Htra2 Cas9-KO Strategy

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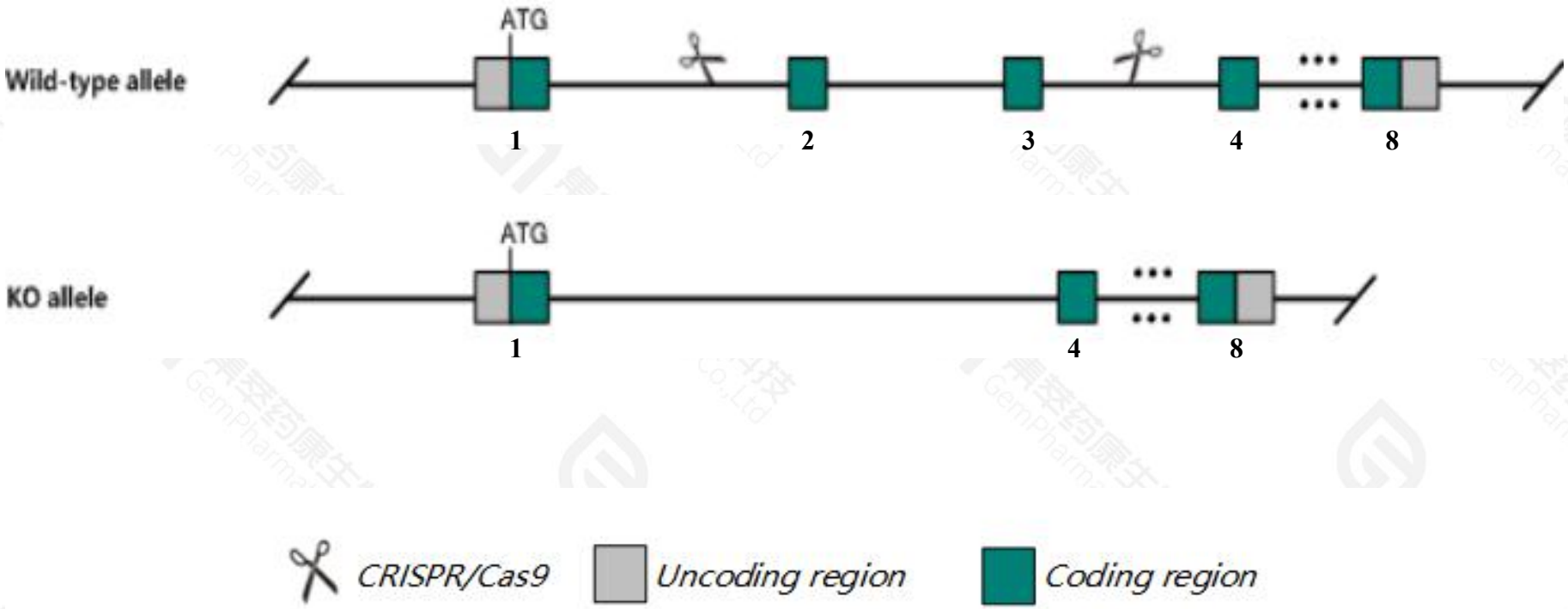
Design Date: 2021-12-30

Project Overview

Project Name	***Htra2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Htra2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Htra2* gene has 11 transcripts. According to the structure of *Htra2* gene, exon2-exon3 of *Htra2-201*(ENSMUST00000089645.13) transcript is recommended as the knockout region.The region contains 400bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Htra2* 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, homozygous mutations of this gene cause progressive parkinsonian symptoms, loss of striatal neurons, spleen and thymus atrophy, failure to thrive, and death before 40 days of age.

➢ The KO region is close to *Loxl3* and *Aup1* gene. Knockout the region may affect the function of *Loxl3* and *Aup1* gene.

➢ The *Htra2* gene is located on the Chr6. 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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Htra2 HtrA serine peptidase 2 [Mus musculus (house mouse)]

Gene ID: 64704, updated on 21-Feb-2021

Summary

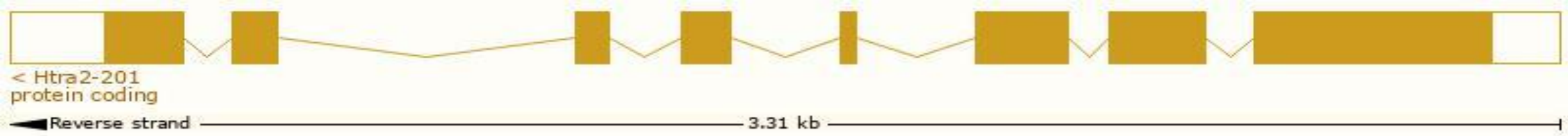
Official Symbol	Htra2 provided by MGI
Official Full Name	HtrA serine peptidase 2 provided byMGI
Primary source	MGI:MGI:1928676
See related	Ensembl:ENSMUSG00000068329
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI481710, Htr, O, Omi, Pr, Prss25, mnd, mnd2
Expression	Ubiquitous expression in liver E14.5 (RPKM 40.6), liver E14 (RPKM 36.8) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 11 transcripts,all transcripts are shown below:

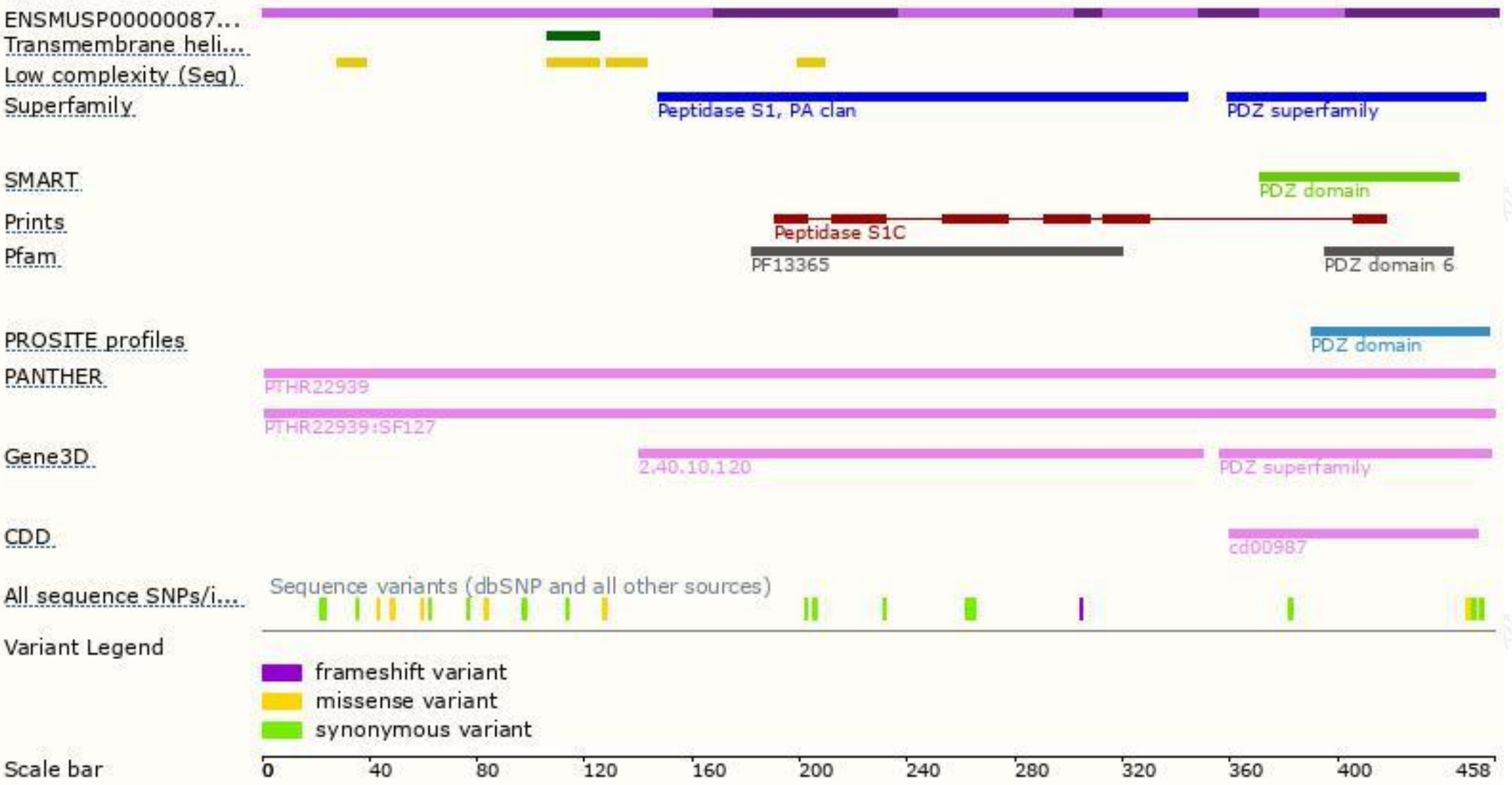
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Htra2-201	ENSMUST00000089645.13	1725	458aa	Protein coding	CCDS20267		TSL:1 , GENCODE basic , APPRIS P1 ,
Htra2-203	ENSMUST00000113963.8	1629	426aa	Protein coding	-		TSL:5 , GENCODE basic ,
Htra2-202	ENSMUST00000113962.8	1305	361aa	Protein coding	-		TSL:5 , GENCODE basic ,
Htra2-206	ENSMUST00000134606.8	883	294aa	Protein coding	-		CDS 5' and 3' incomplete , TSL:1 ,
Htra2-208	ENSMUST00000150217.2	746	249aa	Protein coding	-		CDS 5' and 3' incomplete , TSL:5 ,
Htra2-204	ENSMUST00000122955.8	1605	328aa	Nonsense mediated decay	-		TSL:5 ,
Htra2-205	ENSMUST00000132099.8	745	137aa	Nonsense mediated decay	-		CDS 5' incomplete , TSL:3 ,
Htra2-211	ENSMUST00000204281.2	768	No protein	Processed transcript	-		TSL:5 ,
Htra2-210	ENSMUST00000203339.2	650	No protein	Processed transcript	-		TSL:3 ,
Htra2-209	ENSMUST00000154829.8	2512	No protein	Retained intron	-		TSL:1 ,
Htra2-207	ENSMUST00000144058.4	1108	No protein	Retained intron	-		TSL:5 ,

The strategy is based on the design of *Htra2-201* transcript,the transcription is shown below:

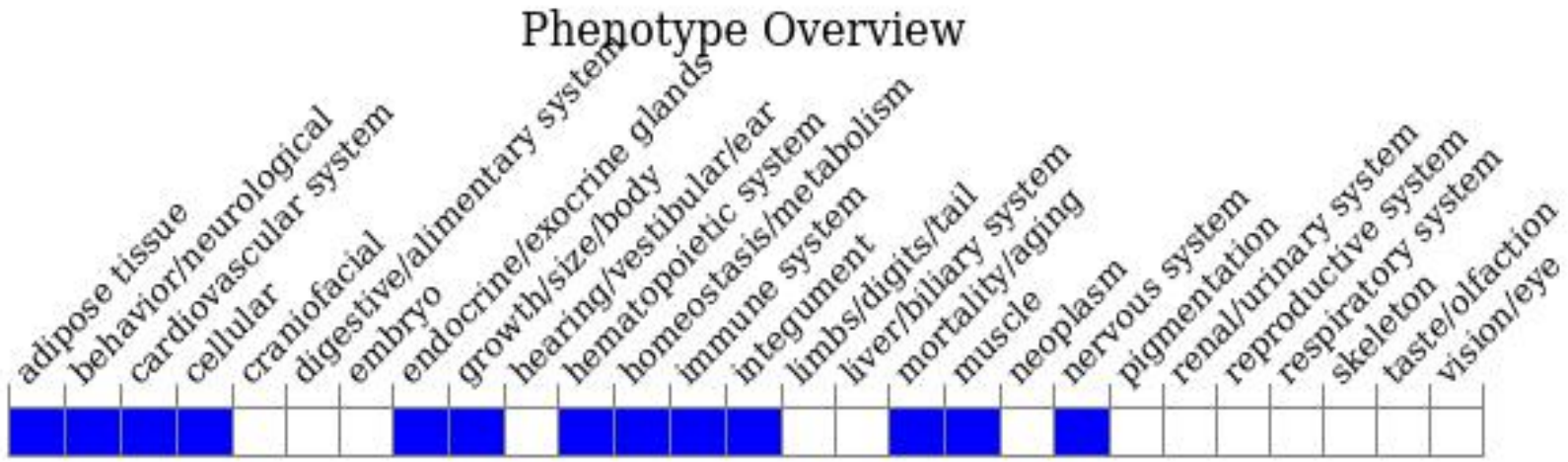


Genomic location distribution

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,homozygous mutations of this gene cause progressive parkinsonian symptoms, loss of striatal neurons, spleen and thymus atrophy, failure to thrive, and death before 40 days of age.

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