

Tdrkh Cas9-CKO Strategy

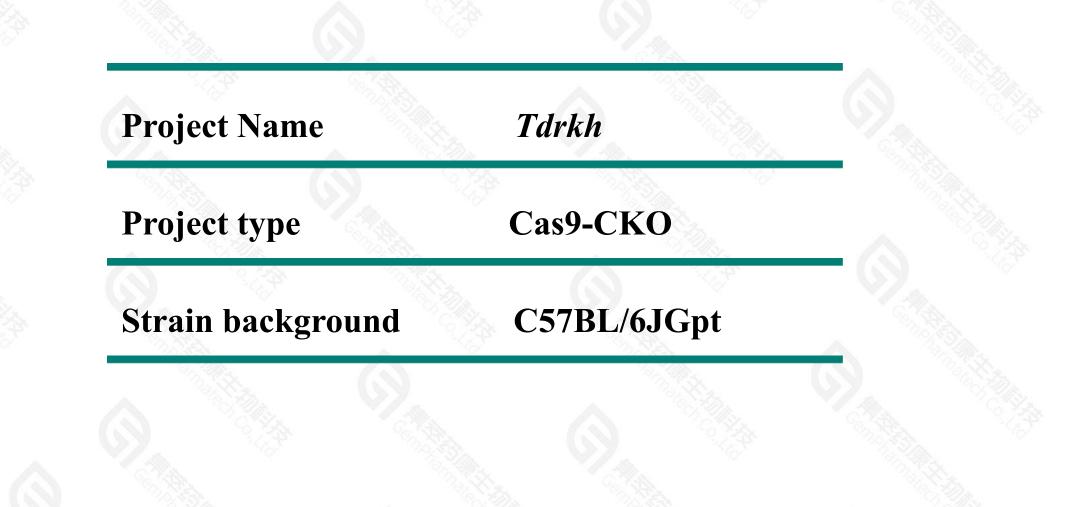
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Reviewer: Huan wang

Design Date: 2021-9-26

Project Overview



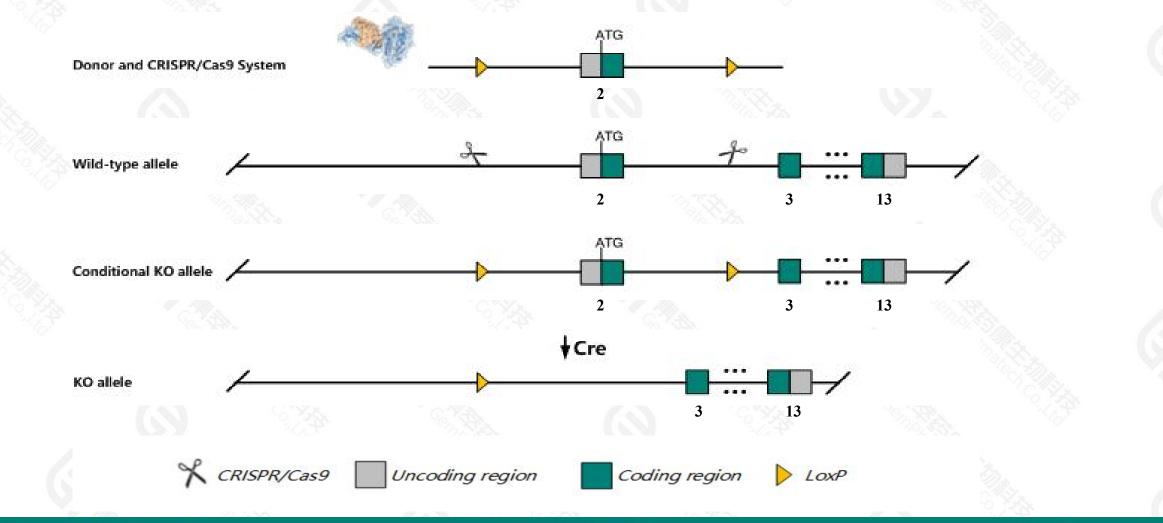


Conditional Knockout strategy

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



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Technical routes



> The *Tdrkh* gene has 8 transcripts. According to the structure of *Tdrkh* gene, exon2 of *Tdrkh-202*(ENSMUST00000166032.8) transcript is recommended as the knockout region. The region contains start codon ATG.Knock out the region will result in disruption of protein function.

> In this project we use CRISPR/Cas9 technology to modify *Tdrkh* 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

> 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.



- > According to the existing MGI data, mice homozygous for a knock-out allele exhibit male infertility associated with arrested male meiosis, massive double-strand breaks and impaired piRNA biogenesis.
- > The *Tdrkh* gene is located on the Chr3. 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.
- > The KO region contains functional region of the Gm42464 gene.Knockout the region will affect the function of Gm42464 gene.
- > 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 existing technological level.

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Gene information (NCBI)

Tdrkh tudor and KH domain containing protein [Mus musculus (house mouse)]

Gene ID: 72634, updated on 17-Dec-2020

Summary

Official Symbol	Tdrkh provided by MGI
Official Full Name	tudor and KH domain containing protein provided by MGI
Primary source	MGI:MGI:1919884
See related	Ensembl:ENSMUSG0000041912
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	2700091C21Rik, Tdrd, Tdrd2
Expression	Biased expression in testis adult (RPKM 43.0), CNS E18 (RPKM 3.9) and 5 other tissuesSee more
Orthologs	human all

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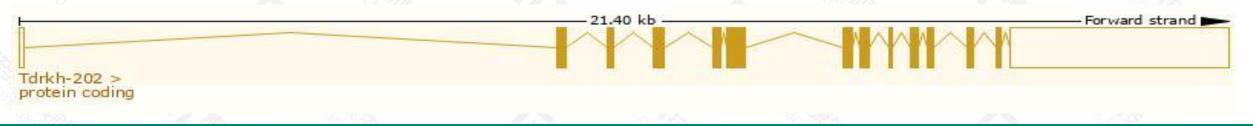
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Transcript information (Ensembl)

The gene has 8 transcripts, all transcripts are shown below:

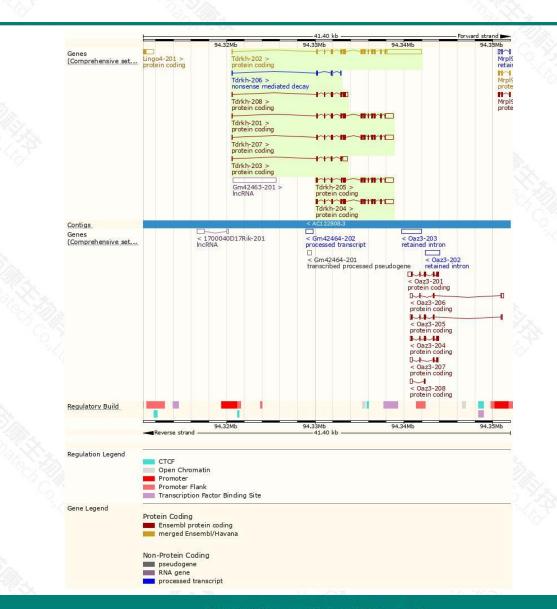
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tdrkh-202	ENSMUST00000166032.8	5692	<u>560aa</u>	Protein coding	CCDS38534		TSL:1 , GENCODE basic , APPRIS P1 ,
Tdrkh-207	ENSMUST00000197901.5	2652	<u>560aa</u>	Protein coding	CCDS38534		TSL:5 , GENCODE basic , APPRIS P1 ,
Tdrkh-201	ENSMUST0000045245.10	2642	<u>560aa</u>	Protein coding	CCDS38534		TSL:1, GENCODE basic, APPRIS P1,
Tdrkh-204	ENSMUST00000196606.5	2586	<u>556aa</u>	Protein coding	-		TSL:5 , GENCODE basic ,
Tdrkh-205	ENSMUST00000197495.2	2476	<u>515aa</u>	Protein coding	-		TSL:5 , GENCODE basic ,
Tdrkh-208	ENSMUST00000200486.5	1231	<u>296aa</u>	Protein coding	-		TSL:3 , GENCODE basic ,
Tdrkh-203	ENSMUST00000196386.5	1050	<u>140aa</u>	Protein coding	-2		TSL:1 , GENCODE basic ,
Tdrkh-206	ENSMUST00000197876.5	476	<u>50aa</u>	Nonsense mediated decay	-		TSL:2,

The strategy is based on the design of *Tdrkh-202* transcript, the transcription is shown below:



Genomic location distribution



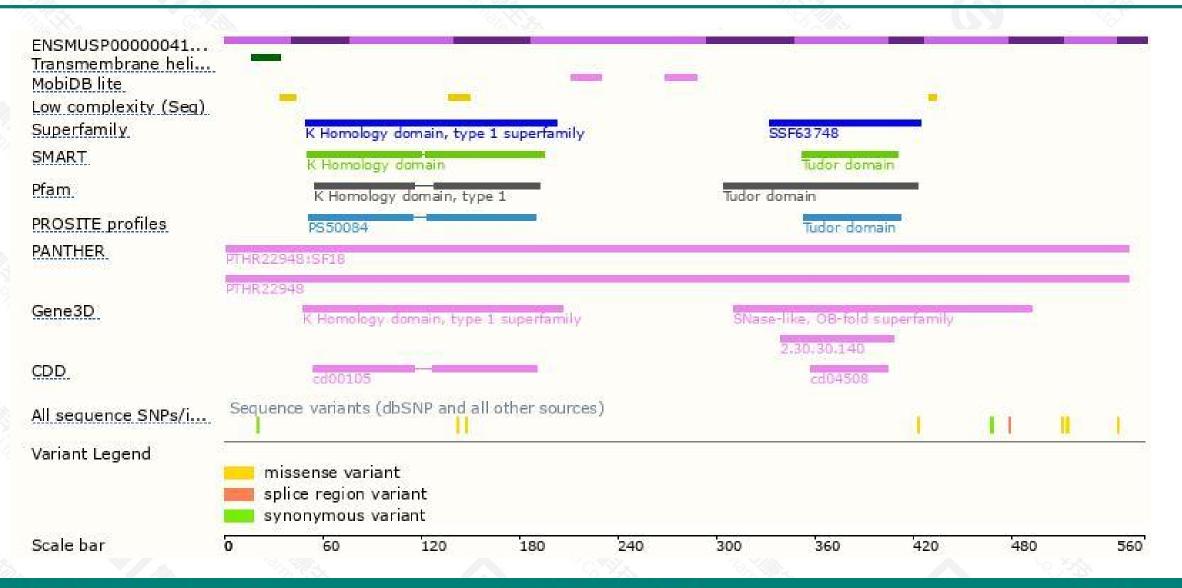


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Protein domain



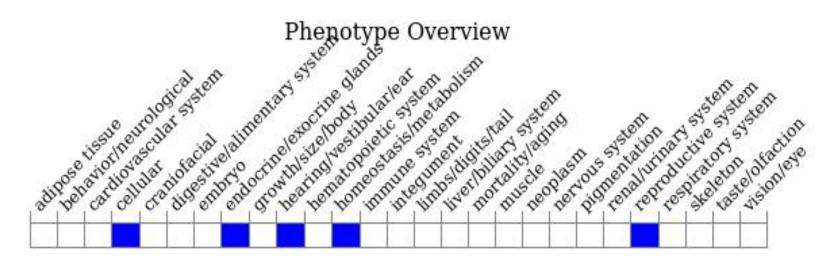


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Mouse phenotype description(MGI)



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 knock-out allele exhibit male infertility associated with arrested male meiosis, massive double-strand breaks and impaired piRNA biogenesis.

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



