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Kcnh6 Cas9-CKO Strategy

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Design Date: 2023-6-21

Overview

Target Gene Name

- Kcnh6

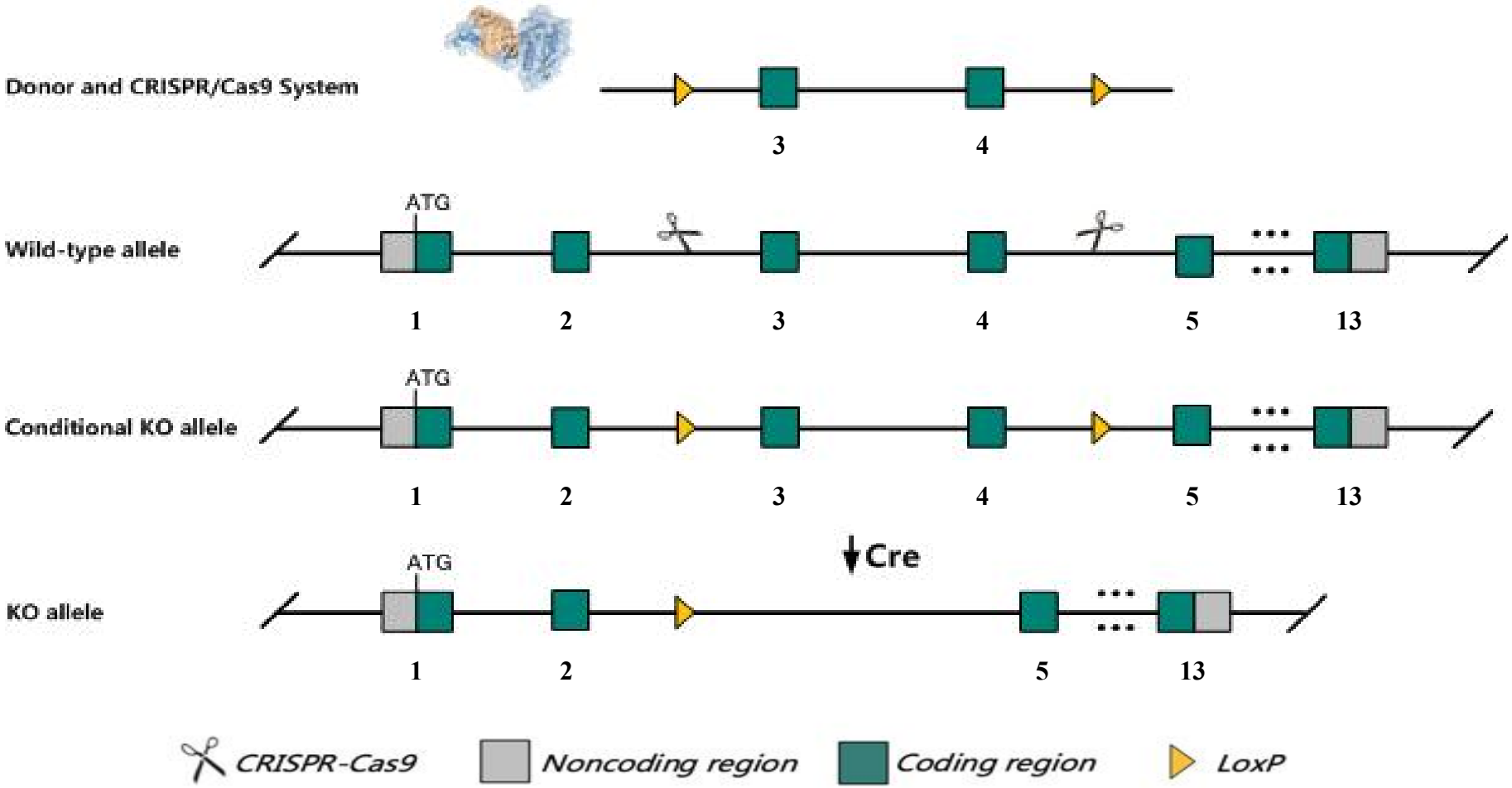
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Kcnh6* gene.

Technical Information

- The *Kcnh6* gene has 4 transcripts. According to the structure of *Kcnh6* gene, exon3-exon4 of *Kcnh6*-201 (ENSMUST00000001965.14) transcript is recommended as the knockout region. The region contains 368bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Kcnh6* gene. The brief process is as follows: CRISPR-Cas9 system and Donor 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.
- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Gene Information

Kcnh6 potassium voltage-gated channel, subfamily H (eag-related), member 6 [Mus musculus (house mouse)]

Gene ID: 192775, updated on 18-May-2023

Summary

Official Symbol	Kcnh6 provided by MGI
Official Full Name	potassium voltage-gated channel, subfamily H (eag-related), member 6 provided by MGI
Primary source	MGI:MGI:2684139
See related	Ensembl:ENSMUSG00000001901
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	m-erg2
Summary	Predicted to enable inward rectifier potassium channel activity. Predicted to be involved in ion transmembrane transport; regulation of heart rate by cardiac conduction; and regulation of ventricular cardiac muscle cell membrane repolarization. Predicted to be integral component of plasma membrane. Is expressed in several structures, including gut; male reproductive gland or organ; nervous system; retina; and skin. Used to study glucose metabolism disease. Orthologous to human KCNH6 (potassium voltage-gated channel subfamily H member 6). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Broad expression in colon adult (RPKM 1.9), frontal lobe adult (RPKM 1.4) and 17 other tissues See more
Orthologs	human all

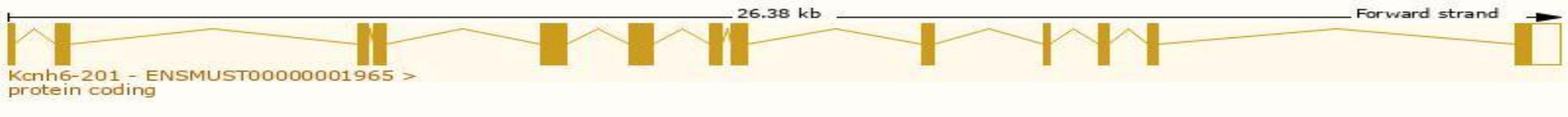
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 4 transcripts,all transcripts are shown below:

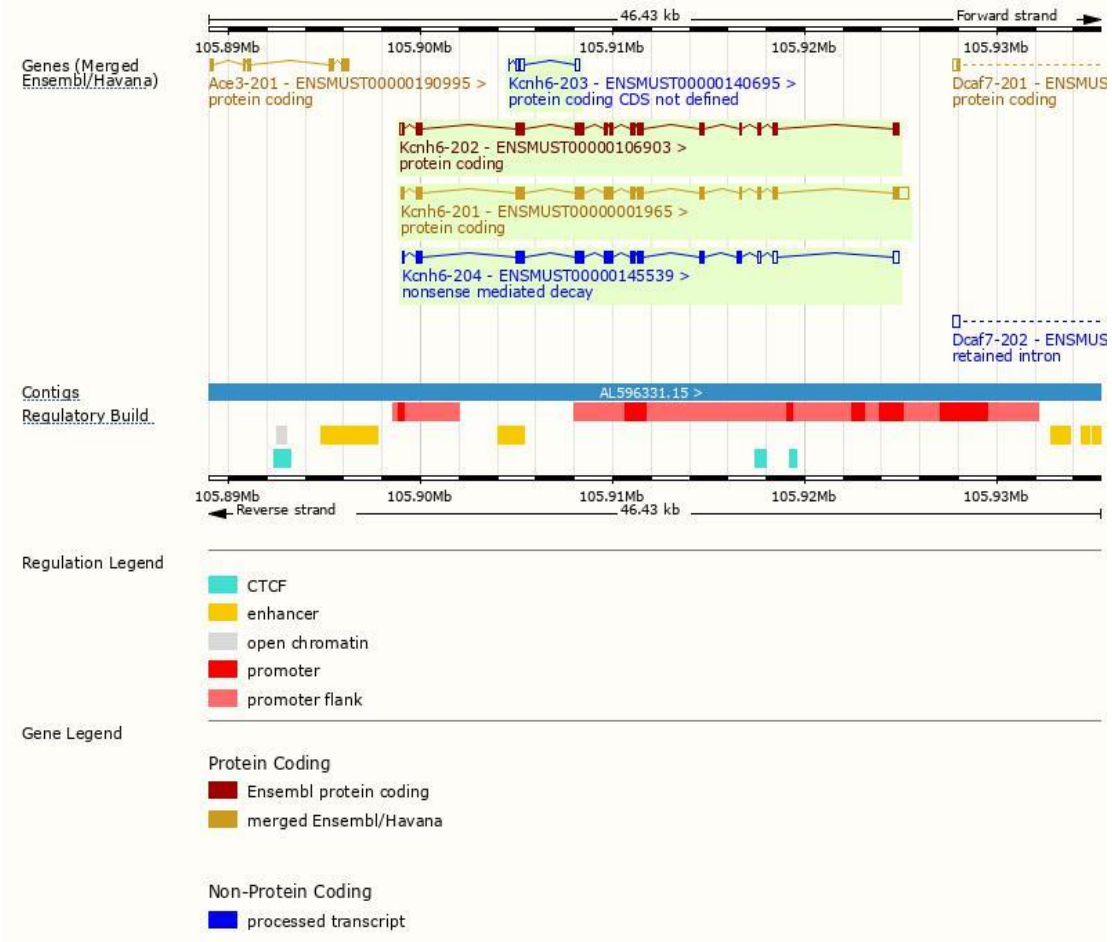
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Kcnh6-201	ENSMUST00000001965.14	3368	950aa	Protein coding	CCDS25345		A single transcript chosen for a gene which is the most conserved, most highly expressed, has the longest coding sequence and is represented in other key resources, such as NCBI and UniProt. This is defined in detail on http://www.ensembl.org/info/genome/genebuild/canonical.html Ensembl Canonical , The GENCODE set is the gene set for human and mouse. GENCODE basic , APPRIS P1 , TSL:1 ,
Kcnh6-202	ENSMUST00000106903.8	2816	807aa	Protein coding			The GENCODE set is the gene set for human and mouse. GENCODE basic , TSL:5 ,
Kcnh6-204	ENSMUST00000145539.2	2959	775aa	Nonsense mediated decay			TSL:1 ,
Kcnh6-203	ENSMUST00000140695.2	633	No protein	Protein coding CDS not defined			TSL:3 ,

The strategy is based on the design of *Kcnh6*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org

Genomic Information



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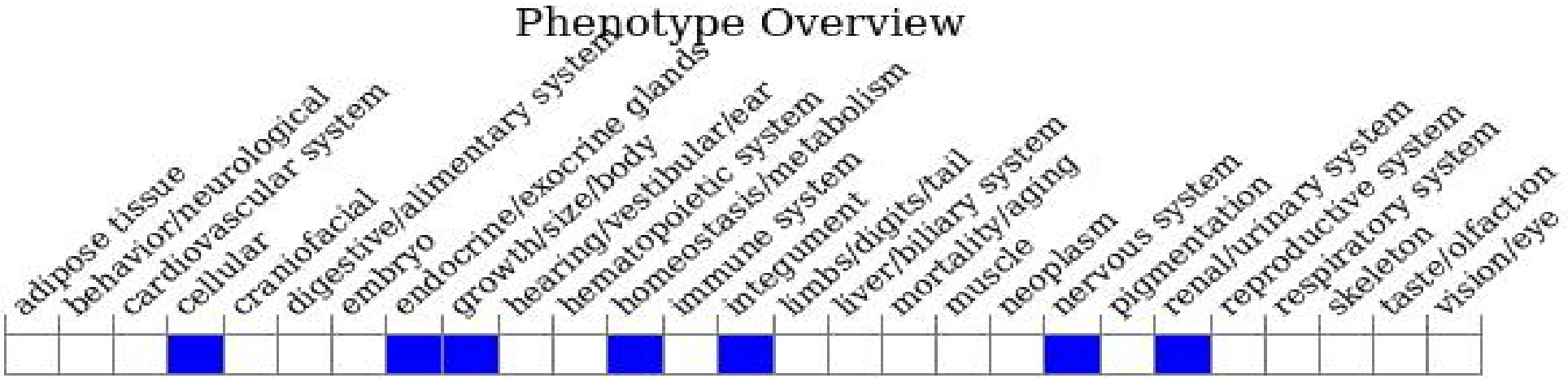
Source: : https://www.ensembl.org

Protein Information

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

Mouse Phenotype Information (MGI)



- Nullizygous mice show a phenotype changing from hyperinsulinemia to hypoinsulinemia and diabetes. Islets from young mice show high intracellular calcium levels and insulin hypersecretion, whereas adult islets show increased ER stress and apoptosis, loss of beta cell mass and insulin hyposecretion.

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

Important Information

- *Kcnh6* is located on Chr11. 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 risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.