Dnaja1 Cas9-KO Strategy

Designer: Zihe Cui

Reviewer: Xueting Zhang

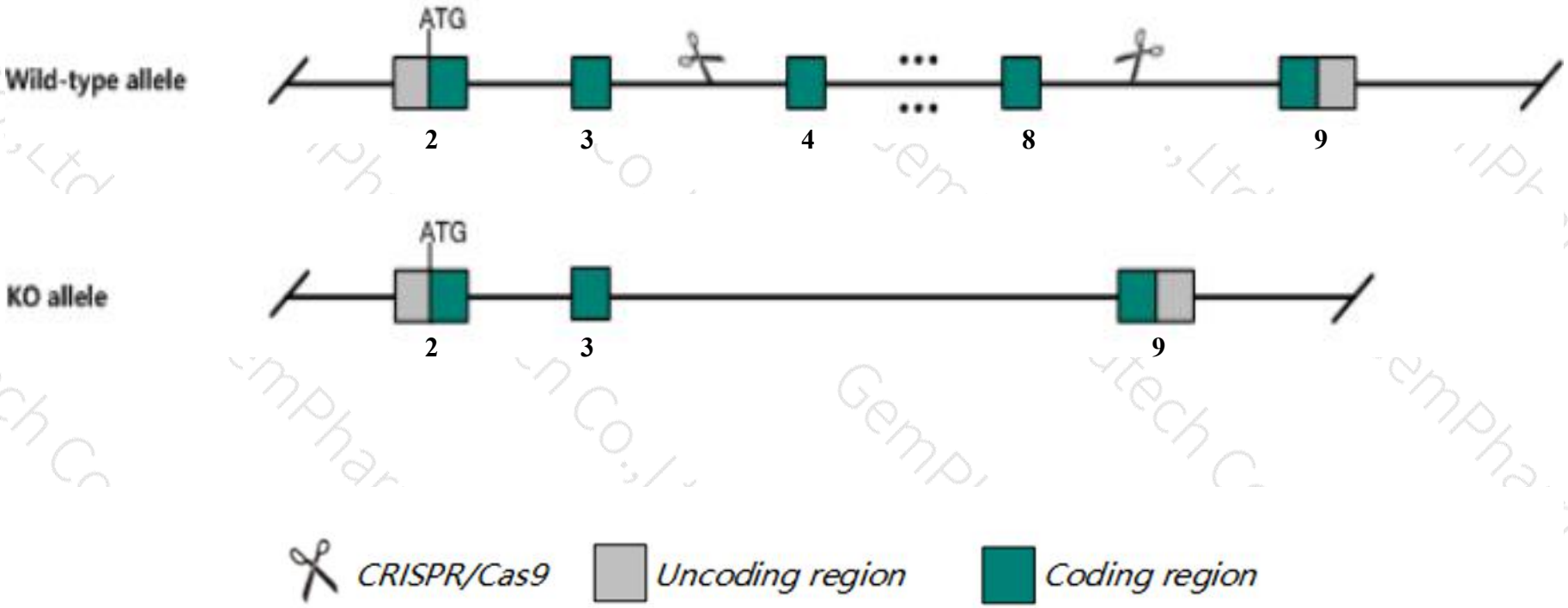
Design Date: 2020-7-22

Project Overview

Project Name	***Dnaja1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Dnaja1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Dnaja1* gene has 10 transcripts. According to the structure of *Dnaja1* gene, exon4-exon8 of *Dnaja1-210*(ENSMUST00000164233.7) transcript is recommended as the knockout region.The region contains 665bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Dnaja1* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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,male mice homozygous for a knock-out allele exhibit decreased postnatal growth and reduced fertility with severe defects in late stages of spermatogenesis that involve aberrant androgen receptor signaling in Sertoli cells and disruption of Sertoli-germ cell adherens junctions.

➢ The N-terminal of *Dnaja1* gene will remain several amino acids ,it may remain the partial function of *Dnaja1* gene.

➢ The *Dnaja1* gene is located on the Chr4. 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）

集萃药康 GemPharmatech

Dnaja1 DnaJ heat shock protein family (Hsp40) member A1 [Mus musculus (house mouse)]

Gene ID: 15502, updated on 13-Mar-2020

Summary

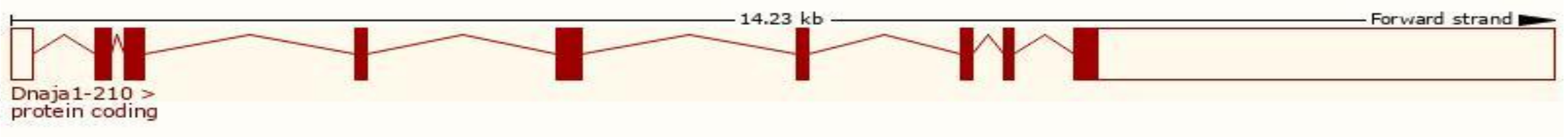
Official Symbol	Dnaja1 provided by MGI
Official Full Name	DnaJ heat shock protein family (Hsp40) member A1 provided byMGI
Primary source	MGI:MGI:1270129
See related	Ensembl:ENSMUSG00000028410
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	HSJ-2, Hsj2, Nedd7
Summary	The protein encoded by this gene is a member of the DnaJ family, whose members act as cochaperones of heat shock protein 70. Heat shock proteins facilitate protein folding, trafficking, prevention of aggregation, and proteolytic degradation. Members of this family are characterized by a highly conserved N-terminal J domain, a glycine/phenylalanine-rich region, four CxxCxGxG zinc finger repeats, and a C-terminal substrate-binding domain. The J domain mediates the interaction with heat shock protein 70 to recruit substrates and regulate ATP hydrolysis activity. Mice deficient for this gene display reduced levels of activation－induced deaminase, an enzyme that deaminates deoxycytidine at the immunoglobulin genes during immune responses. In addition, mice lacking this gene exhibit severe defects in spermatogenesis. Several pseudogenes of this gene are found on other chromosomes. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Sep 2015]
Expression	Broad expression in CNS E11.5 (RPKM 48.9), CNS E14 (RPKM 41.5) and 19 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

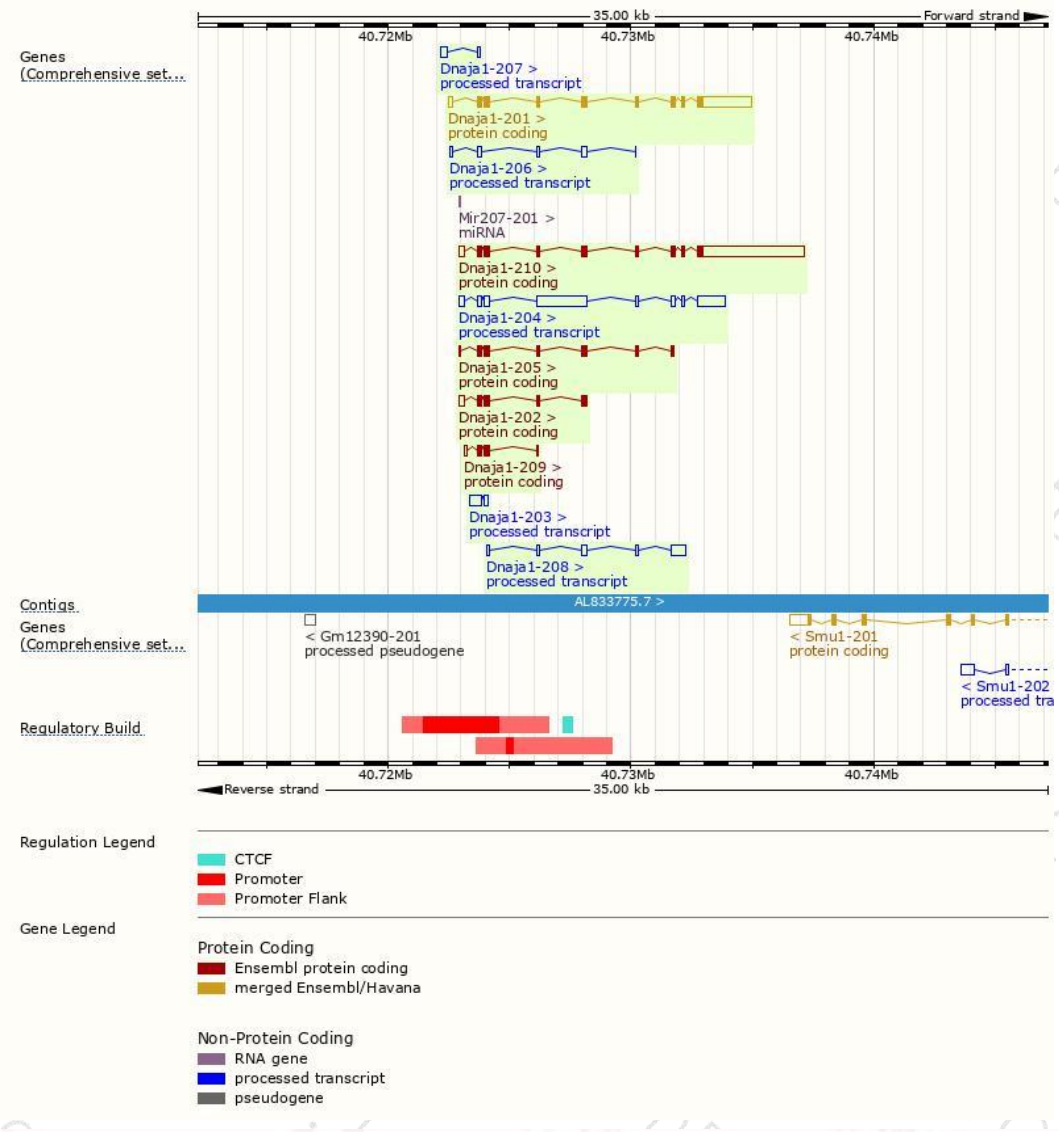
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dnaja1-210	ENSMUST00000164233.7	5622	397aa	Protein coding	CCDS18049	P63037 Q5NTY0	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Dnaja1-201	ENSMUST00000030118.9	3376	397aa	Protein coding	CCDS18049	P63037 Q5NTY0	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Dnaja1-205	ENSMUST00000137246.7	916	278aa	Protein coding	-	B1AXY1	CDS 3' incomplete TSL:5
Dnaja1-202	ENSMUST00000125442.7	826	208aa	Protein coding	-	B1AXY0	CDS 3' incomplete TSL:2
Dnaja1-209	ENSMUST00000149794.1	533	128aa	Protein coding	-	B1AXX9	CDS 3' incomplete TSL:5
Dnaja1-204	ENSMUST00000129204.7	4119	No protein	Processed transcript	-	-	TSL:5
Dnaja1-208	ENSMUST00000148976.1	1130	No protein	Processed transcript	-	-	TSL:2
Dnaja1-203	ENSMUST00000126020.1	622	No protein	Processed transcript	-	-	TSL:2
Dnaja1-206	ENSMUST00000137476.7	571	No protein	Processed transcript	-	-	TSL:3
Dnaja1-207	ENSMUST00000139406.1	379	No protein	Processed transcript	-	-	TSL:2

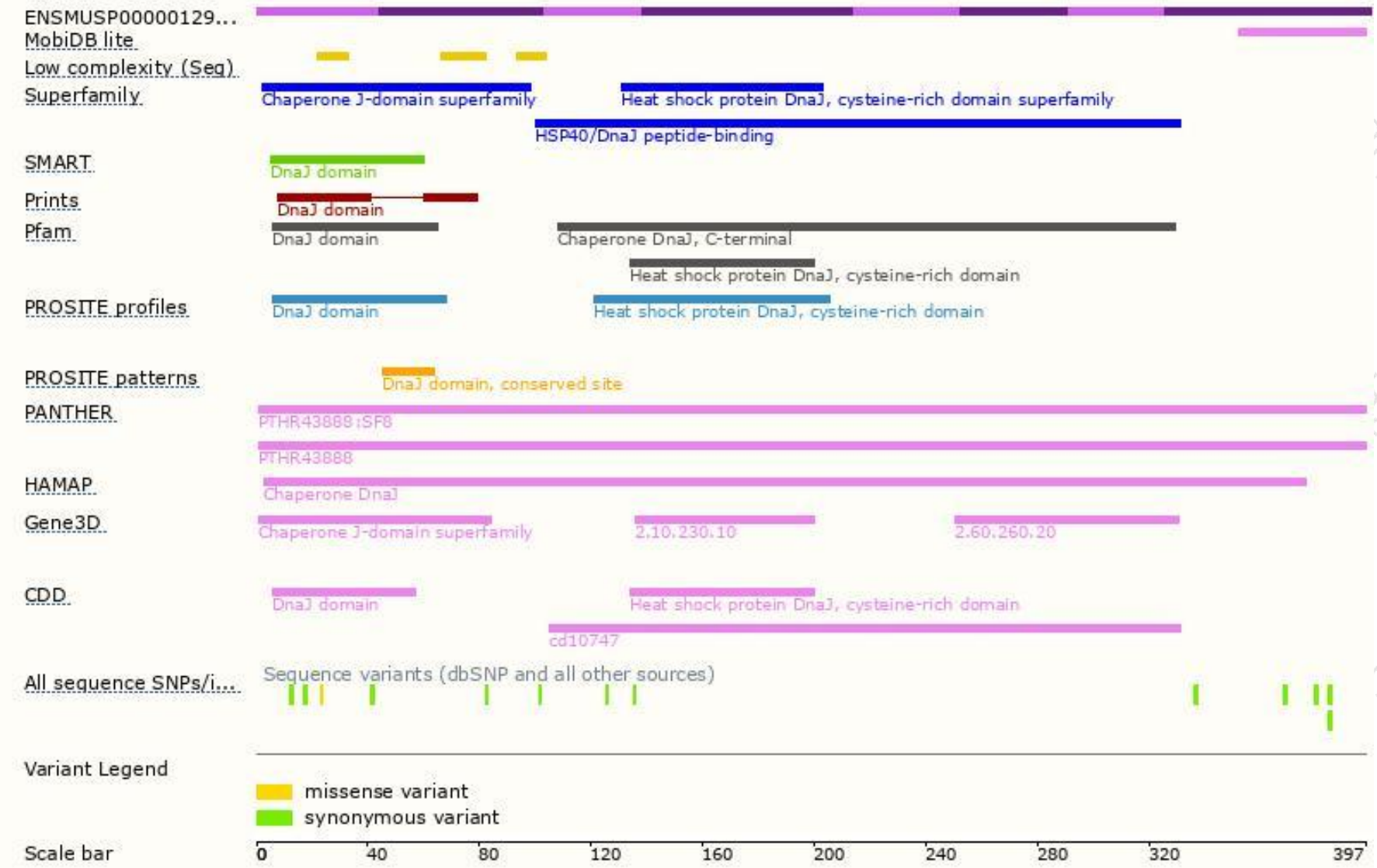
The strategy is based on the design of *Dnaja1-210* transcript,the transcription is shown below:



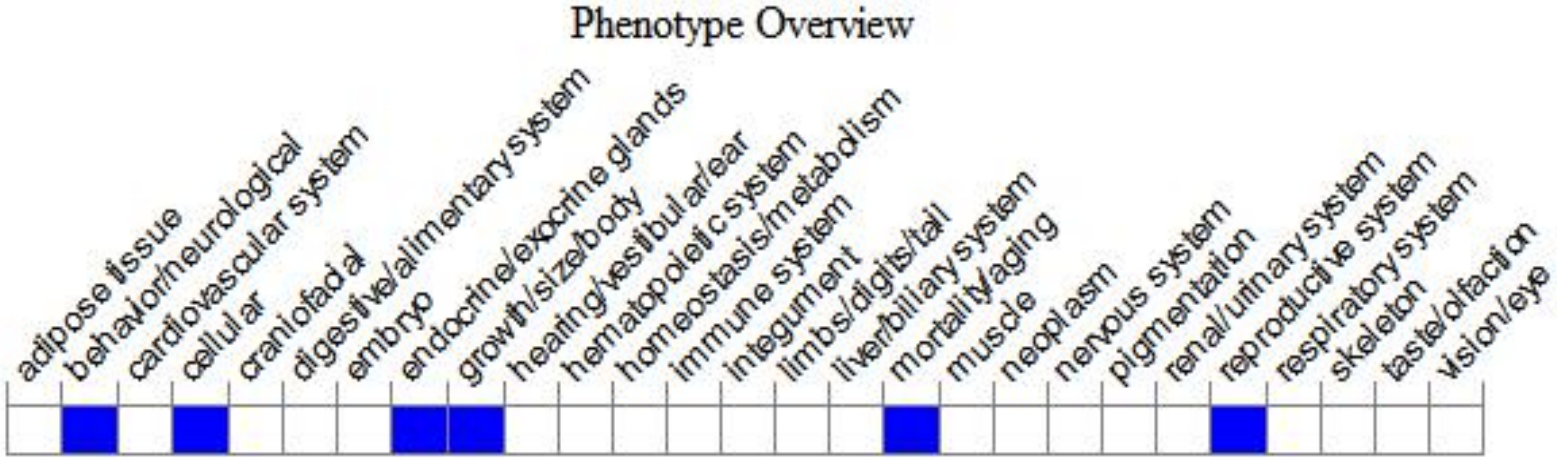
Genomic location distribution



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/).

According to the existing MGI data,male mice homozygous for a knock-out allele exhibit decreased postnatal growth and reduced fertility with severe defects in late stages of spermatogenesis that involve aberrant androgen receptor signaling in Sertoli cells and disruption of Sertoli-germ cell adherens junctions.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd