Cpne2 Cas9-CKO Strategy

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Project Overview

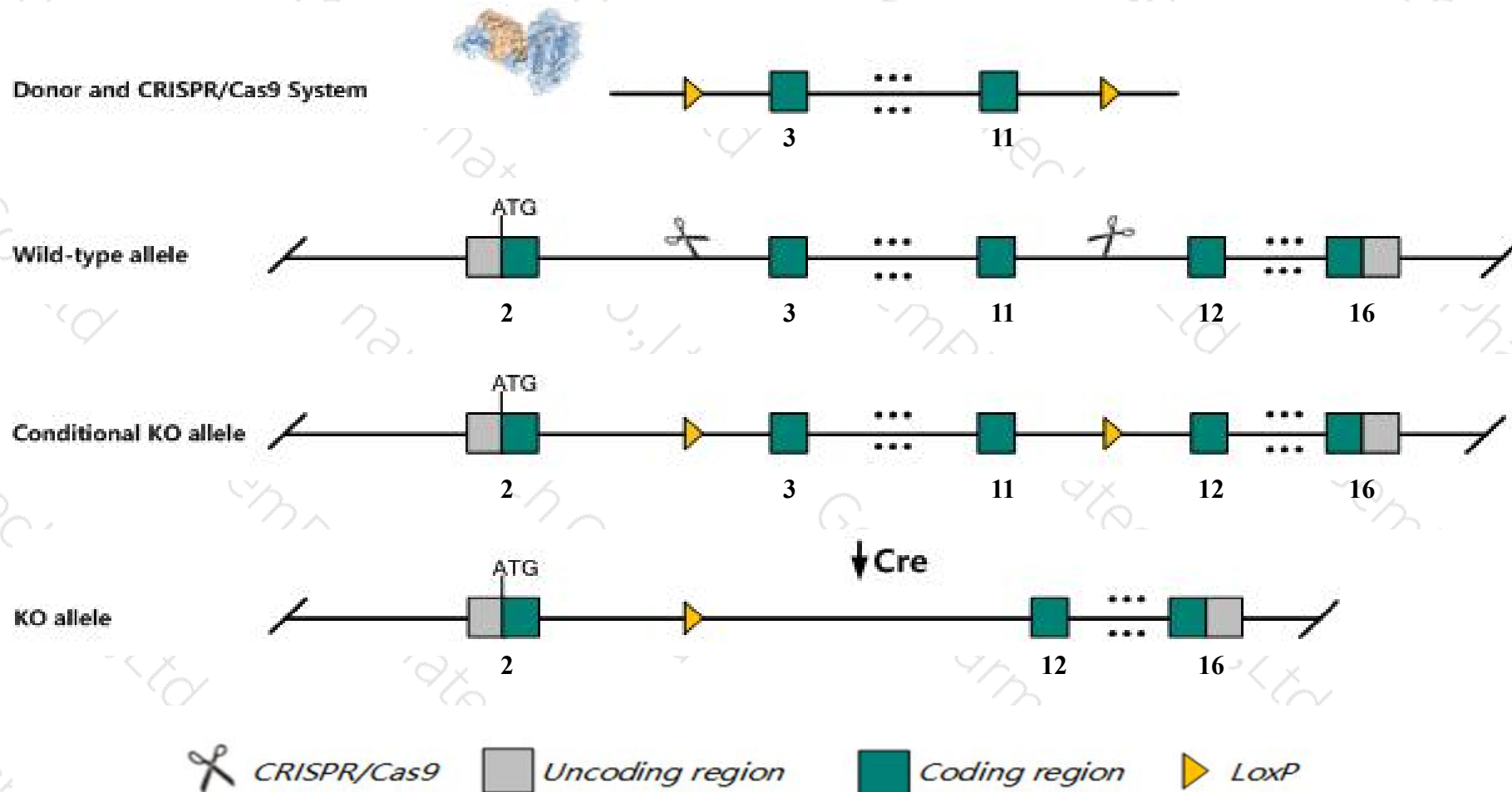|	
Project Name	*Cpne2*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cpne2* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System — 3, 11

Wild-type allele — ATG — 2, 3, 11, 12, 16

Conditional KO allele — ATG — 2, 3, 11, 12, 16

KO allele — ATG — Cre — 2, 12, 16

CRISPR/Cas9   Uncoding region   Coding region   LoxP

Technical routes

➢ The *Cpne2* gene has 6 transcripts. According to the structure of *Cpne2* gene, exon3-exon11 of *Cpne2-201* (ENSMUST00000048653.9) transcript is recommended as the knockout region.The region contains Ens 881bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Cpne2* 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

➤ The *Cpne2* gene is located on the Chr8. 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 *Cpne2*-204 may not be affected.

➤ 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）

Cpne2 copine II [Mus musculus (house mouse)]

Gene ID: 234577, updated on 13-Mar-2020

▲ Summary

|	
Official Symbol	Cpne2 provided by MGI
Official Full Name	copine II provided by MGI
Primary source	MGI:MGI:2387578
See related	Ensembl:ENSMUSG00000034361
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	3322401K10Rik
Summary	Calcium-dependent membrane-binding proteins may regulate molecular events at the interface of the cell membrane and cytoplasm. This gene is one of several genes that encodes a calcium-dependent protein containing two N-terminal type II C2 domains and an integrin A domain-like sequence in the C-terminus. [provided by RefSeq, Jul 2008]
Expression	Ubiquitous expression in colon adult (RPKM 18.5), subcutaneous fat pad adult (RPKM 17.3) and 27 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 6 transcripts,all transcripts are shown below:

| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cpne2-201	ENSMUST00000048653.9	2342	548aa	Protein coding	CCDS22543	P59108	TSL:1 GENCODE basic APPRIS P2
Cpne2-202	ENSMUST00000109537.1	2121	547aa	Protein coding	-	A0A0R4J1D0	TSL:1 GENCODE basic APPRIS ALT1
Cpne2-206	ENSMUST00000212550.1	388	61aa	Nonsense mediated decay	-	A0A1D5RLP0	CDS 5' incomplete TSL:3
Cpne2-203	ENSMUST00000136550.7	833	No protein	Processed transcript	-	-	TSL:1
Cpne2-204	ENSMUST00000156377.1	655	No protein	Retained intron	-	-	TSL:3
Cpne2-205	ENSMUST00000156894.1	655	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Cpne2-201* transcript,the transcription is shown below:
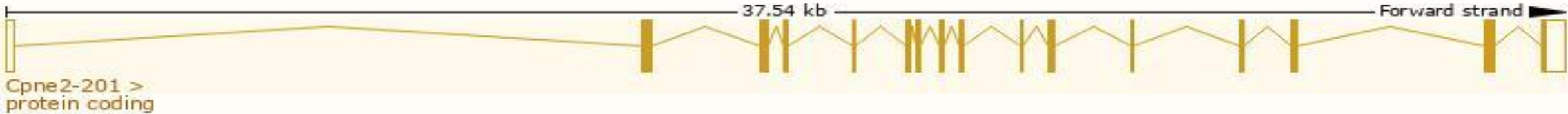
Genomic location distribution
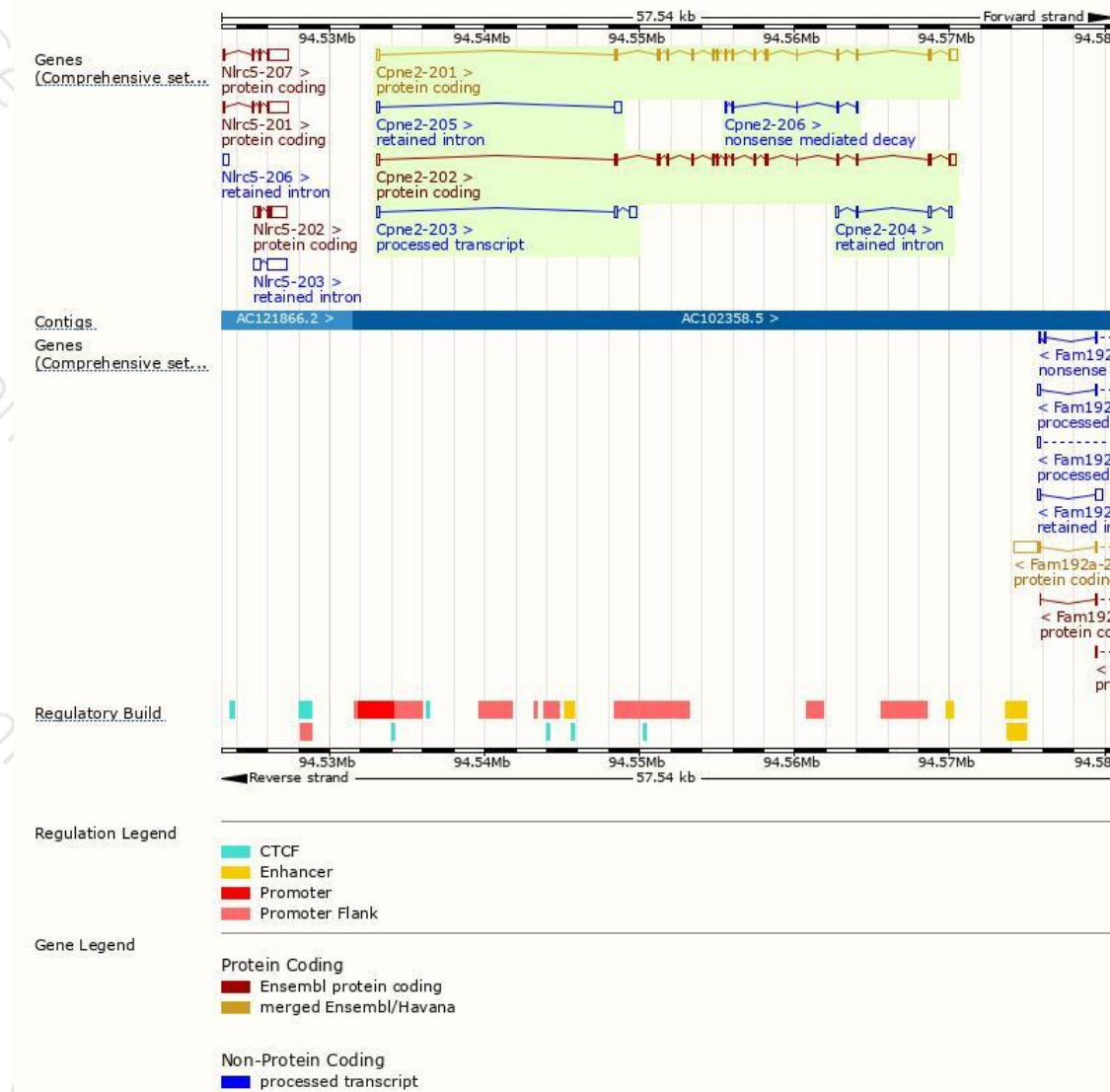
Protein domain
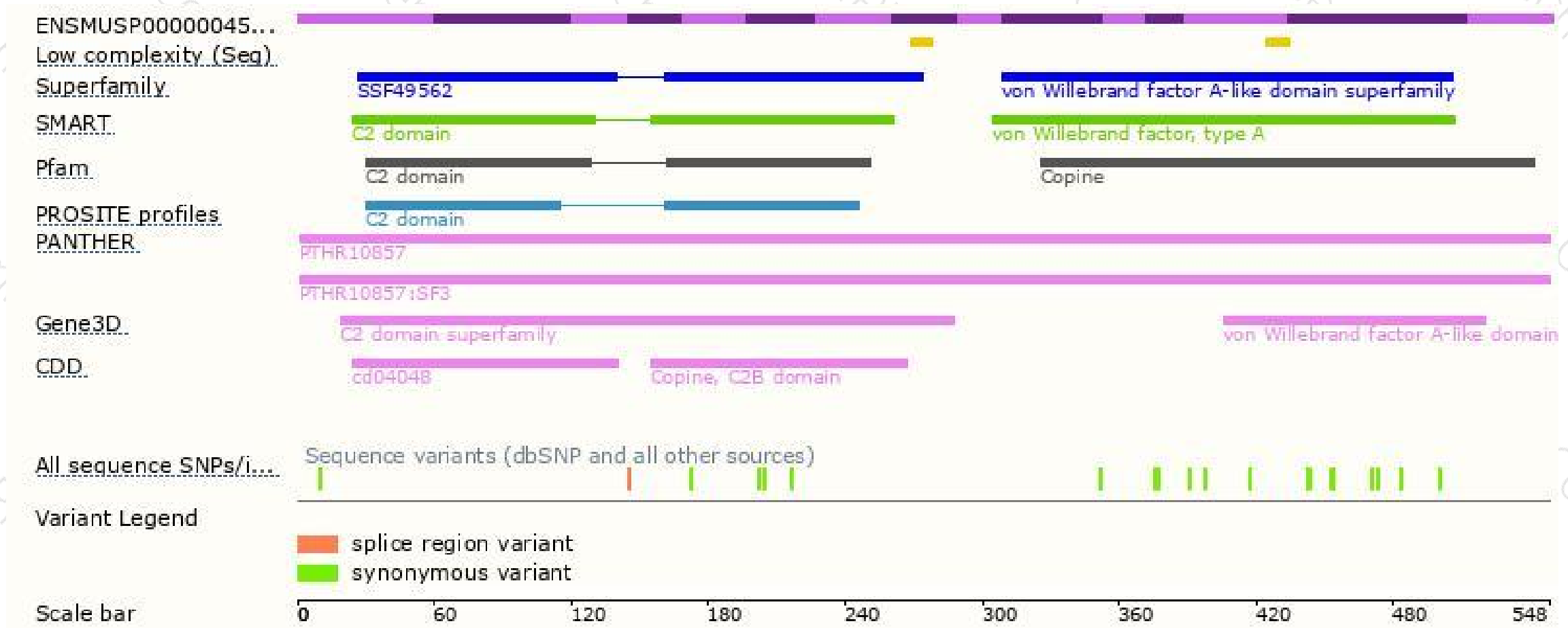
If you have any questions, you are welcome to inquire.
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