

Spast Cas9-KO Strategy

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Project Overview

Project Name

Spast

Project type

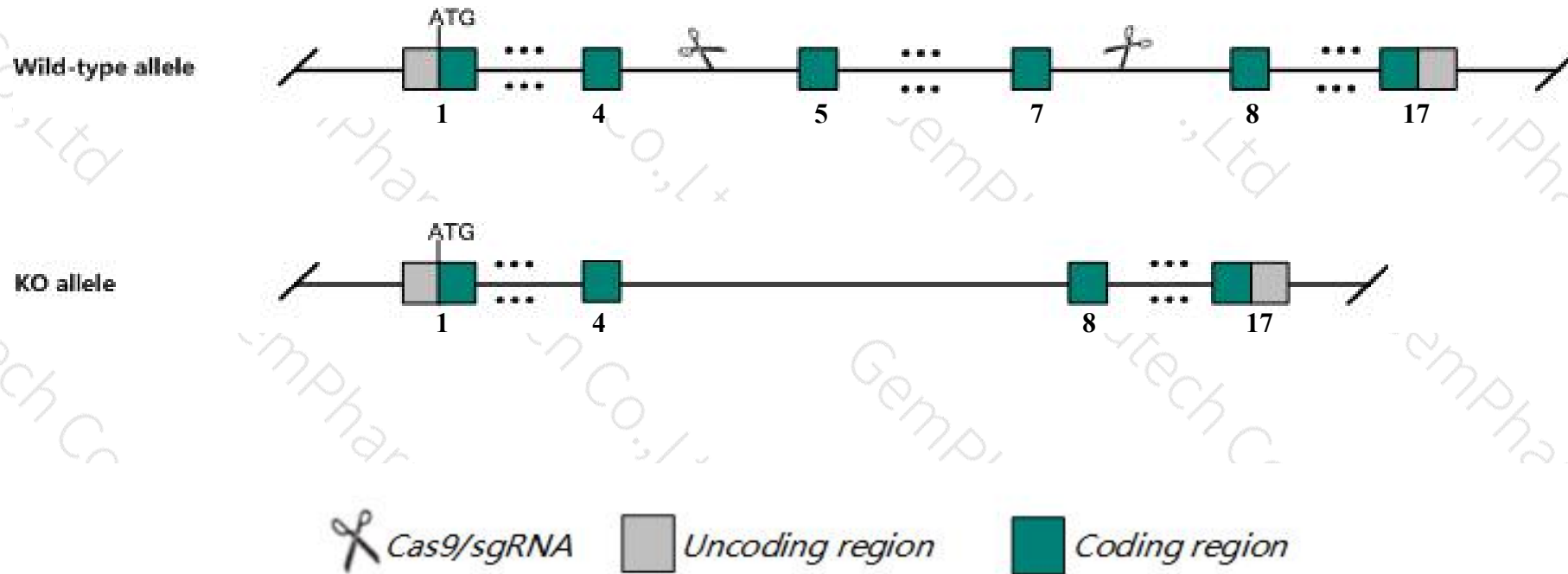
Cas9-KO

Strain background

C57BL/6J

Knockout strategy

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



- The *Spast* gene has 5 transcripts. According to the structure of *Spast* gene, exon5-exon7 of *Spast-201* (ENSMUST00000024869.7) transcript is recommended as the knockout region. The region contains 416bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Spast* 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.

- According to the existing MGI data, Mice homozygous for a mutation in this gene are sterile and display progressive axonopathy with focal axonal swellings and late onset gait abnormalities.
- The *Spast* gene is located on the Chr17. 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)

Spast spastin [*Mus musculus* (house mouse)]

Gene ID: 50850, updated on 24-Oct-2019

Summary

- Official Symbol
- Spast provided by MGI
- Official Full Name
- spastin provided by MGI
- Primary source
- MGI:MGI:1858896
- See related
- Ensembl:ENSMUSG00000024068
- 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
- Spg4; mKIAA1083
- Expression
- Broad expression in CNS E18 (RPKM 14.2), whole brain E14.5 (RPKM 14.2) and 28 other tissues [See more](#)
- Orthologs
- [human](#) [all](#)

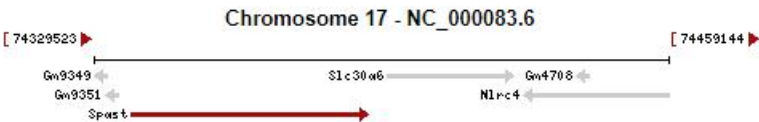
Genomic context

Location: 17; 17 E2

Exon count: 18

See Spast in [Genome Data Viewer](#)

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	17	NC_000083.6 (74337923..74391115)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	17	NC_000083.5 (74738327..74790453)

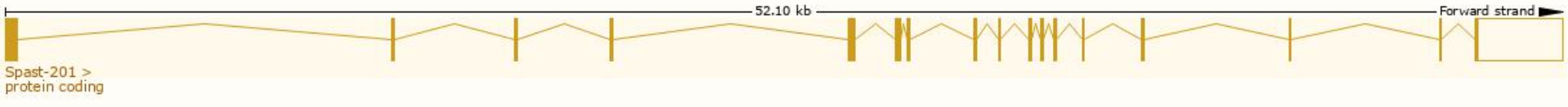


Transcript information (Ensembl)

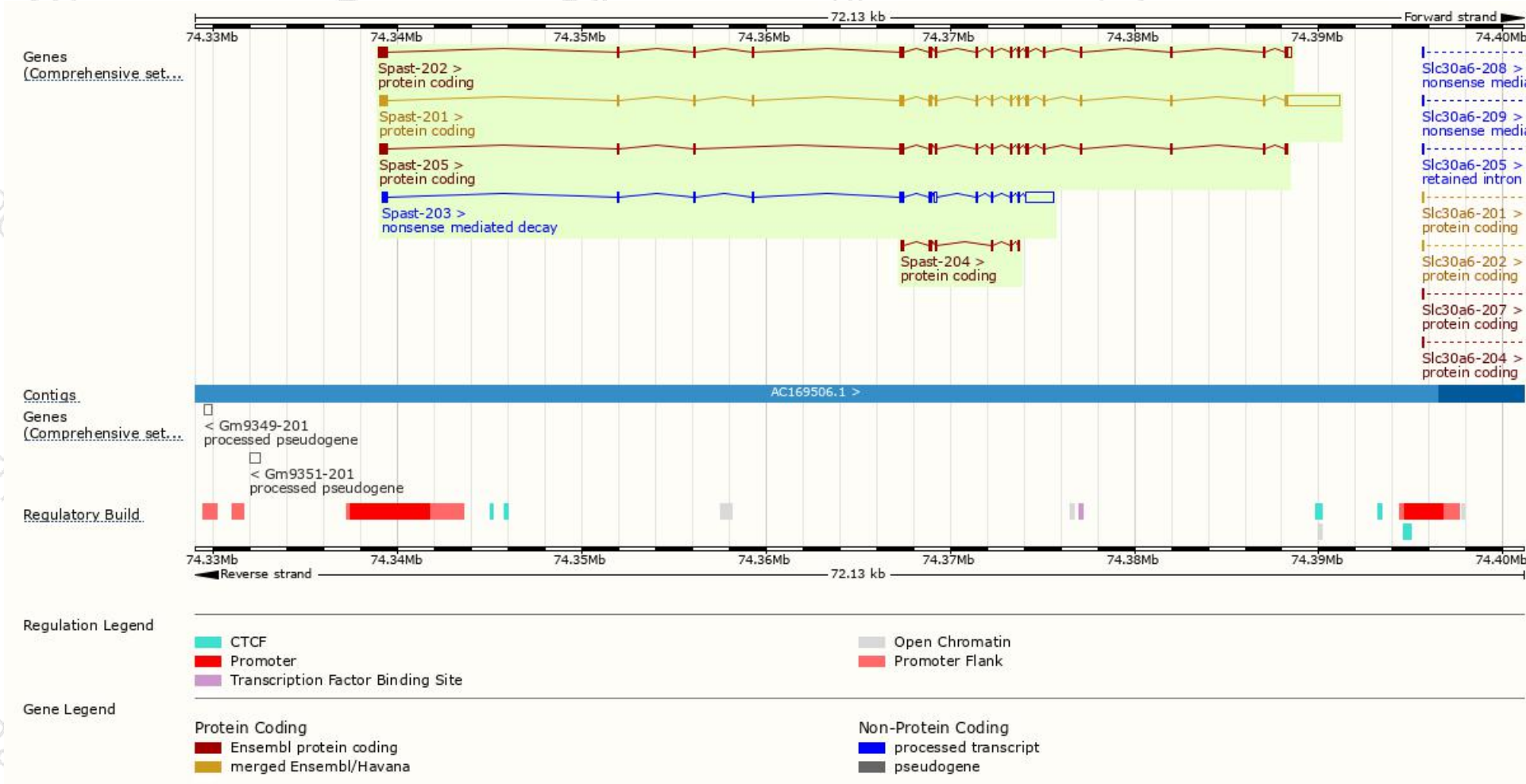
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Spast-201	ENSMUST00000024869.7	4672	614aa	Protein coding	CCDS50181	Q9QYY8	TSL:1 GENCODE basic APPRIS P2
Spast-202	ENSMUST000000224711.1	2066	613aa	Protein coding	-	A0A286YE25	GENCODE basic APPRIS ALT2
Spast-205	ENSMUST000000233611.1	1746	581aa	Protein coding	-	A0A3B2WC92	GENCODE basic APPRIS ALT2
Spast-204	ENSMUST000000233554.1	573	191aa	Protein coding	-	A0A3B2WBA7	CDS 5' and 3' incomplete
Spast-203	ENSMUST000000225549.1	2897	300aa	Nonsense mediated decay	-	A0A286YCJ4	CDS 5' incomplete

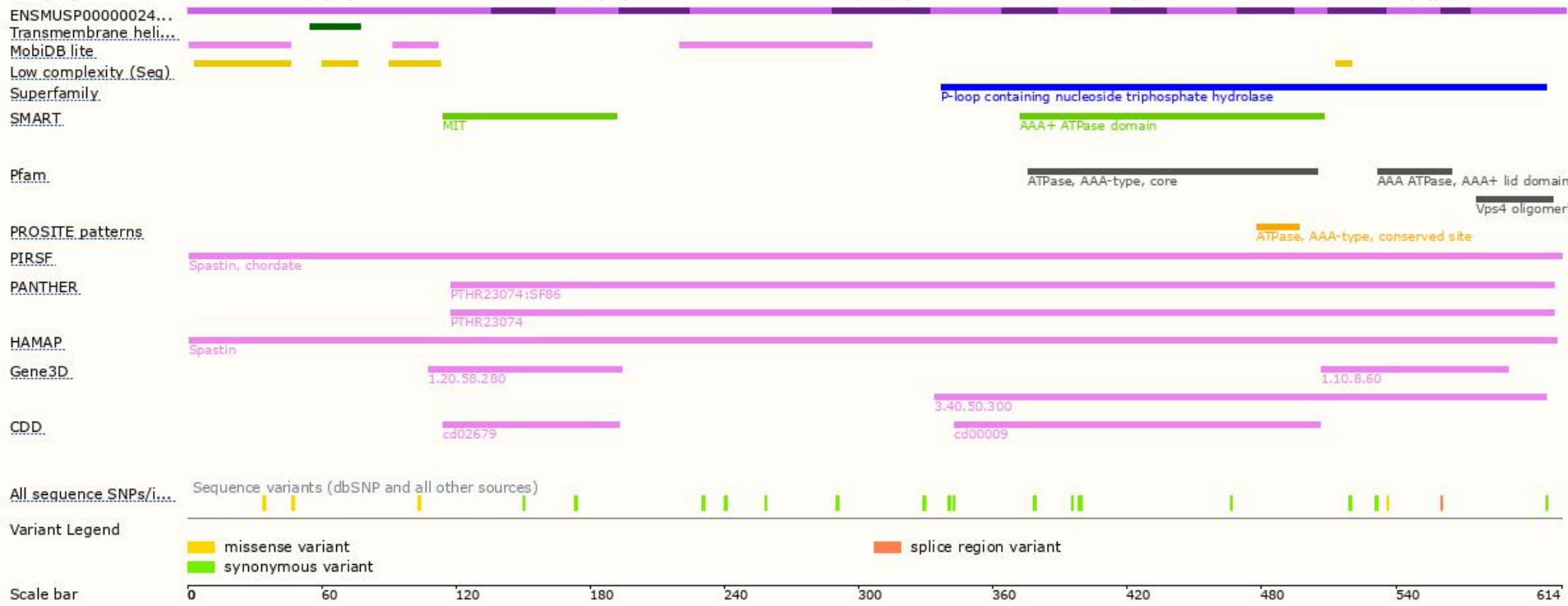
The strategy is based on the design of *Spast-201* transcript,The transcription is shown below



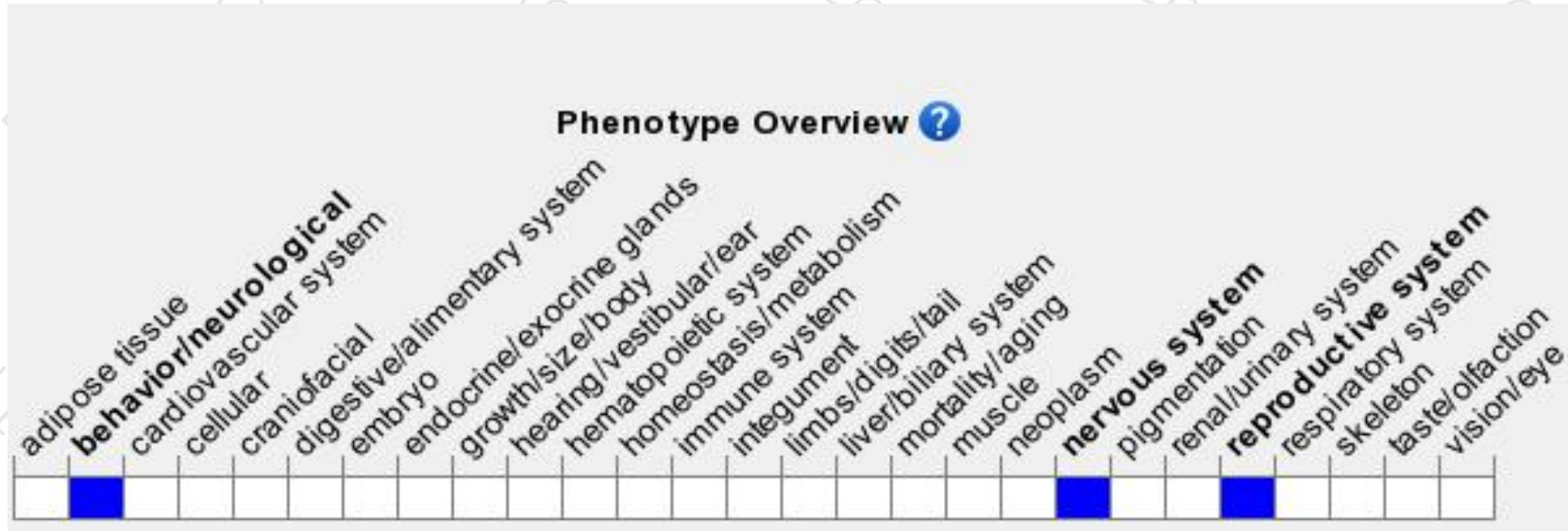
Genomic location distribution



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, Mice homozygous for a mutation in this gene are sterile and display progressive axonopathy with focal axonal swellings and late onset gait abnormalities.

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

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