

***Sptbn1* Cas9-CKO Strategy**

Designer:

Yanhua Shen

Design Date:

2019-7-26

Project Overview

Project Name

Sptbn1

Project type

Cas9-CKO

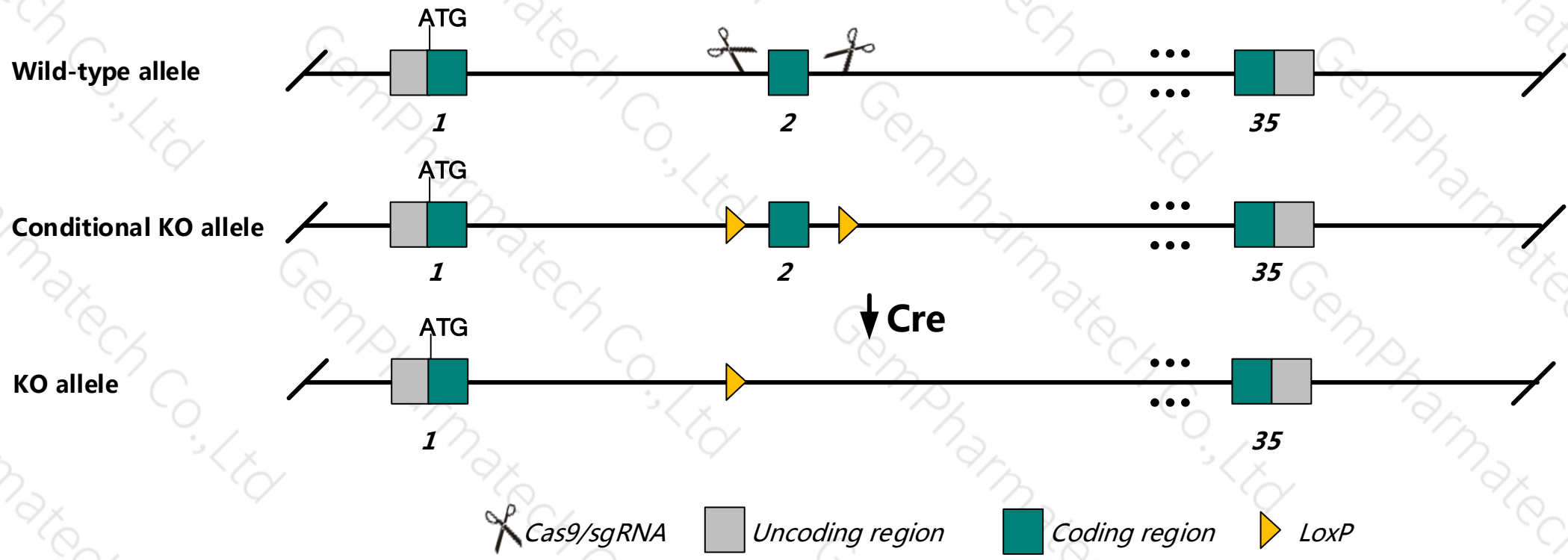
Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sptbn1* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System



- The *Sptbn1* gene has 8 transcripts, According to the structure of *Sptbn1* gene, exon2 of *Sptbn1*-202(ENSMUST00000011877.12) transcript is recommended as the knockout region. The region contains the 152bp key functional area of coding sequence. Knock out the region, result in destruction of protein
- In this project we use CRISPR/Cas9 technology to modify *Sptbn1* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector 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 or cell types.

- According to the existing MGI data , Homozygous inactivation of this gene leads to mid-gestational lethality due to gastrointestinal, liver, neural, and cardiac defects, whereas heterozygotes survive until adulthood and spontaneously develop cancers in several organs.
- Transcript *Sptbn1*-205,206,207,208 may not be affected.
- The *Sptbn1* gene is located on the Chr11. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Sptbn1 spectrin beta, non-erythrocytic 1 [*Mus musculus* (house mouse)]

Gene ID: 20742, updated on 25-Jun-2019

Summary

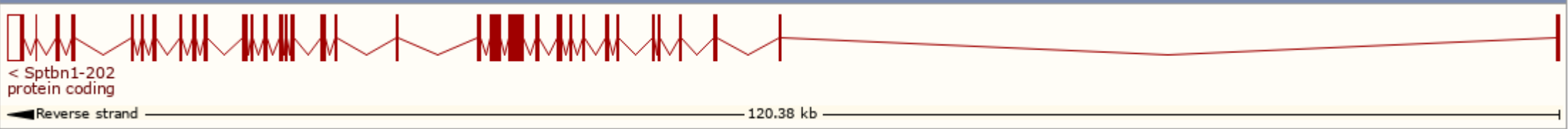
Official Symbol	Sptbn1 provided by MGI
Official Full Name	spectrin beta, non-erythrocytic 1 provided by MGI
Primary source	MGI:MGI:98388
See related	Ensembl:ENSMUSG00000020315
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	elf1; elf3; SPTB2; Spnb2; Spnb-2; AL033301; mKIAA4049; 9930031C03Rik
Expression	Ubiquitous expression in lung adult (RPKM 47.5), frontal lobe adult (RPKM 32.2) and 25 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

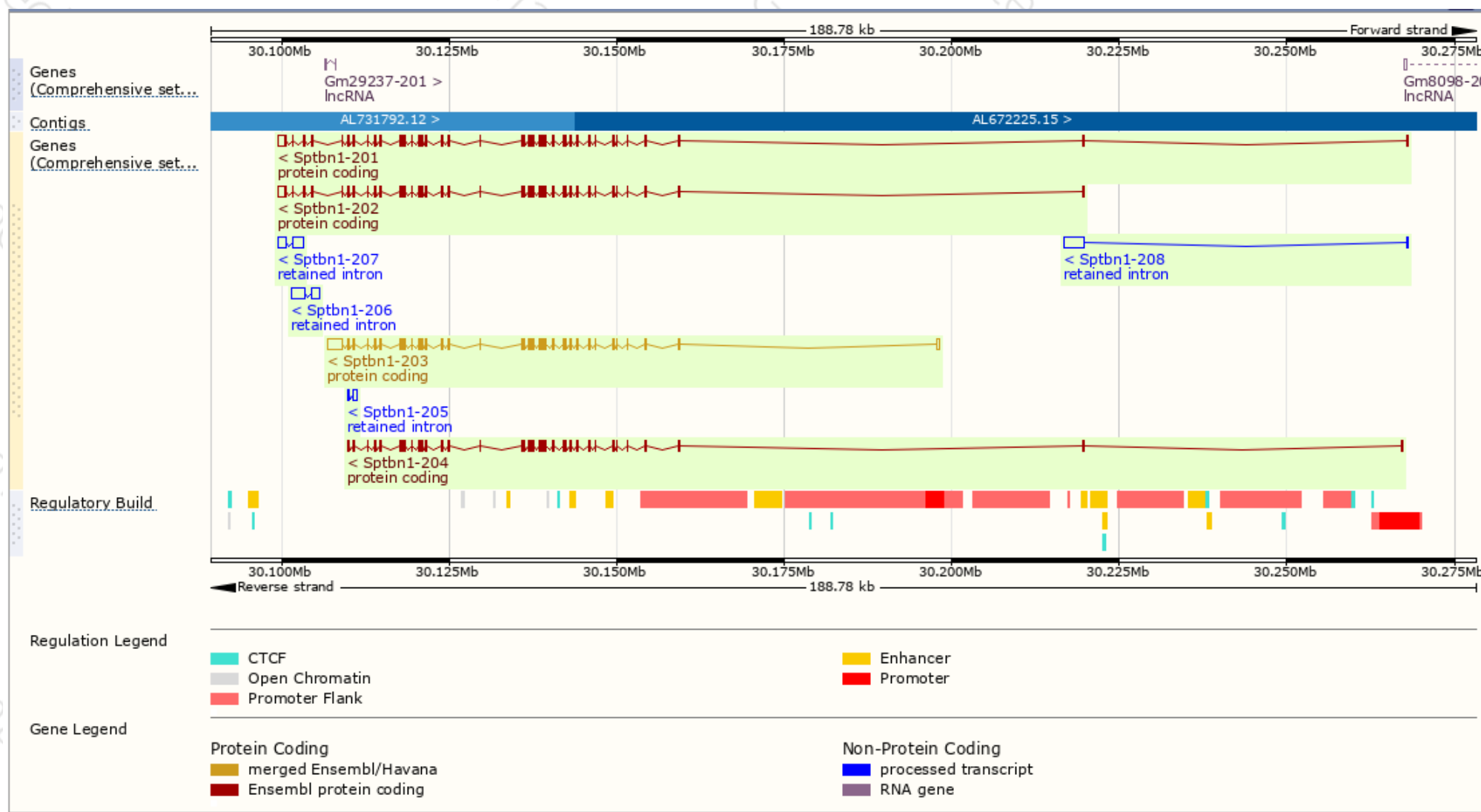
The gene has 8 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sptbn1-203	ENSMUST00000102838.9	9107	2154aa	Protein coding	CCDS24506	Q62261	TSL:1 GENCODE basic APPRIS P3
Sptbn1-201	ENSMUST00000006629.13	8461	2363aa	Protein coding	CCDS36123	Q62261	TSL:5 GENCODE basic APPRIS ALT1
Sptbn1-202	ENSMUST00000011877.12	8238	2363aa	Protein coding	CCDS36123	Q62261	TSL:1 GENCODE basic APPRIS ALT1
Sptbn1-204	ENSMUST00000124231.1	6524	2092aa	Protein coding	-	A0A0A0MQG2	CDS 3' incomplete TSL:1
Sptbn1-208	ENSMUST00000149117.1	3342	No protein	Retained intron	-	-	TSL:1
Sptbn1-206	ENSMUST00000133466.1	3084	No protein	Retained intron	-	-	TSL:1
Sptbn1-207	ENSMUST00000145315.1	2956	No protein	Retained intron	-	-	TSL:1
Sptbn1-205	ENSMUST00000127209.1	744	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Sptbn1*-202 transcript, The transcription is shown below



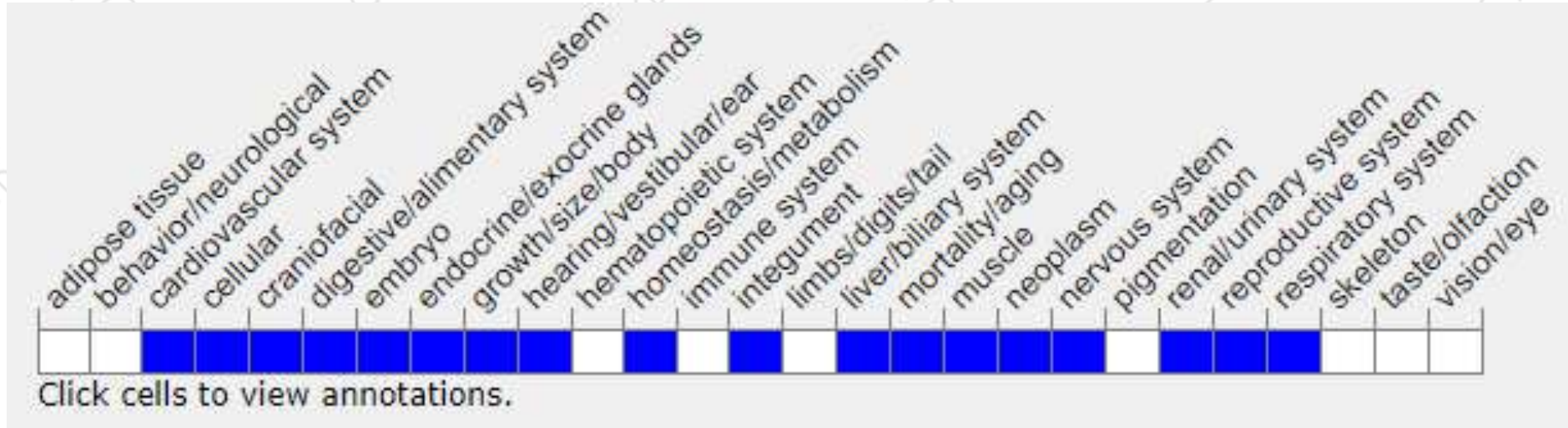
Genomic location distribution



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/>) .

Homozygous inactivation of this gene leads to mid-gestational lethality due to gastrointestinal, liver, neural, and cardiac defects, whereas heterozygotes survive until adulthood and spontaneously develop cancers in several organs.

If you have any questions, you are welcome to inquire.
Tel: 025-5864 1534

