Sptb Cas9-CKO Strategy

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Design Date: 2021-7-12

Project Overview

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

Conditional Knockout strategy

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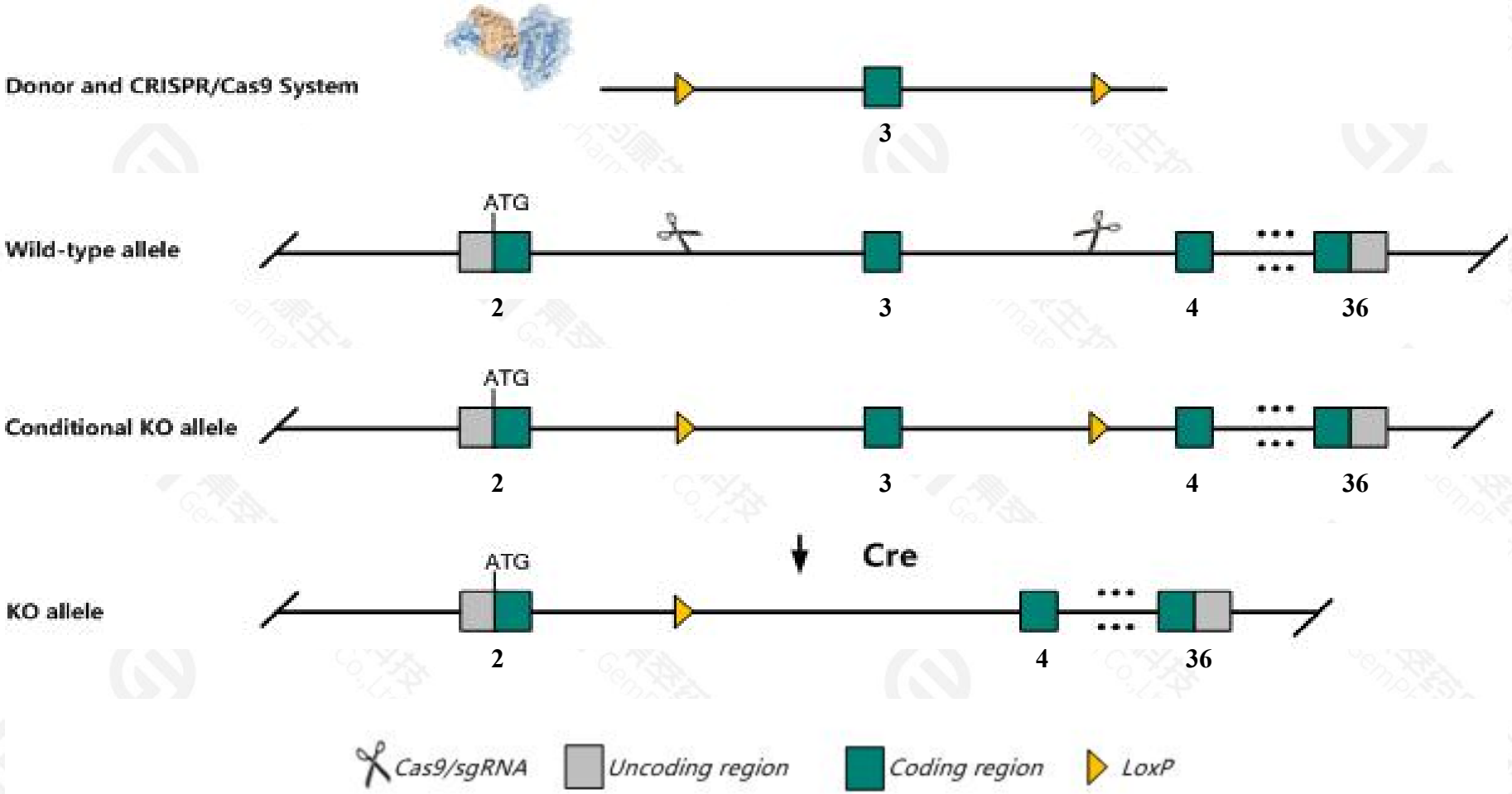
This model will use CRISPR/Cas9 technology to edit the *Sptb* gene. The schematic diagram is as follows:



Technical routes

The *Sptb* gene has 3 transcripts. According to the structure of *Sptb* gene, exon3 of *Sptb-201*(ENSMUST00000021458.13) transcript is recommended as the knockout region.The region contains 152bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Sptb* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor was constructed.Cas9, sgRNA 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 was 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,homozygotes for a spontaneous mutation exhibit a severe microcytic anemia with erythrocyte fragility, hepatomegaly, and jaundice. Mutants die within a few days of birth. Heterozygotes are mildly anemic.

The *Sptb* gene is located on the Chr12. 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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Sptb spectrin beta, erythrocytic [Mus musculus (house mouse)]

Gene ID: 20741, updated on 25-Sep-2020

Summary

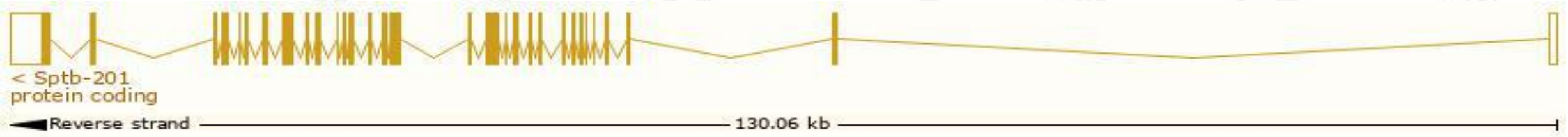
Official Symbol	Sptb provided by MGI
Official Full Name	spectrin beta, erythrocytic provided byMGI
Primary source	MGI:MGI:98387
See related	Ensembl:ENSMUSG00000021061
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI842465, D330027P03Rik, Gm1301, Sp, Spn, Spnb-1, Spnb1, ja, jaundiced, mKIAA4219
Expression	Biased expression in cerebellum adult (RPKM 27.2), liver E14.5 (RPKM 17.4) and 13 other tissuesSee more
Orthologs	human all

Transcript information Ensembl

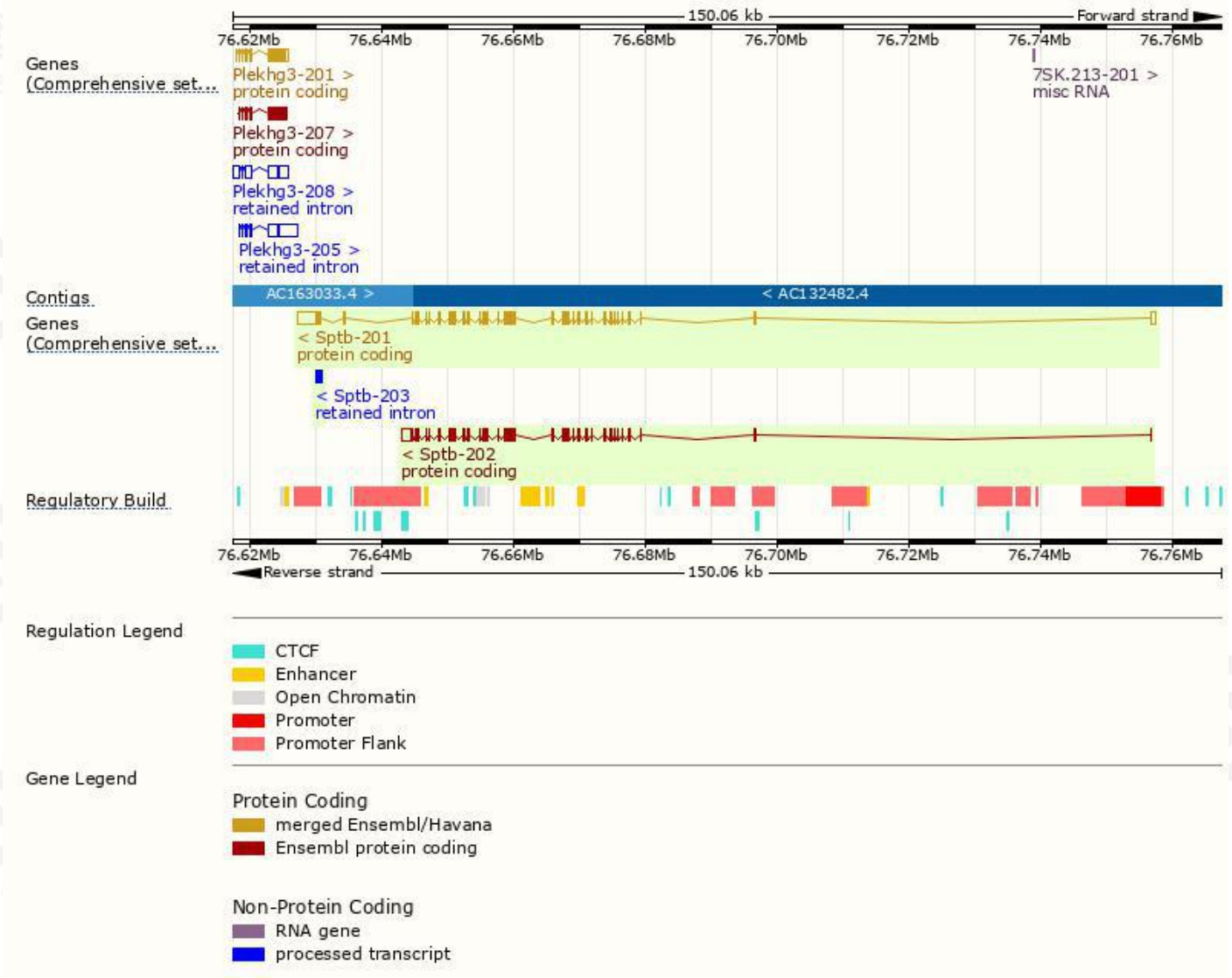
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sptb-201	ENSMUST00000021458.13	10394	2329aa	Protein coding	CCDS36477		TSL:1 , GENCODE basic , APPRIS P1 ,
Sptb-202	ENSMUST00000166101.2	8084	2137aa	Protein coding	-		TSL:5 , GENCODE basic ,
Sptb-203	ENSMUST00000170532.2	532	No protein	Retained intron	-		TSL:1 ,

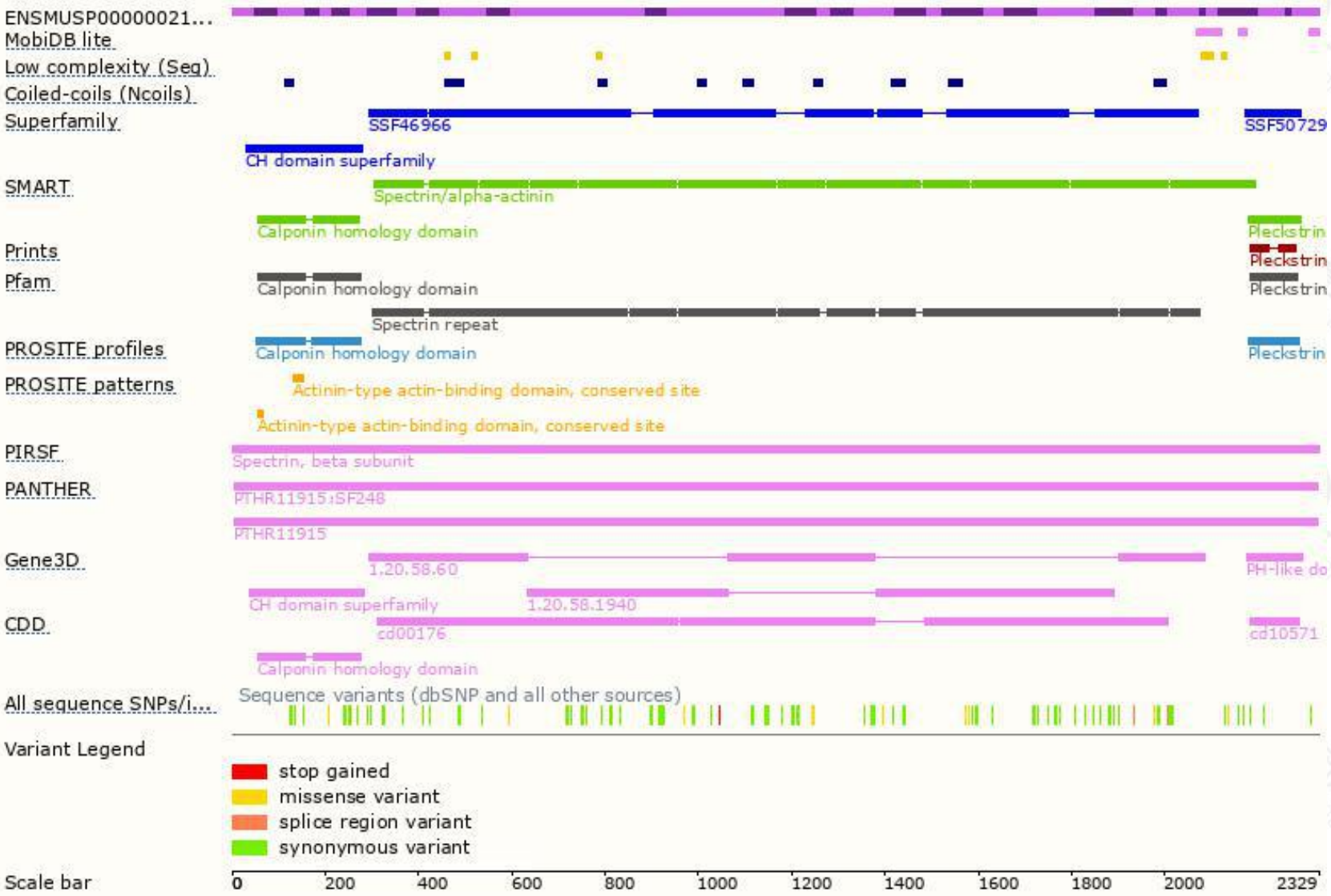
The strategy is based on the design of *Sptb-201* transcript,the transcription is shown below:



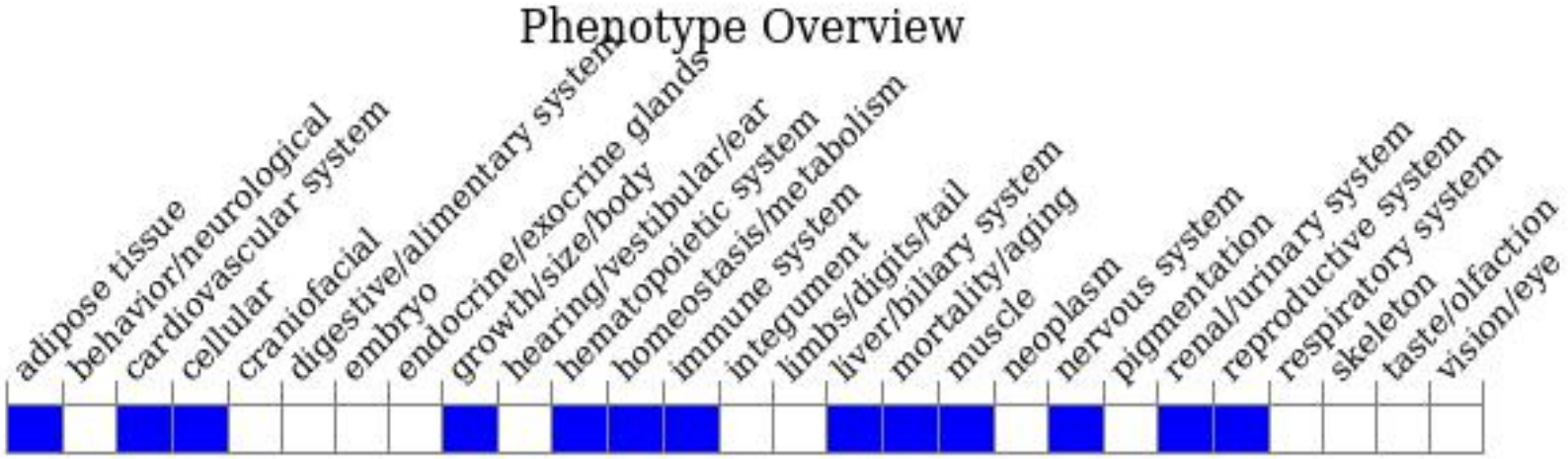
Genomic location distribution



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/).

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

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