

Pdgfra Cas9-CKO Strategy

Designer:

Shilei Zhu

Design Date:

2021-7-8

Project Overview

Project Name

Pdgfra

Project type

Cas9-CKO

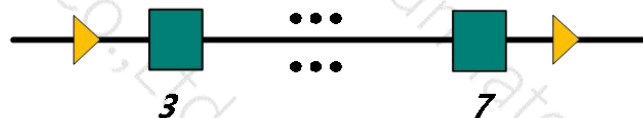
Strain background

C57BL/6JGpt

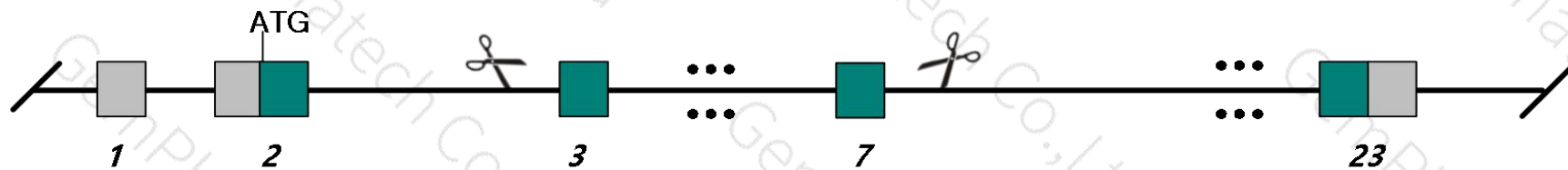
Conditional Knockout strategy

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

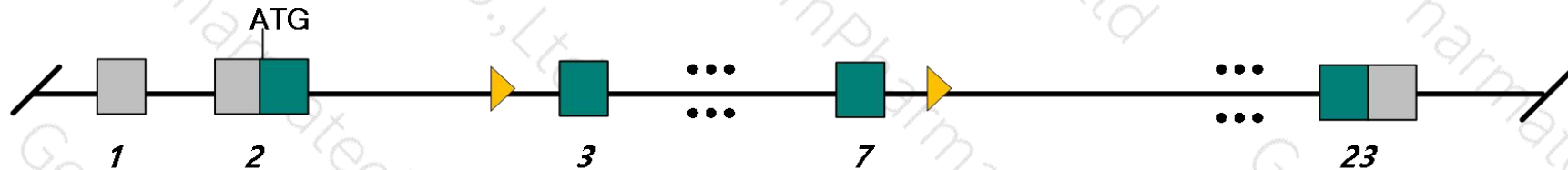
Donor and CRISPR/Cas9 System



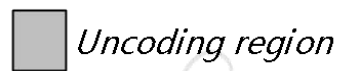
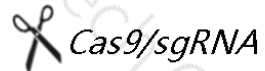
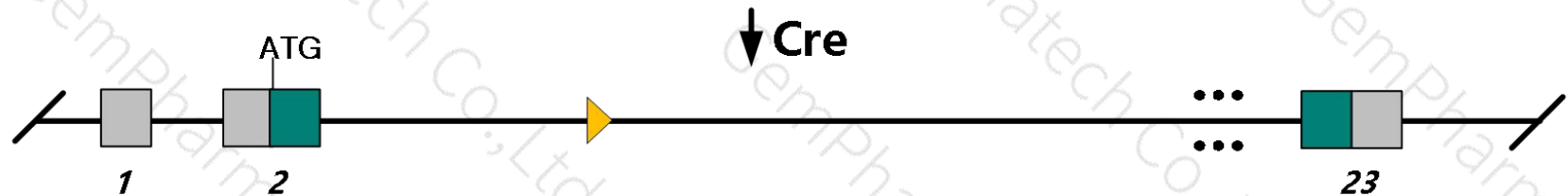
Wild-type allele



Conditional KO allele



KO allele



- The *Pdgfra* gene has 9 transcripts. According to the structure of *Pdgfra* gene, exon3-exon7 of *Pdgfra-202* (ENSMUST00000168162.4) transcript is recommended as the knockout region. The region contains 1072bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Pdgfra* 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.

- According to the existing MGI data, Homozygotes for targeted null mutations exhibit incomplete cephalic closure, increased apoptosis of neural crest cells, impaired myotome and testis formation, abnormal mucosal linings, thoracic skeletal defects, and midgestational lethality.
- The *Pdgfra* 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.
- 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)



Pdgfra platelet derived growth factor receptor, alpha polypeptide [Mus musculus (house mouse)]

Gene ID: 18595, updated on 9-Apr-2019

Summary



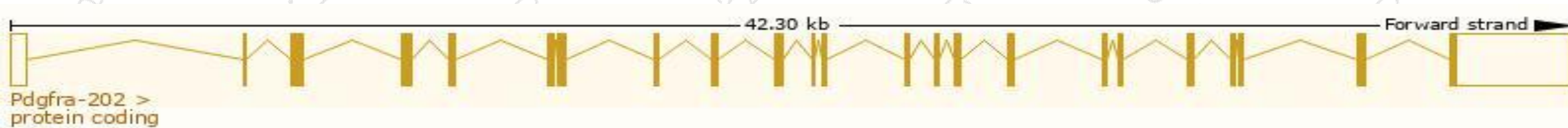
Official Symbol	Pdgfra provided by MGI
Official Full Name	platelet derived growth factor receptor, alpha polypeptide provided by MGI
Primary source	MGI:MGI:97530
See related	Ensembl:ENSMUSG00000029231
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Al115593, CD140a, Pdgfr-2
Summary	This gene encodes a member of the receptor tyrosine kinase family of proteins. Binding of platelet-derived growth factor protein ligands to this receptor triggers receptor dimerization and autophosphorylation, resulting in the activation of several downstream signaling pathways. Signaling through the encoded receptor plays a role in gastrulation and the development of nearly all organ systems. Mice lacking a functional copy of this gene reportedly exhibit defects in lung, skeleton, testis and the central nervous system, and die soon after birth. Alternative splicing and intronic polyadenylation of gene transcripts have been implicated in muscle regeneration and fibrosis in adult mice. [provided by RefSeq, Jan 2017]
Expression	Broad expression in limb E14.5 (RPKM 29.6), lung adult (RPKM 20.2) and 19 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

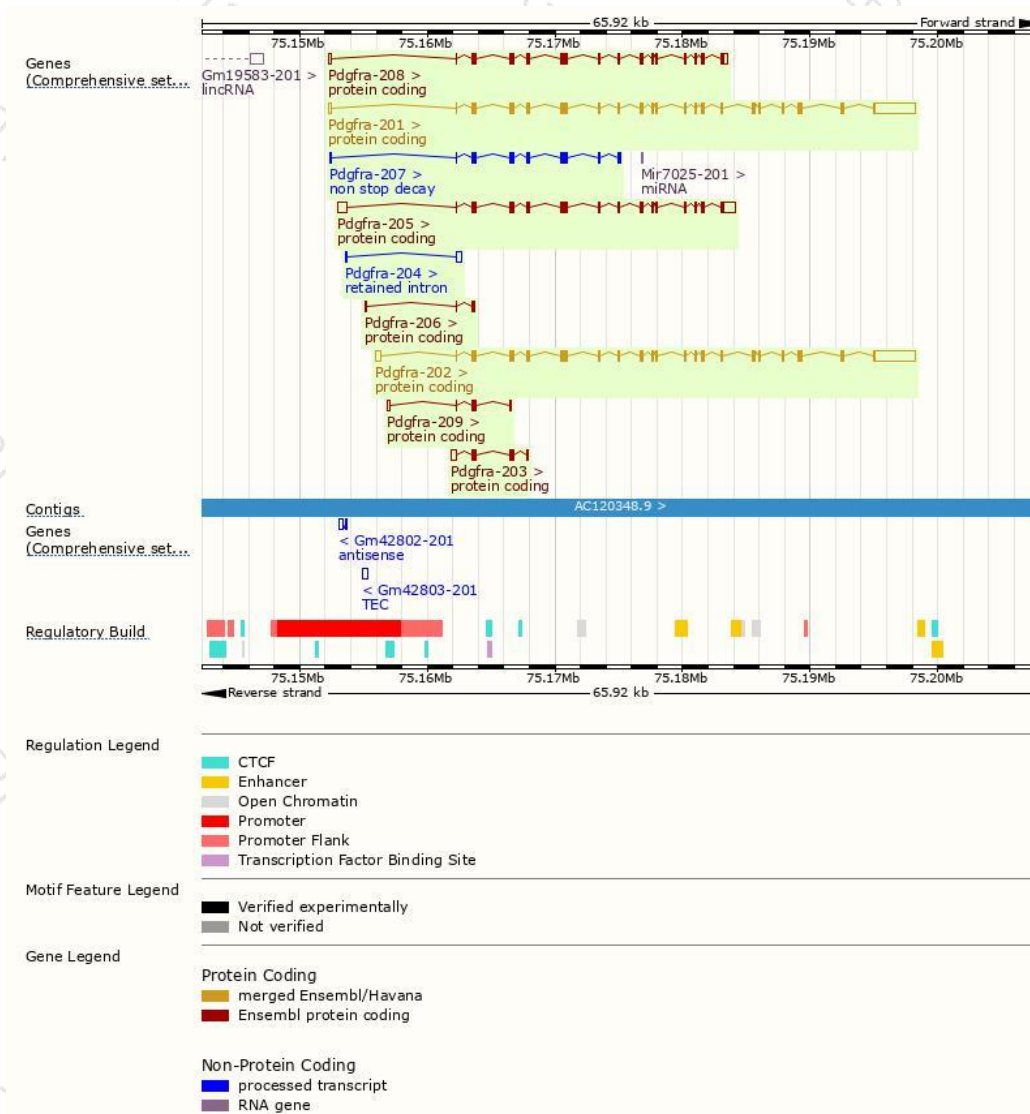
The gene has 9 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pdgfra-202	ENSMUST00000168162.4	6834	1089aa	Protein coding	CCDS19351	P26618	TSL:1 GENCODE basic APPRIS P1
Pdgfra-201	ENSMUST00000000476.14	6549	1089aa	Protein coding	CCDS19351	P26618	TSL:1 GENCODE basic APPRIS P1
Pdgfra-205	ENSMUST00000201711.3	4088	790aa	Protein coding	-	P26618	TSL:1 GENCODE basic
Pdgfra-208	ENSMUST00000202681.3	2876	790aa	Protein coding	-	P26618	TSL:1 GENCODE basic
Pdgfra-203	ENSMUST00000200822.1	1138	243aa	Protein coding	-	A0A0J9YVF3	CDS 3' incomplete TSL:5
Pdgfra-209	ENSMUST00000202992.3	615	139aa	Protein coding	-	A0A0J9YUD9	CDS 3' incomplete TSL:3
Pdgfra-206	ENSMUST00000202161.3	442	88aa	Protein coding	-	A0A0J9YV49	CDS 3' incomplete TSL:2
Pdgfra-207	ENSMUST00000202186.3	1561	481aa	Non stop decay	-	A0A0J9YV87	TSL:1
Pdgfra-204	ENSMUST00000201241.1	603	No protein	Retained intron	-	-	TSL:1

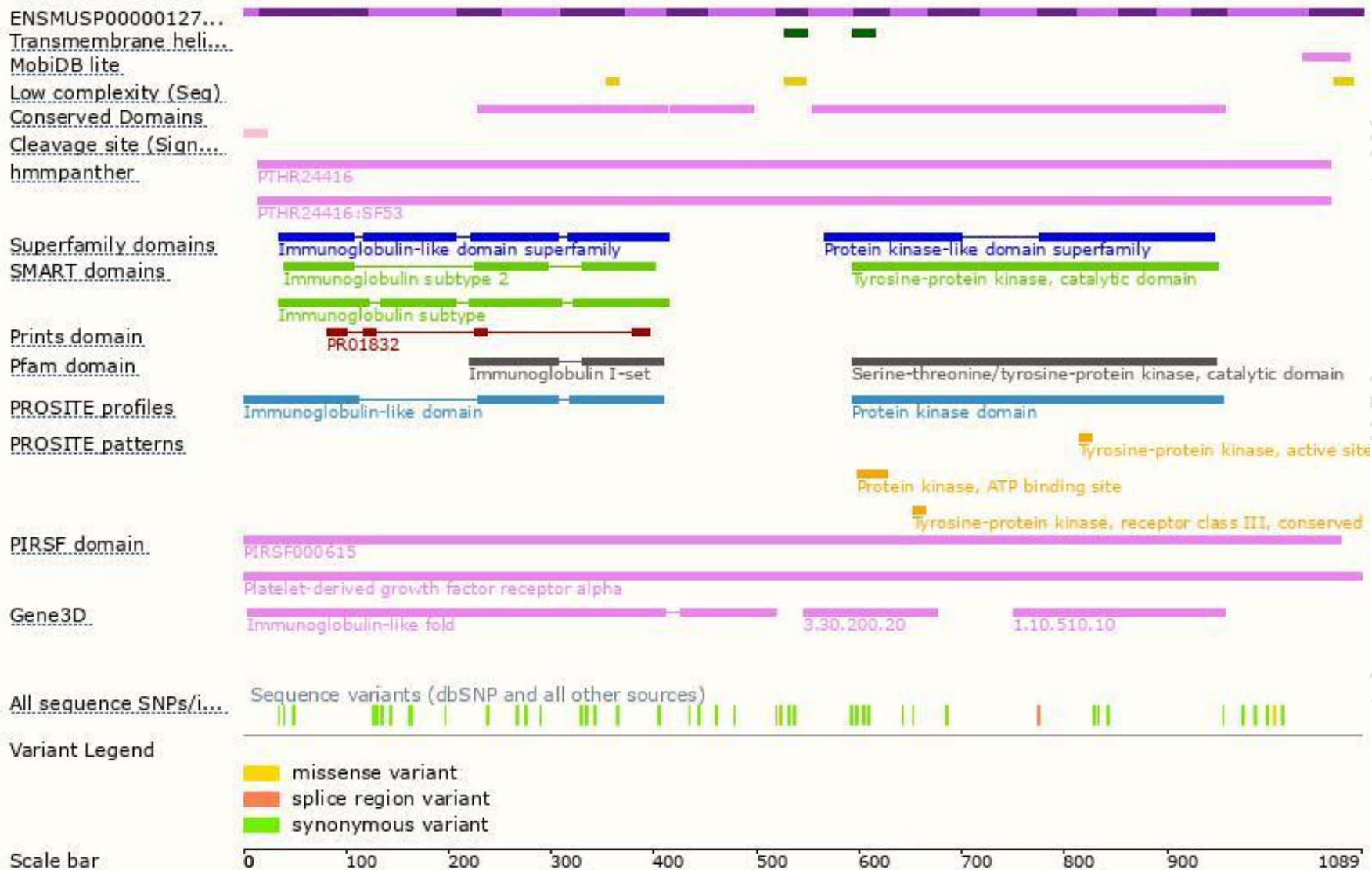
The strategy is based on the design of *Pdgfra-202* transcript, The transcription is shown below



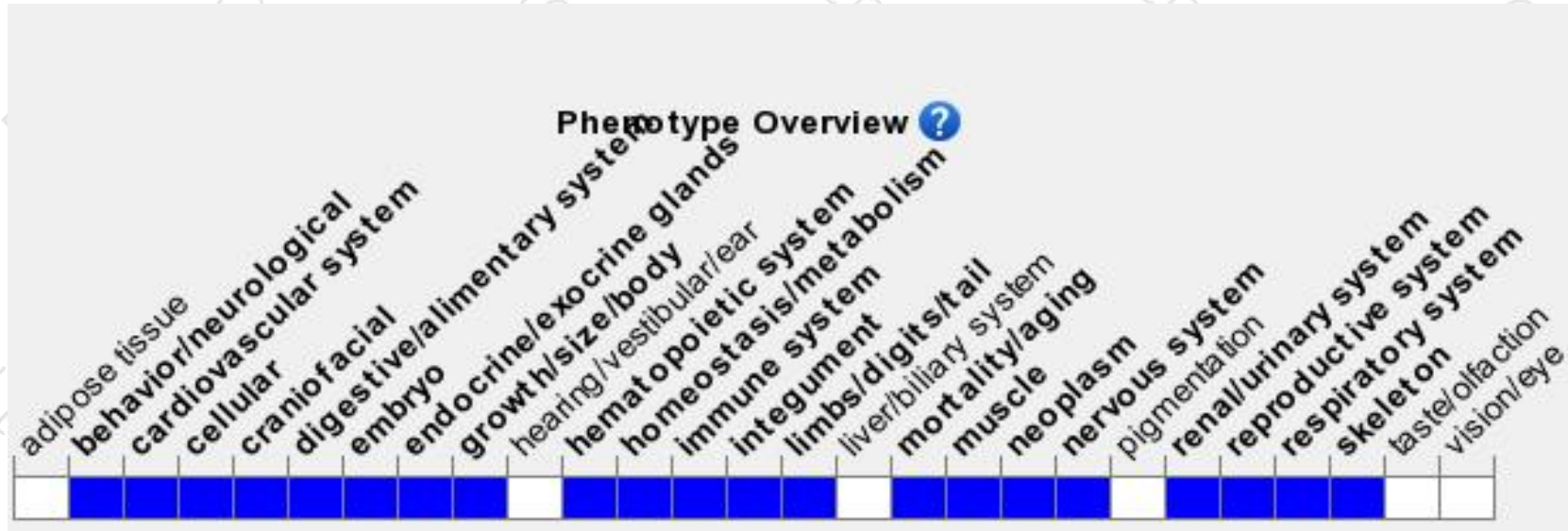
Genomic location distribution



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, Homozygotes for targeted null mutations exhibit incomplete cephalic closure, increased apoptosis of neural crest cells, impaired myotome and testis formation, abnormal mucosal linings, thoracic skeletal defects, and midgestational lethality.

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

Tel: 400-9660890

