Robo4 Cas9-KO Strategy

Designer:Xueting Zhang
Reviewer:Yanhua Shen
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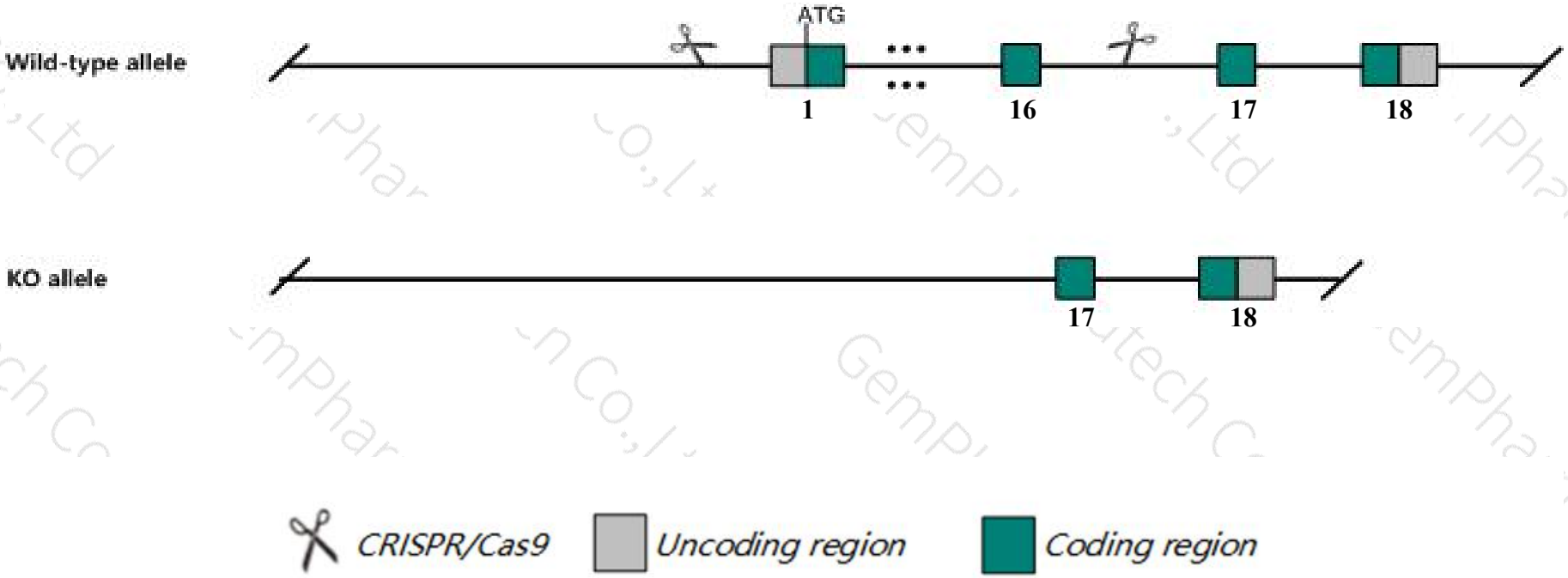
Project Overview

Project Name	***Robo4***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Robo4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Robo4* gene has 6 transcripts. According to the structure of *Robo4* gene, exon1-exon16 of *Robo4-205* (ENSMUST00000214185.1) transcript is recommended as the knockout region.The region contains start codon ATG. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Robo4* gene. The brief process is as follows: CRISPR/Cas9 system

Notice

- According to the existing MGI data,Mice homozygous for a reporter/null allele display enhanced VEGF-induced endothelial migration, tube formation and vascular permeability, and show increased pathologic angiogenesis and vascular leak in models of oxygen-induced retinopathy and choroidal neovascularization.
- Transcript *Robo4*-204 may not be affected.
- The *Robo4* gene is located on the Chr9. 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 gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Robo4 roundabout guidance receptor 4 [*Mus musculus* (house mouse)]

Gene ID: 74144, updated on 4-Dec-2019

Summary

Official Symbol	Robo4 provided by MGI
Official Full Name	roundabout guidance receptor 4 provided by MGI
Primary source	MGI:MGI:1921394
See related	Ensembl:ENSMUSG00000032125
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	AI593217; 1200012D01Rik
Expression	Broad expression in lung adult (RPKM 31.0), subcutaneous fat pad adult (RPKM 26.6) and 19 other tissues See more
Orthologs	human all

Genomic context

Location: 9; 9 A4

See Robo4 in Genome Data Viewer

Exon count: 17

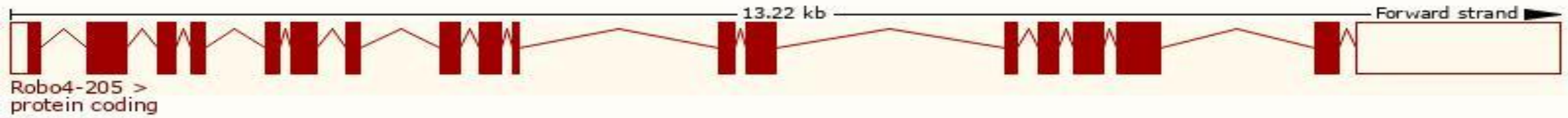
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	9	NC_000075.6 (37401902..37414023)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	9	NC_000075.5 (37209631..37221607)

Transcript information（Ensembl）

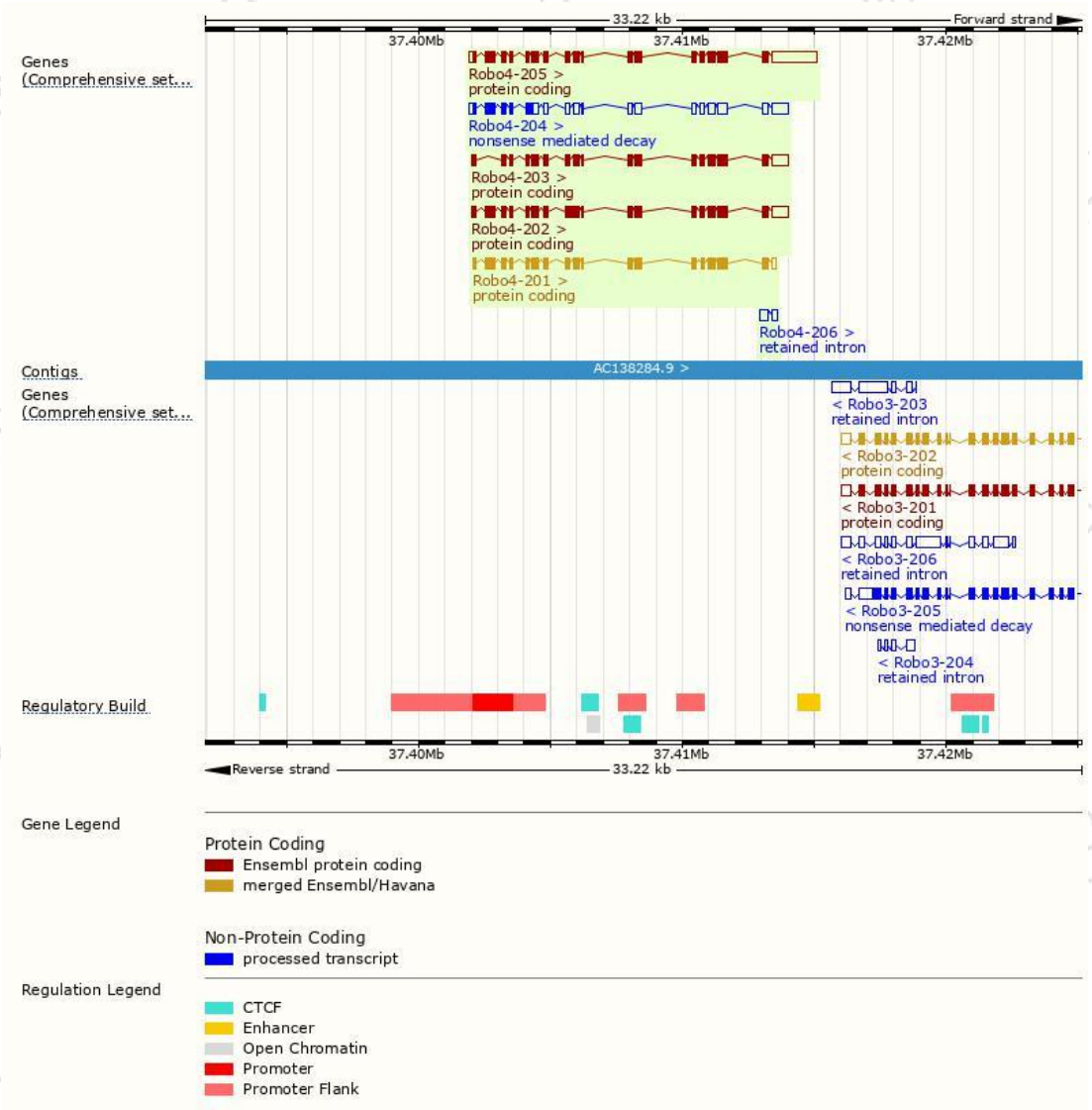
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Robo4-205	ENSMUST00000214185.1	4952	1022aa	Protein coding	CCDS80976	A0A1L1SUF8	TSL:1 GENCODE basic APPRIS ALT2
Robo4-201	ENSMUST00000102895.5	3226	1015aa	Protein coding	CCDS22977	A0A0R4J197	TSL:1 GENCODE basic APPRIS P3
Robo4-202	ENSMUST00000115046.7	3878	1074aa	Protein coding	-	D3Z4M4	TSL:2 GENCODE basic APPRIS ALT2
Robo4-203	ENSMUST00000115048.8	3392	911aa	Protein coding	-	E9QN68	TSL:1 GENCODE basic
Robo4-204	ENSMUST00000156972.1	3947	318aa	Nonsense mediated decay	-	A0A1L1SSV2	TSL:2
Robo4-206	ENSMUST00000215777.1	530	No protein	Retained intron	-	-	TSL:2

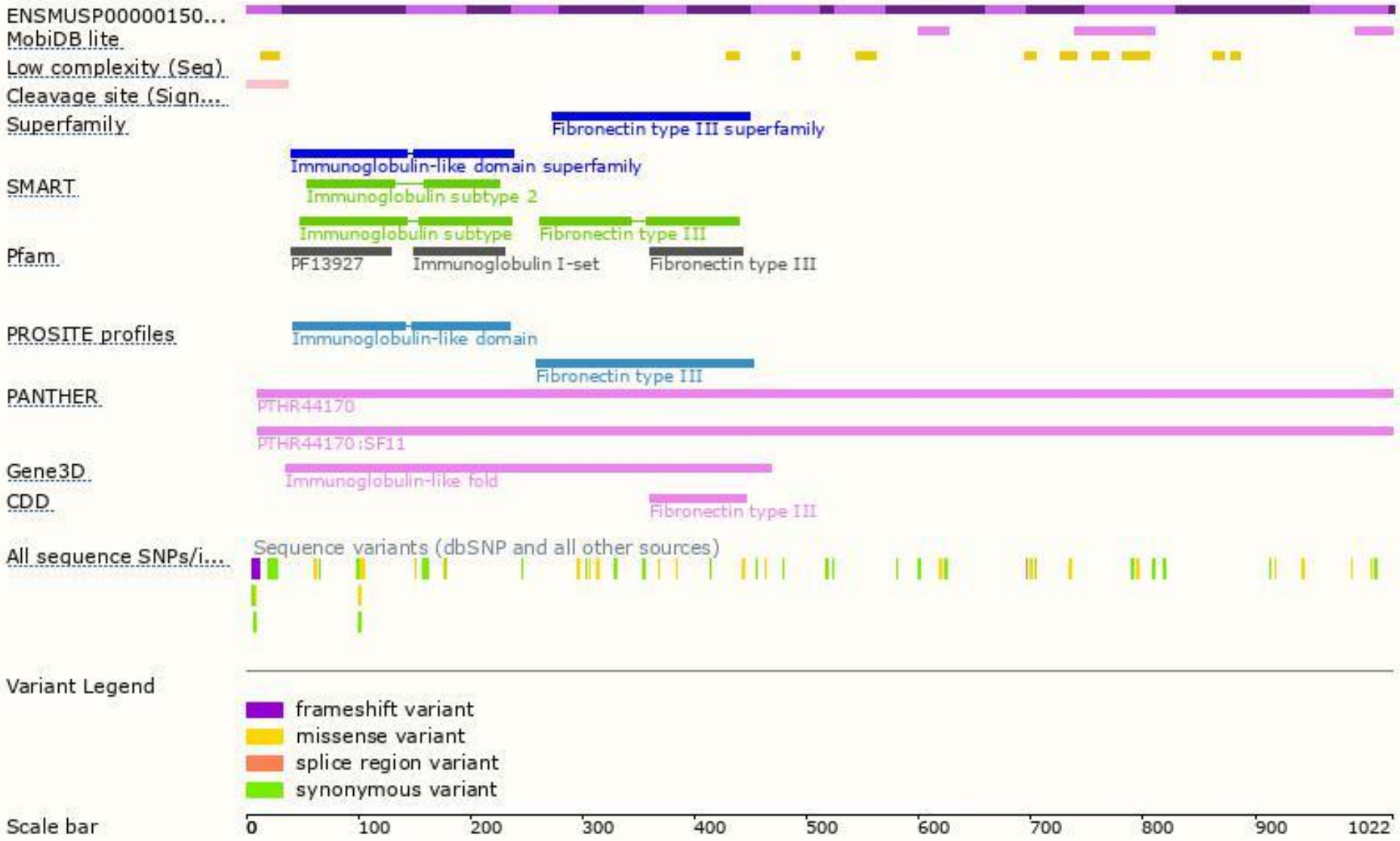
The strategy is based on the design of *Robo4-205* transcript,The transcription is shown below



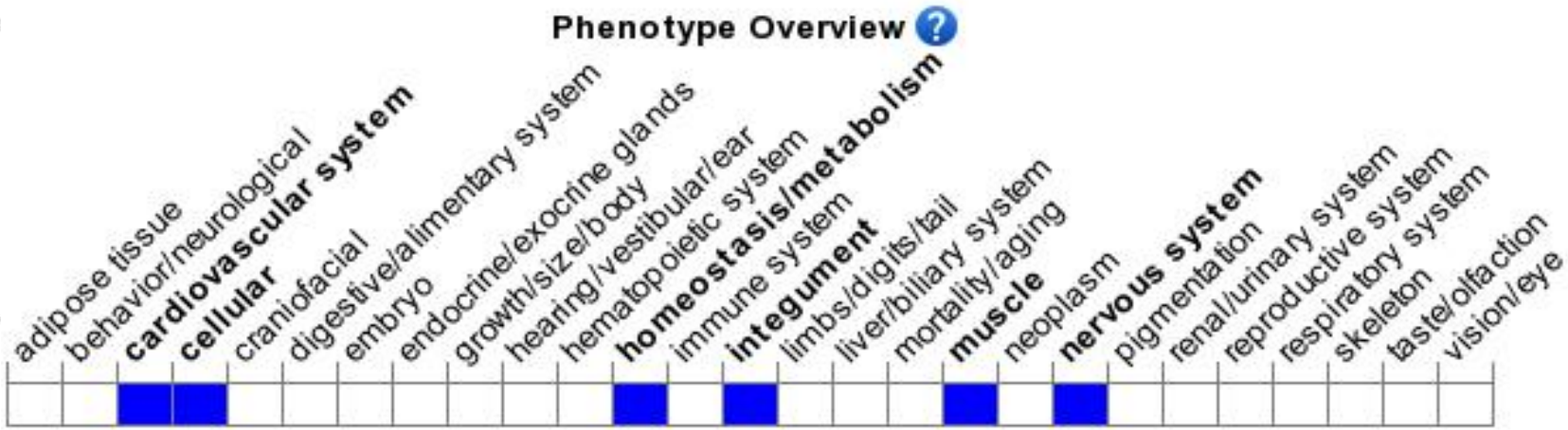
Genomic location distribution



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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GemPharmatech

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

Tel: 400-9660890

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GemPharmatech Co.,Ltd