Speg Cas9-CKO Strategy

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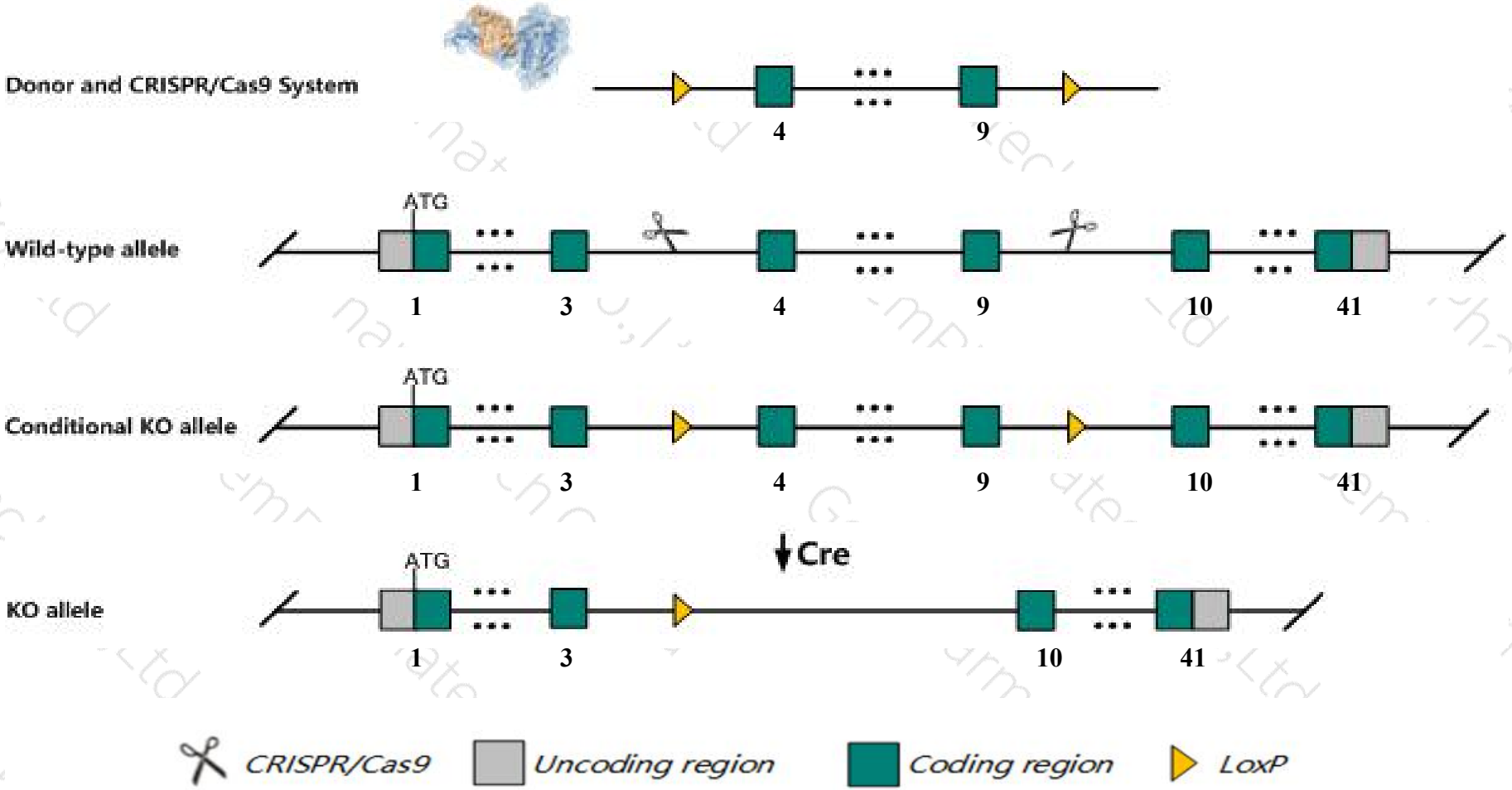
Project Overview

Project Name	***Speg***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional 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 and Donor 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.

➢ The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

➢ 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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

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

Summary

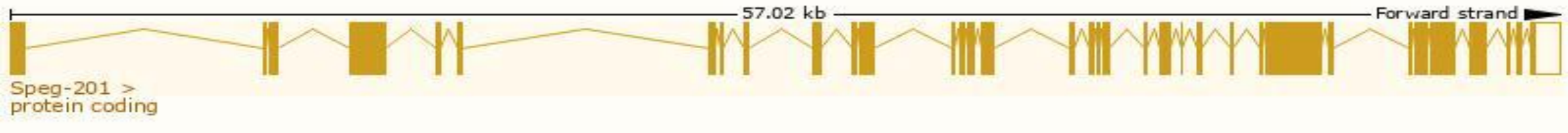
Official Symbol	Speg provided by MGI
Official Full Name	SPEG complex locus provided byMGI
Primary source	MGI:MGI:109282
See related	Ensembl:ENSMUSG00000026207
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW125581, Apeg1, BPEG, D1Bwg1450e, 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	human all

Transcript information（Ensembl）

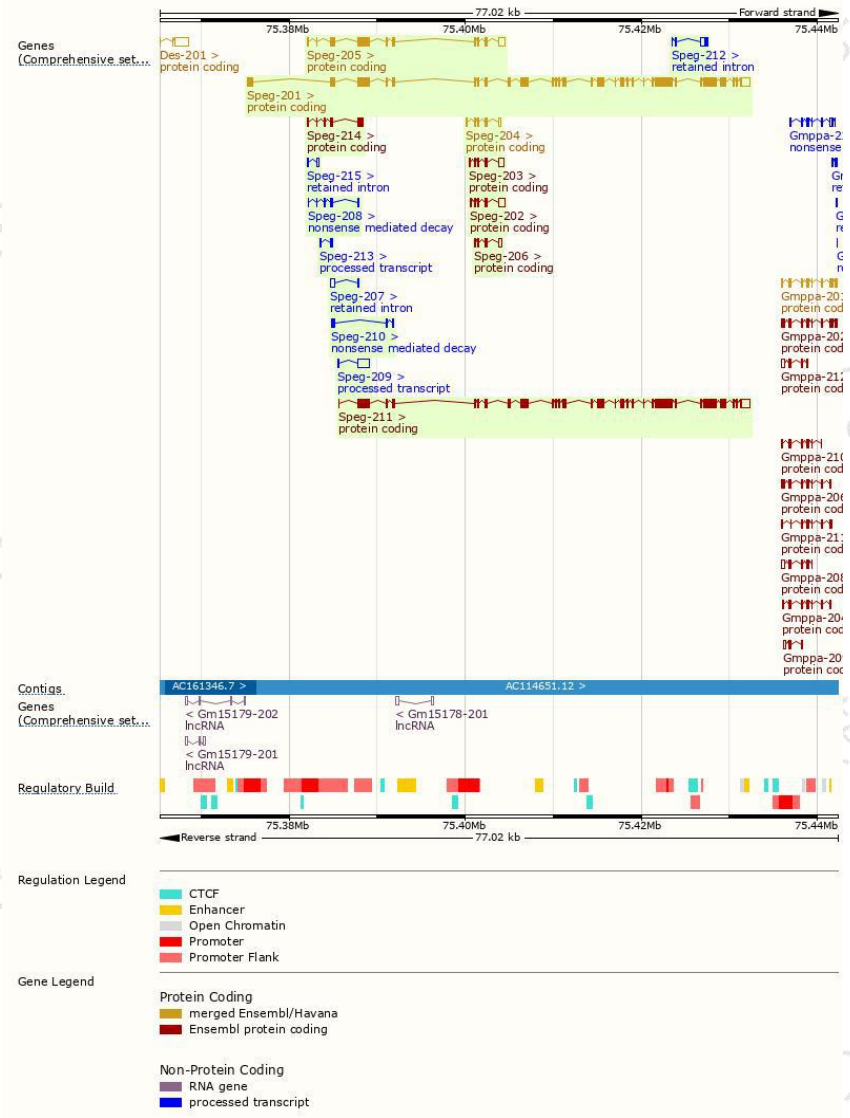
The gene has 15 transcripts,all transcripts are shown below:

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	861aa	Protein coding	CCDS48292	Q62407	TSL:1 GENCODE basic
Speg-202	ENSMUST00000113587.7	1254	113aa	Protein coding	CCDS48293	A0A0R4J1J0	TSL:5 GENCODE basic
Speg-203	ENSMUST00000113588.7	1078	113aa	Protein coding	CCDS48293	A0A0R4J1J0	TSL:5 GENCODE basic
Speg-206	ENSMUST00000122266.2	917	113aa	Protein coding	CCDS48293	A0A0R4J1J0	TSL:1 GENCODE basic
Speg-204	ENSMUST00000113589.7	833	113aa	Protein coding	CCDS48293	A0A0R4J1J0	TSL:2 GENCODE basic
Speg-211	ENSMUST00000137868.7	9885	3010aa	Protein coding	-	A0A087WSE3	CDS 5' incomplete TSL:1
Speg-214	ENSMUST00000148515.7	932	301aa	Protein coding	-	D3Z7T0	CDS 3' incomplete TSL:5
Speg-210	ENSMUST00000132228.1	643	132aa	Nonsense mediated decay	-	F6RU40	CDS 5' incomplete TSL:2
Speg-208	ENSMUST00000125306.1	463	84aa	Nonsense mediated decay	-	D6RI69	TSL:5
Speg-209	ENSMUST00000132222.1	1472	No protein	Processed transcript	-	-	TSL:2
Speg-213	ENSMUST00000146705.1	346	No protein	Processed transcript	-	-	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

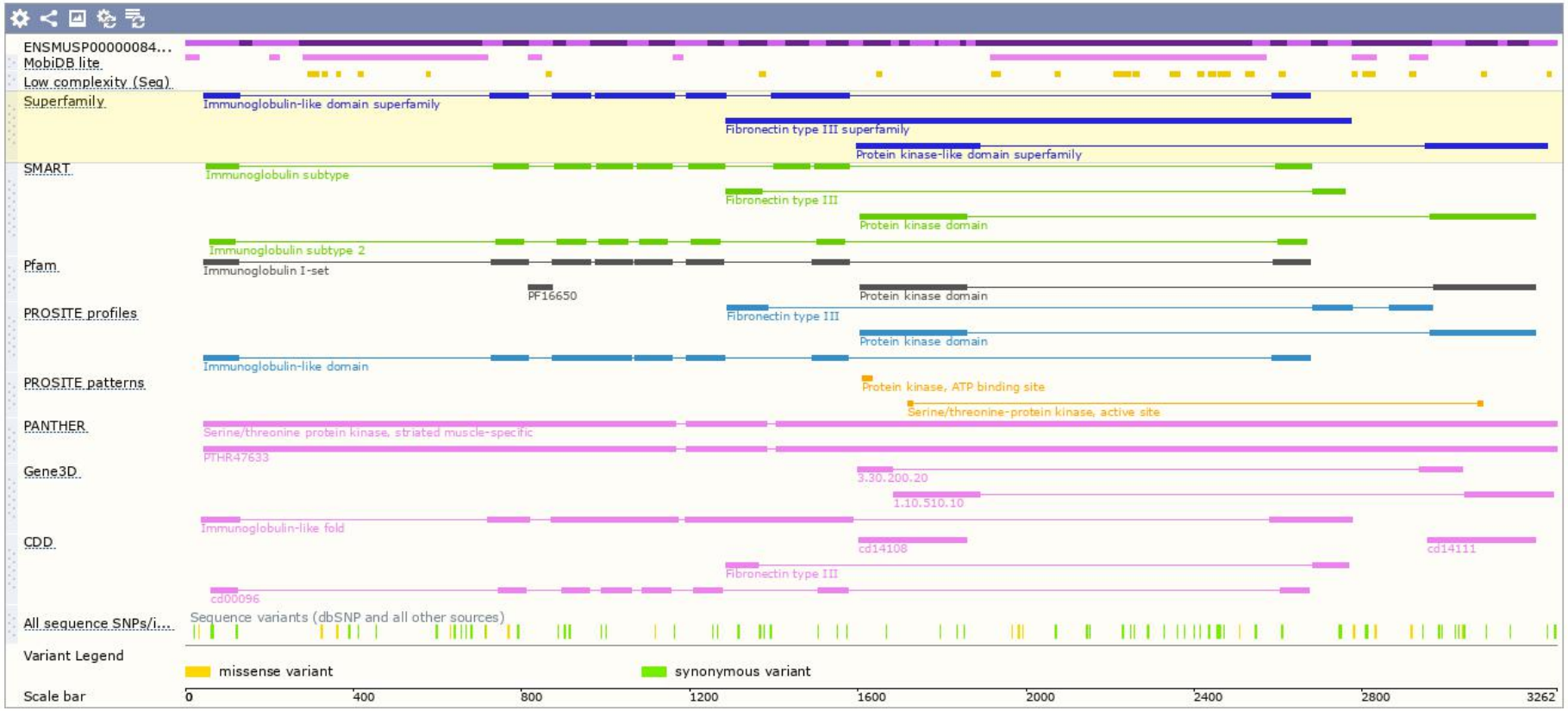
The strategy is based on the design of *Speg-201* transcript,the transcription is shown below:



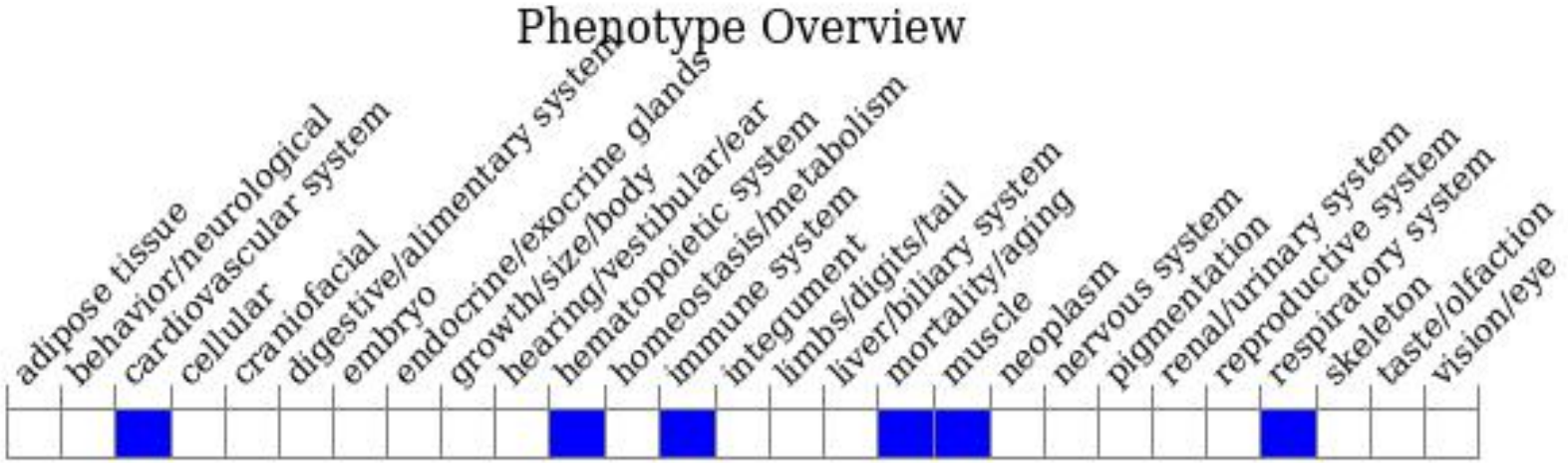
Genomic location distribution



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.

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