Pikfyve Cas9-KO Strategy

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Project Overview

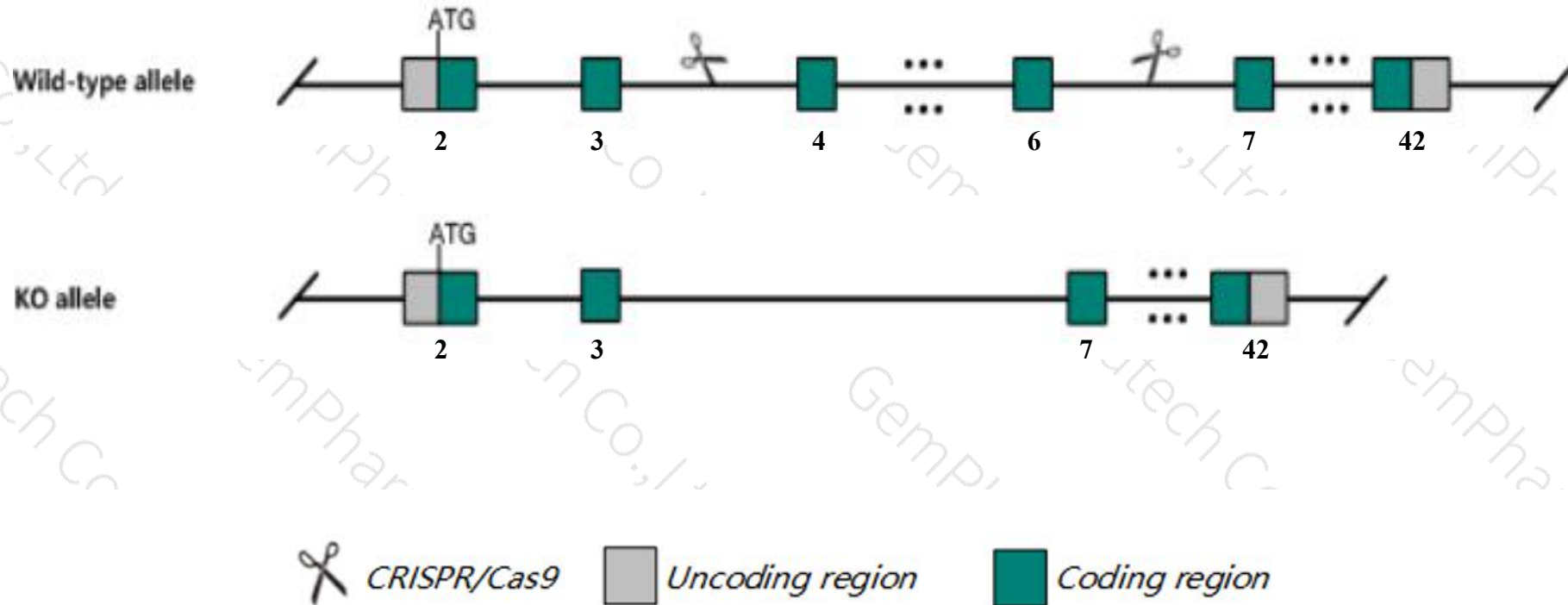|	
Project Name	*Pikfyve*
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

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

Technical routes

➢ The *Pikfyve* gene has 11 transcripts. According to the structure of *Pikfyve* gene, exon4-exon6 of *Pikfyve-202* (ENSMUST00000097707.4) transcript is recommended as the knockout region.The region contains 499bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Pikfyve* gene. The brief process is as follows: CRISPR/Cas9 syster

Notice

➢ According to the existing MGI data,mice homozygous for a null allele die prior to implantation with reduced numbers of inner cell mass and trophectoderm cells and blastocoele abnormalities. mice homozygous for a second null allele show embryonic lethality between somite formation and embryo turning with abnormal visceral endoderm.

➢ The *Pikfyve* gene is located on the Chr1. 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）

Pikfyve phosphoinositide kinase, FYVE type zinc finger containing [Mus musculus (house mouse)]

Gene ID: 18711, updated on 13-Mar-2020

Summary

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Official Symbol	Pikfyve provided by MGI
Official Full Name	phosphoinositide kinase, FYVE type zinc finger containing provided by MGI
Primary source	MGI:MGI:1335106
See related	Ensembl:ENSMUSG00000025949
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	5230400C17Rik, Pip5k, Pip5k3, Pipk5k3, PipkIII, p235
Expression	Ubiquitous expression in CNS E11.5 (RPKM 3.4), CNS E18 (RPKM 3.3) 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
Pikfyve-201	ENSMUST00000081154.13	11237	2052aa	Protein coding	CCDS35601	D3Z5N5	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Pikfyve-202	ENSMUST00000097707.4	6641	2097aa	Protein coding	CCDS78600	Q9Z1T6	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Pikfyve-209	ENSMUST00000190058.6	3277	158aa	Nonsense mediated decay	-	A0A087WQI1	TSL:5
Pikfyve-203	ENSMUST00000185263.6	443	66aa	Nonsense mediated decay	-	A0A087WS78	TSL:3
Pikfyve-211	ENSMUST00000213081.1	1790	No protein	Processed transcript	-	-	TSL:5
Pikfyve-208	ENSMUST00000189925.6	1303	No protein	Processed transcript	-	-	TSL:1
Pikfyve-205	ENSMUST00000186404.6	4266	No protein	Retained intron	-	-	TSL:5
Pikfyve-207	ENSMUST00000188799.6	4083	No protein	Retained intron	-	-	TSL:1
Pikfyve-206	ENSMUST00000187579.1	2250	No protein	Retained intron	-	-	TSL:1
Pikfyve-204	ENSMUST00000185317.6	1044	No protein	Retained intron	-	-	TSL:1
Pikfyve-210	ENSMUST00000190847.1	651	No protein	Retained intron	-	-	TSL:5

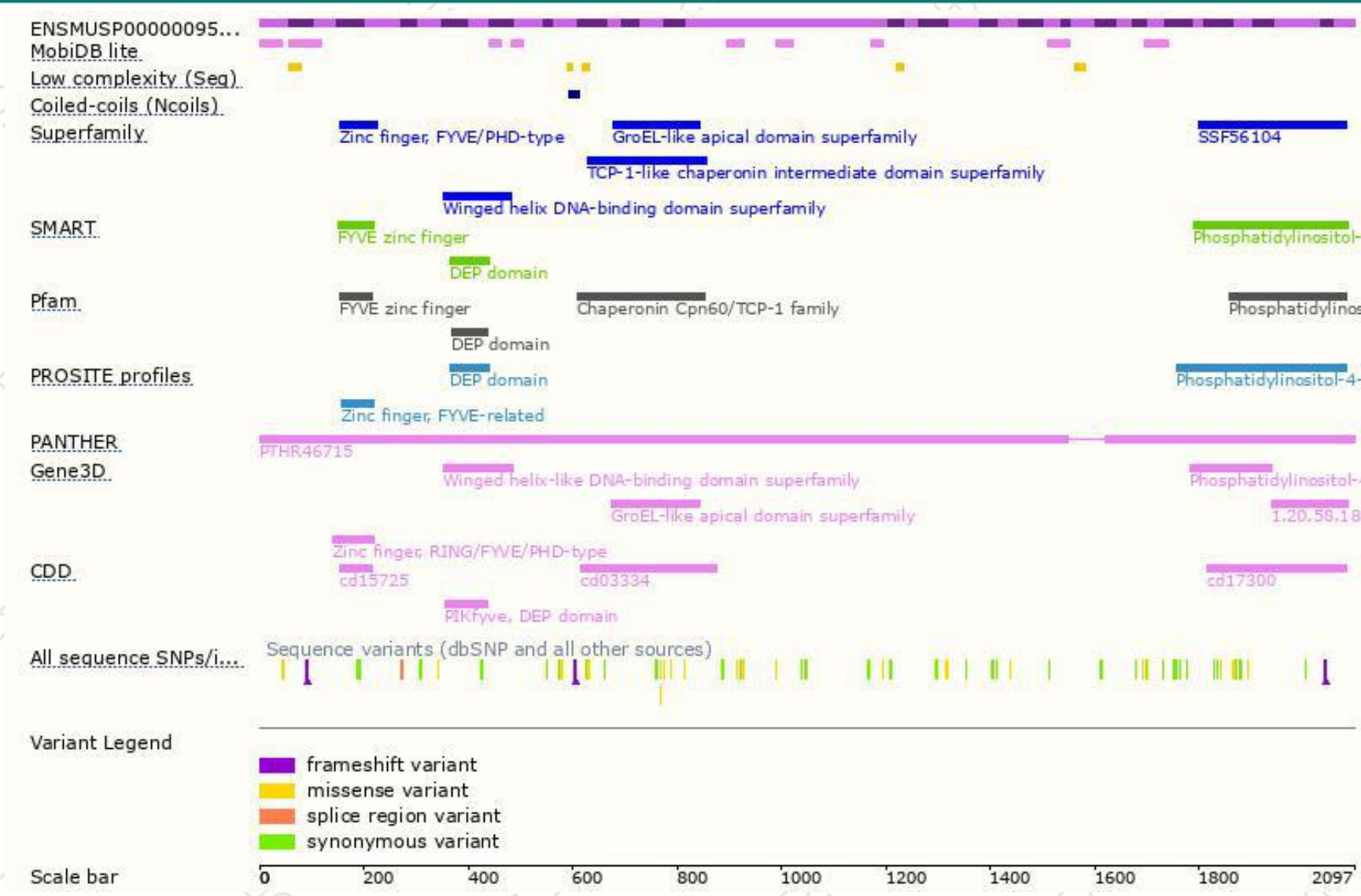The strategy is based on the design of *Pikfyve-202* transcript,the transcription is shown below:
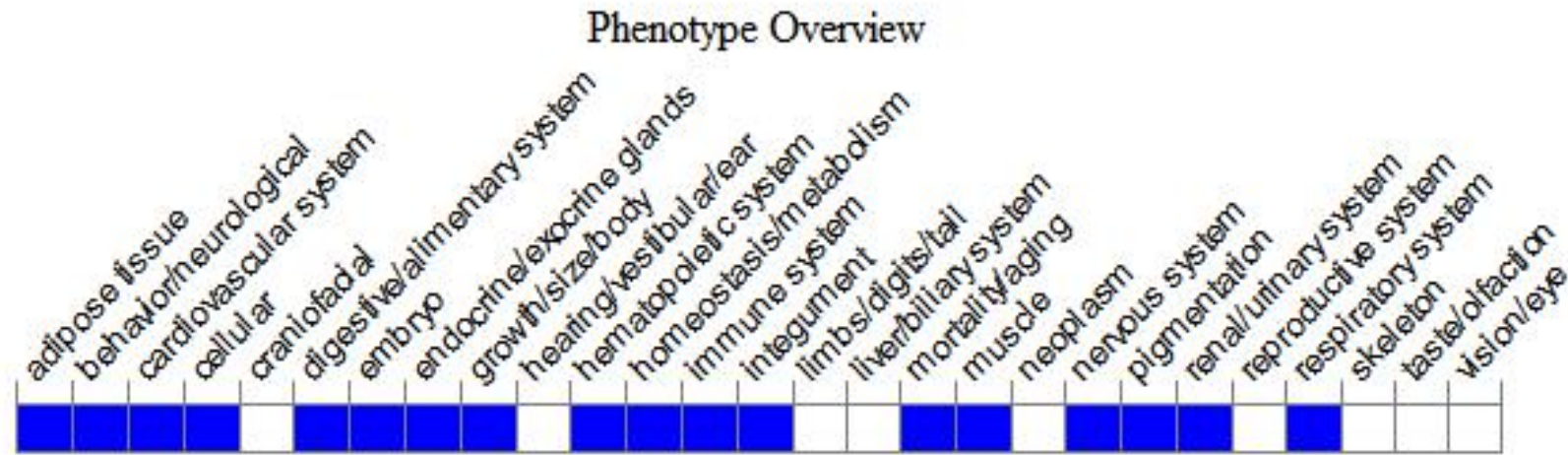
Genomic location distribution
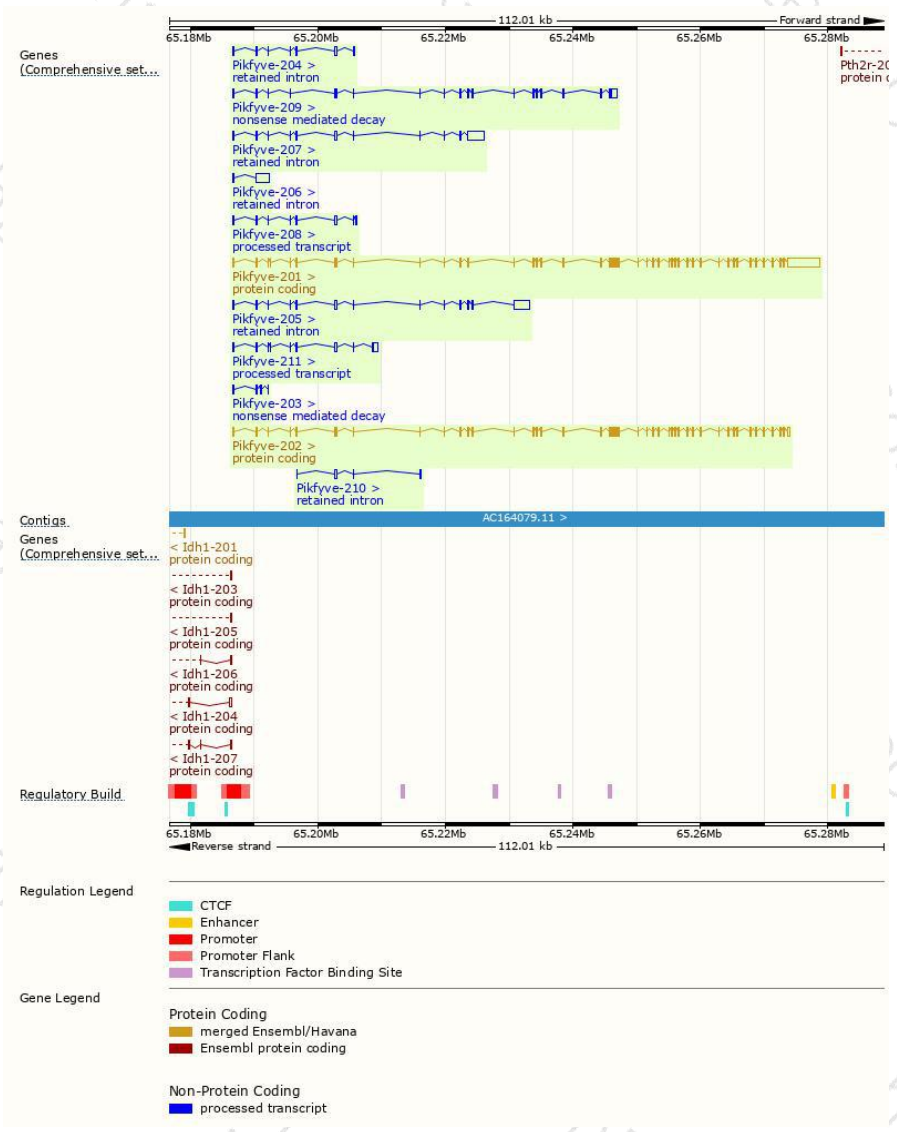
Protein domain

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/).

According to the existing MGI data,mice homozygous for a null allele die prior to implantation with reduced numbers of inner cell mass and trophectoderm cells and blastocoele abnormalities. Mice homozygous for a second null allele show embryonic lethality between somite formation and embryo turning with abnormal visceral endoderm.

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