Dst Cas9-KO Strategy

Designer: Daohua Xu

Reviewer: Huimin Su

Design Date: 2019/11/15

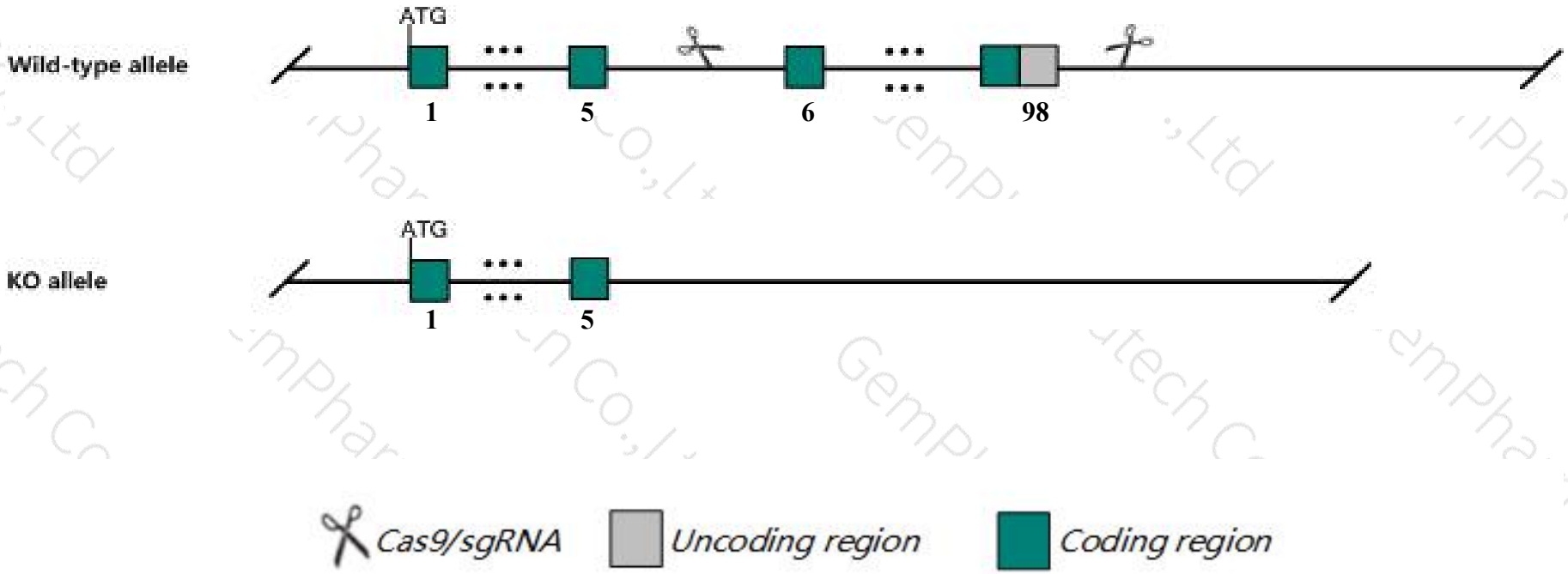
GemPharmatech Co.,Ltd

Project Overview

Project Name	***Dst***
Project type	**Cas9-KO**
Strain background	**C57BL/6J**

Knockout strategy

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



Technical routes

- The *Dst* gene has 20 transcripts. According to the structure of *Dst* gene, exon6-exon98 of *Dst-201* (ENSMUST00000097785.9) transcript is recommended as the knockout region.The region contains most of the coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Dst* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

Notice

- According to the existing MGI data,Mutations in this gene produce peripheral nervous system demyelination resulting in impaired muscle function and shorter lifespan.

- *Gm26788* and *A930005N03Rik* are overlapped with *Dst* gene, so these two gene will be knockout together.

- The *Dst* 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）

集萃药康 GemPharmatech

Dst dystonin [*Mus musculus* (house mouse)]

Gene ID: 13518, updated on 10-Oct-2019

Summary

Official Symbol	Dst provided by MGI
Official Full Name	dystonin provided by MGI
Primary source	MGI:MGI:104627
See related	Ensembl:ENSMUSG00000026131
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	ah; dt; Bpag; BP230; Bpag1; Macf2; nmf203; nmf339; BPAG1-n; AW554249; athetoid; mKIAA0728; 2310001O04Rik; A830042E19Rik
Expression	Ubiquitous expression in cerebellum adult (RPKM 8.8), bladder adult (RPKM 7.5) and 28 other tissues See more
Orthologs	human all

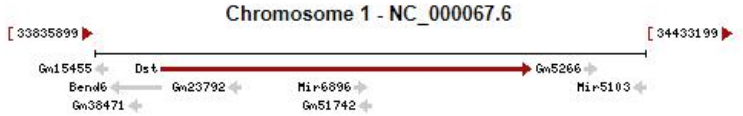
Genomic context

Location: 1 B; 1 12.91 cM

See Dst in Genome Data Viewer

Exon count: 113

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	1	NC_000067.6 (33907888..34308662)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	1	NC_000067.5 (34068670..34365497)

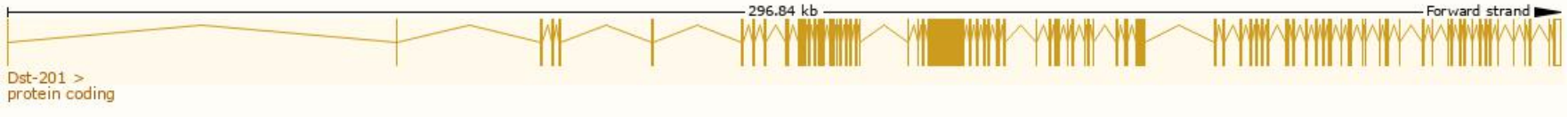


Transcript information（Ensembl）

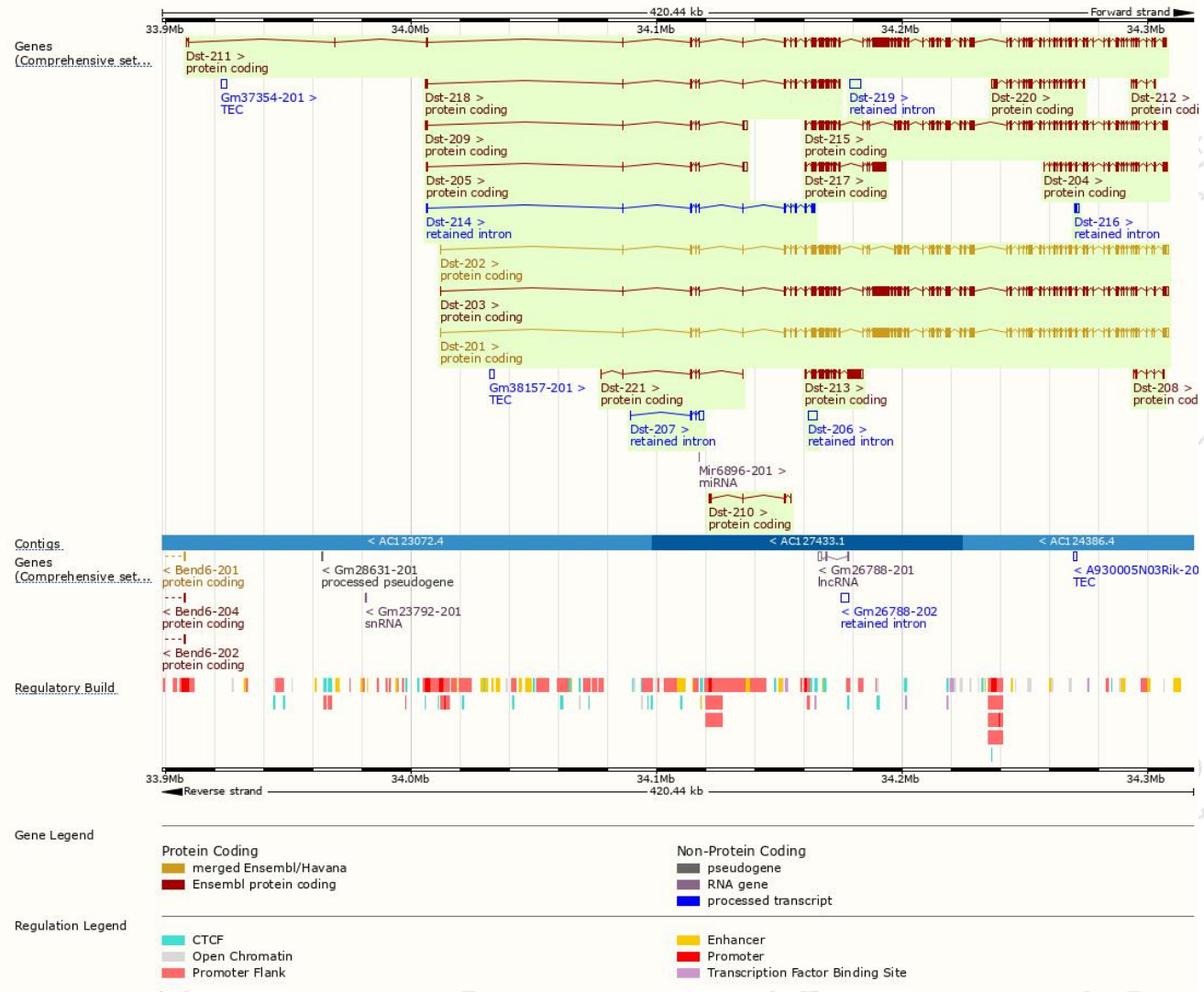
The gene has 20 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dst-201	ENSMUST00000097785.9	23251	7393aa	Protein coding	CCDS35534	Q91ZU6	TSL:1 GENCODE basic APPRIS P4
Dst-211	ENSMUST00000183034.4	23201	7717aa	Protein coding	CCDS69874	S4R1P5	TSL:5 GENCODE basic APPRIS ALT2
Dst-202	ENSMUST00000097786.9	17212	5379aa	Protein coding	CCDS35535	Q91ZU6	TSL:1 GENCODE basic APPRIS ALT2
Dst-213	ENSMUST00000183302.5	8788	2639aa	Protein coding	CCDS69875	Q91ZU6	TSL:1 GENCODE basic
Dst-203	ENSMUST00000115104.9	23279	7406aa	Protein coding	-	E9Q9X1	TSL:5 GENCODE basic APPRIS ALT2
Dst-215	ENSMUST00000185269.6	16340	5175aa	Protein coding	-	A0A087WSP0	TSL:5 GENCODE basic
Dst-217	ENSMUST00000185897.6	8961	2987aa	Protein coding	-	A0A087WPR7	CDS 3' incomplete TSL:5
Dst-220	ENSMUST00000194192.2	6039	1674aa	Protein coding	-	A0A1D5RLZ3	CDS 3' incomplete TSL:5
Dst-204	ENSMUST00000182018.7	5729	1638aa	Protein coding	-	S4R1Y6	CDS 5' incomplete TSL:5
Dst-218	ENSMUST00000187486.6	4539	1471aa	Protein coding	-	A0A087WRB8	CDS 3' incomplete TSL:5
Dst-209	ENSMUST00000182697.7	2704	274aa	Protein coding	-	A0A0A6YX28	TSL:1 GENCODE basic
Dst-205	ENSMUST00000182314.7	2643	282aa	Protein coding	-	A0A0A6YXR1	TSL:1 GENCODE basic
Dst-210	ENSMUST00000183006.4	1539	386aa	Protein coding	-	-	CDS 3' incomplete TSL:5
Dst-212	ENSMUST00000183100.7	786	262aa	Protein coding	-	S4R2A8	CDS 5' and 3' incomplete TSL:3
Dst-208	ENSMUST00000182507.3	776	259aa	Protein coding	-	S4R2C6	CDS 5' and 3' incomplete TSL:2
Dst-221	ENSMUST00000239001.1	537	153aa	Protein coding	-	-	CDS 3' incomplete
Dst-219	ENSMUST00000189952.1	4342	No protein	Retained intron	-	-	TSL:NA
Dst-206	ENSMUST00000182335.1	3478	No protein	Retained intron	-	-	TSL:NA
Dst-214	ENSMUST00000183331.7	2728	No protein	Retained intron	-	-	TSL:5
Dst-207	ENSMUST00000182410.2	2027	No protein	Retained intron	-	-	TSL:1
Dst-216	ENSMUST00000185736.1	1394	No protein	Retained intron	-	-	TSL:1

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

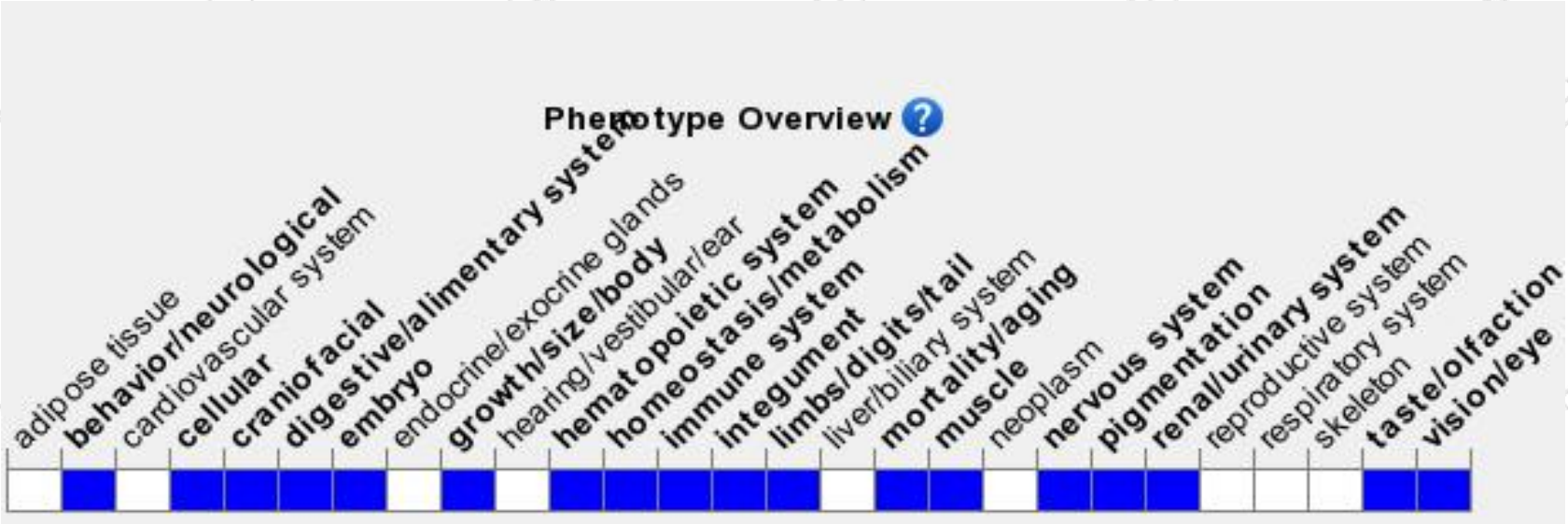


Genomic location distribution



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/).

According to the existing MGI data,Mutations in this gene produce peripheral nervous system demyelination resulting in impaired muscle function and shorter lifespan.

集萃药康
GemPharmatech

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

Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd

GemPharmatech Co.,Ltd