Plekhg5 Cas9-CKO Strategy

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Project Overview

Project Name	***Plekhg5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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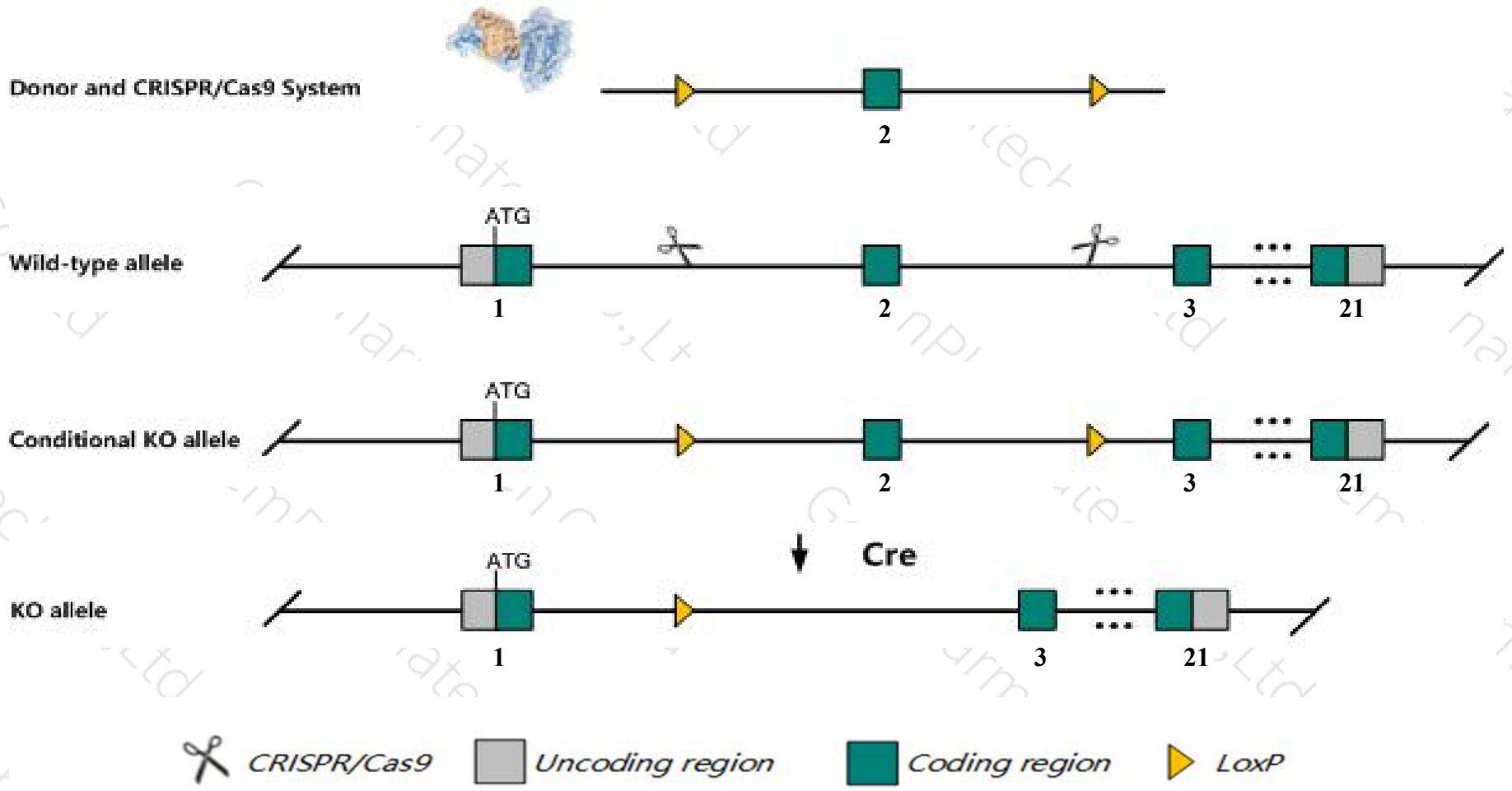
This model will use CRISPR/Cas9 technology to edit the *Plekhg5* gene. The schematic diagram is as follows:



Technical routes

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- The *Plekhg5* gene has 7 transcripts. According to the structure of *Plekhg5* gene, exon2 of *Plekhg5-202* (ENSMUST00000105661.9) transcript is recommended as the knockout region.The region contains 130bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Plekhg5* 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, mice homozygous for a knock-out allele display angiogenic defects that affect multiple organs, including sparser coronary and kidney arterial systems that appear to deficient in small diameter vessels while the major coronary and kidney arteries remain intact.

- The *Plekhg5* gene is located on the Chr4. 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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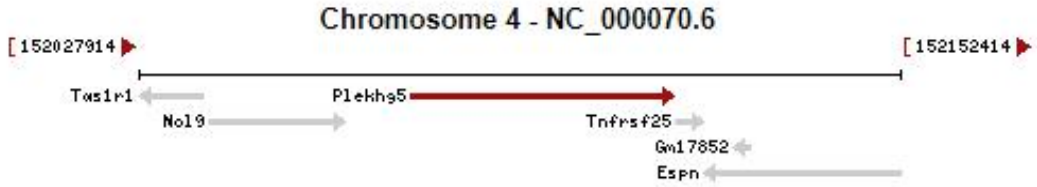
Plekhg5 pleckstrin homology domain containing, family G (with RhoGef domain) member 5 [*Mus musculus* (house mouse)]

Gene ID: 269608, updated on 24-Oct-2019

Summary

Official Symbol	Plekhg5 provided by MGI
Official Full Name	pleckstrin homology domain containing, family G (with RhoGef domain) member 5 provided by MGI
Primary source	MGI:MGI:2652860
See related	Ensembl:ENSMUSG00000039713
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	Syx; BC023181
Summary	This gene encodes a protein belonging to the Rho guanine exchange factor (GEF) family of proteins, which activate GTPases by replacing GDP with GTP. This family member is a RhoA GEF that plays a role in endothelial cell migration and tube formation. It is required for angiogenesis and may function in neuronal cell differentiation. Alternative splicing of this gene results in multiple transcript variants. [provided by RefSeq, Oct 2013]
Expression	Ubiquitous expression in lung adult (RPKM 14.6), ovary adult (RPKM 13.6) and 28 other tissues See more
Orthologs	human all

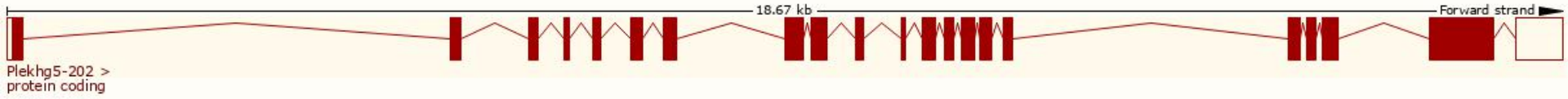


Transcript information（Ensembl）

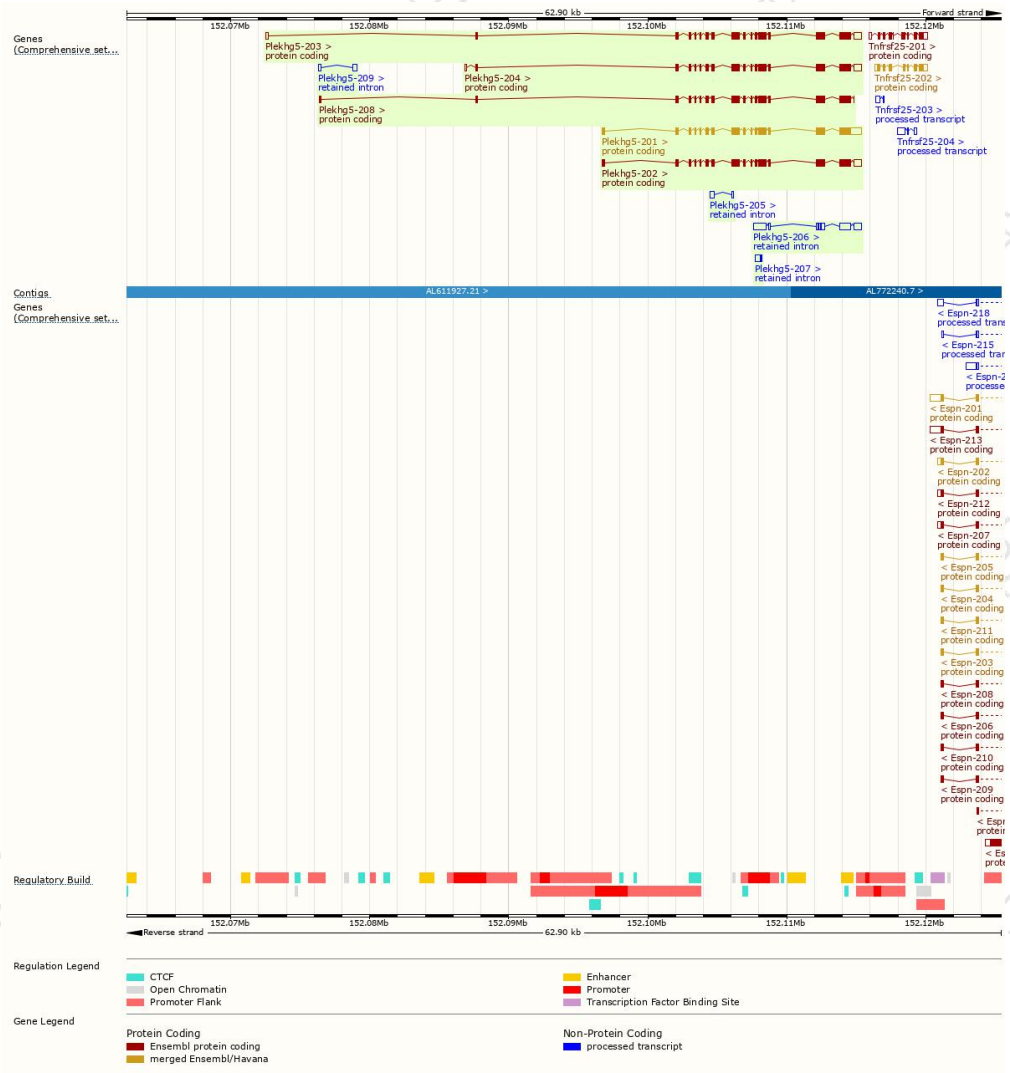
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Plekhg5-202	ENSMUST00000105661.9	3842	1073aa	Protein coding	CCDS18987	A0A2X0SG37 Q66T02	TSL:1 GENCODE basic APPRIS P2
Plekhg5-201	ENSMUST00000084115.3	4113	1071aa	Protein coding	-	Q66T02	TSL:2 GENCODE basic APPRIS ALT2
Plekhg5-203	ENSMUST00000105662.7	3933	1041aa	Protein coding	-	A0A0A0MQC2	TSL:5 GENCODE basic APPRIS ALT2
Plekhg5-204	ENSMUST00000118648.7	3818	1060aa	Protein coding	-	B1AS67	TSL:5 GENCODE basic APPRIS ALT2
Plekhg5-208	ENSMUST00000239025.1	3312	1103aa	Protein coding	-	-	GENCODE basic APPRIS ALT2
Plekhg5-206	ENSMUST00000140085.1	2792	No protein	Retained intron	-	-	TSL:1
Plekhg5-209	ENSMUST00000239128.1	485	No protein	Retained intron	-	-	-
Plekhg5-207	ENSMUST00000142412.1	387	No protein	Retained intron	-	-	TSL:3
Plekhg5-205	ENSMUST00000123615.1	384	No protein	Retained intron	-	-	TSL:3

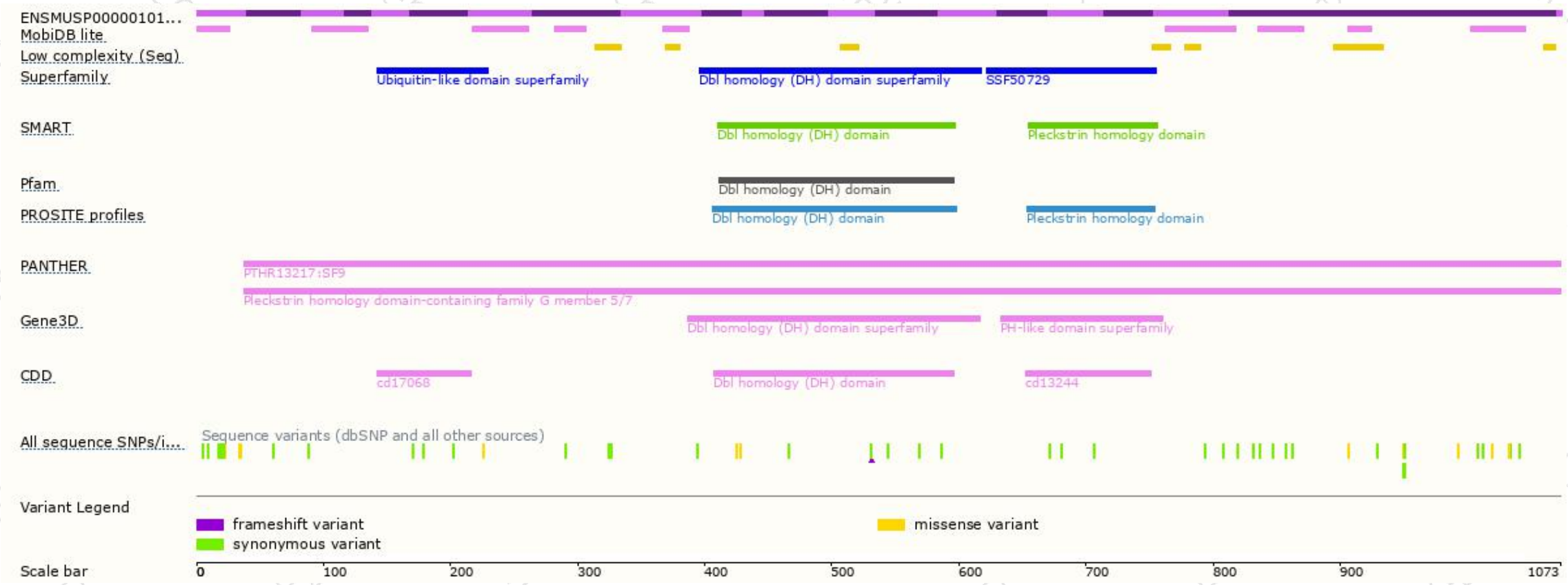
The strategy is based on the design of *Plekhg5-202* transcript, the transcription is shown below:



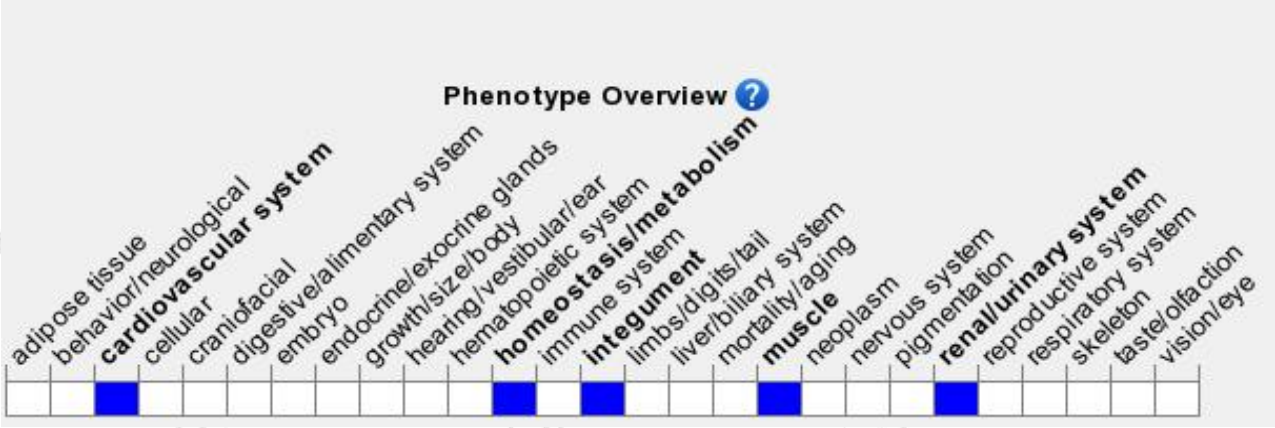
Genomic location distribution



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, mice homozygous for a knock-out allele display angiogenic defects that affect multiple organs, including sparser coronary and kidney arterial systems that appear to deficient in small diameter vessels while the major coronary and kidney arteries remain intact.

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