Akt1s1 Cas9-CKO Strategy

Designer: Huan Wang

Reviewer: Yumeng Wang

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Project Overview

Project Name	***Akt1s1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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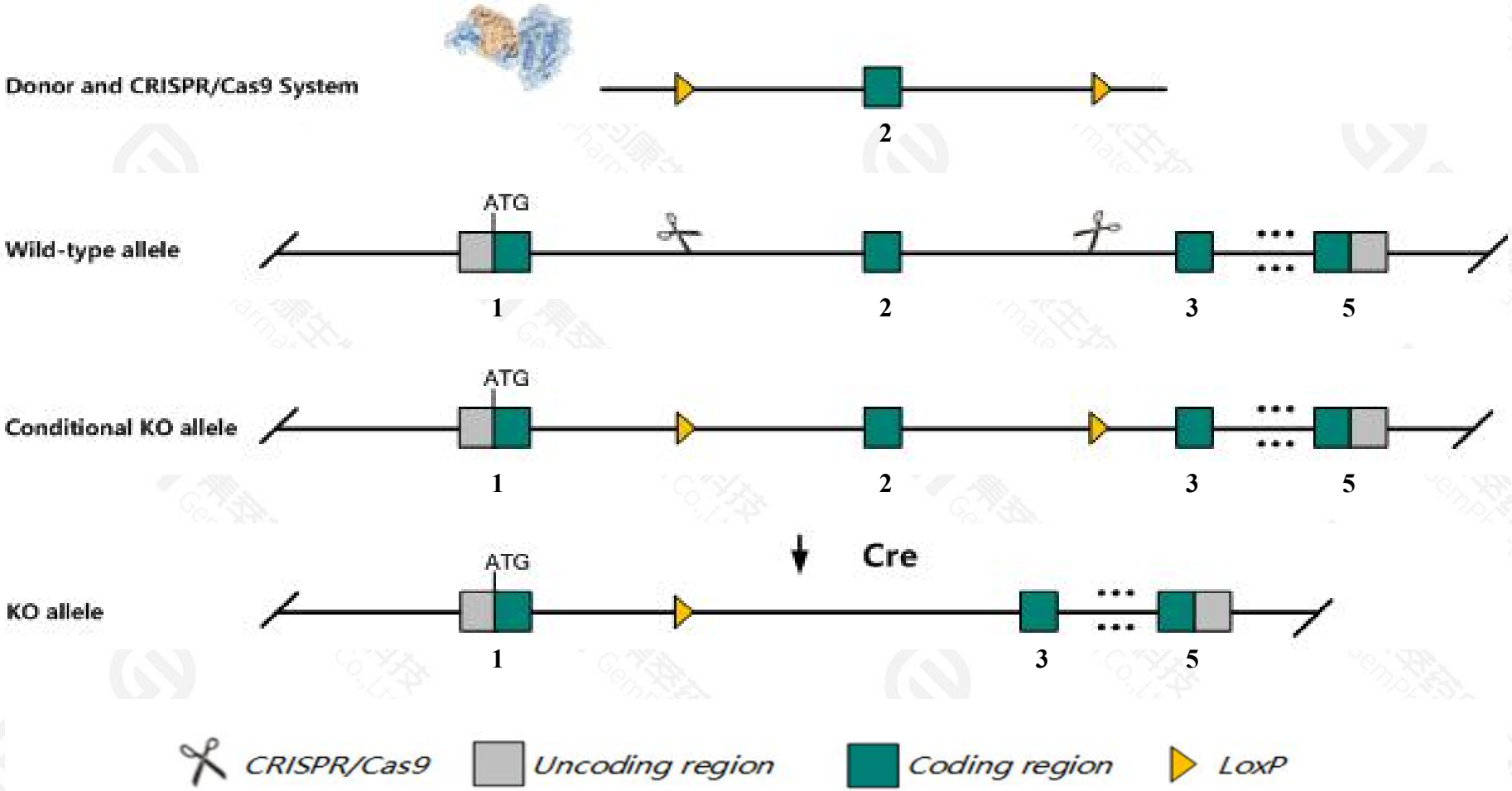
This model will use CRISPR/Cas9 technology to edit the *Akt1s1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Akt1s1* gene has 12 transcripts. According to the structure of *Akt1s1* gene, exon2 of *Akt1s1-203*(ENSMUST00000107882.7) transcript is recommended as the knockout region.The region contains 389bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Akt1s1* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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

➢ According to the existing MGI data,mice homozygous for a knock-out allele exhibit increased susceptibility to induced ischemic brain injury.

➢ The KO region is about 3kb and 3.5kb from *Tbc1d17* and *Pnkp* gene. Knockout the region may affect the function of *Tbc1d17* and *Pnkp* gene .

➢ The *Akt1s1* gene is located on the Chr7. 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）

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Akt1s1 AKT1 substrate 1 (proline-rich) [Mus musculus (house mouse)]

Gene ID: 67605, updated on 13-Mar-2020

Summary

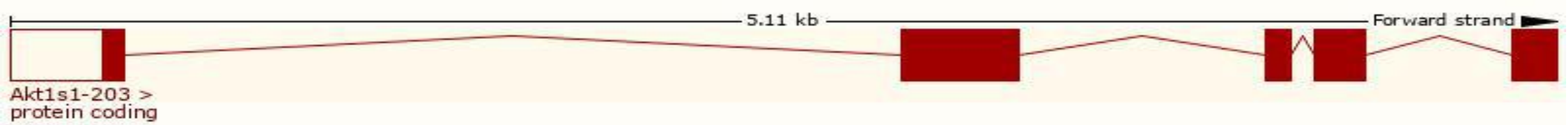
Official Symbol	Akt1s1 provided by MGI
Official Full Name	AKT1 substrate 1 (proline-rich) provided by MGI
Primary source	MGI:MGI:1914855
See related	Ensembl:ENSMUSG00000011096
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1110012J22Rik, AI227026, Lobe, Lobel, PRAS40
Expression	Ubiquitous expression in adrenal adult (RPKM 52.6), ovary adult (RPKM 41.8) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

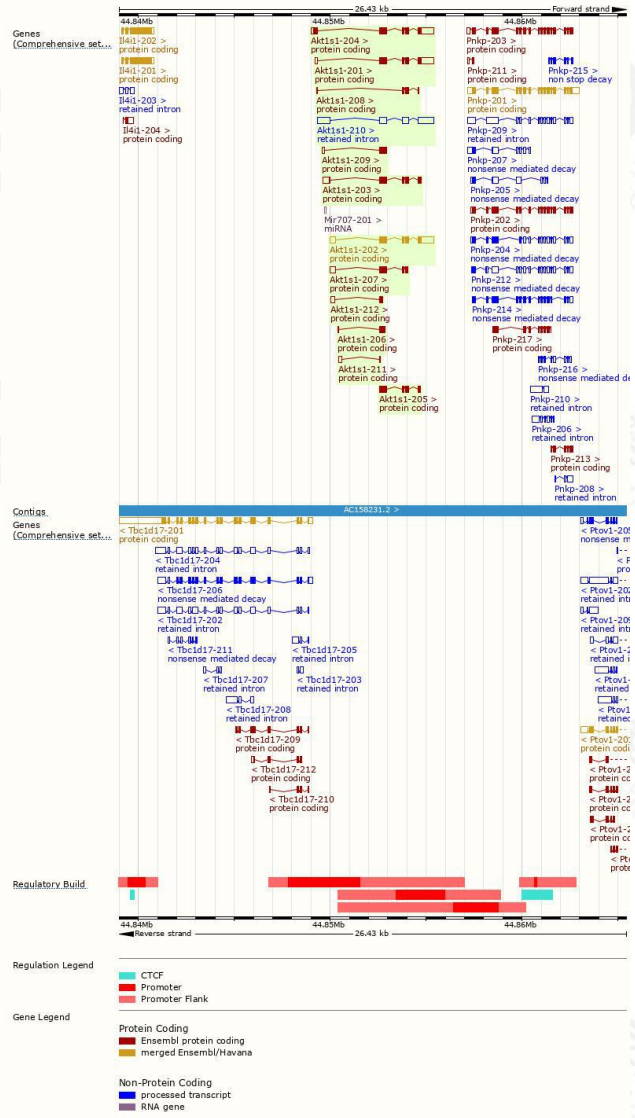
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Akt1s1-202	ENSMUST00000107880.8	1757	257aa	Protein coding	CCDS21218	Q9D1F4	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Akt1s1-201	ENSMUST00000054343.14	1592	257aa	Protein coding	CCDS21218	Q9D1F4	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Akt1s1-203	ENSMUST00000107882.7	1160	283aa	Protein coding	CCDS71954	E9QKI5	TSL:3 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Akt1s1-204	ENSMUST00000107885.7	1812	328aa	Protein coding	-	E9QKI4	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Akt1s1-207	ENSMUST00000141311.7	835	193aa	Protein coding	-	D3YWZ1	CDS 3' incomplete TSL:2
Akt1s1-205	ENSMUST00000127783.1	750	247aa	Protein coding	-	F6R8S6	CDS 3' incomplete TSL:5
Akt1s1-209	ENSMUST00000150335.1	485	123aa	Protein coding	-	D3Z4R0	CDS 3' incomplete TSL:2
Akt1s1-212	ENSMUST00000208384.1	392	57aa	Protein coding	-	A0A140LJJ3	CDS 3' incomplete TSL:2
Akt1s1-208	ENSMUST00000142880.2	333	111aa	Protein coding	-	A0A140LHA0	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Akt1s1-206	ENSMUST00000136232.1	332	95aa	Protein coding	-	D3Z4W3	CDS 3' incomplete TSL:2
Akt1s1-211	ENSMUST00000207223.1	212	14aa	Protein coding	-	A0A140LHN7	CDS 3' incomplete TSL:3
Akt1s1-210	ENSMUST00000152091.7	2207	No protein	Retained intron	-	-	TSL:1

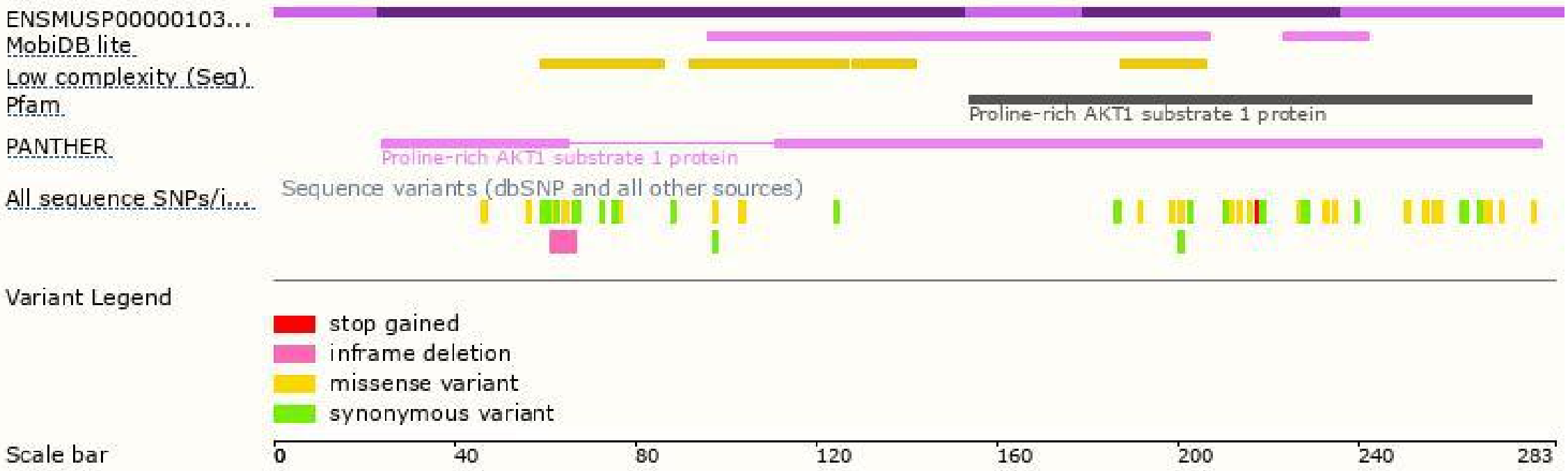
The strategy is based on the design of *Akt1s1-203* transcript,the transcription is shown below:



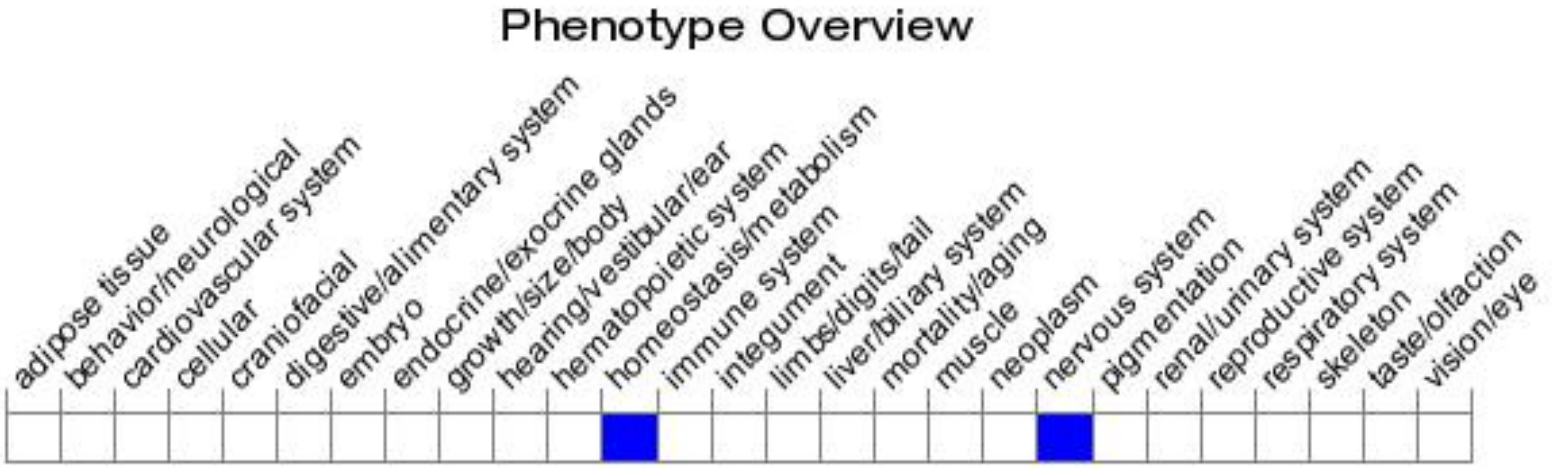
Genomic location distribution



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 exhibit increased susceptibility to induced ischemic brain injury.

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

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GemPharmatech Co.,Ltd