Dgkd Cas9-KO Strategy

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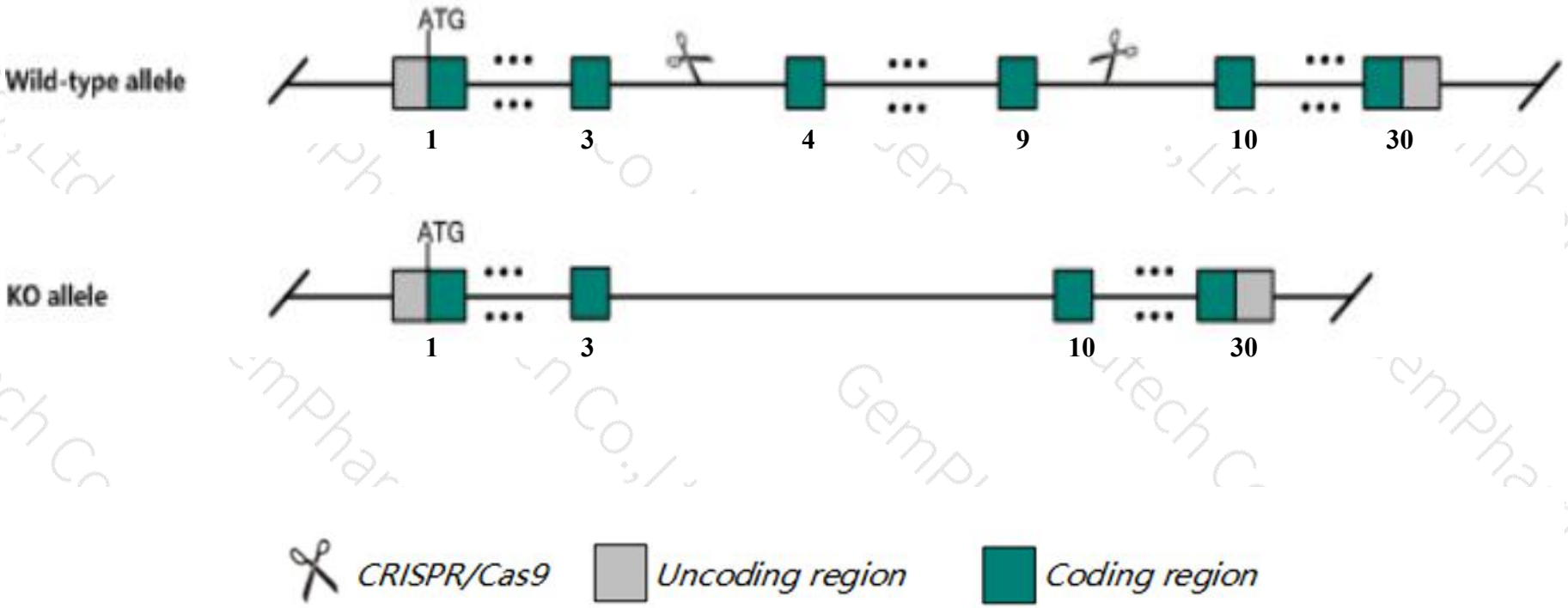
Design Date: 2020-7-24

Project Overview

Project Name	***Dgkd***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Dgkd* gene. The schematic diagram is as follows:



Technical routes

➢ The *Dgkd* gene has 7 transcripts. According to the structure of *Dgkd* gene, exon4-exon9 of *Dgkd-201*(ENSMUST00000027517.13) transcript is recommended as the knockout region.The region contains 737bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Dgkd* gene. The brief process is as follows: CRISPR/Cas9 system 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

Notice

➢ According to the existing MGI data,mice homozygous for a null allele are born with open eyelids and reduced body size, develop respiratory distress and die within 24 hrs of birth. Half of mice homozygous for a hypomorphic gene trap allele exhibit abnormal epileptic discharges and seizureswhile 9% of aging homozygotes develop tumors.

➢ Transcript *Dgkd*-202, *Dgkd*-203 and *Dgkd*-205 may not be affected.

➢ The *Dgkd* gene is located on the Chr1. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Dgkd diacylglycerol kinase, delta [Mus musculus (house mouse)]

Gene ID: 227333, updated on 13-Mar-2020

Summary

Official Symbol	Dgkd provided by MGI
Official Full Name	diacylglycerol kinase, delta provided byMGI
Primary source	MGI:MGI:2138334
See related	Ensembl:ENSMUSG00000070738
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	AI841987, D330025K09, DGKdelta, dgkd-2
Expression	Ubiquitous expression in thymus adult (RPKM 36.4), spleen adult (RPKM 18.4) and 28 other tissuesSee more
Orthologs	human all

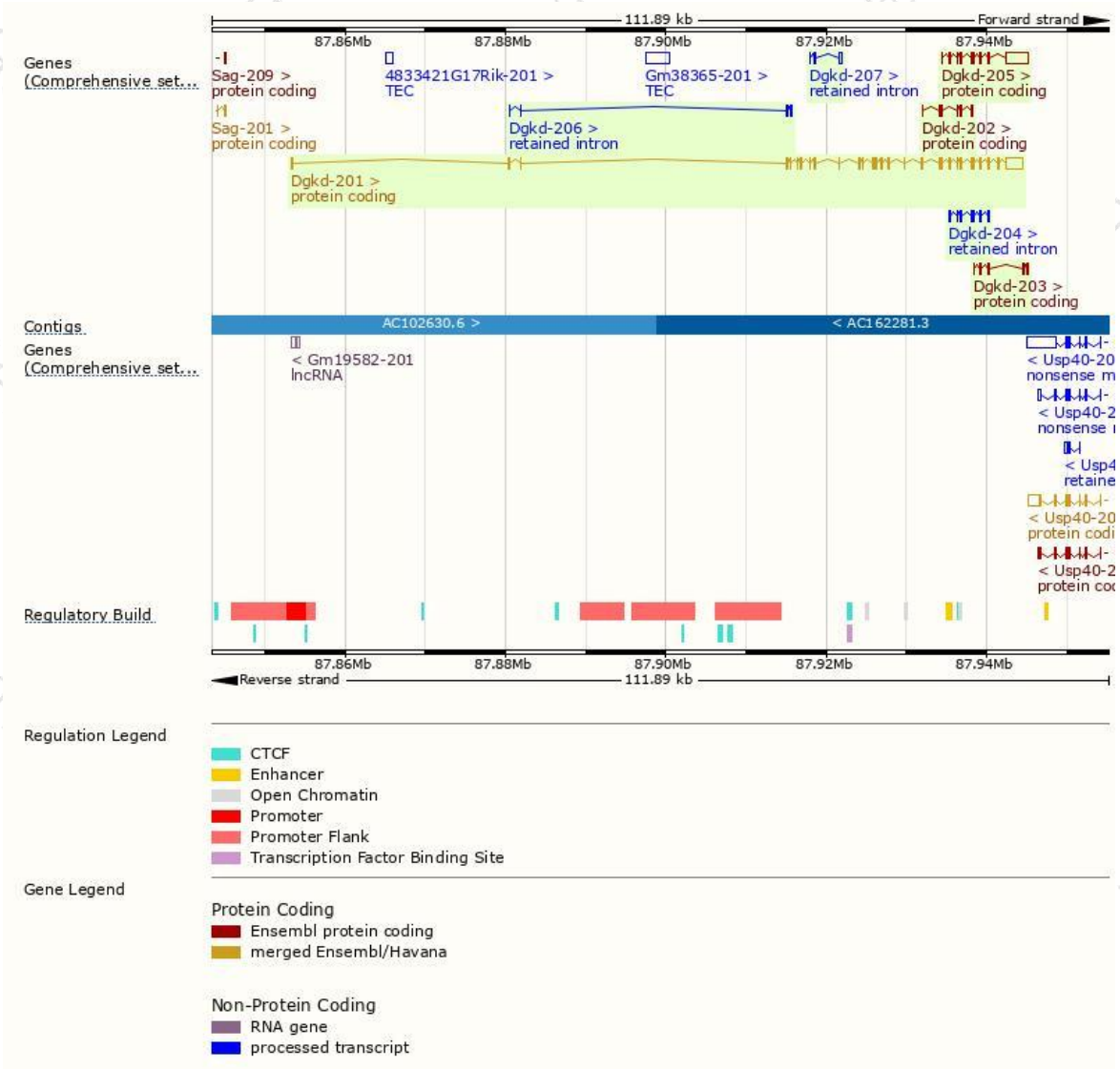
Transcript information（Ensembl）

The gene has 7 transcripts,all transcripts are shown below:

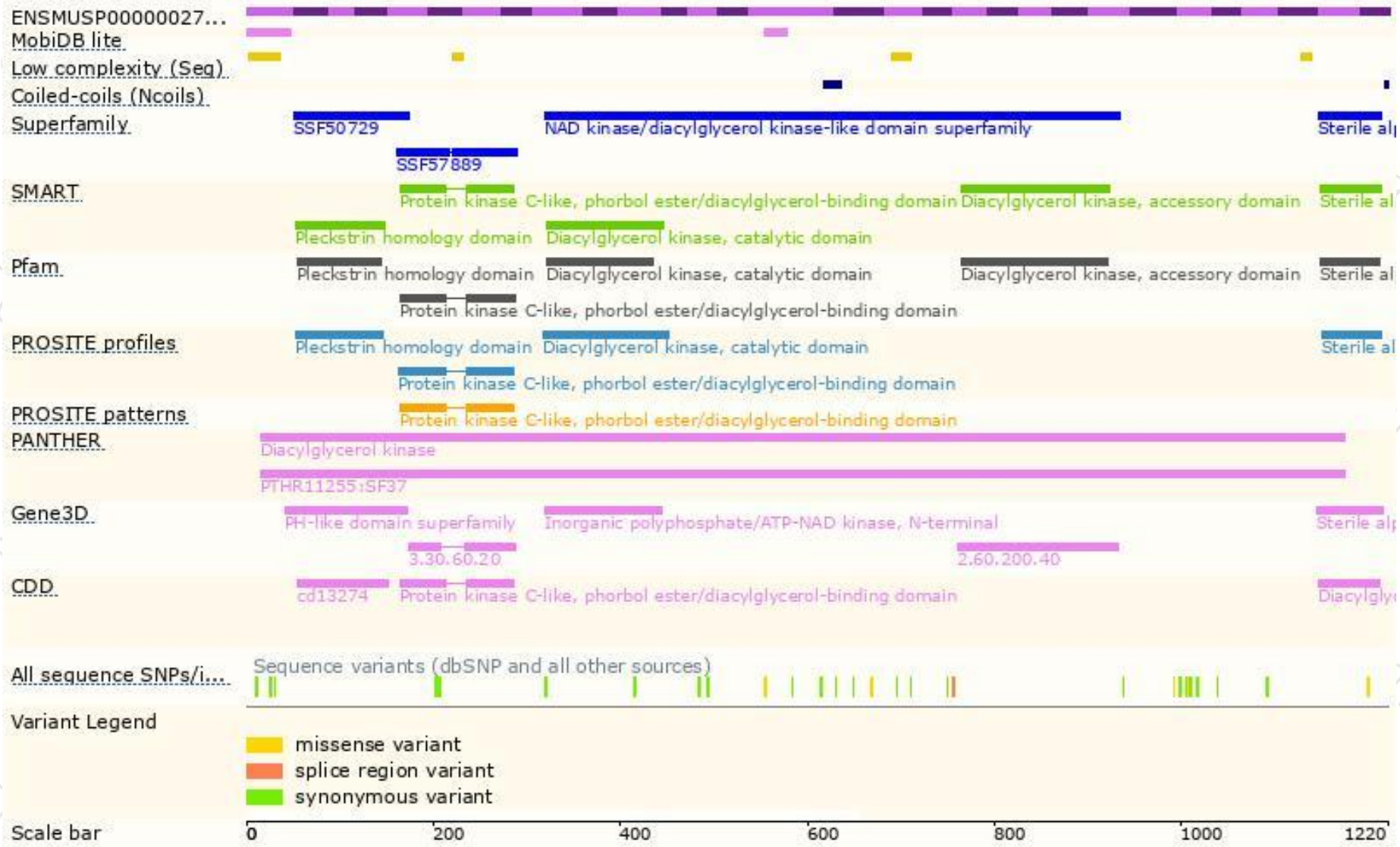
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dgkd-201	ENSMUST00000027517.13	5695	1220aa	Protein coding	CCDS48312	E9PUQ8	TSL:5 GENCODE basic APPRIS P1
Dgkd-205	ENSMUST00000190061.6	3759	364aa	Protein coding	-	A0A087WP73	CDS 5' incomplete TSL:5
Dgkd-203	ENSMUST00000189448.1	716	197aa	Protein coding	-	A0A087WP49	CDS 5' incomplete TSL:3
Dgkd-202	ENSMUST00000185260.6	653	218aa	Protein coding	-	A0A087WQ75	CDS 5' and 3' incomplete TSL:3
Dgkd-204	ENSMUST00000189726.1	870	No protein	Retained intron	-	-	TSL:3
Dgkd-207	ENSMUST00000191589.1	659	No protein	Retained intron	-	-	TSL:3
Dgkd-206	ENSMUST00000190243.1	483	No protein	Retained intron	-	-	TSL:2

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

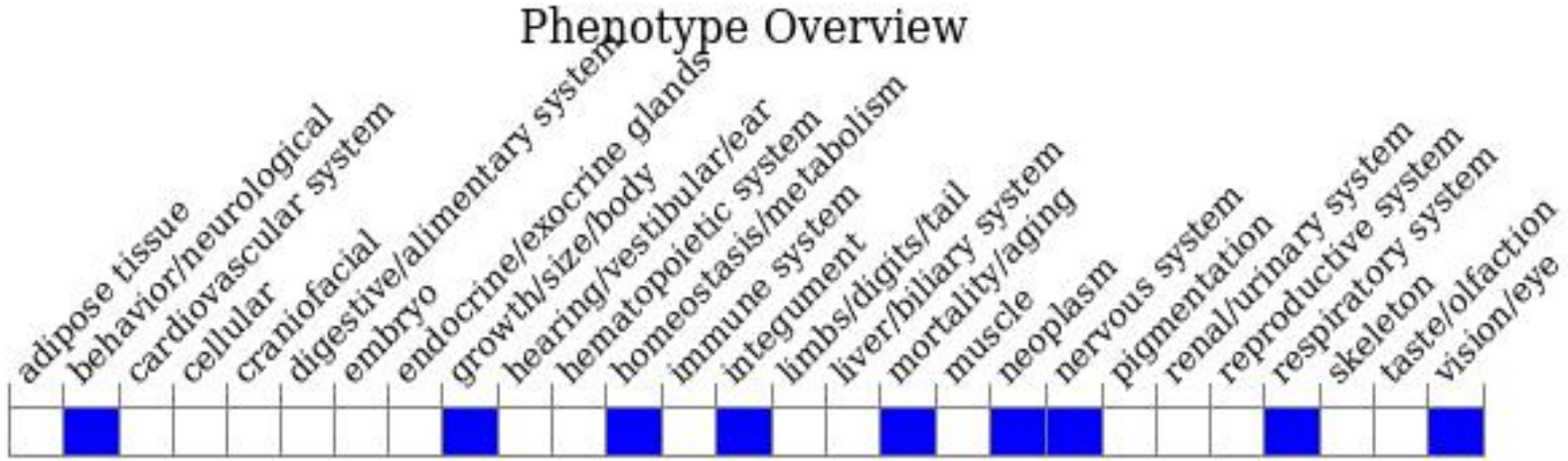
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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If you have any questions, you are welcome to inquire.

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