Pqlc3 Cas9-CKO Strategy

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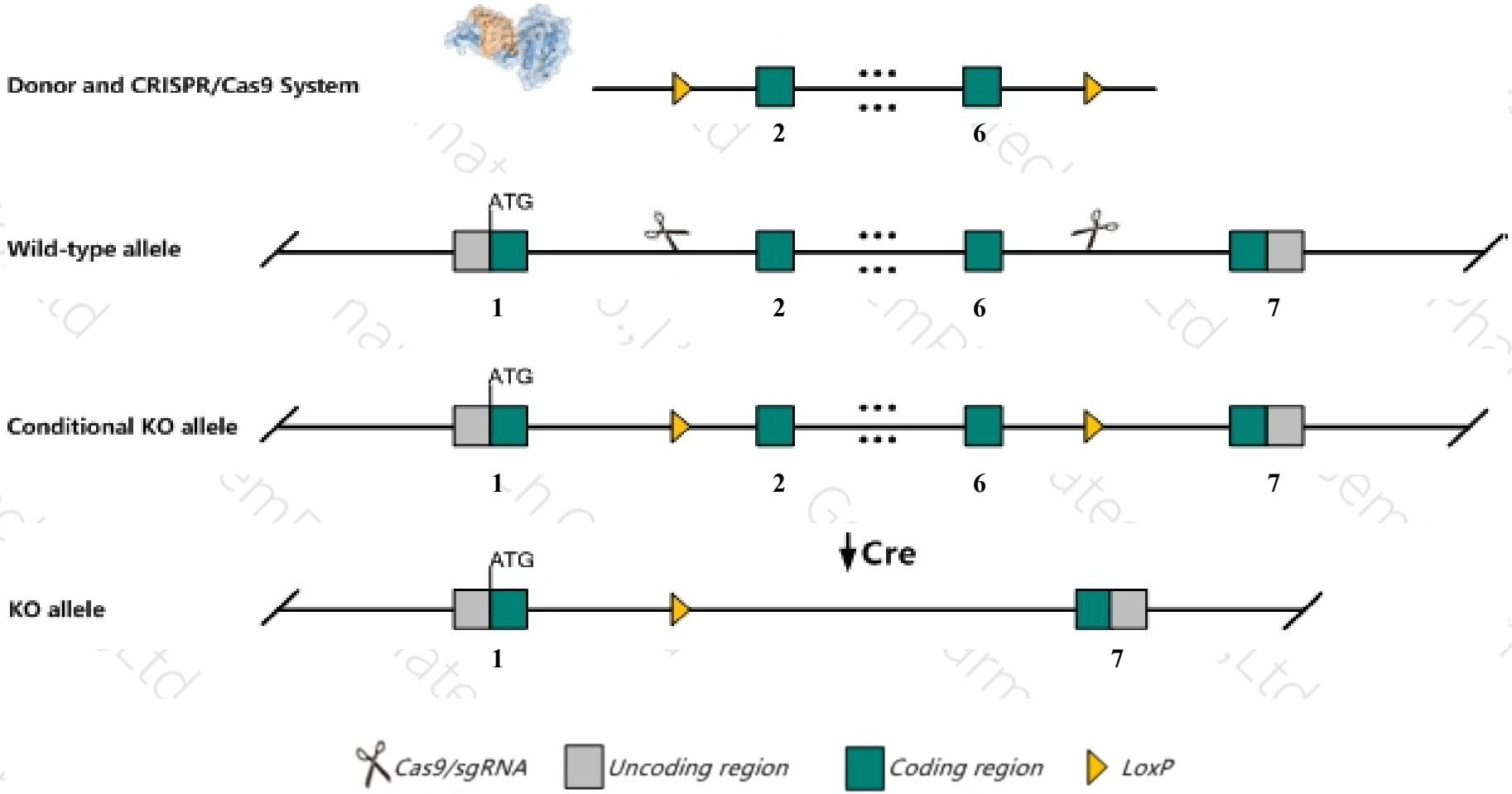
Project Overview

Project Name	***Pqlc3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pqlc3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Pqlc3* gene has 8 transcripts. According to the structure of *Pqlc3* gene, exon2-exon6 of *Pqlc3-201*(ENSMUST00000054536.10) transcript is recommended as the knockout region.The region contains 374bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Pqlc3* 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 *Pqlc3* gene is located on the Chr12. 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.

➢Transcript 203,204 CDS 3' incomplete the influences is unknown.

➢ 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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Pqlc3 PQ loop repeat containing [Mus musculus (house mouse)]

Gene ID: 217430, updated on 13-Mar-2020

Summary

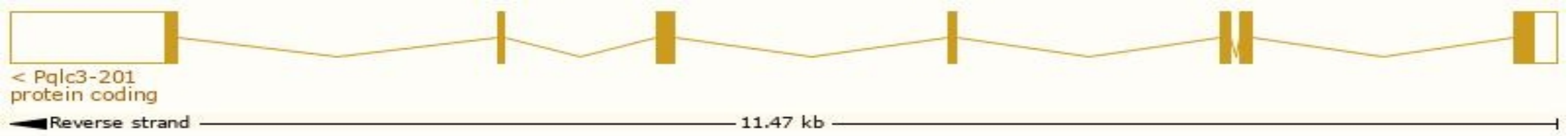
Official Symbol	Pqlc3 provided by MGI
Official Full Name	PQ loop repeat containing provided byMGI
Primary source	MGI:MGI:2444067
See related	Ensembl:ENSMUSG00000045679
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	C78076, E030024M05Rik, Slc66a3
Expression	Broad expression in subcutaneous fat pad adult (RPKM 7.3), bladder adult (RPKM 5.8) and 25 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

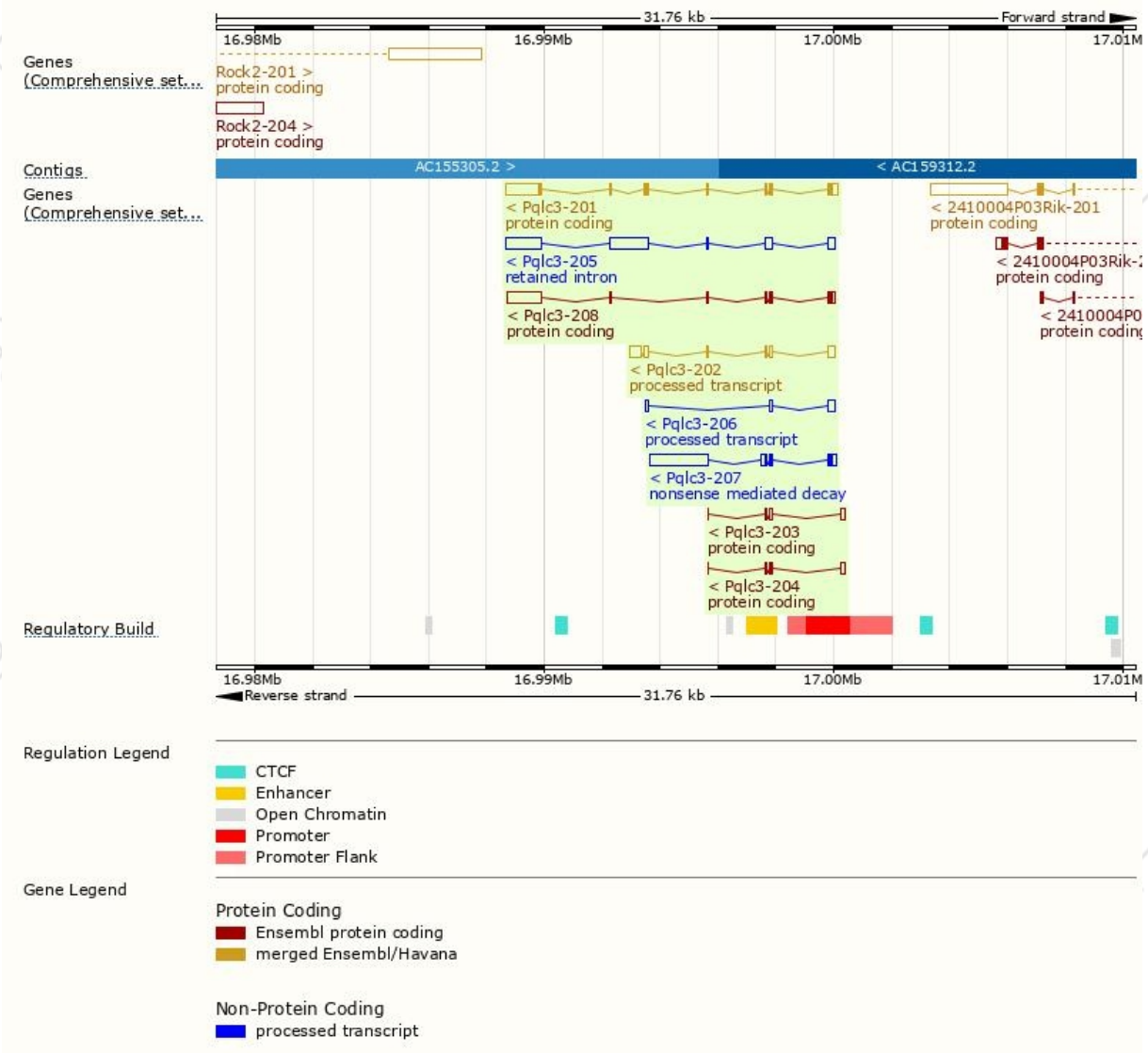
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pqlc3-201	ENSMUST00000054536.10	1928	202aa	Protein coding	CCDS25824	Q8C6U2	TSL:1 GENCODE basic APPRIS P1
Pqlc3-208	ENSMUST00000222203.1	1706	120aa	Protein coding	-	A0A1Y7VLY9	TSL:5 GENCODE basic
Pqlc3-203	ENSMUST00000134938.7	363	12aa	Protein coding	-	A0A1Y7VIU4	CDS 3' incomplete TSL:3
Pqlc3-204	ENSMUST00000137960.1	316	34aa	Protein coding	-	A0A1Y7VMC5	CDS 3' incomplete TSL:3
Pqlc3-207	ENSMUST00000156360.1	2585	104aa	Nonsense mediated decay	-	Q3UU83	TSL:1
Pqlc3-202	ENSMUST00000067572.12	922	No protein	Processed transcript	-	-	TSL:1
Pqlc3-206	ENSMUST00000141232.7	361	No protein	Processed transcript	-	-	TSL:3
Pqlc3-205	ENSMUST00000140933.1	3045	No protein	Retained intron	-	-	TSL:5

The strategy is based on the design of *Pqlc3-201* 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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