

# Speg Cas9-KO Strategy

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Reviewer: Rui Xiong

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# **Project Overview**



**Project Name** 

Speg

**Project type** 

Cas9-KO

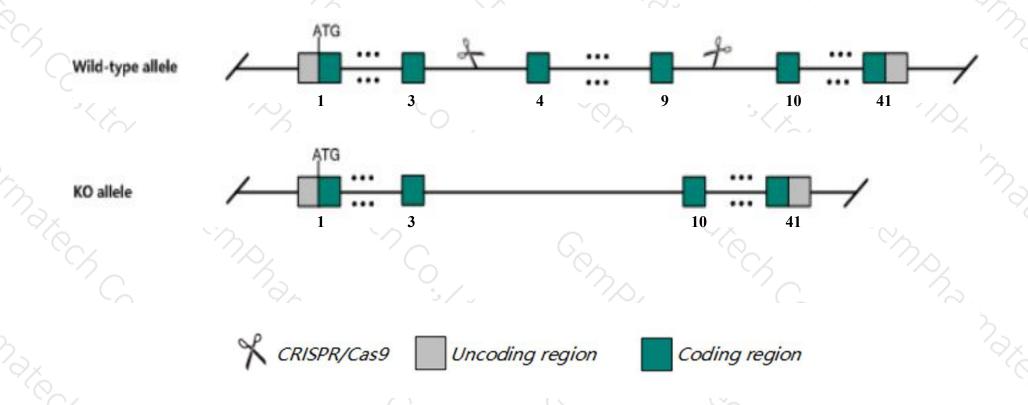
Strain background

C57BL/6JGpt

# **Knockout strategy**



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



### **Technical routes**



- > The *Speg* gene has 15 transcripts. According to the structure of *Speg* gene, exon4-exon9 of *Speg-201*(ENSMUST00000087122.11) transcript is recommended as the knockout region. The region contains 2075bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Speg* 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**



- $\rightarrow$  Gm15178-201 will be deleted.
- > According to the existing MGI data,mice homozygous for a knock-out allele die during the early postnatal period with enlarged, dilated hearts, and decreased cardiac function.
- The *Speg* 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)



Speq SPEG complex locus [Mus musculus (house mouse)]

Gene ID: 11790, updated on 13-Mar-2020

#### ▲ Summary

☆ ?

Official Symbol Speq provided by MGI

Official Full Name SPEG complex locus provided by MGI

Primary source MGI:MGI:109282

See related Ensembl: ENSMUSG00000026207

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 AW125581, Apeq1, BPEG, D1Bwq1450e, SPEGalpha, SPEGbeta, mKIAA1297

Summary This gene encodes a protein with similarity to members of the myosin light chain kinase family. This protein family is required for

myocyte cytoskeletal development. Studies have determined that a lack of this protein affected myocardial development. Multiple

alternatively spliced transcript variants that encode different protein isoforms have been defined. [provided by RefSeq, Mar 2010]

Expression Broad expression in heart adult (RPKM 14.9), cortex adult (RPKM 9.1) and 23 other tissuesSee more

Orthologs <u>human all</u>

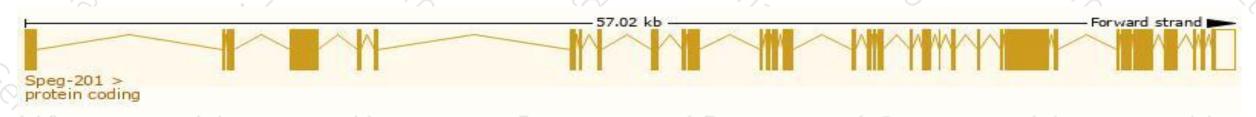
# Transcript information (Ensembl)



#### The gene has 15 transcripts, all transcripts are shown below:

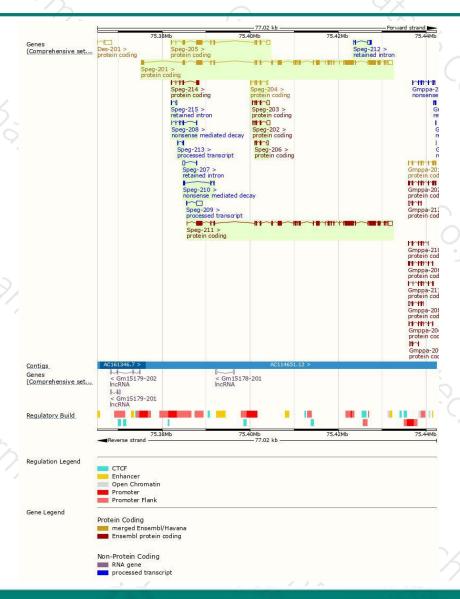
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Speg-201	ENSMUST00000087122.11	10801	3262aa	Protein coding	CCDS35626	E9QQ25	TSL:1 GENCODE basic APPRIS P1
Speg-205	ENSMUST00000113590.7	3393	<u>861aa</u>	Protein coding	CCDS48292	Q62407	TSL:1 GENCODE basic
Speg-202	ENSMUST00000113587.7	1254	113aa	Protein coding	CCD548293	A0A0R4J1J0	TSL:5 GENCODE basic
Speg-203	ENSMUST00000113588.7	1078	113aa	Protein coding	CCDS48293	A0A0R4J1J0	TSL:5 GENCODE basic
Speg-206	ENSMUST00000122266.2	917	<u>113aa</u>	Protein coding	CCDS48293	A0A0R4J1J0	TSL:1 GENCODE basic
Speg-204	ENSMUST00000113589.7	833	113aa	Protein coding	CCDS48293	A0A0R4J1J0	TSL:2 GENCODE basic
peg-211	ENSMUST00000137868.7	9885	3010aa	Protein coding	н ,	A0A087WSE3	CDS 5' incomplete TSL:1
Speg-214	ENSMUST00000148515.7	932	<u>301aa</u>	Protein coding	4	D3Z7T0	CDS 3' incomplete TSL:5
peg-210	ENSMUST00000132228.1	643	132aa	Nonsense mediated decay	-	F6RU40	CDS 5' incomplete TSL:2
Speg-208	ENSMUST00000125306.1	463	<u>84aa</u>	Nonsense mediated decay	-	D6RI69	TSL:5
Speg-209	ENSMUST00000132222.1	1472	No protein	Processed transcript	2	2	TSL:2
Speg-213	ENSMUST00000146705.1	346	No protein	Processed transcript	=	1.51	TSL:2
Speg-212	ENSMUST00000143679.1	735	No protein	Retained intron	=	-	TSL:3
Speg-207	ENSMUST00000125118.1	616	No protein	Retained intron	-	-	TSL:2
Speg-215	ENSMUST00000187214.1	295	No protein	Retained intron	-	-	TSL:5

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



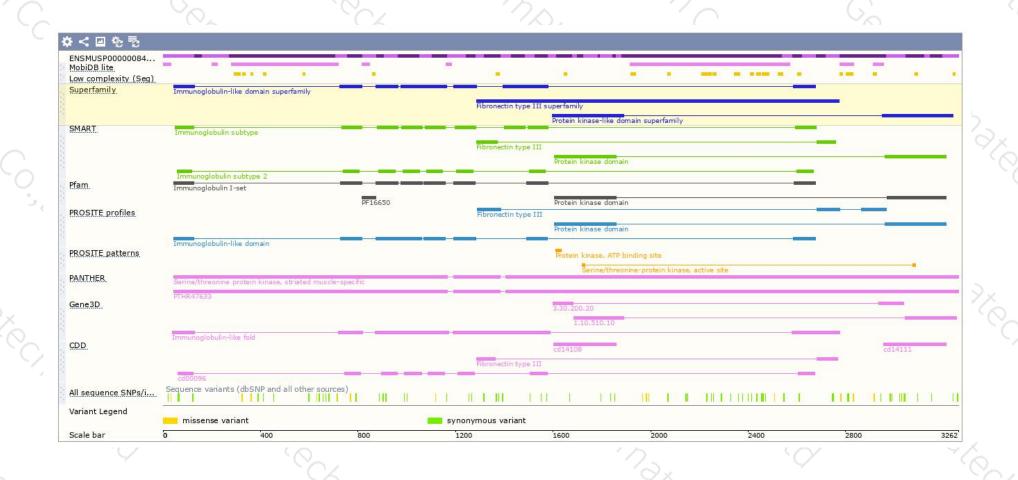
## Genomic location distribution





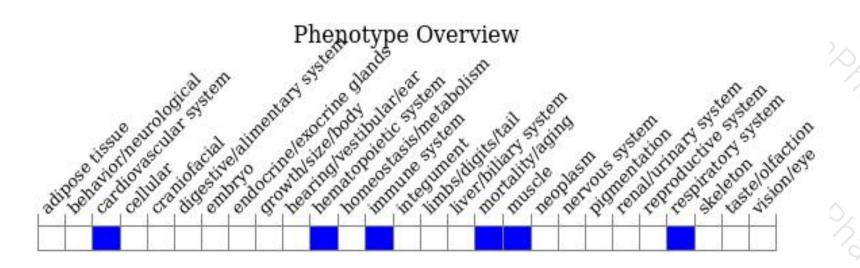
### Protein domain





# 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 knock-out allele die during the early postnatal period with enlarged, dilated hearts, and decreased cardiac function.



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





