Cnst Cas9-CKO Strategy

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Project Overview

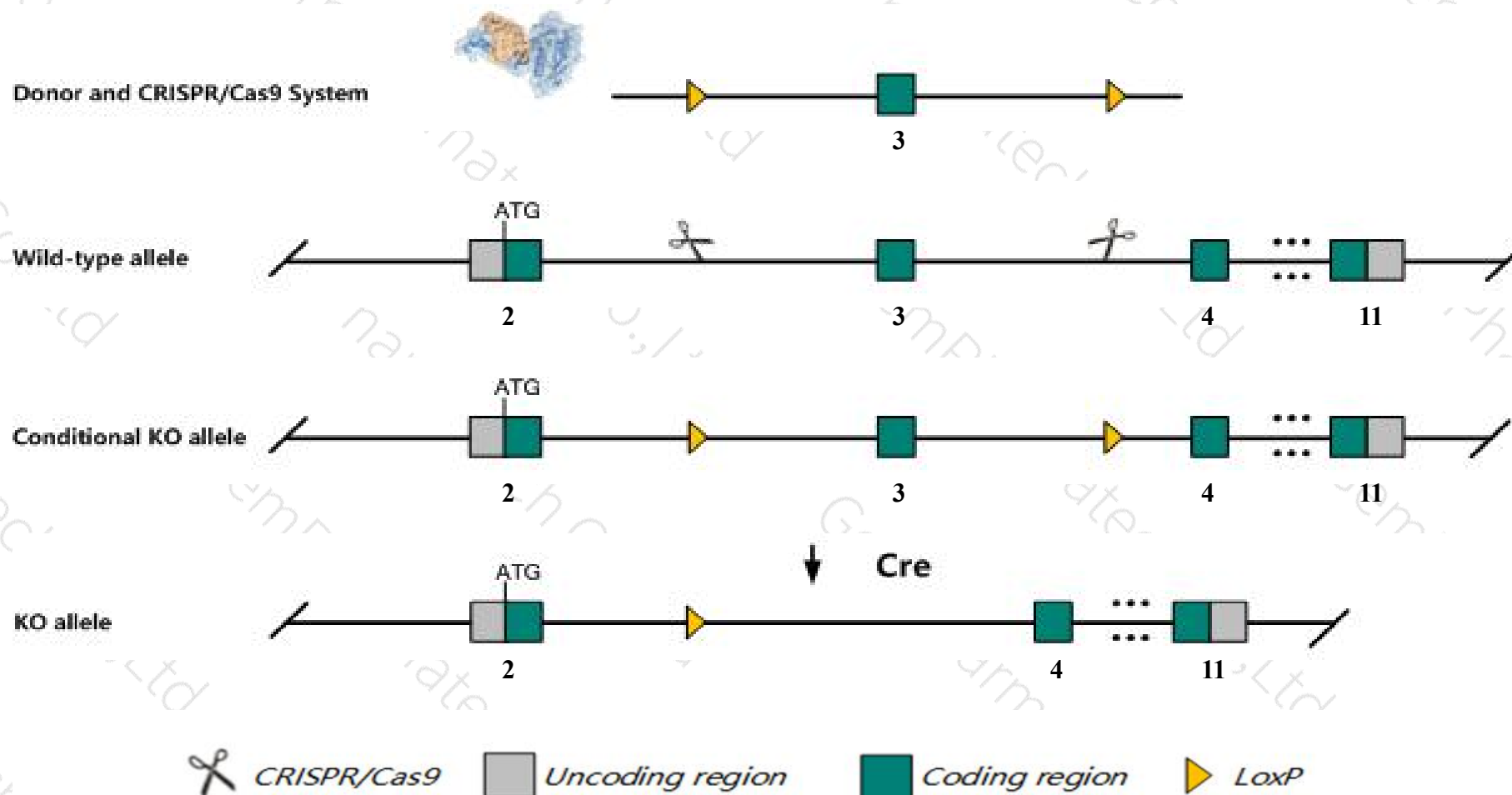|	
Project Name	*Cnst*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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

CRISPR/Cas9   Uncoding region   Coding region   LoxP

Technical routes

➢ The *Cnst* gene has 4 transcripts. According to the structure of *Cnst* gene, exon3 of *Cnst-201* (ENSMUST00000040706.8) transcript is recommended as the knockout region.The region contains Ens 182bp coding sequence. Knock out the region will result in disruption of protein function.

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

➢ Transcript *Cnst*-203 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）

Cnst consortin, connexin sorting protein [Mus musculus (house mouse)]

Gene ID: 226744, updated on 13-Mar-2020

Summary

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Official Symbol	Cnst provided by MGI
Official Full Name	consortin, connexin sorting protein provided by MGI
Primary source	MGI:MGI:2445141
See related	Ensembl:ENSMUSG00000038949
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	9630058J23Rik
Expression	Ubiquitous expression in cerebellum adult (RPKM 11.4), heart adult (RPKM 11.2) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cnst-201	ENSMUST00000040706.8	4709	711aa	Protein coding	CCDS15562	Q8CBC4	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 P1
Cnst-202	ENSMUST00000143270.1	617	No protein	Processed transcript	-	-	TSL:3
Cnst-203	ENSMUST00000144370.1	3717	No protein	Retained intron	-	-	TSL:1
Cnst-204	ENSMUST00000153962.1	1350	No protein	Retained intron	-	-	TSL:1

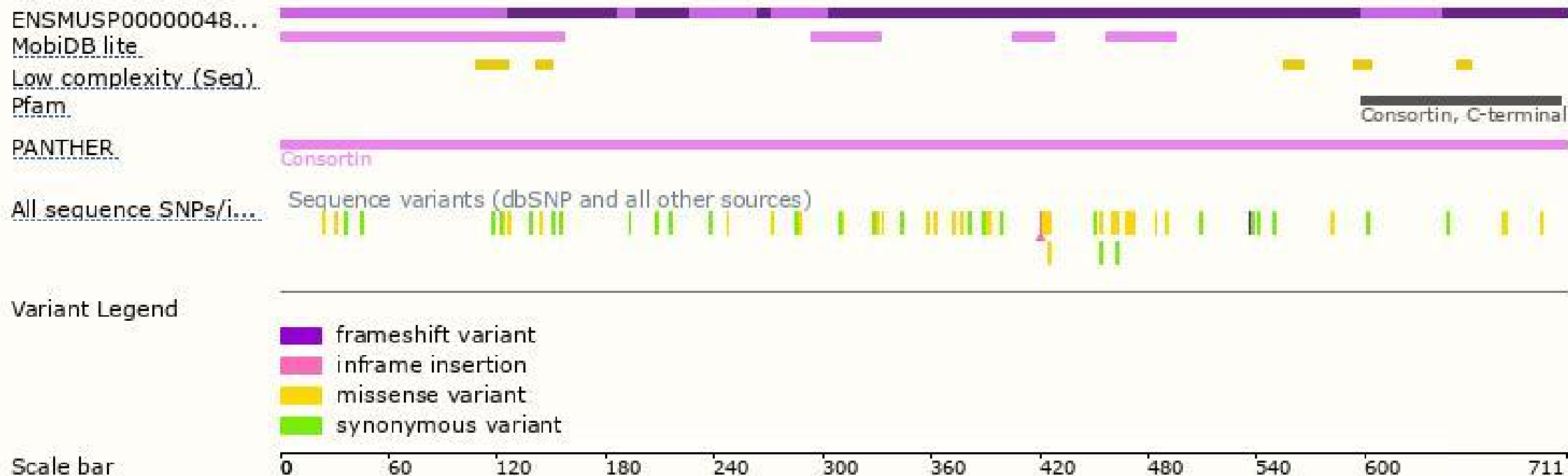The strategy is based on the design of *Cnst-201* transcript,the transcription is shown below:
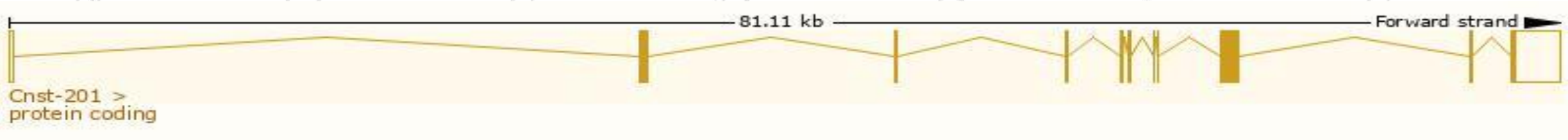
Genomic location distribution
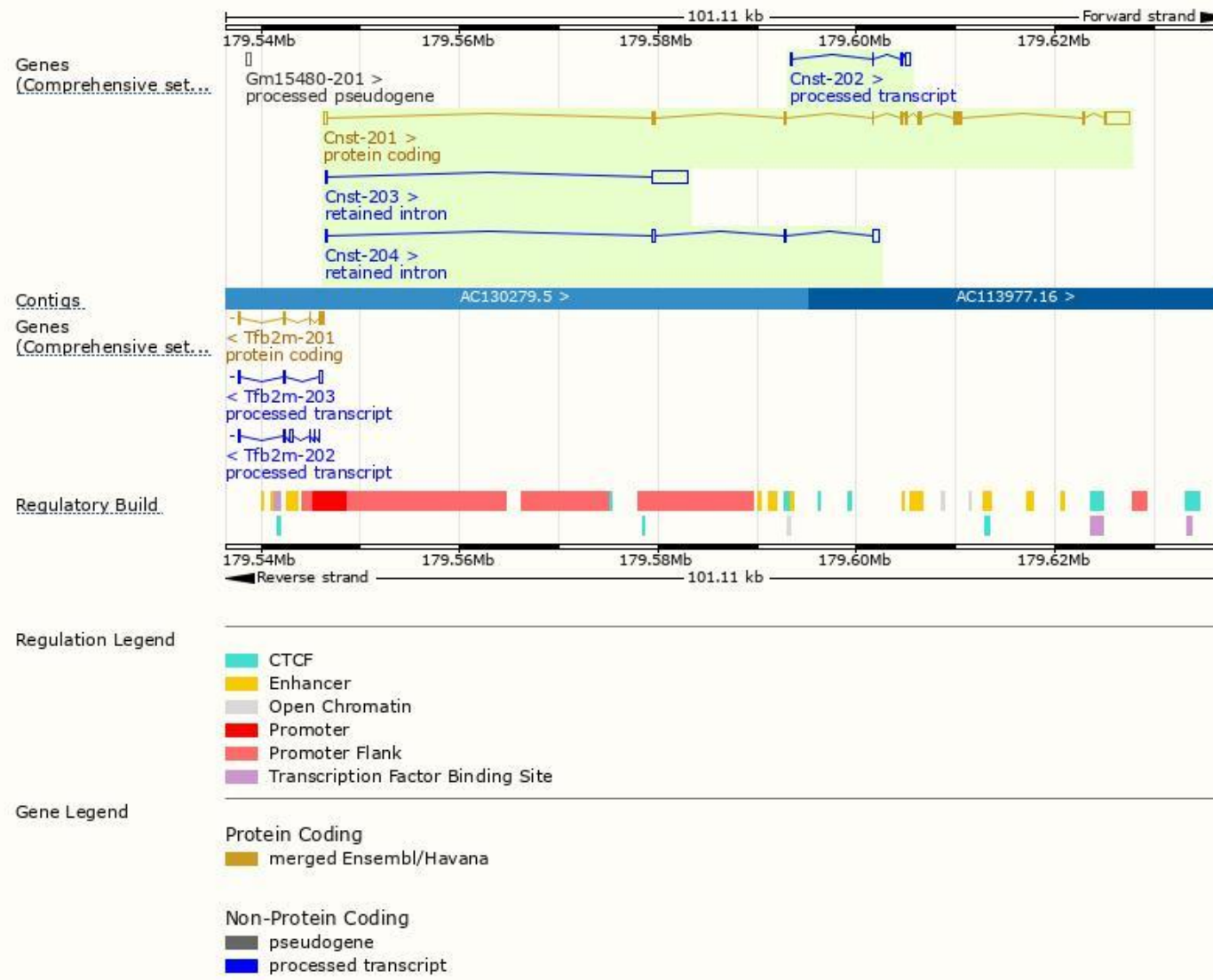
Protein domain

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