Rsf1 Cas9-CKO Strategy

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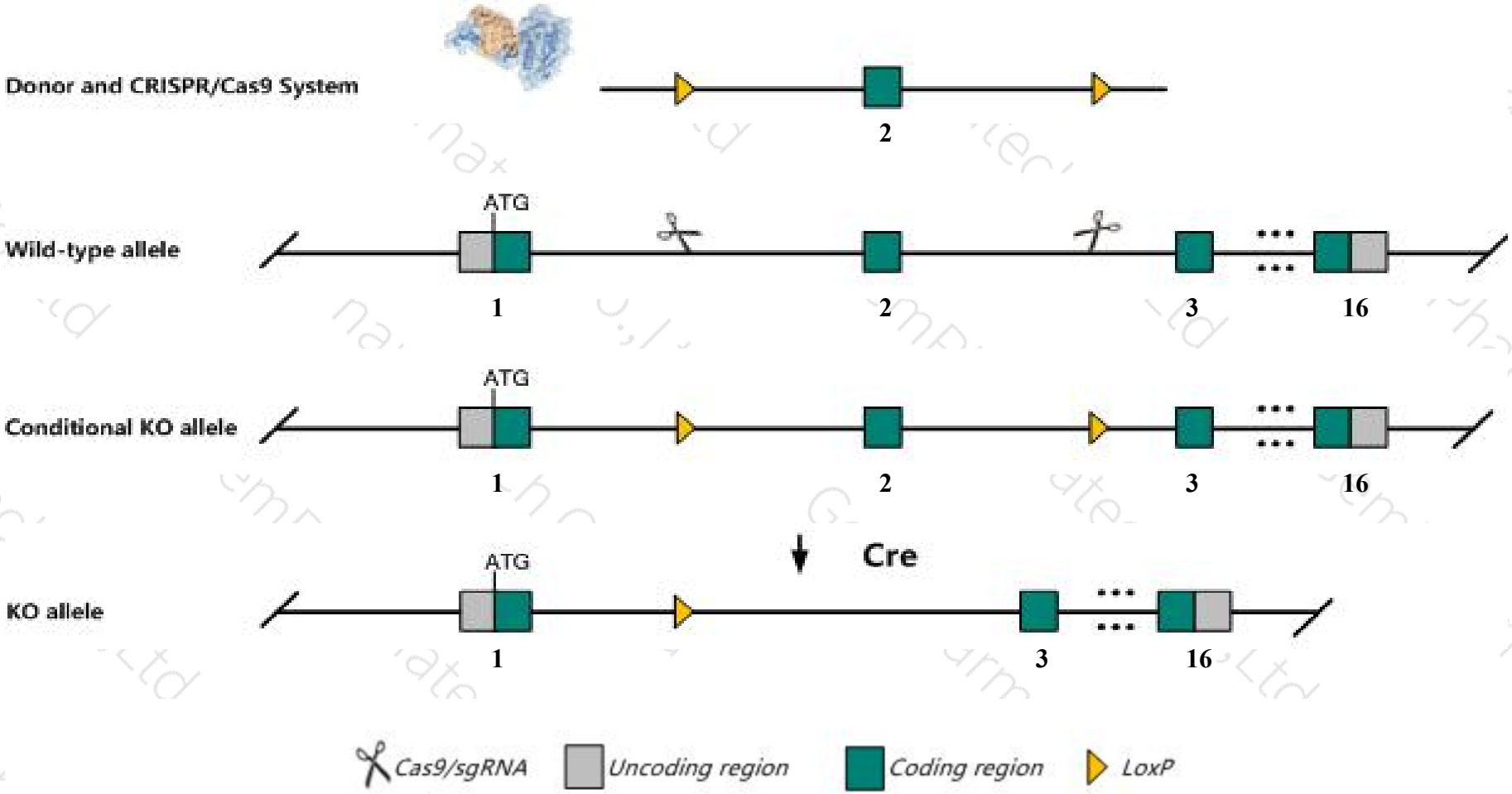
Project Overview

Project Name	***Rsf1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rsf1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Rsf1* gene has 8 transcripts. According to the structure of *Rsf1* gene, exon2 of *Rsf1-201*(ENSMUST00000107153.2) transcript is recommended as the knockout region.The region contains 92bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Rsf1* 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 and cell types.

Notice

➢ According to the existing MGI data,mice homozygous for a conditional allele activated in neurons exhibit increased susceptibility to etoposide-induced neuron apoptosis with failure to repair double-strand breaks.

➢ The partial intron of *Rsf1os1* gene will be deleted together in this strategy.

➢ Transcript *Rsf1*-202&204&205&208 may not be affected.

➢ The KO region contains functional region of the Rsf1 gene.Knockout the region may affect the function of Rsf1os1 gene.

➢ The *Rsf1* 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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Rsf1 remodeling and spacing factor 1 [Mus musculus (house mouse)]

Gene ID: 233532, updated on 13-Mar-2020

Summary

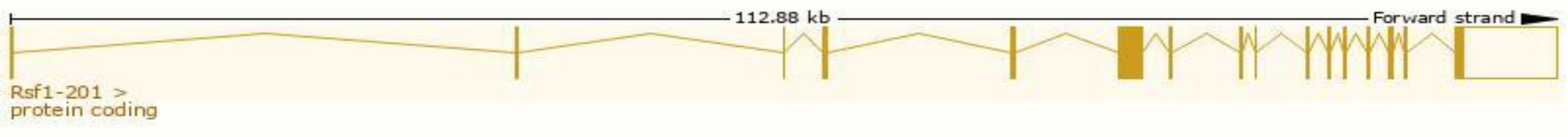
Official Symbol	Rsf1 provided by MGI
Official Full Name	remodeling and spacing factor 1 provided by MGI
Primary source	MGI:MGI:2682305
See related	Ensembl:ENSMUSG00000035623
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4832420A03Rik, C030033M12Rik, Gm164, Hbxap, XAP8, p325
Expression	Broad expression in CNS E11.5 (RPKM 4.7), CNS E14 (RPKM 4.1) and 24 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

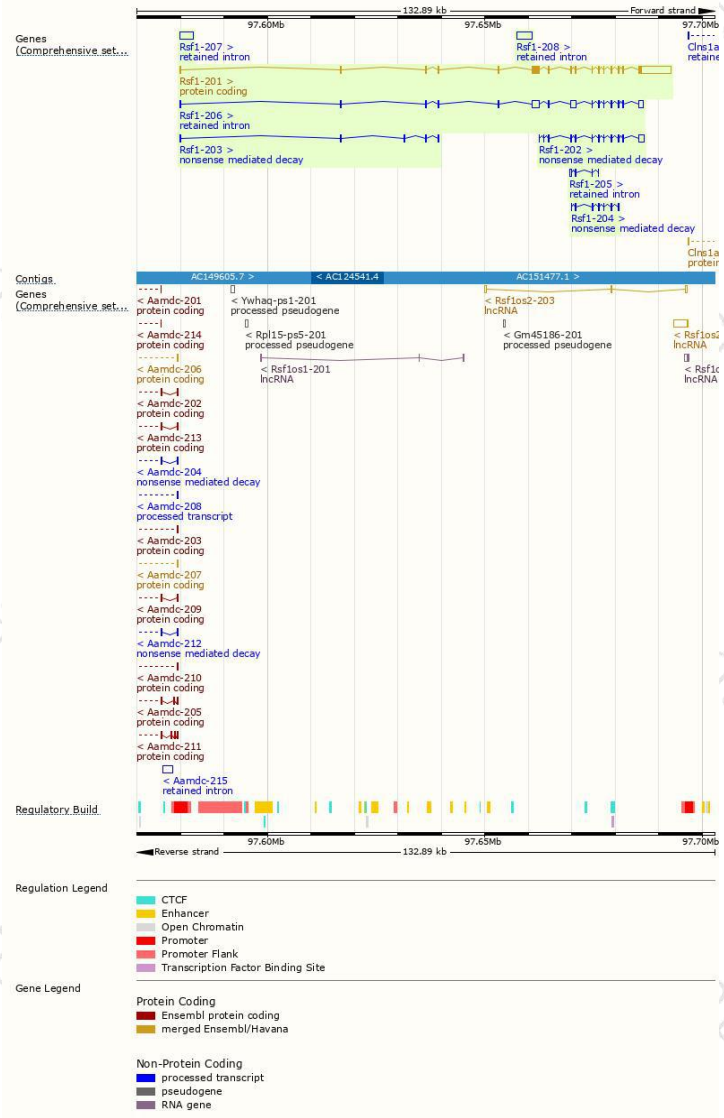
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rsf1-201	ENSMUST00000107153.2	11124	1441aa	Protein coding	CCDS40025	E9PWW9	TSL:5 GENCODE basic APPRIS P1
Rsf1-202	ENSMUST00000123731.7	2493	14aa	Nonsense mediated decay	-	A0A0U1RNQ5	CDS 5' incomplete TSL:5
Rsf1-204	ENSMUST00000135270.2	764	121aa	Nonsense mediated decay	-	A0A0U1RP31	CDS 5' incomplete TSL:5
Rsf1-203	ENSMUST00000127891.2	754	109aa	Nonsense mediated decay	-	A0A0U1RPA7	CDS 5' incomplete TSL:3
Rsf1-206	ENSMUST00000156060.7	5695	No protein	Retained intron	-	-	TSL:2
Rsf1-208	ENSMUST00000206866.1	3635	No protein	Retained intron	-	-	TSL:NA
Rsf1-207	ENSMUST00000205536.1	3085	No protein	Retained intron	-	-	TSL:NA
Rsf1-205	ENSMUST00000150288.6	542	No protein	Retained intron	-	-	TSL:5

The strategy is based on the design of *Rsf1-201* transcript,the transcription is shown below:

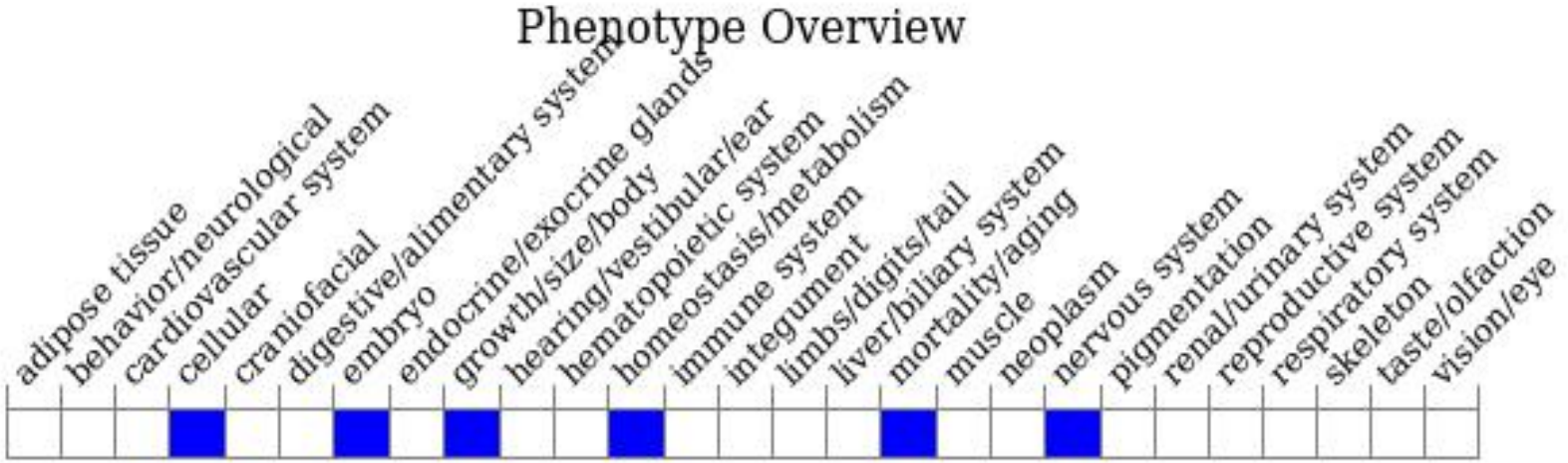


Genomic location distribution



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 conditional allele activated in neurons exhibit increased susceptibility to etoposide-induced neuron apoptosis with failure to repair double-strand breaks.

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

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