Pacrgl Cas9-CKO Strategy

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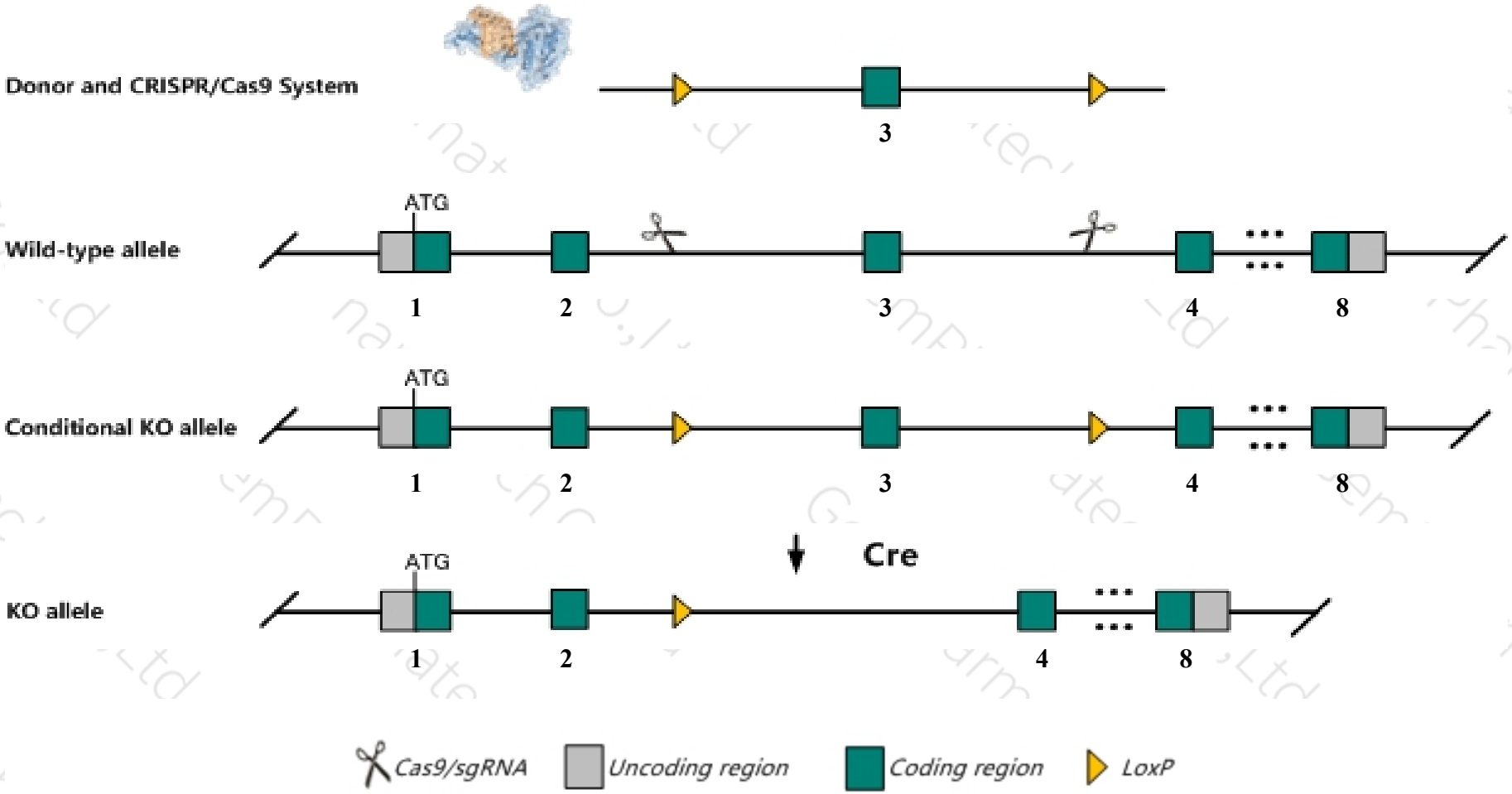
Design Date: 2020-11-17

Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Pacrgl* gene has 9 transcripts. According to the structure of *Pacrgl* gene, exon3 of *5730480H06Rik-205*(ENSMUST00000196950.4) transcript is recommended as the knockout region.The region contains 68bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Pacrgl* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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

➢ The KO region contains functional region of the Pacrgl gene.Knockout the region may affect the function of 5730480H06Rik gene.

➢ The *Pacrgl* 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）

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Pacrgl PARK2 co-regulated-like [Mus musculus (house mouse)]

Gene ID: 66768, updated on 13-Mar-2020

Summary

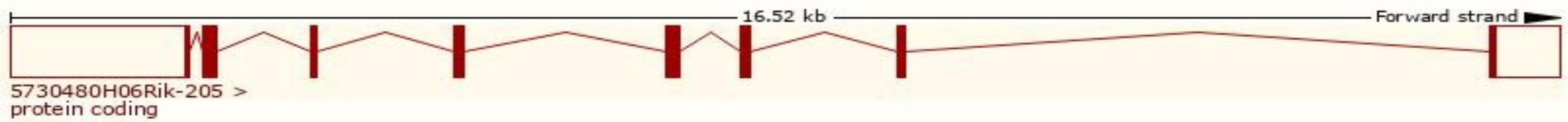
Official Symbol	Pacrgl provided by MGI
Official Full Name	PARK2 co-regulated-like provided byMGI
Primary source	MGI:MGI:1914018
See related	Ensembl:ENSMUSG00000029089
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4933428G09Rik
Expression	Broad expression in testis adult (RPKM 12.7), CNS E11.5 (RPKM 4.1) and 21 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

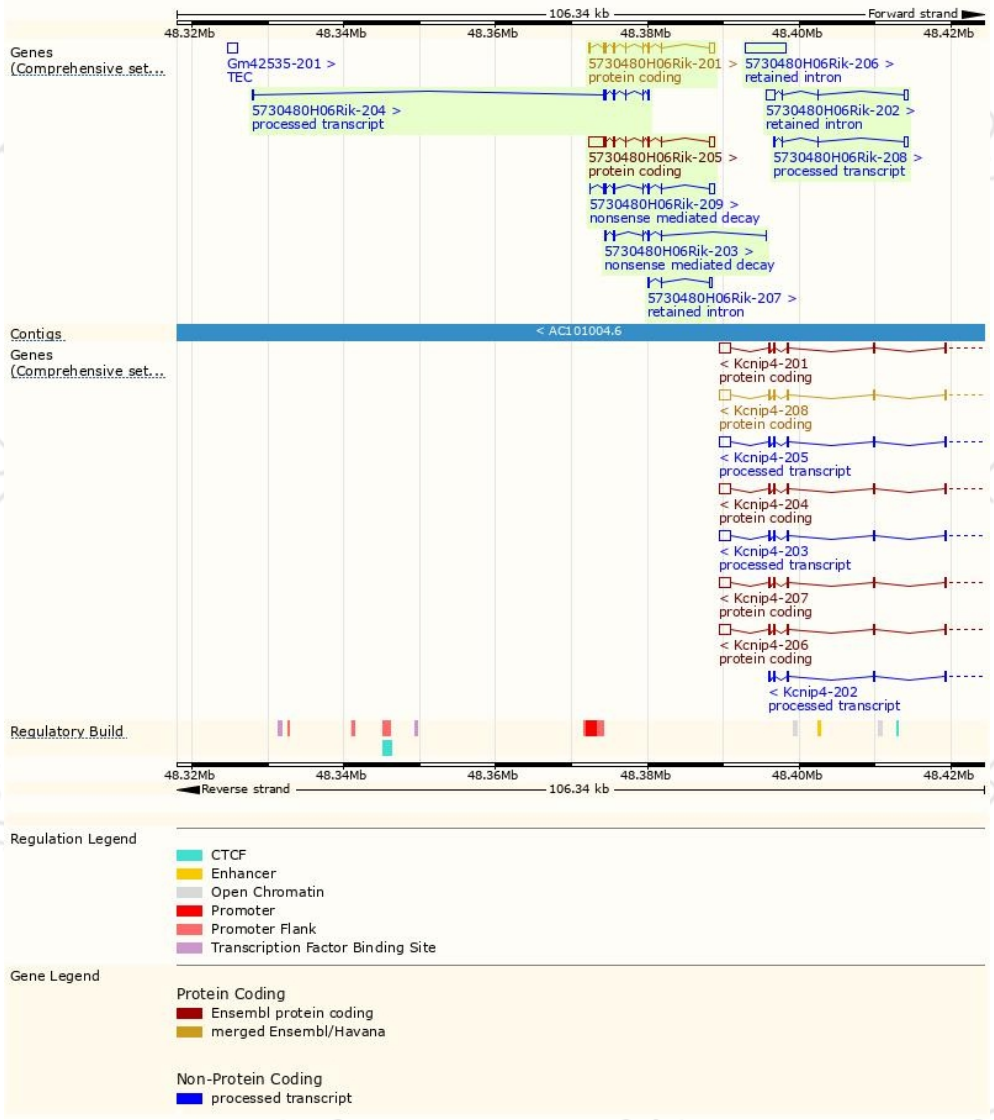
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
5730480H06Rik-205	ENSMUST00000196950.4	3300	248aa	Protein coding	CCDS19281	Q9D3X5	TSL:1 GENCODE basic APPRIS P1
5730480H06Rik-201	ENSMUST00000030968.6	1616	248aa	Protein coding	CCDS19281	Q9D3X5	TSL:1 GENCODE basic APPRIS P1
5730480H06Rik-209	ENSMUST00000200566.4	1422	93aa	Nonsense mediated decay	-	A0A0G2JE33	TSL:5
5730480H06Rik-203	ENSMUST00000195960.4	549	59aa	Nonsense mediated decay	-	A0A0G2JE98	CDS 5' incomplete TSL:2
5730480H06Rik-204	ENSMUST00000196604.1	705	No protein	Processed transcript	-	-	TSL:3
5730480H06Rik-208	ENSMUST00000199818.1	587	No protein	Processed transcript	-	-	TSL:3
5730480H06Rik-206	ENSMUST00000197072.1	5405	No protein	Retained intron	-	-	TSL:NA
5730480H06Rik-202	ENSMUST00000176521.5	1702	No protein	Retained intron	-	-	TSL:1
5730480H06Rik-207	ENSMUST00000197915.1	648	No protein	Retained intron	-	-	TSL:3

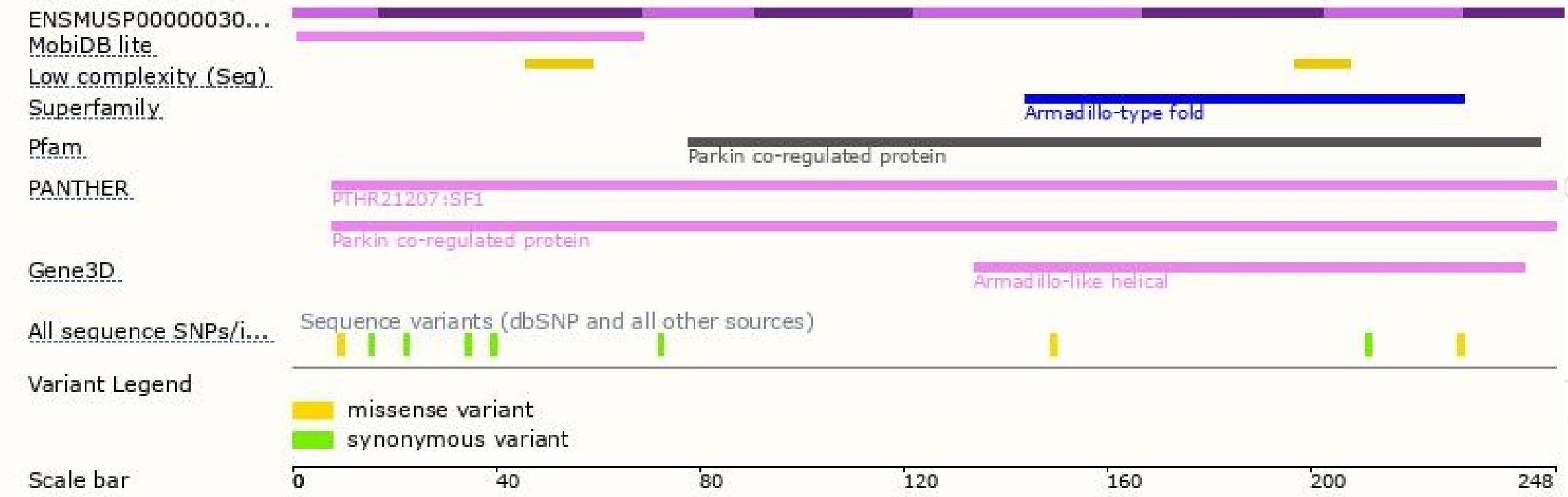
The strategy is based on the design of *5730480H06Rik-205* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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