Vegfa Cas9-CKO Strategy

Designer: Huan Fan
Reviewer: Huan Wang

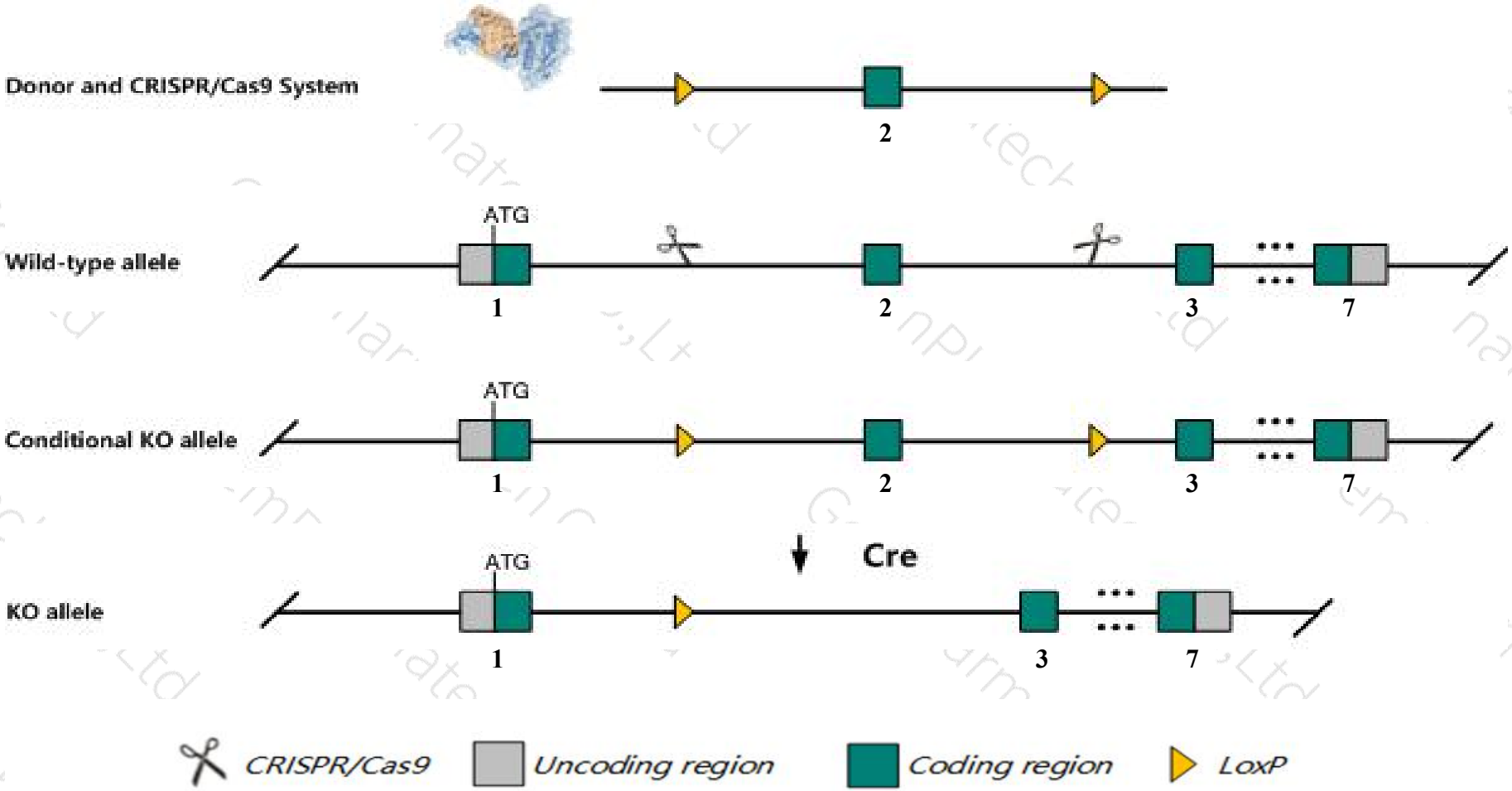
Design Date: 2019-10-30

Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Vegfa* gene has 13 transcripts. According to the structure of *Vegfa* gene, exon2 of *Vegfa-202* (ENSMUST00000071648.11) transcript is recommended as the knockout region.The region contains 49bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Vegfa* gene. The brief process is as follows:CRISPR/Cas9 system 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 will be 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,Hetero- or homozygous null mutants show embryonic lethality with impaired angiogenesis and blood-island formation. Mutants selectively expressing isoform 120 or 188 exhibit vascular outgrowth/patterning defects or impaired arterial development, respectively.

- The *Vegfa* gene is located on the Chr17. 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）

集萃药康 GemPharmatech

Vegfa vascular endothelial growth factor A [Mus musculus (house mouse)]

Gene ID: 22339, updated on 25-Mar-2019

Summary

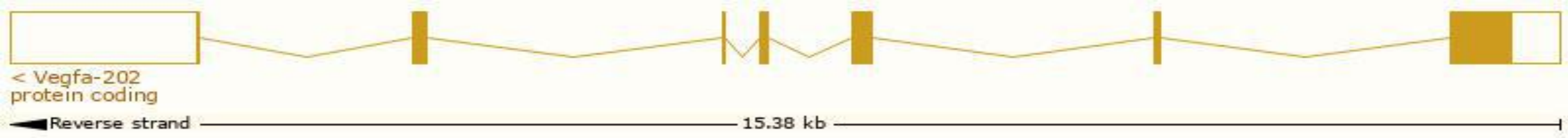
Official Symbol	Vegfa provided by MGI
Official Full Name	vascular endothelial growth factor A provided byMGI
Primary source	MGI:MGI:103178
See related	Ensembl:ENSMUSG00000023951
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Vegf, Vpf
Summary	This gene is a member of the PDGF/VEGF growth factor family. It encodes a heparin-binding protein, which exists as a disulfide-linked homodimer. This growth factor induces proliferation and migration of vascular endothelial cells, and is essential for both physiological and pathological angiogenesis. Disruption of this gene in mice resulted in abnormal embryonic blood vessel formation. This gene is upregulated in many known tumors and its expression is correlated with tumor stage and progression. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. There is also evidence for alternative translation initiation from upstream non-AUG (CUG) codons resulting in additional isoforms. A recent study showed that a C-terminally extended isoform is produced by use of an alternative in-frame translation termination codon via a stop codon readthrough mechanism, and that this isoform is antiangiogenic. Expression of some isoforms derived from the AUG start codon is regulated by a small upstream open reading frame, which is located within an internal ribosome entry site.[provided by RefSeq, Nov 2015]
Expression	Broad expression in lung adult (RPKM 187.9), adrenal adult (RPKM 89.7) and 24 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

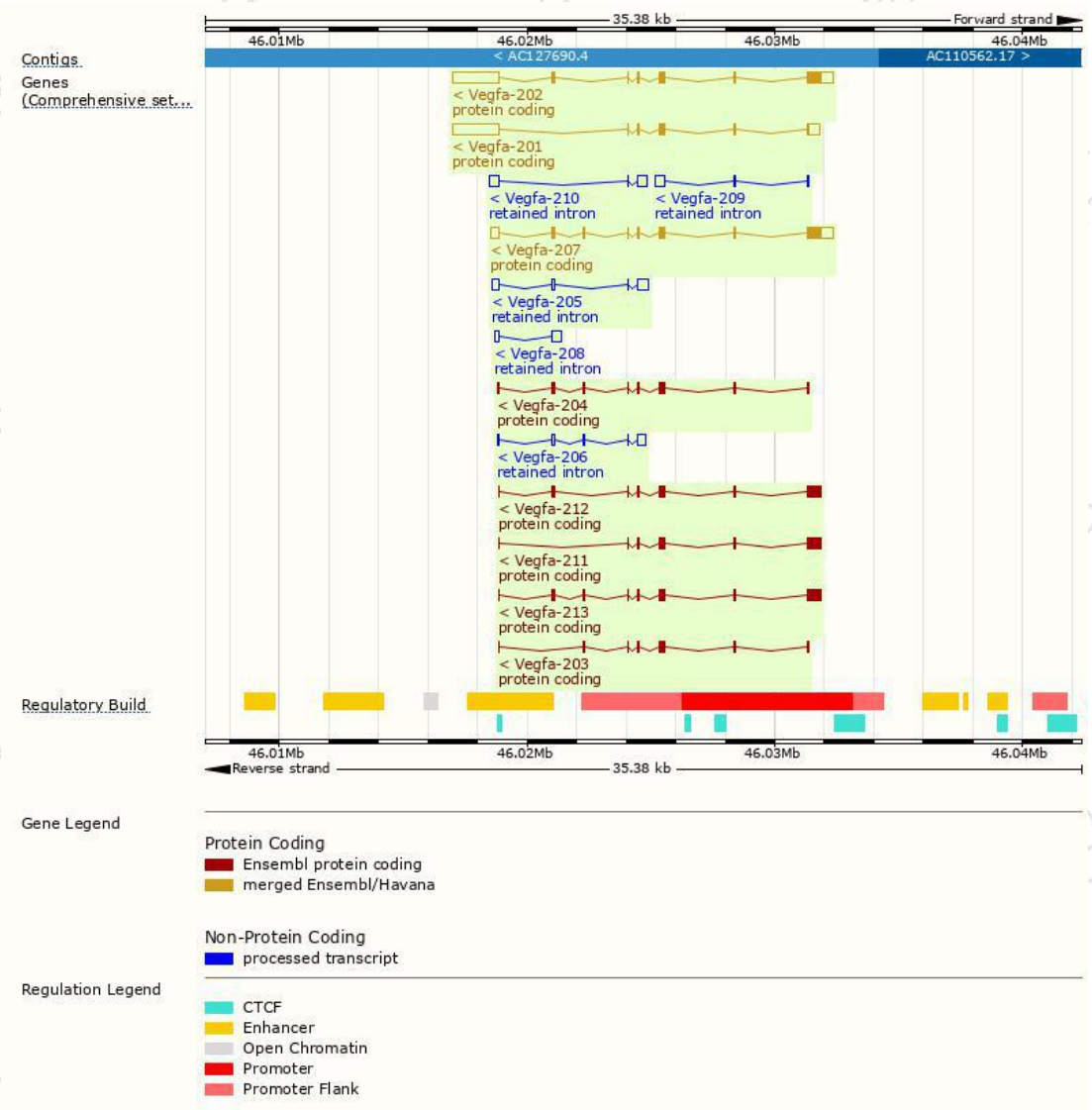
The gene has 13 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Vegfa-202	ENSMUST00000071648.11	3451	368aa	Protein coding	CCDS28818	F8WH10	TSL:1 GENCODE basic
Vegfa-201	ENSMUST00000024747.13	2765	146aa	Protein coding	CCDS70817	Q00731	TSL:1 GENCODE basic
Vegfa-207	ENSMUST00000142351.8	1973	392aa	Protein coding	CCDS28816	F8WH81	TSL:1 GENCODE basic
Vegfa-213	ENSMUST00000217017.1	1179	392aa	Protein coding	CCDS28816	A0A1L1SVG2	TSL:1 GENCODE basic
Vegfa-212	ENSMUST00000214739.1	1107	368aa	Protein coding	CCDS28818	Q00731	TSL:1 GENCODE basic
Vegfa-211	ENSMUST00000167860.8	975	324aa	Protein coding	CCDS28817	F6ZE01	TSL:1 GENCODE basic
Vegfa-204	ENSMUST00000113520.7	654	208aa	Protein coding	-	E9Q4N8	TSL:5 GENCODE basic APPRIS P1
Vegfa-203	ENSMUST00000113519.1	513	170aa	Protein coding	-	E9Q4N9	TSL:5 GENCODE basic
Vegfa-205	ENSMUST00000133605.7	874	No protein	Retained intron	-	-	TSL:2
Vegfa-210	ENSMUST00000150327.7	852	No protein	Retained intron	-	-	TSL:2
Vegfa-206	ENSMUST00000142321.1	640	No protein	Retained intron	-	-	TSL:3
Vegfa-208	ENSMUST00000143985.1	550	No protein	Retained intron	-	-	TSL:2
Vegfa-209	ENSMUST00000146149.1	463	No protein	Retained intron	-	-	TSL:1

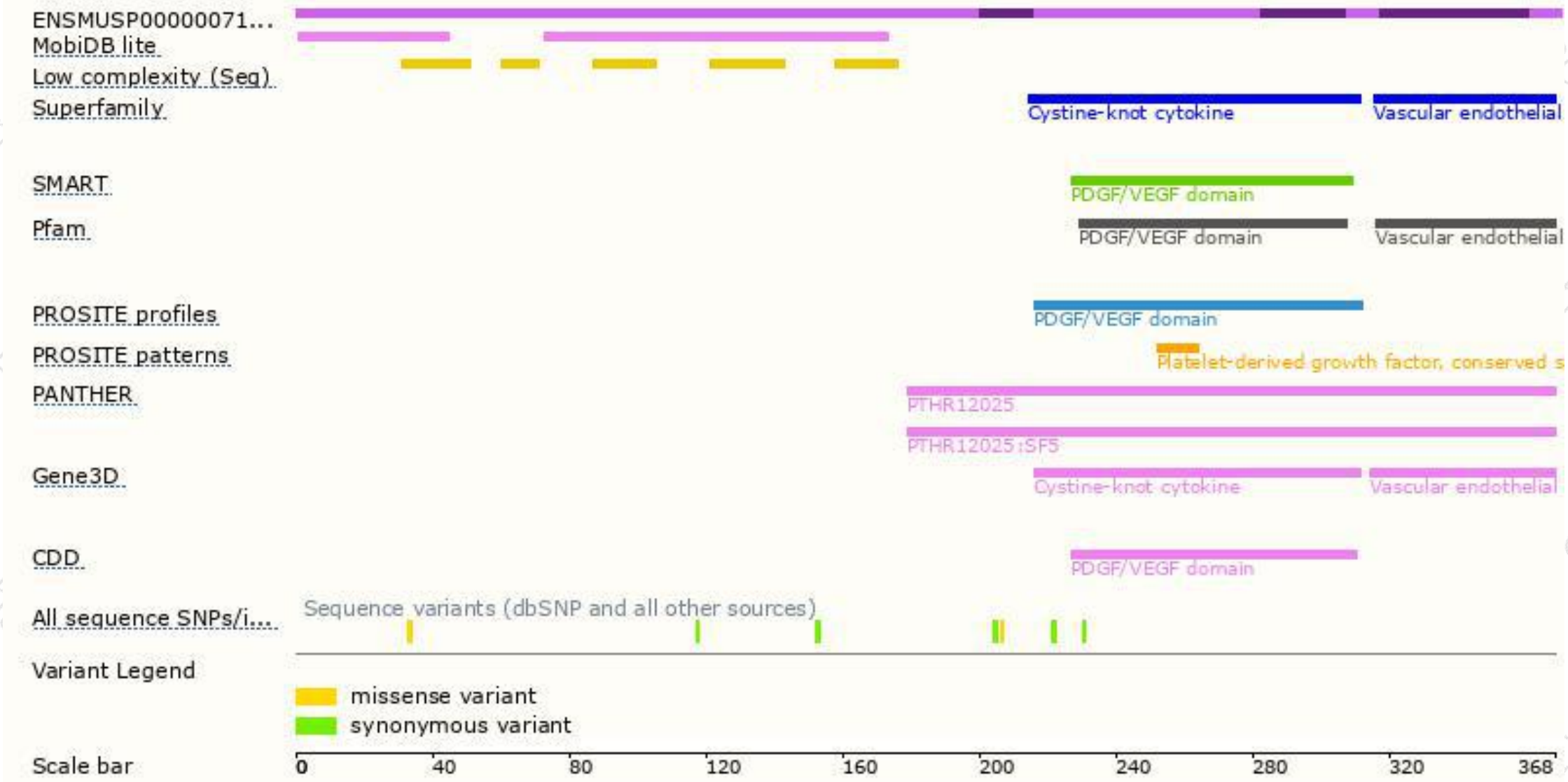
The strategy is based on the design of *Vegfa-202* transcript,The transcription is shown below



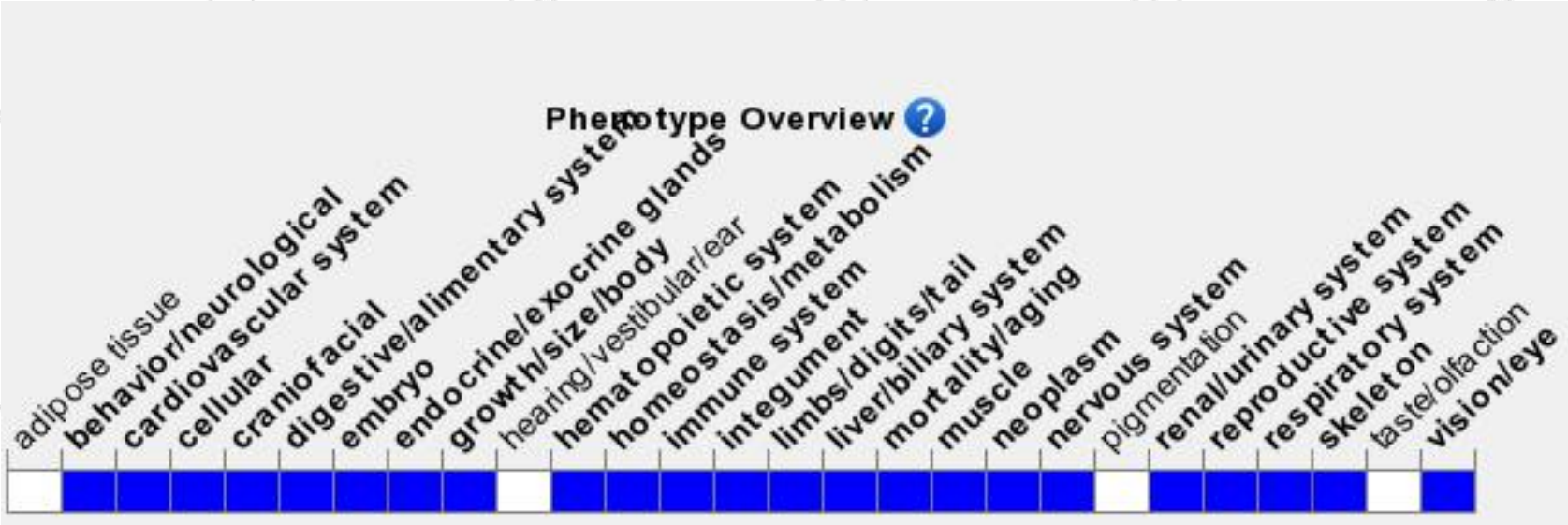
Genomic location distribution



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/).

According to the existing MGI data,Hetero- or homozygous null mutants show embryonic lethality with impaired angiogenesis and blood-island formation. Mutants selectively expressing isoform 120 or 188 exhibit vascular outgrowth/patterning defects or impaired arterial development, respectively.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd