GemPharmatech™

Kcnt1 Cas9-KO Strategy

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Overview

Target Gene Name

- Kcnt1

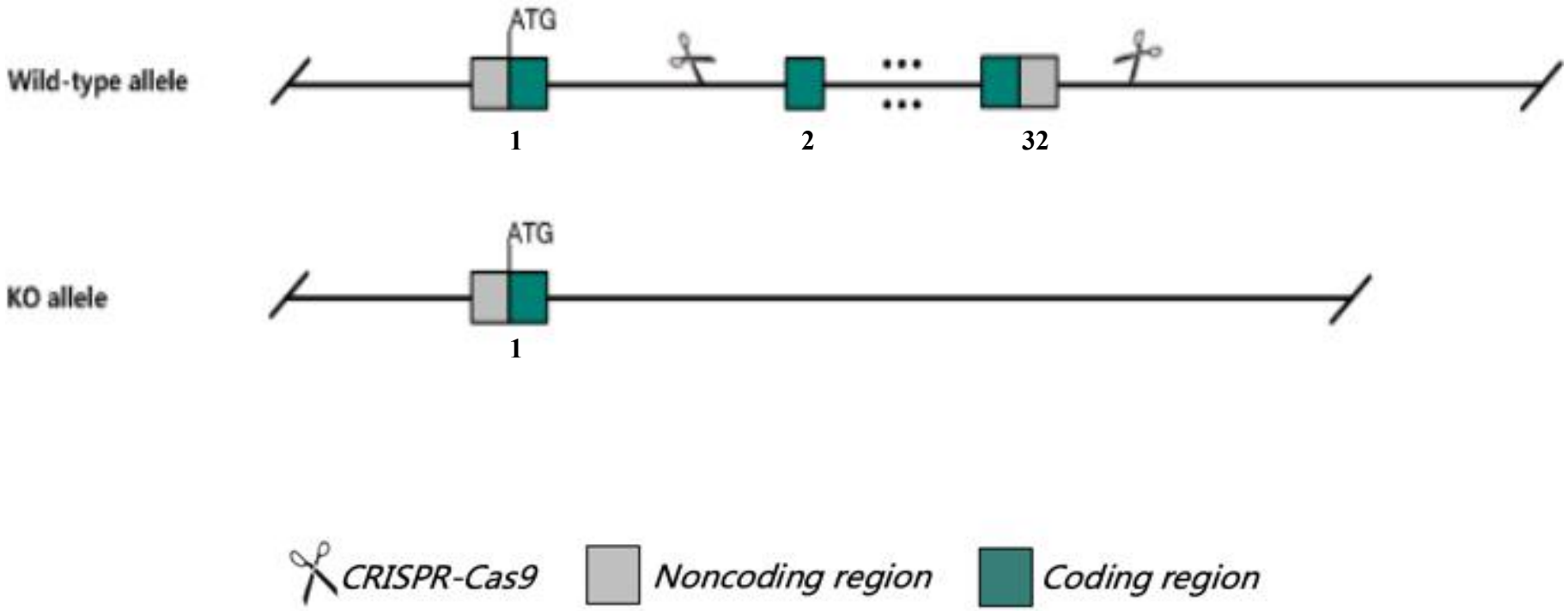
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Technical Information

- The *Kcnt1* gene has 15 transcripts. According to the structure of *Kcnt1* gene, exon2-exon32 of *Kcnt1*-201 (ENSMUST00000037580.13) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Kcnt1* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs were microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.

Gene Information

Kcnt1 potassium channel, subfamily T, member 1 [Mus musculus (house mouse)]

Gene ID: 227632, updated on 19-Mar-2019

Summary

Official Symbol	Kcnt1 provided by MGI
Official Full Name	potassium channel, subfamily T, member 1 provided by MGI
Primary source	MGI:MGI:1924627
See related	Ensembl:ENSMUSG00000058740
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	C030030G16Rik, Slack, Slo2.2, slo2
Summary	This gene encodes a member of the Slo potassium channel family that has shown to be activated by both sodium and chloride ions. This channel represents the largest potassium channel subunit yet identified. This channel may be important in development and pain signaling. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Oct 2014]
Expression	Biased expression in cerebellum adult (RPKM 46.3), frontal lobe adult (RPKM 20.5) and 6 other tissues See more
Orthologs	human all

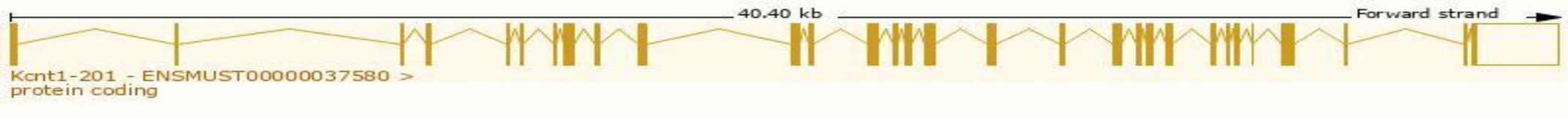
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 15 transcripts,all transcripts are shown below:

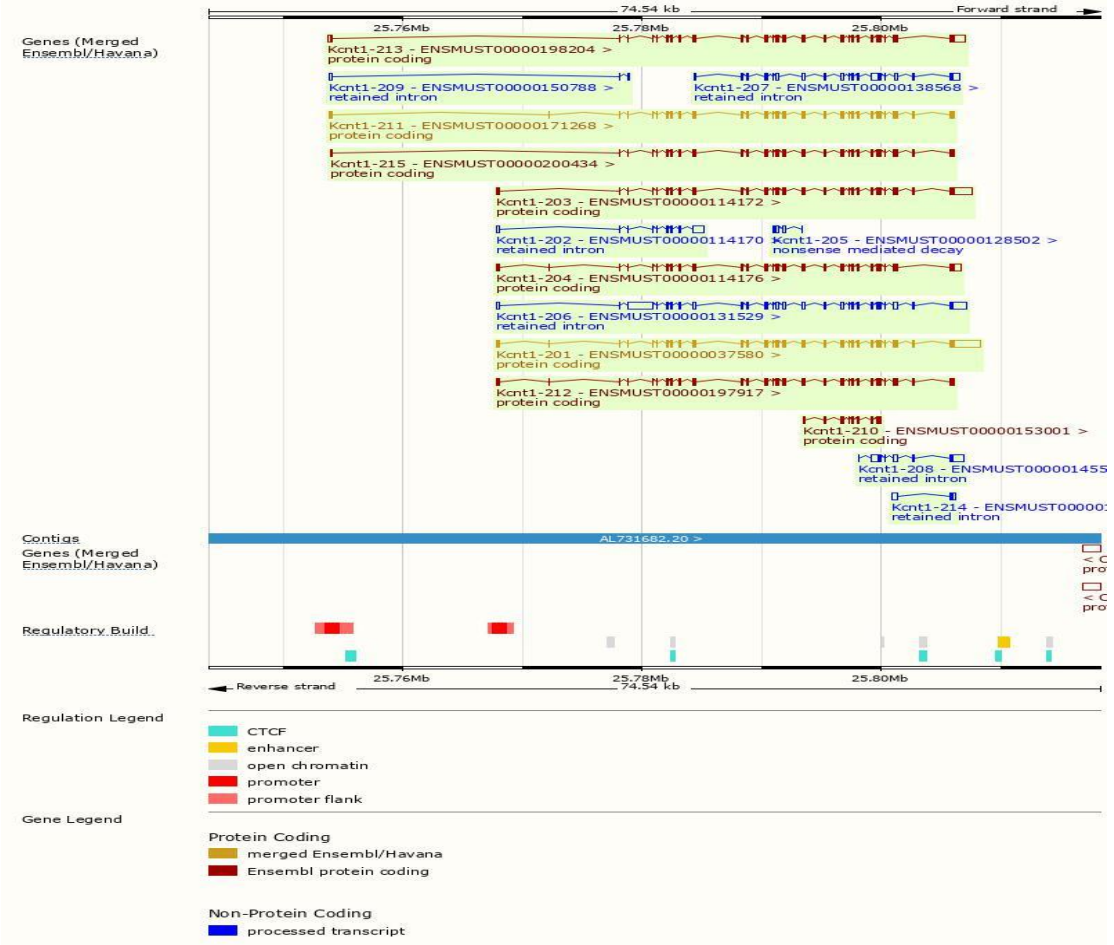
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Kcnt1-201	ENSMUST00000037580.12	5881	1238aa	Protein coding	CCDS15794	A2AHB8	TSL:1 GENCODE basic APPRIS P3
Kcnt1-203	ENSMUST00000114172.5	5219	1224aa	Protein coding	CCDS79757	Q6ZPR4	TSL:1 GENCODE basic APPRIS ALT2
Kcnt1-211	ENSMUST00000171268.7	3771	1218aa	Protein coding	CCDS50537	C0KTP6	TSL:1 GENCODE basic APPRIS ALT2
Kcnt1-213	ENSMUST00000198204.4	4714	1183aa	Protein coding	-	A0A0G2JER3	TSL:5 GENCODE basic APPRIS ALT2
Kcnt1-204	ENSMUST00000114176.8	4166	1217aa	Protein coding	-	A2AHB7	TSL:5 GENCODE basic APPRIS ALT2
Kcnt1-212	ENSMUST00000197917.4	3711	1236aa	Protein coding	-	A2AHB9	TSL:5 GENCODE basic APPRIS ALT2
Kcnt1-215	ENSMUST00000200434.4	3609	1202aa	Protein coding	-	A0A0G2JG99	TSL:5 GENCODE basic APPRIS ALT2
Kcnt1-210	ENSMUST00000153001.2	990	330aa	Protein coding	-	A0A0G2JDW4	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Kcnt1-205	ENSMUST00000128502.2	592	36aa	Nonsense mediated decay	-	A0A0G2JG91	CDS 5' incomplete TSL:5
Kcnt1-206	ENSMUST00000131529.5	6639	No protein	Retained intron	-	-	TSL:2
Kcnt1-207	ENSMUST00000138568.7	3299	No protein	Retained intron	-	-	TSL:2
Kcnt1-208	ENSMUST00000145544.5	2120	No protein	Retained intron	-	-	TSL:2
Kcnt1-202	ENSMUST00000114170.7	1673	No protein	Retained intron	-	-	TSL:2
Kcnt1-214	ENSMUST00000199269.1	795	No protein	Retained intron	-	-	TSL:3
Kcnt1-209	ENSMUST00000150788.5	532	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Kcnt1*-201 transcript, the transcription is shown below:



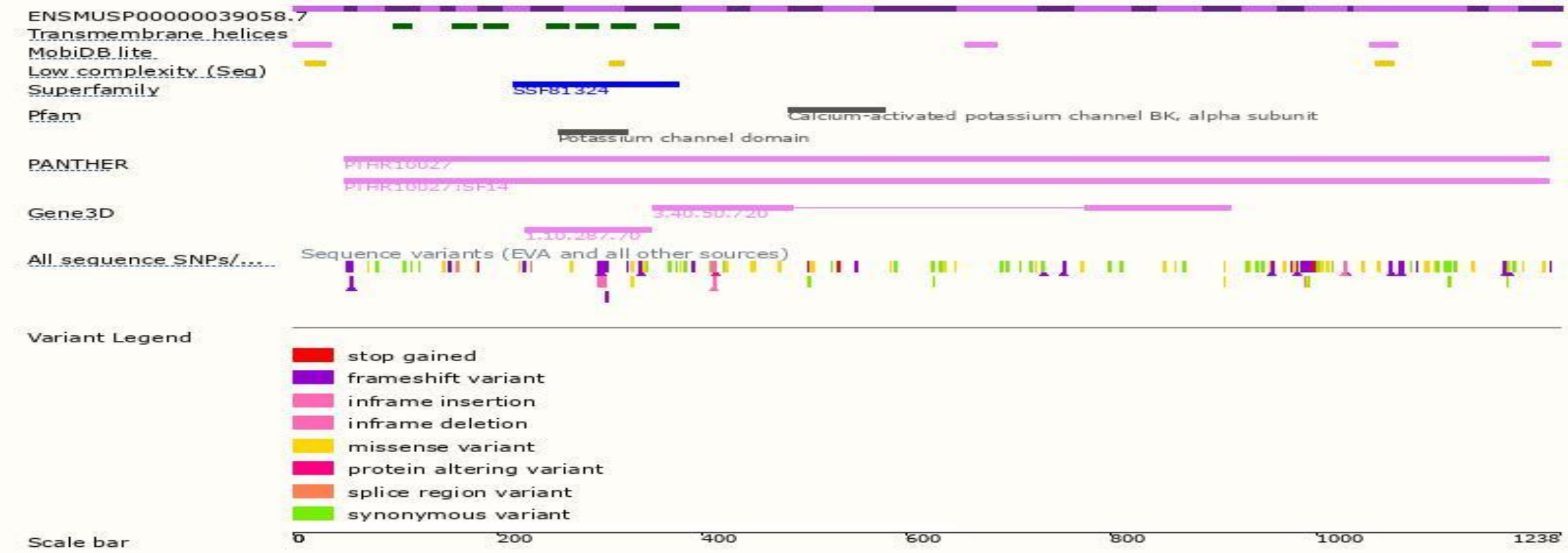
Source: https://www.ensembl.org

Genomic Information



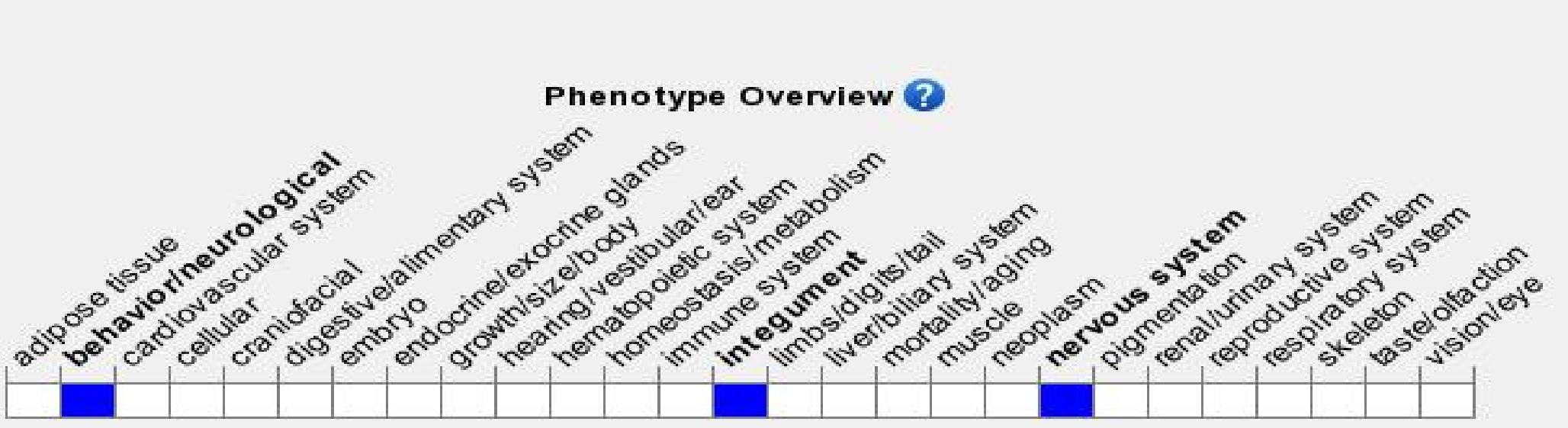
Source: : https://www.ensembl.org

Protein Information



Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Mice homozygous for a knock-out allele exhibit impaired action potential firing in sensory neurons and increased mechanical hypersensitivity in neuropathic pain models. Homozygosity for a gain-of-function mutation increases overall excitability of neurons and causes (nocturnal) seizures, hyperactivity and learning deficits.

Source: https://www.informatics.jax.org

Important Information

- *Kcnt1* is located on Chr2. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.