	Mor.-Mateos	Outcome: Out-of-Frame	Lindel	Off-targets for 0-1-2-3-4 mismatches + next to PAM	Genome Browser links to matches sorted by CFD off-target score ☐ exons only ☐ X only
TGCTGCATGAATAACGTGAC *TGG* Enzymes: *Bse1I, MluCI, BmuI, MaeIII, Tsp45I* **Cloning / PCR primers**	91	95	45	26	66	87	0 - 0 - 0 - 4 - 74 0 - 0 - 0 - 0 - 1 78 off-targets	4:intron:Galnt11 4:intron:Itpr2 4:intergenic:Matn1-Ptpru show all...
GCTGCATGAATAACGTGACT *GGG* Enzymes: *Bse1I, Tsp45I, MaeIII, XapI, MluCI, BmuI* **Cloning / PCR primers**	89	94	64	27	52	83	0 - 0 - 1 - 5 - 69 0 - 0 - 0 - 0 - 0 75 off-targets	3:intron:Klhl14 4:intergenic:RGD1561161-Snx18 4:intergenic:5S_rRNA-Peli2 show all...

3’ sgRNAs

http://crispor.tefor.net/crispor.py?batchId=vp78A1LgtL03BrVReOed

TCATAAACCTATGCAGCGTG *TGG* Enzymes: *BtsCI* **Cloning / PCR primers**	88	93	65	67	58	88	0 - 0 - 1 - 5 - 51 0 - 0 - 0 - 1 - 0 57 off-targets	4:intron:Foxp1 3:intron:Fam134b 4:intergenic:Cntn6-Cntn4 show all...
CTGTCATCCACACGCTGCAT *AGG* Enzymes: *ApeKI, Fsp4HI, HpyCH4V* **Cloning / PCR primers**	84	94	56	49	65	70	0 - 0 - 4 - 12 - 89 0 - 0 - 0 - 0 - 0 105 off-targets	2:intergenic:Tvp23b-Cdrt4 4:intergenic:Grifin-Chst12 3:intergenic:ENSRNOG00000048409-Skor2 show all...

Haeussler, M. et al. Evaluation of off-target and on-target scoring algorithms and integration into the guide RNA selection tool CRISPOR. **Genome Biol. 17, 148. doi:10.1186/s13059-016-1012-2**

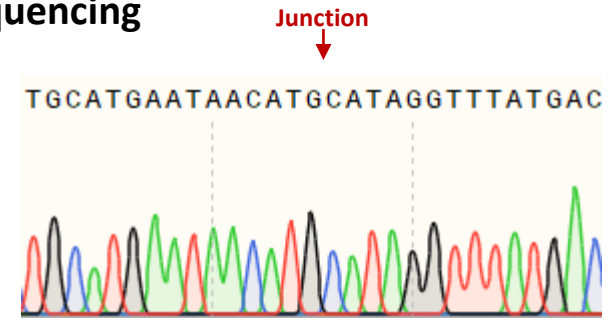
Line Kur8011-536-DEL

F1 genotype

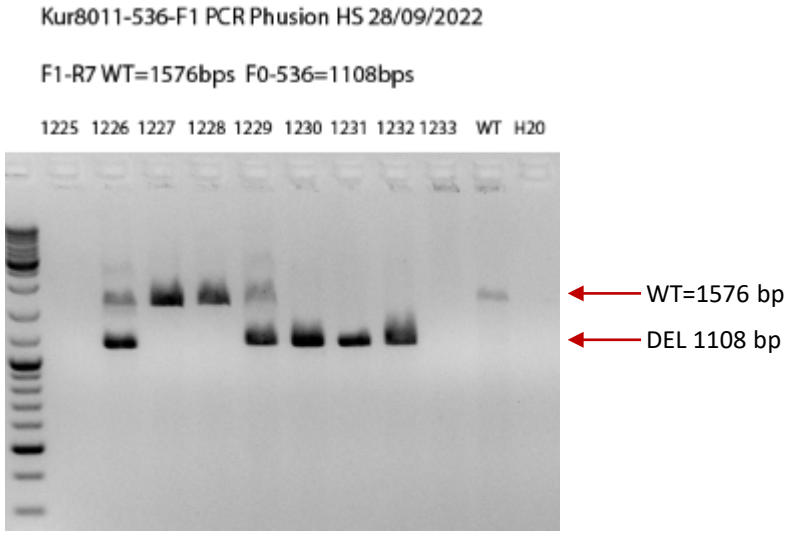
Sequence with PCR F1-R7
Primers are in orange
Insertion in purple

GGATACTTTTGCTGATGAGTAGAGGAGGGGAGTGGGGACTATCTTGCAGCAGGTGATGGGATACACCATGCTGCACAGCTCAGTATGCAGGCAGGGCTCACCTTAGAGAGTGACAAACTCCTAGCATGCAAATATCTACT
GAAGCTTGAGACACTGACAAGGTGACTTAGGCACTTAGAGTAGGGATGGAAGCAGGGCCCTAGAAAAGTCAGGGTACATATTCTGTATACAGAGGGAGGCAGGTGAGAGTCAGAGTCAGACAGAGATGAAATAGGGA
ACCAAAAAAGGATGGGGCGATGATGTCAGAGGAGTGTAGGGCTCCCCCAGAATGGGGAGGGGAAAAGGCATGATATAAAGATGCCATAATGTTCTCTAAGAGTTTAGACCCTTAAATAAAAACCTGTGCTCAATCTGATT
TGCTCTTTGGTGATGTAAAATCCACTGTGAAGGGGAACACTGATTGTGTGTTGCATCCTTATAGCCCTATGAACTAGAAAGGCCATTTAAACATTGTGCTGAATTGCTGCATGAATAACATG--------- DEL JUNCTION----------
CATAGGTTTATGACCACCCTGAGCTTTTACTCAAAGCACACATTTTATATTCTGTTATACCTGGCTATGTGTGGGGTTACAAATACAGAAGTGTTATAATCTGCAAGCATCAAACACCACATGTGATGGGCTTTTCCTATCACTA
GAAAGCCTTGAAATGCATGATTAAACATGCTAAGACTTTCAAAGTAATGCTGTCCACCCACAGAATGTAAGGTGAAACCAGGATAGTTGCACCTAGAATACACACTATTCTGGCTCTTAGAGACGTTTTGTTTTATGCTGTCA
TAATCTCGGTGCTACTTTGTGGATGATAGATACTTGGCTCTTCATGGCACTTGTTTGCTGAACTTAATTTCTTTTTAGGAGGTTATGAACAGATTTATGTTAAATAGCCTCTCTGGATAAAACACCCTGATGCTGGGTAAAGACT
TTCTCTTTTCCCTTCATGATTCCAGTGTGCAGCAGGAGGCAACAAACATGTAGAAAGCTGGTTCTATGGACATAGTAGAAATGTAGGAAGGAATAAAGAGGTTTTGGAGATAGGTCCCTCCAGTGTTCTTTGG

Sanger sequencing



F1 genotyping



FO	Nb F1 born	F1 heterozygote	
		M	F
Kur8011-536	23*	7	7

*15 more F1 born recently

The line Kur8011-536-DEL is established (and transfered to Yann Hérault's team). This line will be cryopreserved.

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Genotyping protocol

cnrs
Université de Strasbourg
Instituts thématiques Inserm Institut national de la santé et de la recherche médicale
CERBM centre européen de recherche en biologie et en médecine gie
Aix*Marseille université

GENOTYPING INSTRUCTIONS

Sequence with PCR F1-R7
Primers are in orange
Insertion in purple

GGATACTTTTGCTGATGAGTAGAGGAGGGGAGTGGGGACTATCTTGCAGCAGGTGATGGGATACACCATGCTGCACAGCTCAGTATGCAGGCAGGGCTCACCTTAGAGAGTGACAAACTCCTAGCATGCAAATATCTACT
GAAGCTTGAGACACTGACAAGGTGACTTAGGCACTTAGAGTAGGGATGGAAGCAGGGCCCTAGAAAAGTCAGGGTACATATTCTGTATACAGAGGGAGGCAGGTGAGAGTCAGAGTCAGACAGAGATGAAATAGGGA
ACCAAAAAAGGATGGGGCGATGATGTCAGAGGAGTGTAGGGCTCCCCCAGAATGGGGAGGGGAAAAGGCATGATATAAAGATGCCATAATGTTCTCTAAGAGTTTAGACCCTTAAATAAAAACCTGTGCTCAATCTGATT
TGCTCTTTGGTGATGTAAAATCCACTGTGAAGGGGAACACTGATTGTGTGTTGCATCCTTATAGCCCTATGAACTAGAAAGGCCATTTAAACATTGTGCTGAATTGCTGCATGAATAACATG--------- DEL JUNCTION----------
CATAGGTTTATGACCACCCTGAGCTTTTACTCAAAGCACACATTTTATATTCTGTTATACCTGGCTATGTGTGGGGTTACAAATACAGAAGTGTTATAATCTGCAAGCATCAAACACCACATGTGATGGGCTTTTCCTATCACTA
GAAAGCCTTGAAATGCATGATTAAACATGCTAAGACTTTCAAAGTAATGCTGTCCACCCACAGAATGTAAGGTGAAACCAGGATAGTTGCACCTAGAATACACACTATTCTGGCTCTTAGAGACGTTTTGTTTTATGCTGTCA
TAATCTCGGTGCTACTTTGTGGATGATAGATACTTGGCTCTTCATGGCACTTGTTTGCTGAACTTAATTTCTTTTTAGGAGGTTATGAACAGATTTATGTTAAATAGCCTCTCTGGATAAAACACCCTGATGCTGGGTAAAGACT
TTCTCTTTTCCCTTCATGATTCCAGTGTGCAGCAGGAGGCAACAAACATGTAGAAAGCTGGTTCTATGGACATAGTAGAAATGTAGGAAGGAATAAAGAGGTTTTGGAGATAGGTCCCTCCAGTGTTCTTTGG

PCR genotyping strategy

Primer ref.	Sequence	Amplification product size for the WT allele	Amplification product for DEL allele line Kur8011-536-DEL
F1	GGGATACTTTTGCTGATGAGTAGAGGAG	1576 bps	1108 bps
R7	CCAAAGAACACTGGAGGGACCTATC		
sq4	TTTACTGCTTGAACCACT	277 bps	
sq5	AGCCCATCACATGTGGTG		

Sequence of the WT PCR (control WT allele) sq4-sq5

TTTACTGCTTGAACCACTGAGACTAAGGCAAGAGCCTGATCTTAACTGTGGCACCAAAGGCCAAAGCAAAGATGGCTCACAAAACAACTACGGGATTTTGATGTTG
TAATTAACAGTGCTGCTTGACCCTGTCATCCACACGCTGCATAGGTTTATGACCACCCTGAGCTTTTACTCAAAGCACACATTTTATATTCTGTTATACCTGGCTATGTG
TGGGGTTACAAATACAGAAGTGTTATAATCTGCAAGCATCAAACACCACATGTGATGGGCT

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GENOTYPING INSTRUCTIONS

PCR Protocol

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

Lysis buffer: 50 µl DNA Extract All Reagents – appliedbiosystems, Ref 4402616 (25 µl Lysis buffer +25 µl stabilising buffer)

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	30
62°C	10s	
68°C	45s	
68°C	5min	1
12°C	5min	1

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REPORT REDACTION & VALIDATION

Report performed on 2022/10 /06

by Marie-Christine BIRLING, PhD

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