Flt4 Cas9-KO Strategy

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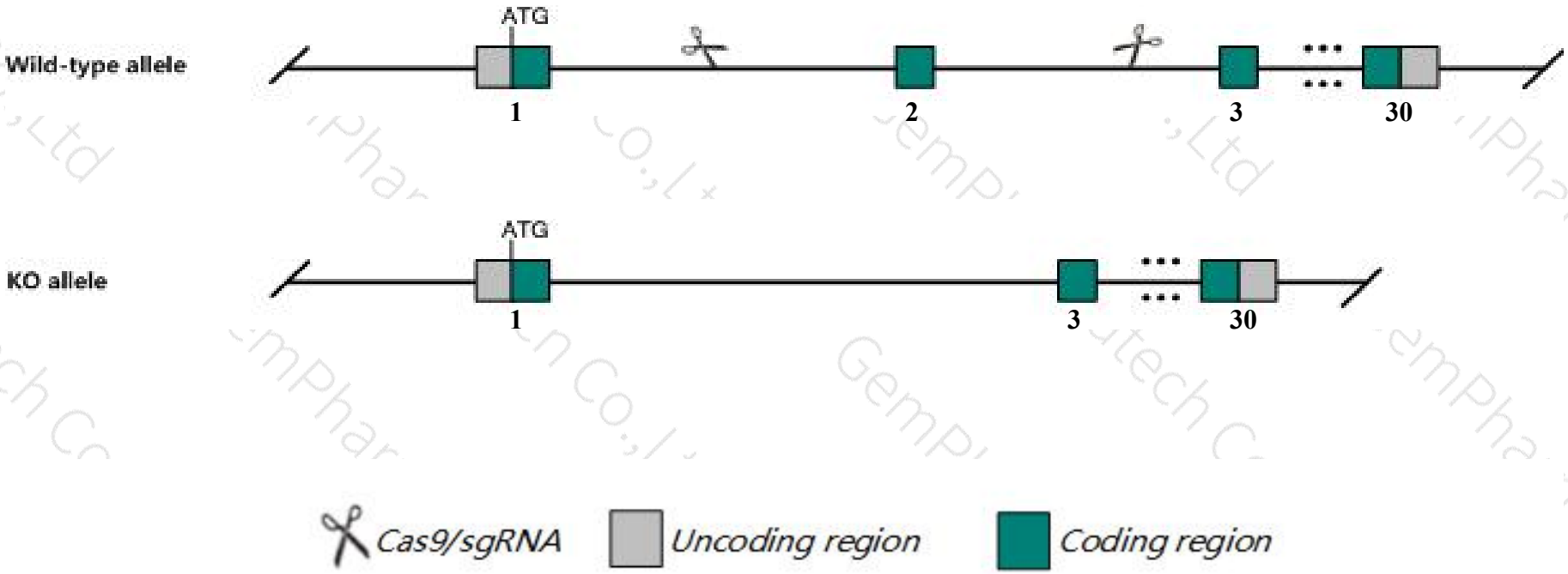
Project Overview

Project Name	***Flt4***
Project type	**Cas9-KO**
Strain background	**C57BL/6J**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Flt4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Flt4* gene has 2 transcripts. According to the structure of *Flt4* gene, exon2 of *Flt4-201* (ENSMUST00000020617.2) transcript is recommended as the knockout region.The region contains 97bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Flt4* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

Notice

- According to the existing MGI data,Embryos homozygous for a targeted null mutation show growth retardation, vascular abnormalities, severe anemia and die from cardiovascular failure at embryonic day 9.5. Heterozygotes for another mutation show abdominal chylous ascites, abnormal lymphaticvessels, and lymphedema.

- The *Flt4* 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 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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Flt4 FMS-like tyrosine kinase 4 [Mus musculus (house mouse)]

Gene ID: 14257, updated on 12-Mar-2019

Summary

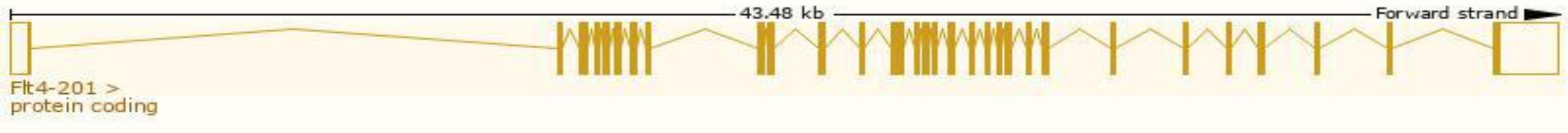
Official Symbol	Flt4 provided by MGI
Official Full Name	FMS-like tyrosine kinase 4 provided by MGI
Primary source	MGI:MGI:95561
See related	Ensembl:ENSMUSG00000020357
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI323512, Chy, Flt-4, VEGFR-3, VEGFR3
Expression	Broad expression in lung adult (RPKM 14.6), ovary adult (RPKM 10.5) and 23 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

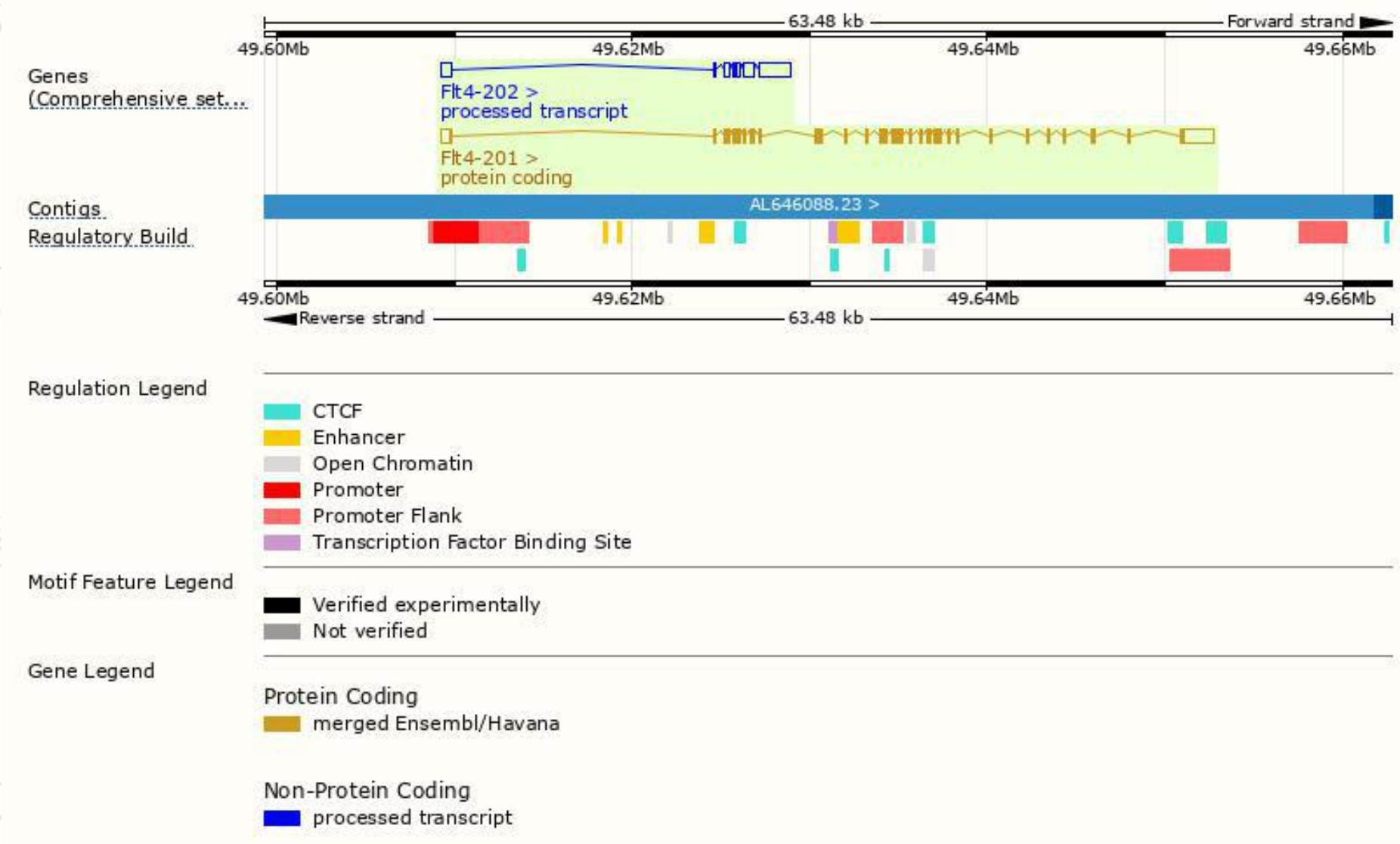
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Flt4-201	ENSMUST00000020617.2	6255	1363aa	Protein coding	CCDS24618	P35917 Q5SU94	TSL:1 GENCODE basic APPRIS P1
Flt4-202	ENSMUST00000152253.1	3587	No protein	Processed transcript	-	-	TSL:1

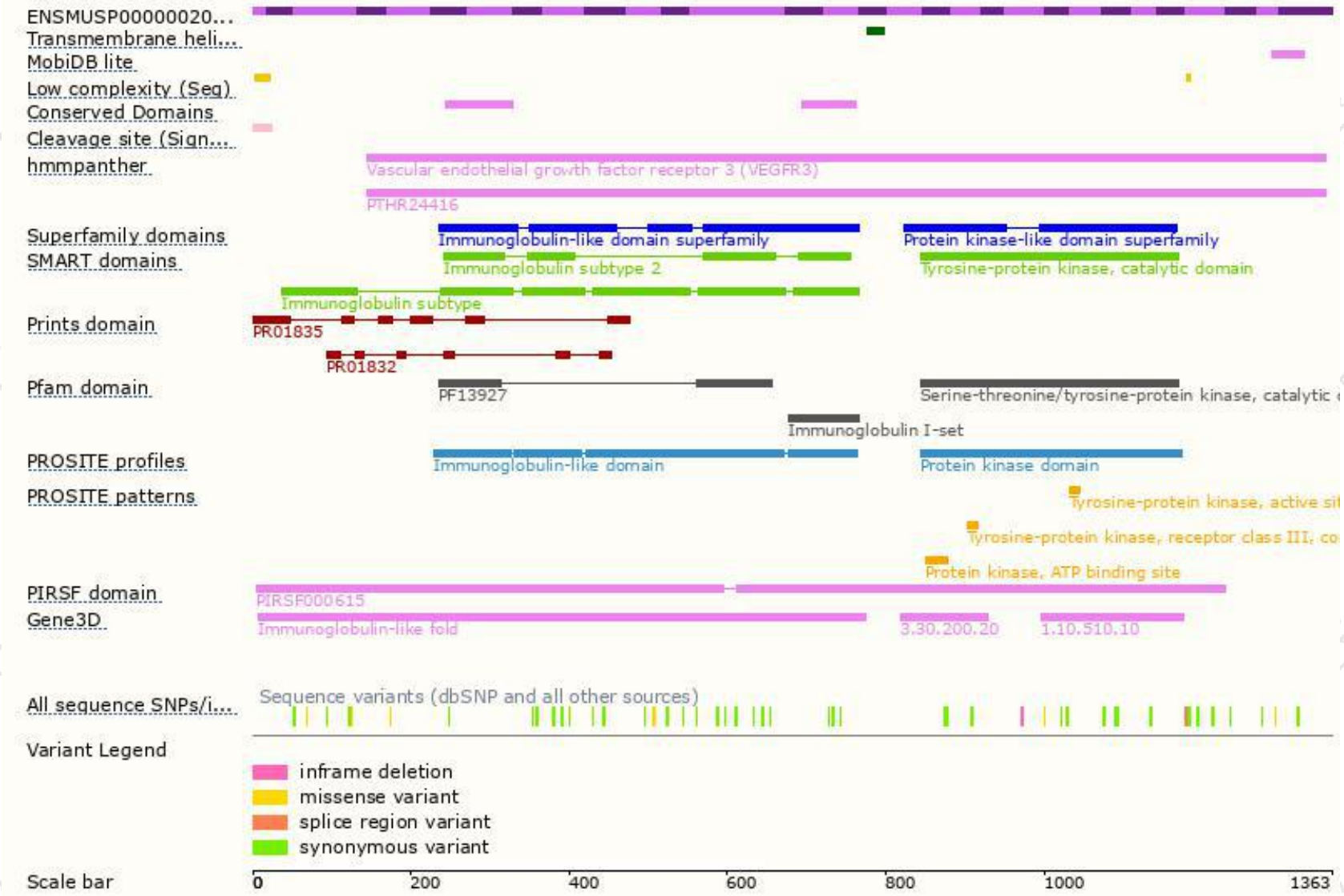
The strategy is based on the design of *Flt4-201* transcript,The transcription is shown below



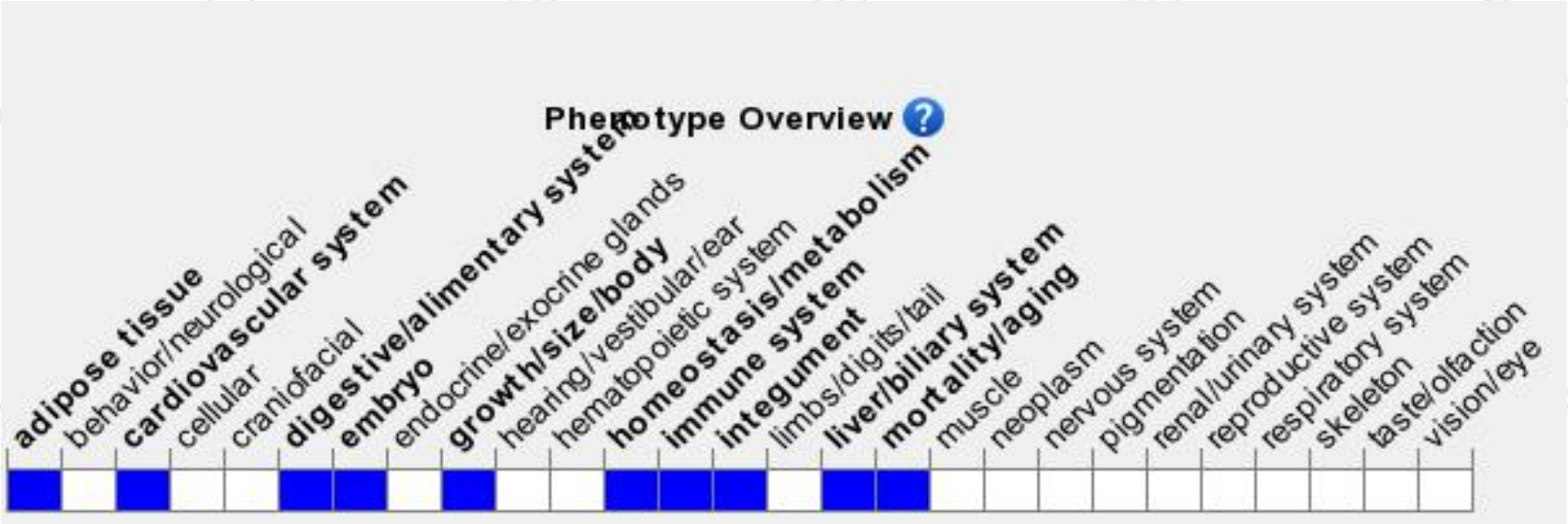
Genomic location distribution



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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