Pik3r4 Cas9-CKO Strategy

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Project Overview

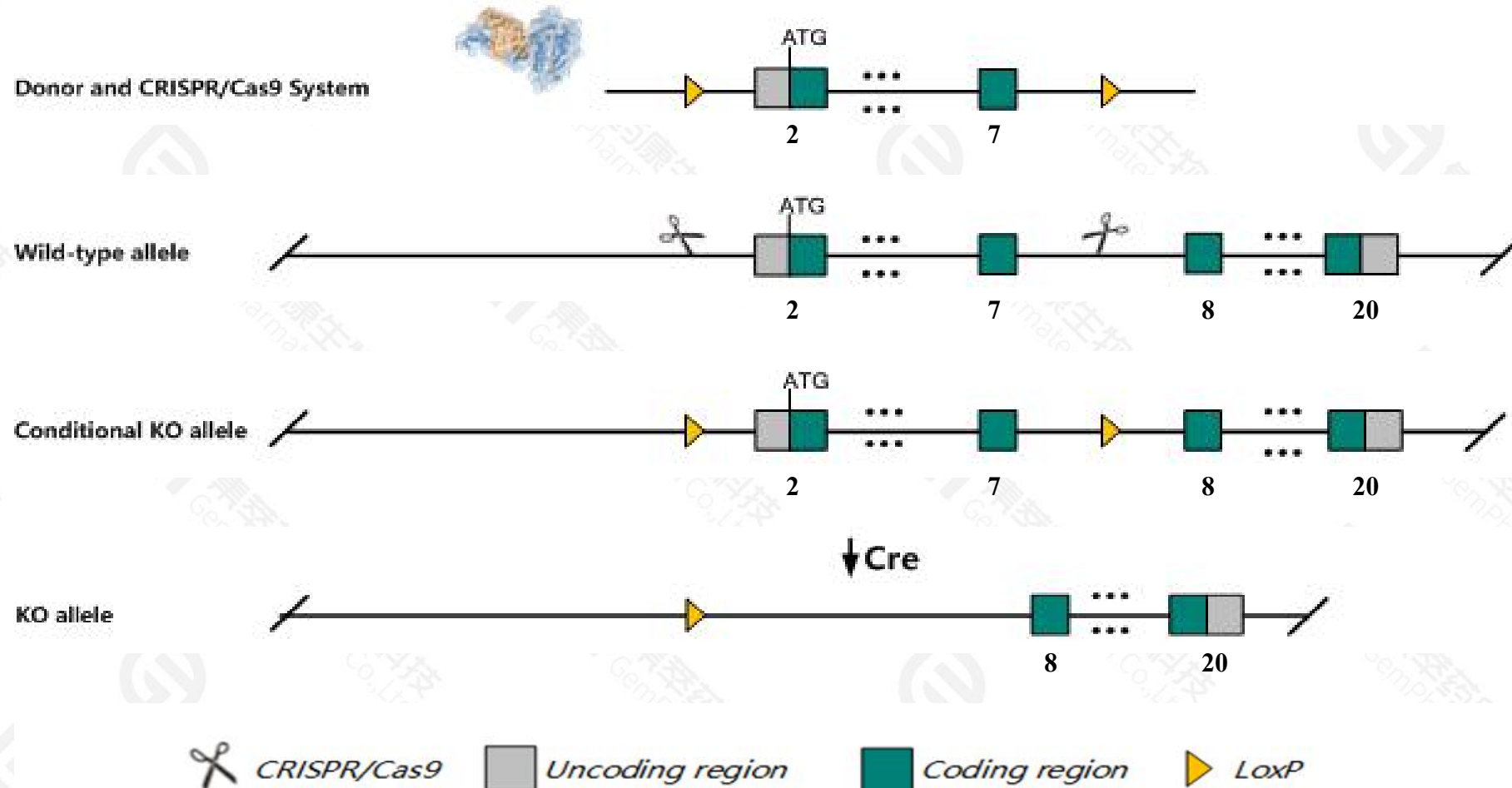|	
Project Name	*Pik3r4*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pik3r4* gene. The schematic diagram is as follows:

Technical routes

➤ The *Pik3r4* gene has 11 transcripts. According to the structure of *Pik3r4* gene, exon2-exon7 of *Pik3r4-210*(ENSMUST00000191268.7) 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 *Pik3r4* 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 exhibit early embryonic lethality before E7.5. Mice homozygous for a conditional allele activated in muscles exhibit symptoms of autophagic vacuolar myopathies.

➢ The *Pik3r4* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

Pik3r4 phosphoinositide-3-kinase regulatory subunit 4 [Mus musculus (house mouse)]

Gene ID: 75669, updated on 26-Jan-2021

Summary

|	
Official Symbol	Pik3r4 provided by MGI
Official Full Name	phosphoinositide-3-kinase regulatory subunit 4 provided by MGI
Primary source	MGI:MGI:1922919
See related	Ensembl:ENSMUSG00000032571
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2210010O15Rik, C730038E05Rik, C85833, D9Ertd418e, Vp, Vps15, p15, p150
Expression	Ubiquitous expression in ovary adult (RPKM 8.1), spleen adult (RPKM 7.8) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 11 transcripts,all transcripts are shown below:

| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pik3r4-210	ENSMUST00000191268.7	4756	1358aa	Protein coding	CCDS40753		TSL:1 , GENCODE basic , APPRIS P1 ,
Pik3r4-201	ENSMUST00000065778.13	4711	1358aa	Protein coding	CCDS40753		TSL:1 , GENCODE basic , APPRIS P1 ,
Pik3r4-206	ENSMUST00000188784.2	1295	305aa	Protein coding	-		TSL:1 , GENCODE basic ,
Pik3r4-202	ENSMUST00000186943.2	770	208aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Pik3r4-208	ENSMUST00000190358.2	611	174aa	Protein coding	-		CDS 3' incomplete , TSL:2 ,
Pik3r4-204	ENSMUST00000187573.7	4929	No protein	Retained intron	-		TSL:1 ,
Pik3r4-203	ENSMUST00000187446.2	4124	No protein	Retained intron	-		TSL:1 ,
Pik3r4-209	ENSMUST00000191117.7	3613	No protein	Retained intron	-		TSL:1 ,
Pik3r4-211	ENSMUST00000214254.2	1358	No protein	Retained intron	-		TSL:1 ,
Pik3r4-207	ENSMUST00000189691.2	969	No protein	Retained intron	-		TSL:2 ,
Pik3r4-205	ENSMUST00000188385.2	634	No protein	Retained intron	-		TSL:3 ,

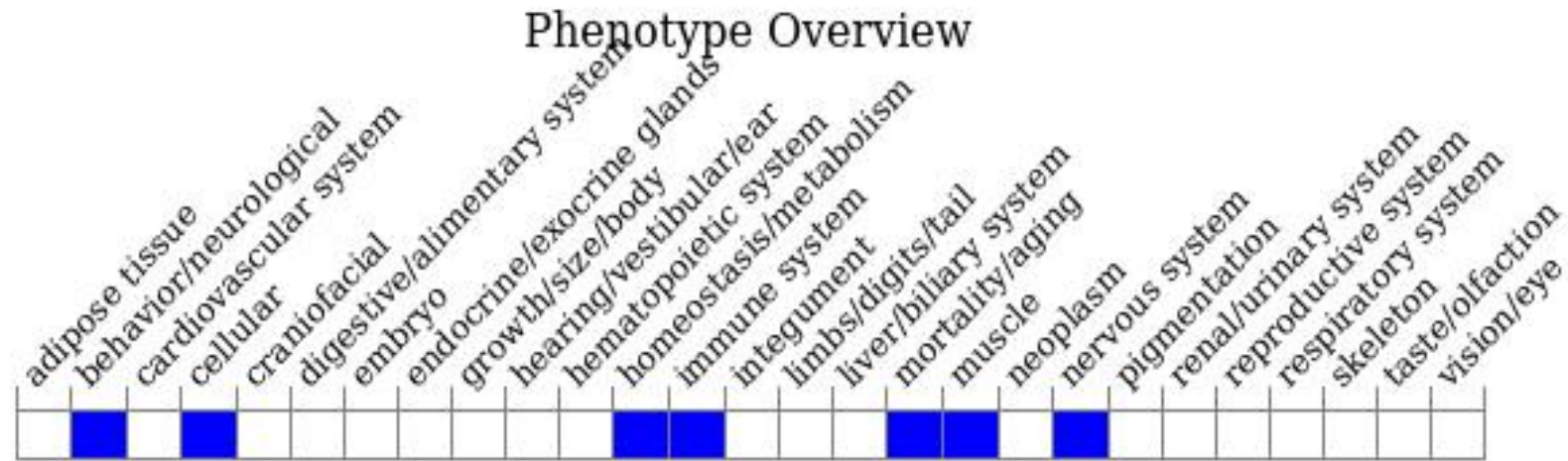The strategy is based on the design of *Pik3r4-210* transcript,the transcription is shown below:

Genomic location distribution
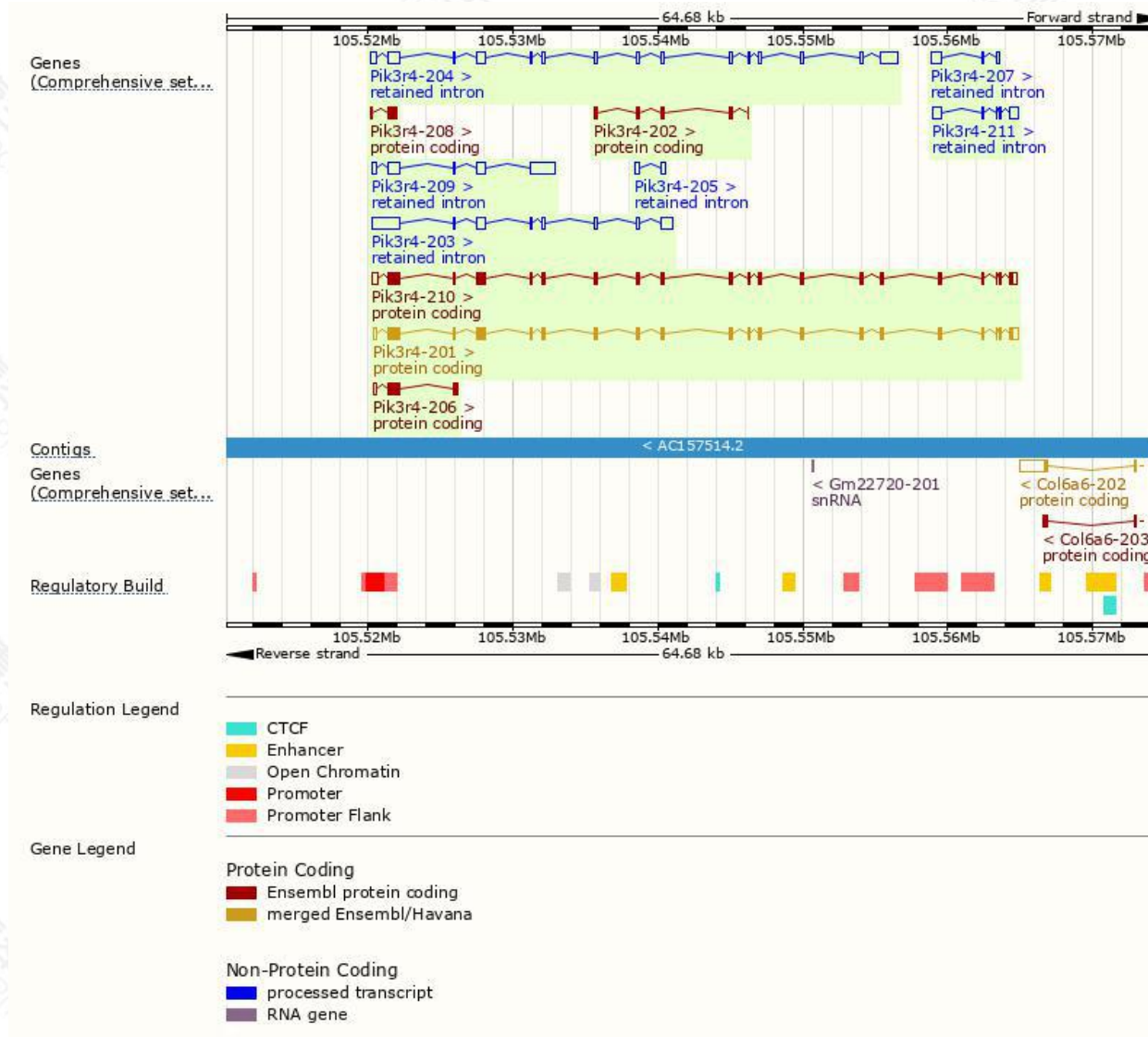
Protein domain
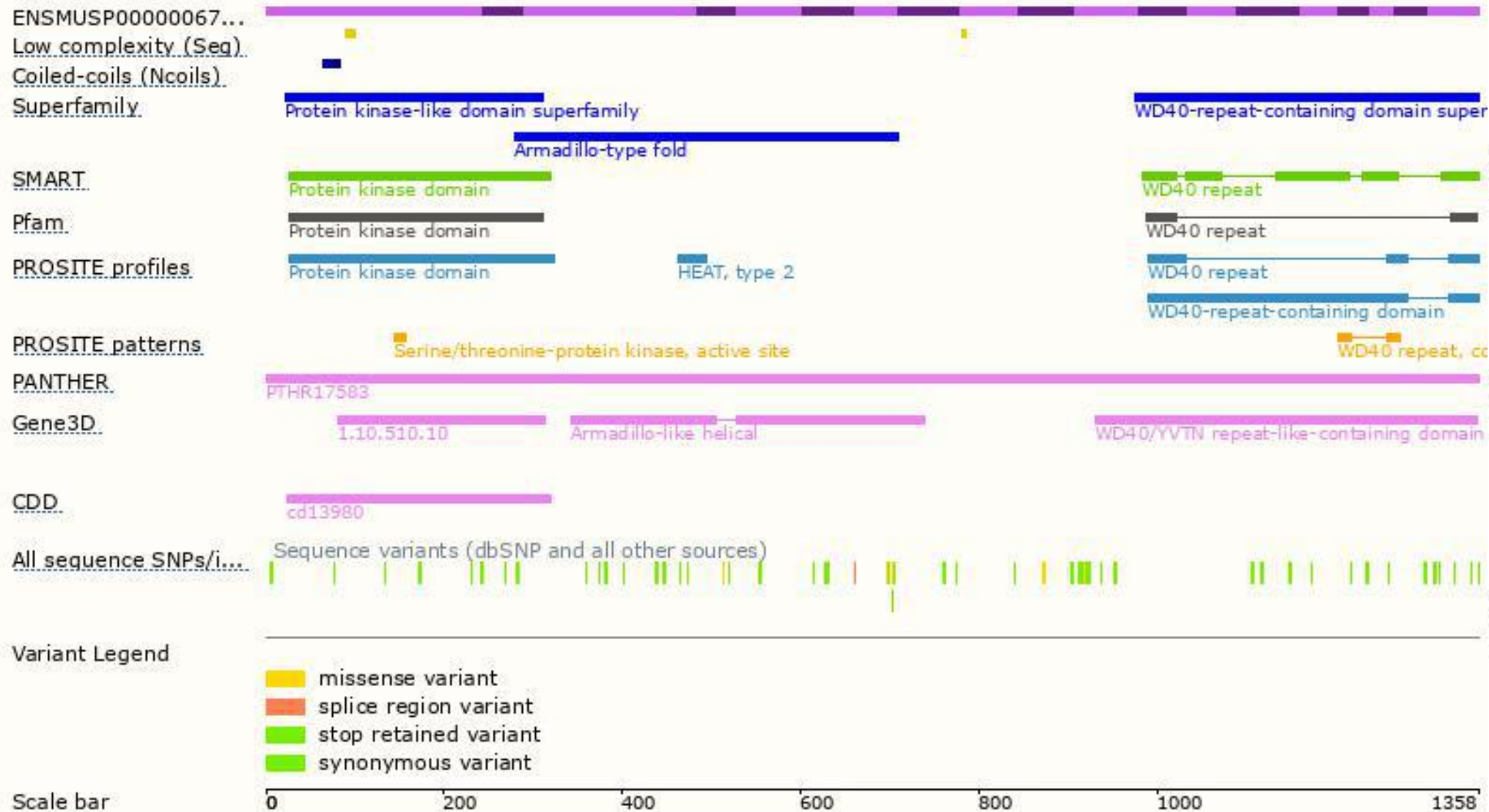
Mouse phenotype description(MGI)

Phenotype Overview

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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