Chmp2a Cas9-CKO Strategy

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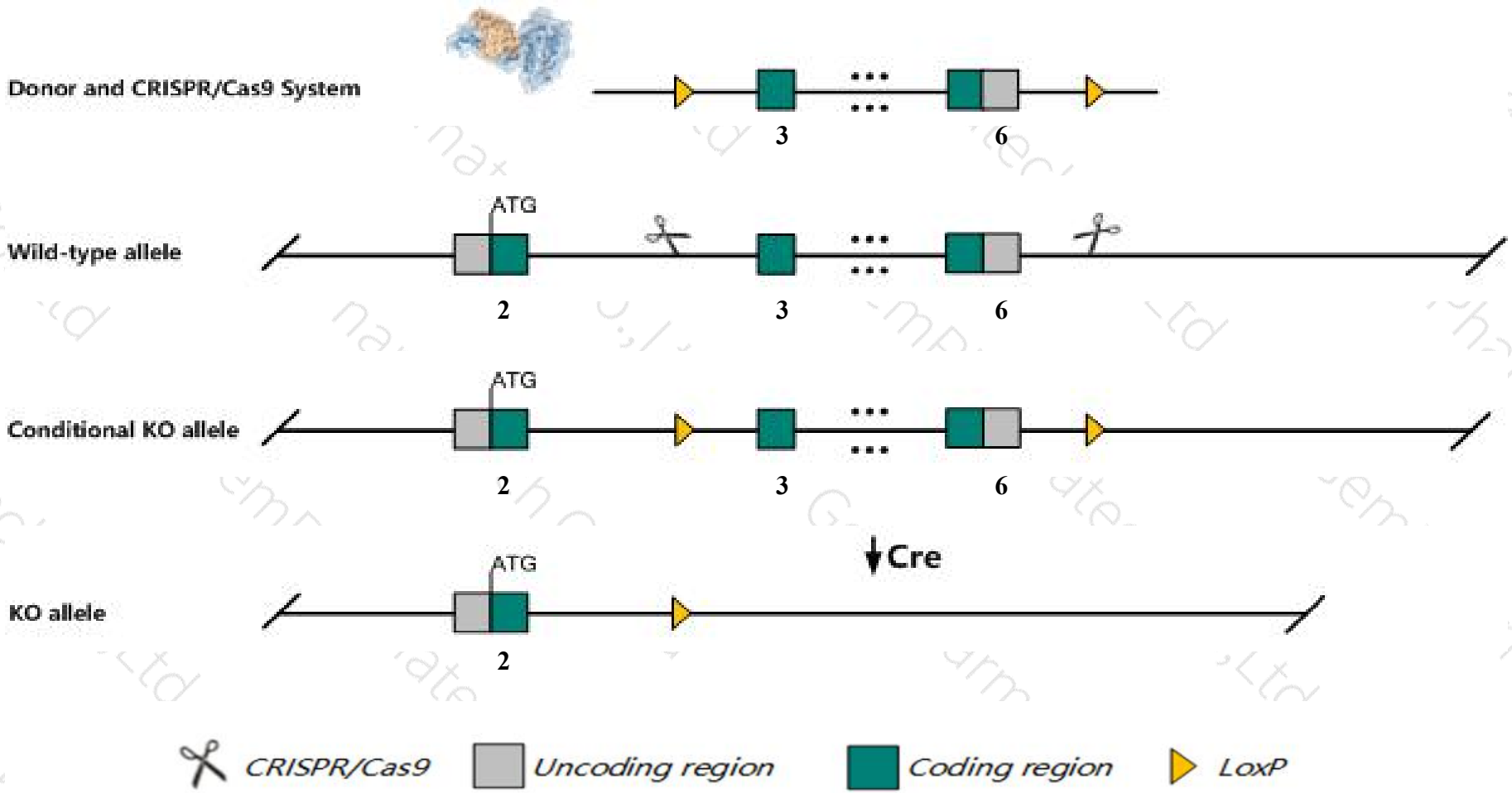
Project Overview

Project Name	***Chmp2a***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Chmp2a* gene. The schematic diagram is as follows:



Technical routes

➢ The *Chmp2a* gene has 6 transcripts. According to the structure of *Chmp2a* gene, exon3-exon6 of *Chmp2a-201*(ENSMUST00000005711.5) transcript is recommended as the knockout region.The region contains most of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Chmp2a* 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 N-terminal of *Chmp2a* gene will remain several amino acids ,it may remain the partial function of *Chmp2a* gene.

➢ The floxed region is near to the C-terminal of *Trim28* gene,this strategy may influence the regulatory function of the C-terminal of *Trim28* gene.

➢ The *Chmp2a* gene is located on the Chr7. 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）

Chmp2a charged multivesicular body protein 2A [Mus musculus (house mouse)]

Gene ID: 68953, updated on 13-Mar-2020

Summary

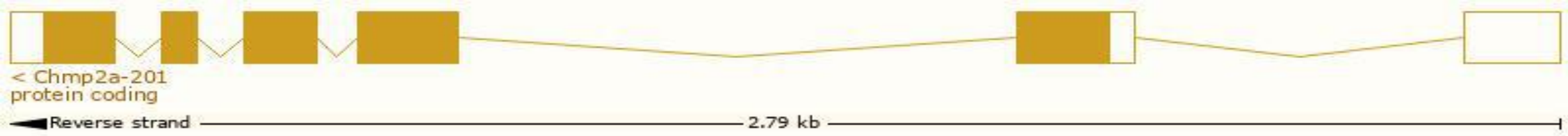
Official Symbol	Chmp2a provided by MGI
Official Full Name	charged multivesicular body protein 2A provided byMGI
Primary source	MGI:MGI:1916203
See related	Ensembl:ENSMUSG00000033916
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1500016L11Rik, mVps2
Expression	Ubiquitous expression in placenta adult (RPKM 67.0), bladder adult (RPKM 65.4) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

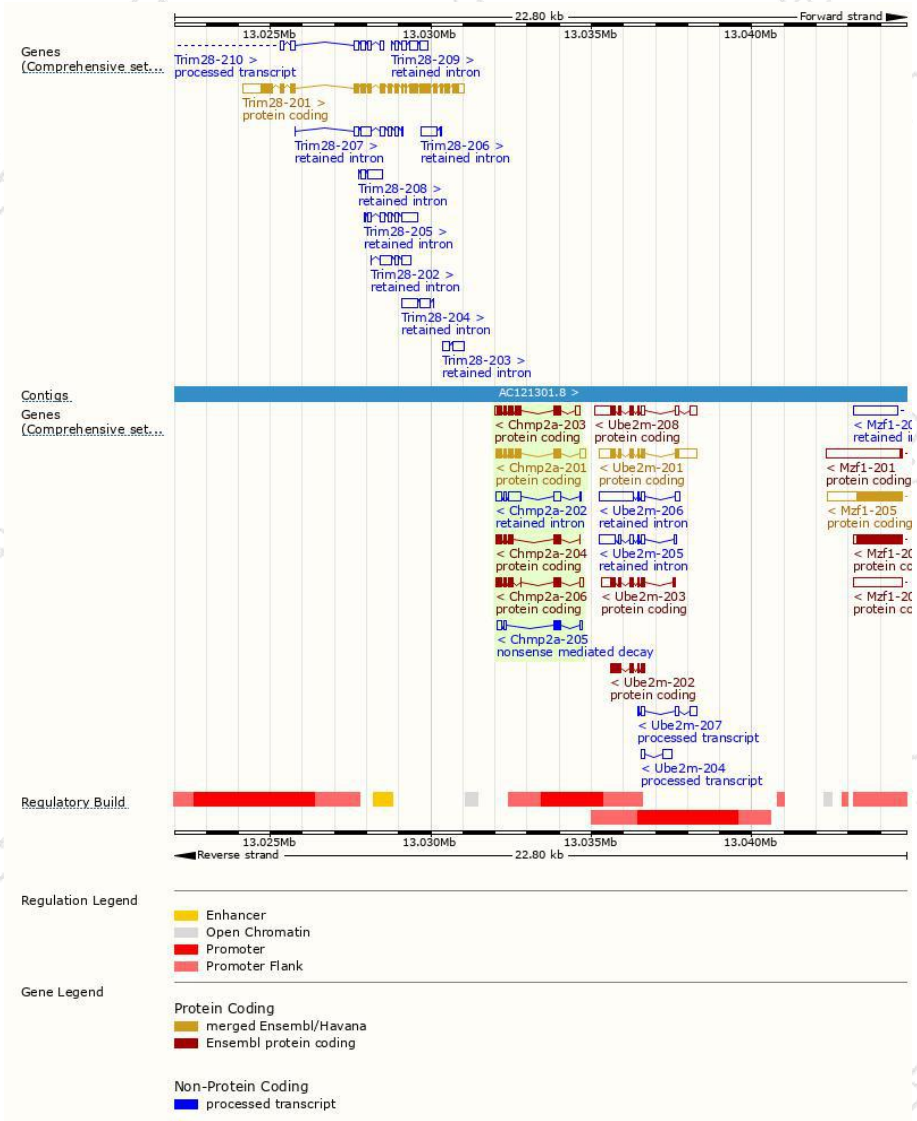
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Chmp2a-201	ENSMUST00000005711.5	949	222aa	Protein coding	CCDS20824	Q9DB34	TSL:1 GENCODE basic APPRIS P1
Chmp2a-203	ENSMUST00000210587.1	930	222aa	Protein coding	CCDS20824	Q9DB34	TSL:3 GENCODE basic APPRIS P1
Chmp2a-206	ENSMUST00000211626.1	726	172aa	Protein coding	-	A0A1B0GR63	TSL:2 GENCODE basic
Chmp2a-204	ENSMUST00000211344.1	606	162aa	Protein coding	-	A0A1B0GT75	TSL:5 GENCODE basic
Chmp2a-205	ENSMUST00000211369.1	521	56aa	Nonsense mediated decay	-	A0A1B0GSH4	TSL:3
Chmp2a-202	ENSMUST00000209487.1	888	No protein	Retained intron	-	-	TSL:5

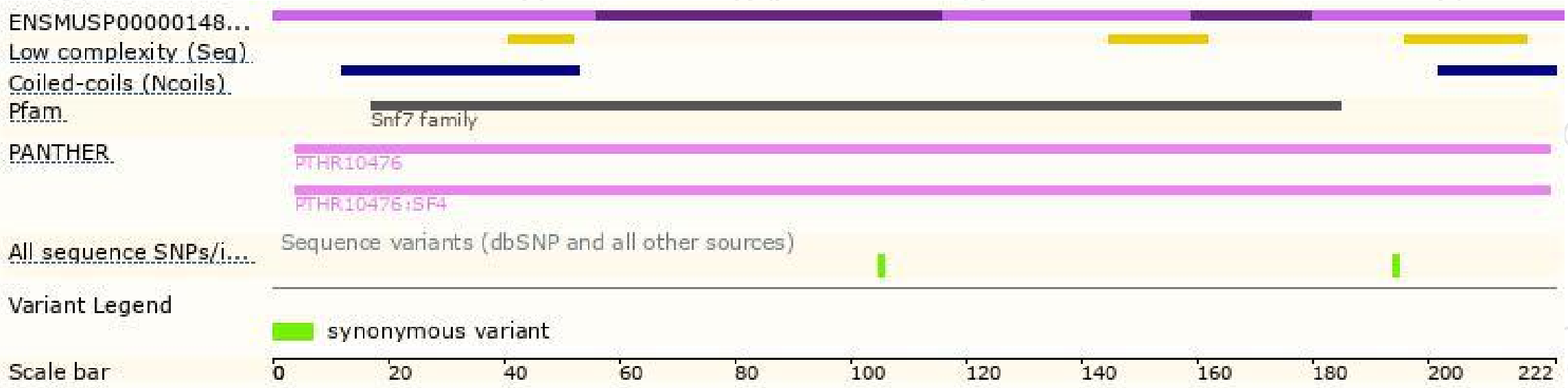
The strategy is based on the design of *Chmp2a-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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