

Dgkd Cas9-KO Strategy

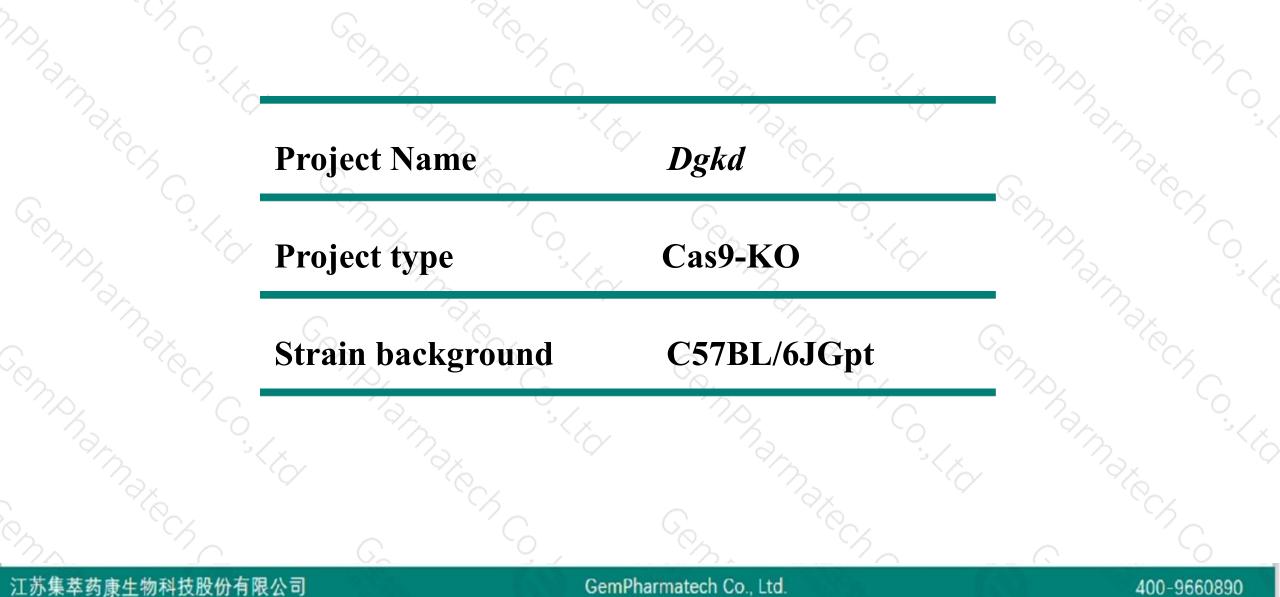
Designer: Zihe Cui

Reviewer: Ruirui Zhang

Design Date: 2020-7-24

Project Overview

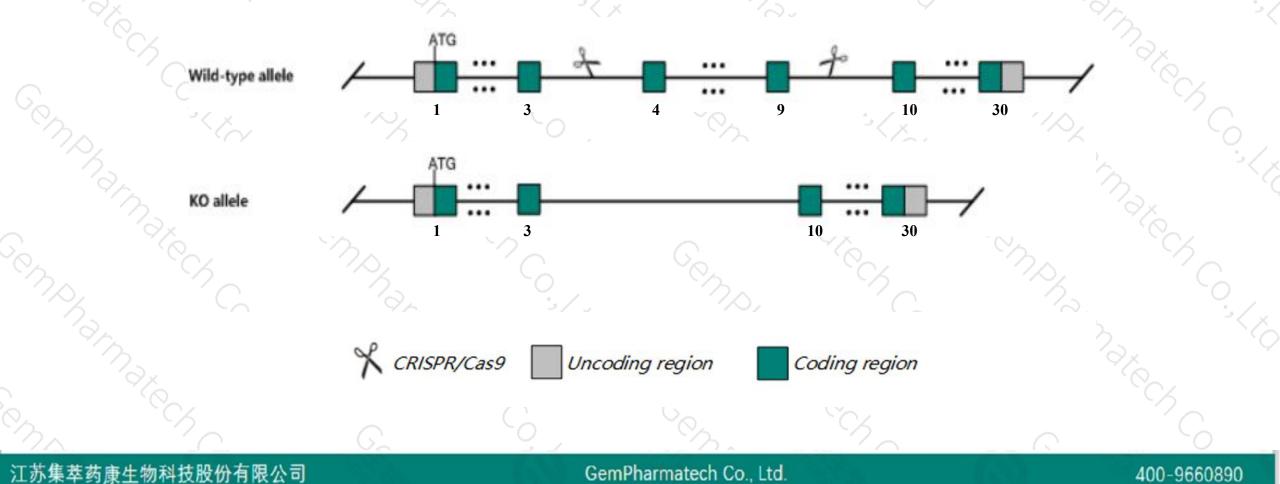




Knockout strategy



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





> 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.



> 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.

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Gene information (NCBI)



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 by <u>MGI</u>
Primary source	MGI:MGI:2138334
See related	Ensembl:ENSMUSG0000070738
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

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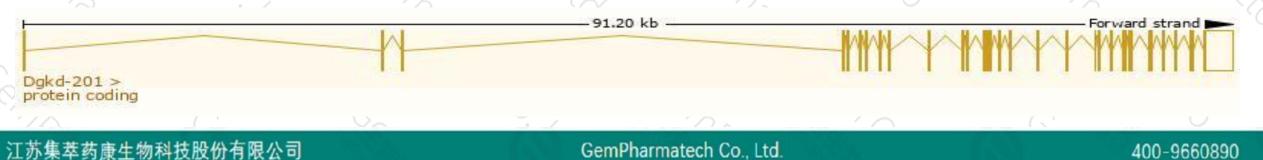
Transcript information (Ensembl)



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

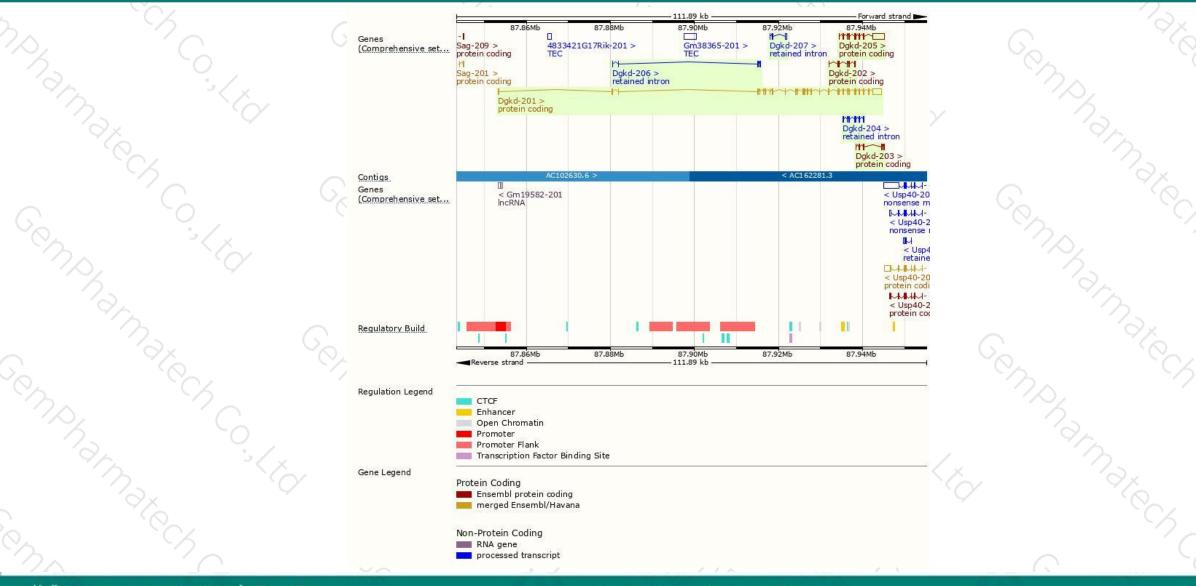
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dgkd-201	ENSMUST0000027517.13	5695	<u>1220aa</u>	Protein coding	CCDS48312	E9PUQ8	TSL:5 GENCODE basic APPRIS P1
Dgkd-205	ENSMUST00000190061.6	3759	<u>364aa</u>	Protein coding	-	A0A087WP73	CDS 5' incomplete TSL:5
Dgkd-203	ENSMUST00000189448.1	716	<u>197aa</u>	Protein coding	828	A0A087WP49	CDS 5' incomplete TSL:3
Dgkd-202	ENSMUST00000185260.6	653	<u>218aa</u>	Protein coding	-	A0A087WQ75	CDS 5' and 3' incomplete TSL:3
Dgkd-204	ENSMUST00000189726.1	870	No protein	Retained intron	-	14	TSL:3
Dgkd-207	ENSMUST00000191589.1	659	No protein	Retained intron	870	8	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





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Protein domain

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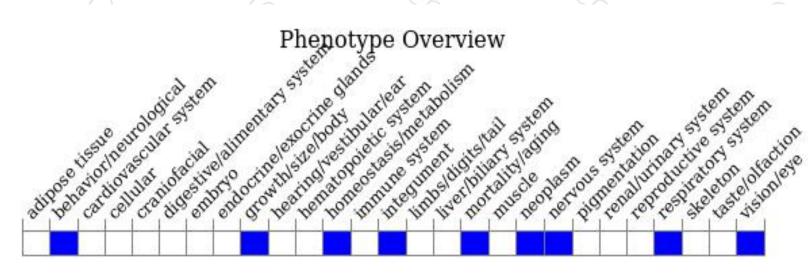


4	ENSMUSP00000027 MobiDB lite Low complexity (Seq)		
	Coiled-coils (Ncoils)		5
	Superfamily	SSF50729 NAD kinase/diacylglycerol kinase-like domain superfamily Sterile all SSF57889	
	SMART	Protein kinase C-like, phorbol ester/diacylglycerol-binding domain Diacylglycerol kinase, accessory domain Sterile al	
		Pleckstrin homology domain Diacylglycerol kinase, catalytic domain	
	Pfam	Pleckstrin homology domain Diacylglycerol kinase, catalytic domain Diacylglycerol kinase, accessory domain Sterile al	
		Protein kinase C-like, phorbol ester/diacylglycerol-binding domain	
	PROSITE profiles	Pleckstrin homology domain Diacylglycerol kinase, catalytic domain Sterile al	
		Protein kinase C-like, phorbol ester/diacylglycerol-binding domain	
	DDOCTTE anthorna		
	PROSITE patterns PANTHER	Protein kinase C-like, phorbol ester/diacylglycerol-binding domain	1960 - 1960 - 1960 - 1960 - 1960 - 1960 - 1960 - 1960 - 1960 - 1960 - 1960 - 1960 - 1960 - 1960 - 1960 - 1960 -
	LOUTIEN.	Diacylglycerol kinase	
		PTHR11255:SF37	
	Gene3D	PH-like domain superfamily Inorganic polyphosphate/ATP-NAD kinase, N-terminal Sterile al;	
		3.30.60.20	
	CDD	cd13274 Protein kinase C-like, phorbol ester/diacylglycerol-binding domain Diacylglycerol-binding domain	\cap
		Collocyd Proceir kinase chine, phorbol ester/diacydiyceror-binding domain	
			3/
	All sequence SNPs/i	Sequence variants (dbSNP and all other sources)	
	Manianth Languard		
	Variant Legend	missense variant	
		splice region variant	
		synonymous variant	
	Scale bar	0 200 400 600 800 1000 1220	
			\smile

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Mouse phenotype description(MGI)





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

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If you have any questions, you are welcome to inquire. Tel: 400-9660890



