Wsb2 Cas9-CKO Strategy

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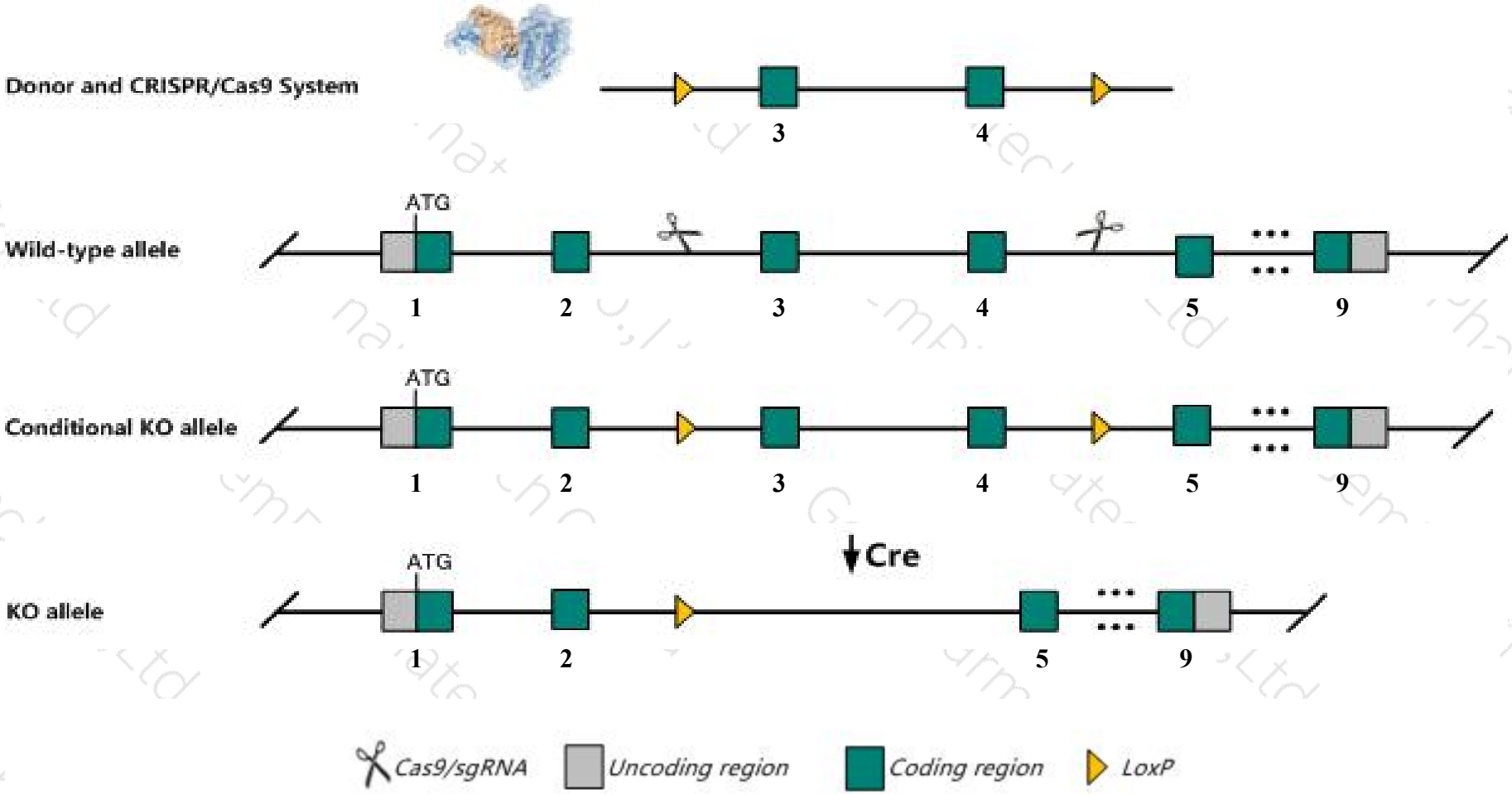
Project Overview

Project Name	***Wsb2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Wsb2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Wsb2* gene has 6 transcripts. According to the structure of *Wsb2* gene, exon3-exon4 of *Wsb2-201*(ENSMUST00000031309.15) transcript is recommended as the knockout region.The region contains 377bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Wsb2* 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

➢ The *Wsb2* gene is located on the Chr5. 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.

➢ The transcript of *Wsb2*-203&204&206 may not be affected.

➢ 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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Wsb2 WD repeat and SOCS box-containing 2 [Mus musculus (house mouse)]

Gene ID: 59043, updated on 13-Mar-2020

Summary

Official Symbol	Wsb2 provided by MGI
Official Full Name	WD repeat and SOCS box-containing 2 provided by MGI
Primary source	MGI:MGI:2144041
See related	Ensembl:ENSMUSG00000029364
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	AA673511, Swip2, WSB-2
Expression	Ubiquitous expression in CNS E18 (RPKM 87.6), cortex adult (RPKM 80.5) and 28 other tissues See more
Orthologs	human all

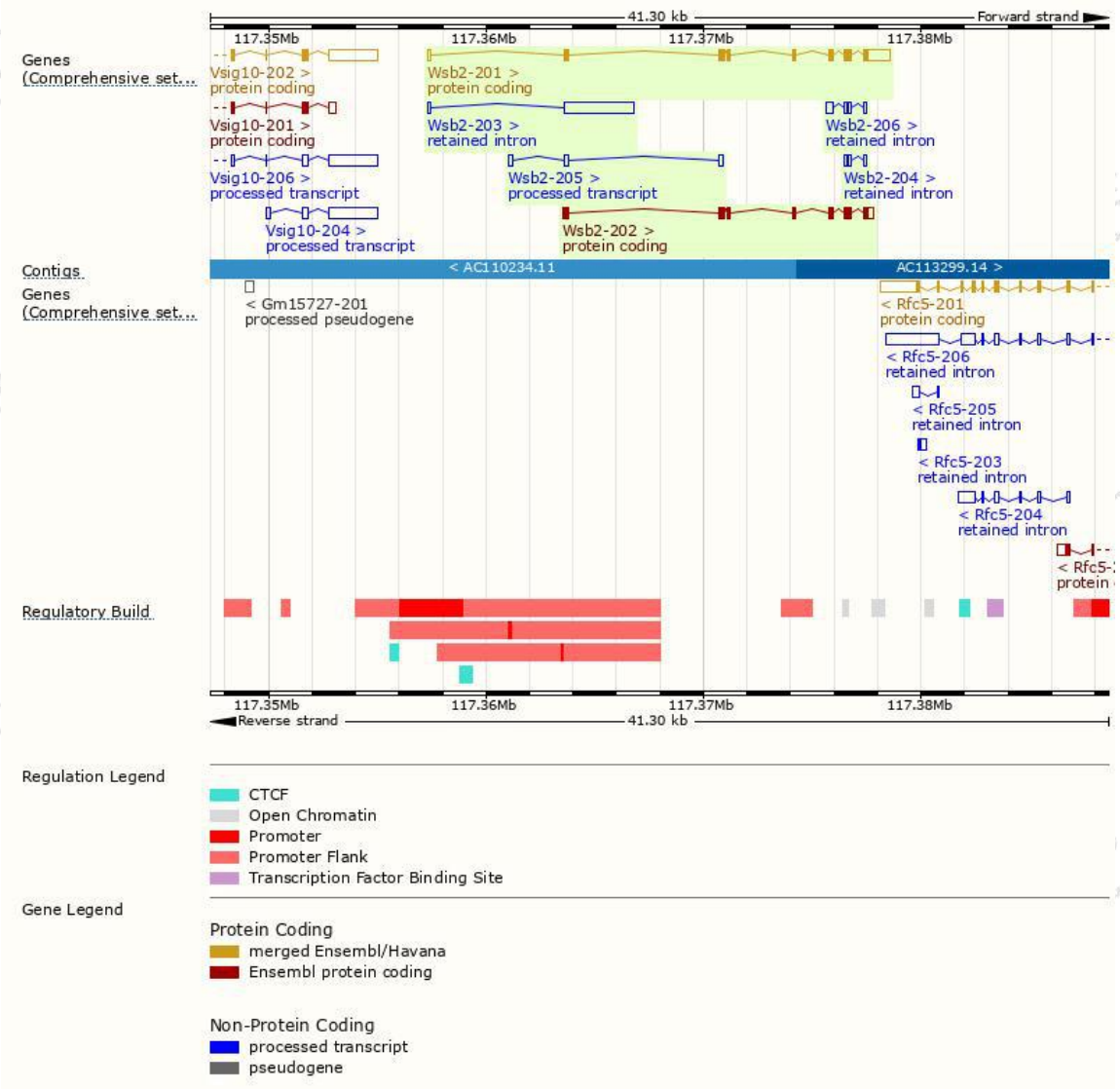
Transcript information（Ensembl）

The gene has 6 transcripts,all transcripts are shown below:

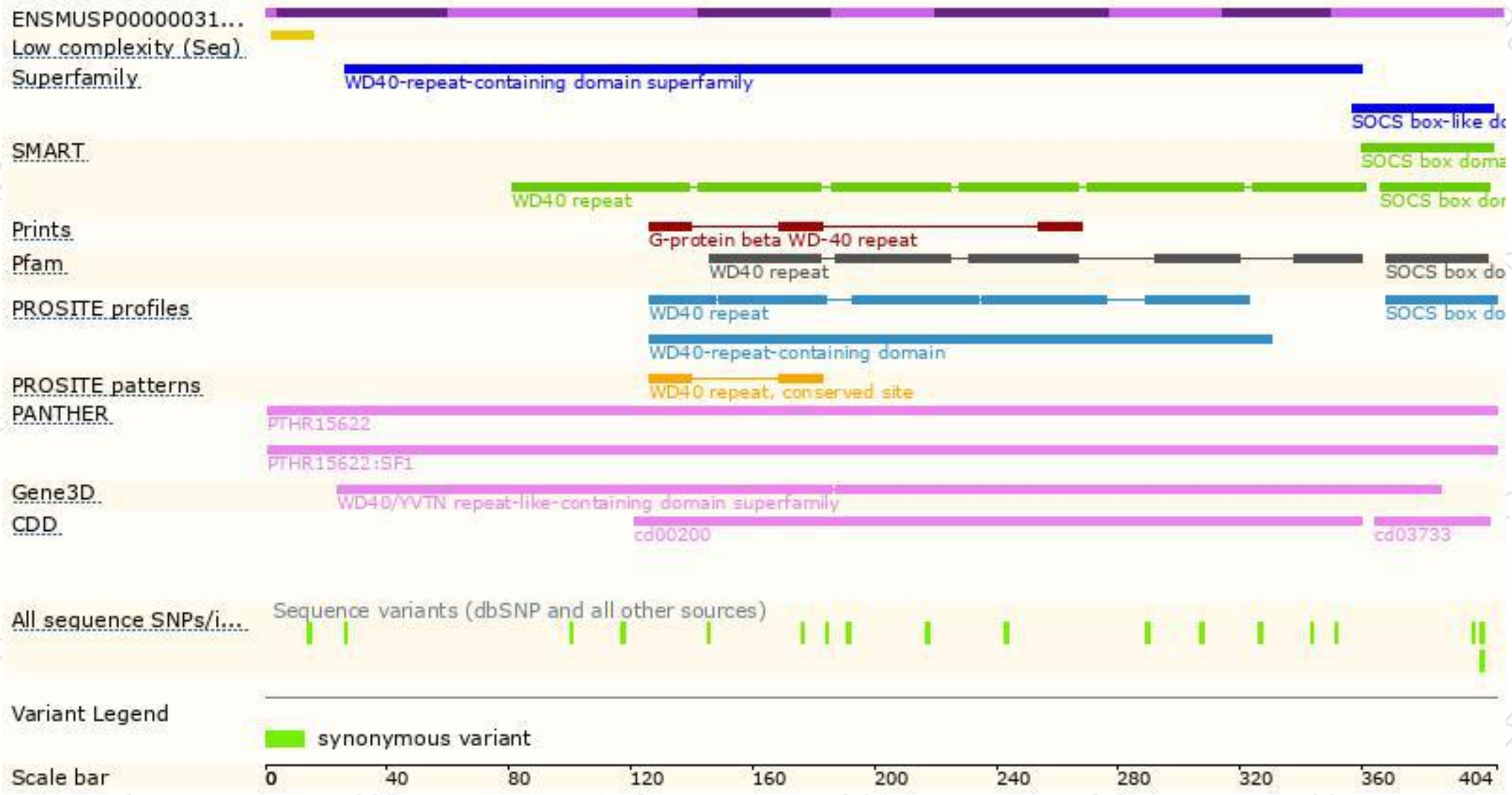
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Wsb2-201	ENSMUST00000031309.15	2421	404aa	Protein coding	CCDS19604	O54929	TSL:1 GENCODE basic APPRIS P2
Wsb2-202	ENSMUST00000111959.1	1526	406aa	Protein coding	-	E9Q8J9	TSL:1 GENCODE basic APPRIS ALT2
Wsb2-205	ENSMUST00000138071.1	585	No protein	Processed transcript	-	-	TSL:3
Wsb2-203	ENSMUST00000125522.1	3357	No protein	Retained intron	-	-	TSL:1
Wsb2-206	ENSMUST00000149669.7	600	No protein	Retained intron	-	-	TSL:2
Wsb2-204	ENSMUST00000136895.1	392	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Wsb2-201* transcript,the transcription is shown below:

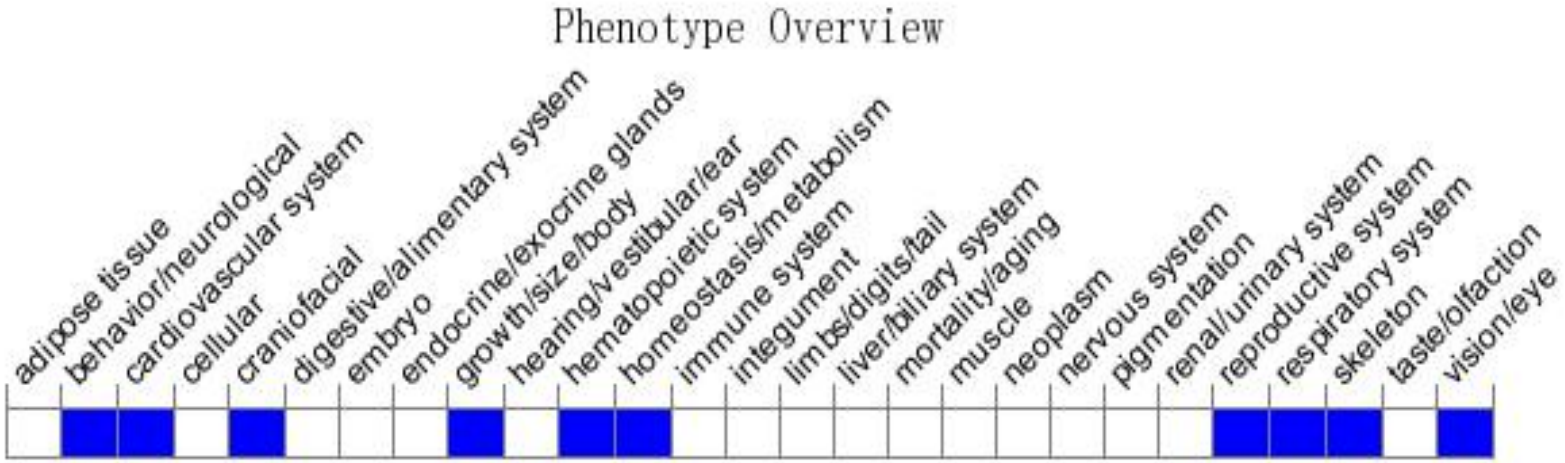
Genomic location distribution



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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