Cc2d2a Cas9-KO Strategy

Designer: Xiaojing Li

Reviewer: JiaYu

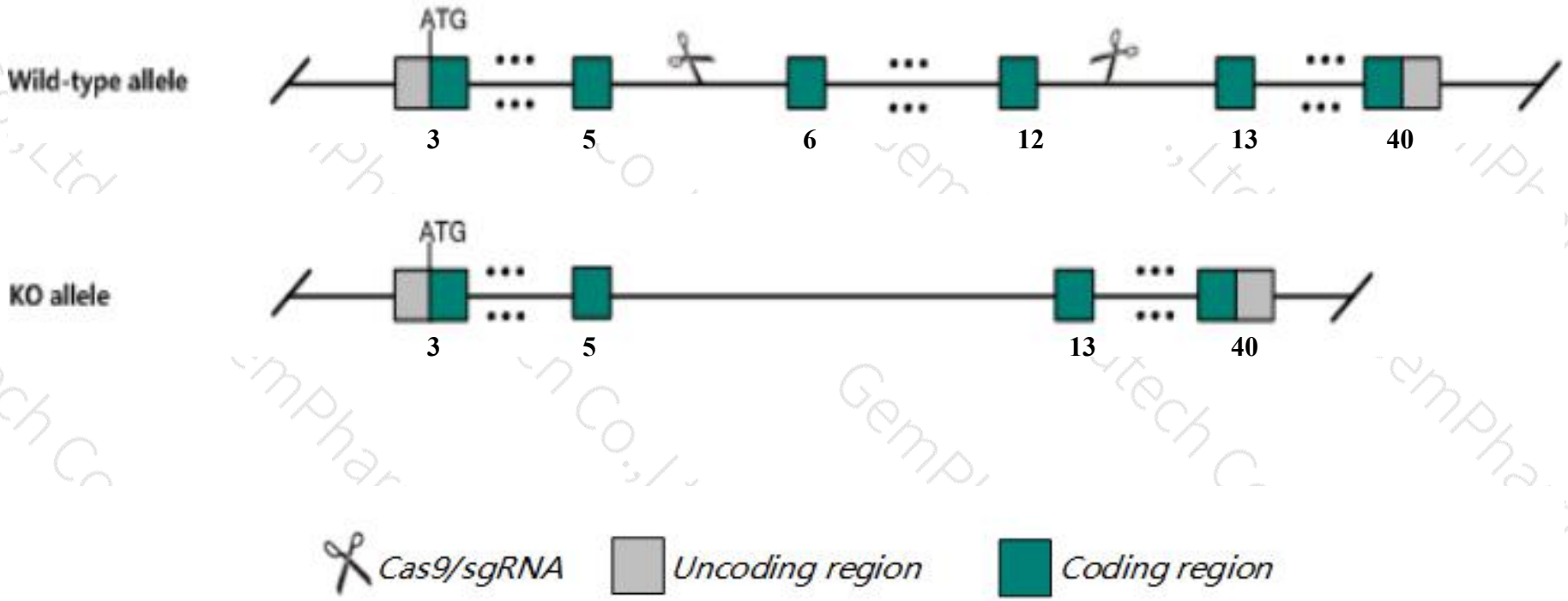
Design Date: 2020-8-20

Project Overview

Project Name	***Cc2d2a***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cc2d2a* gene. The schematic diagram is as follows:



Technical routes

➢ The *Cc2d2a* gene has 4 transcripts. According to the structure of *Cc2d2a* gene, exon6-exon12 of *Cc2d2a-201*(ENSMUST00000048150.14) transcript is recommended as the knockout region.The region contains 902bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Cc2d2a* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice

➢ According to the existing MGI data,mice homozygous for a null allele exhibit embryonic lethality with multiorgan defects related to cilia biogenesis. Homozygotes for a gene trap allele show randomized body axis, holoprosencephaly, and microphthalmia. Homozygotes for an ENU-induced allele show heterotaxia, congenital heart anomalies, kidney and eye defects, polydactyly, and cleft palate.

➢ The *Cc2d2a* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Cc2d2a coiled-coil and C2 domain containing 2A [Mus musculus (house mouse)]

Gene ID: 231214, updated on 13-Mar-2020

Summary

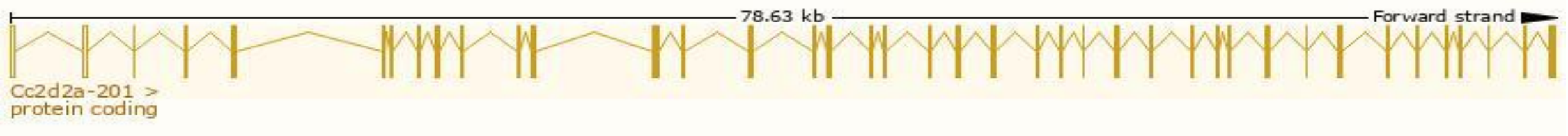
Official Symbol	Cc2d2a provided by MGI
Official Full Name	coiled-coil and C2 domain containing 2A provided byMGI
Primary source	MGI:MGI:1924487
See related	Ensembl:ENSMUSG00000039765
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5730509K17Rik, b2b1035Clo
Expression	Ubiquitous expression in bladder adult (RPKM 4.8), cerebellum adult (RPKM 3.2) and 22 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

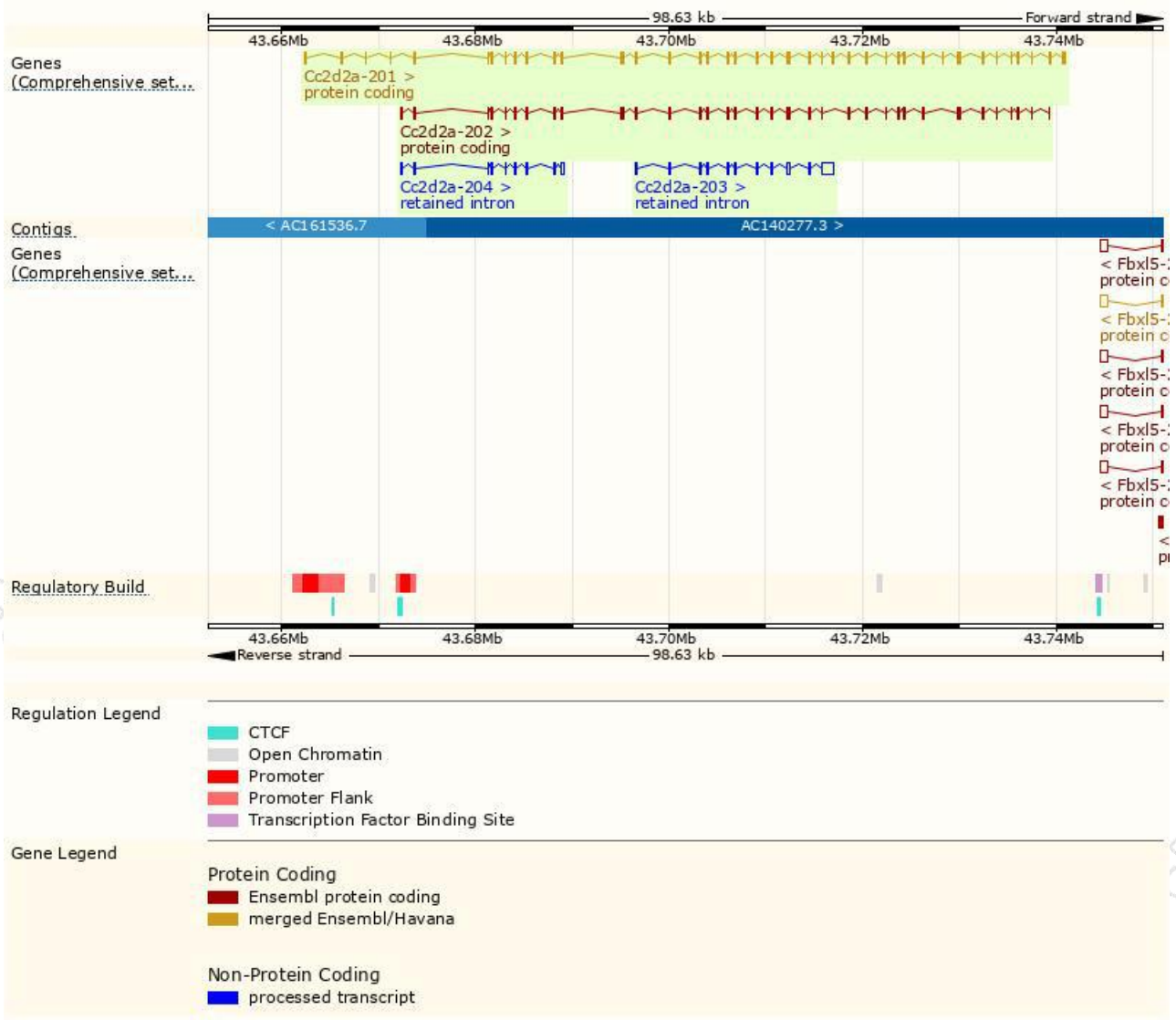
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cc2d2a-201	ENSMUST00000048150.14	5484	1633aa	Protein coding	CCDS39080	Q8CFW7	TSL:1 GENCODE basic APPRIS P1
Cc2d2a-202	ENSMUST00000125866.3	4512	1457aa	Protein coding	-	F6YZ61	CDS 3' incomplete TSL:5
Cc2d2a-203	ENSMUST00000127355.1	2774	No protein	Retained intron	-	-	TSL:1
Cc2d2a-204	ENSMUST00000142303.4	1194	No protein	Retained intron	-	-	TSL:1

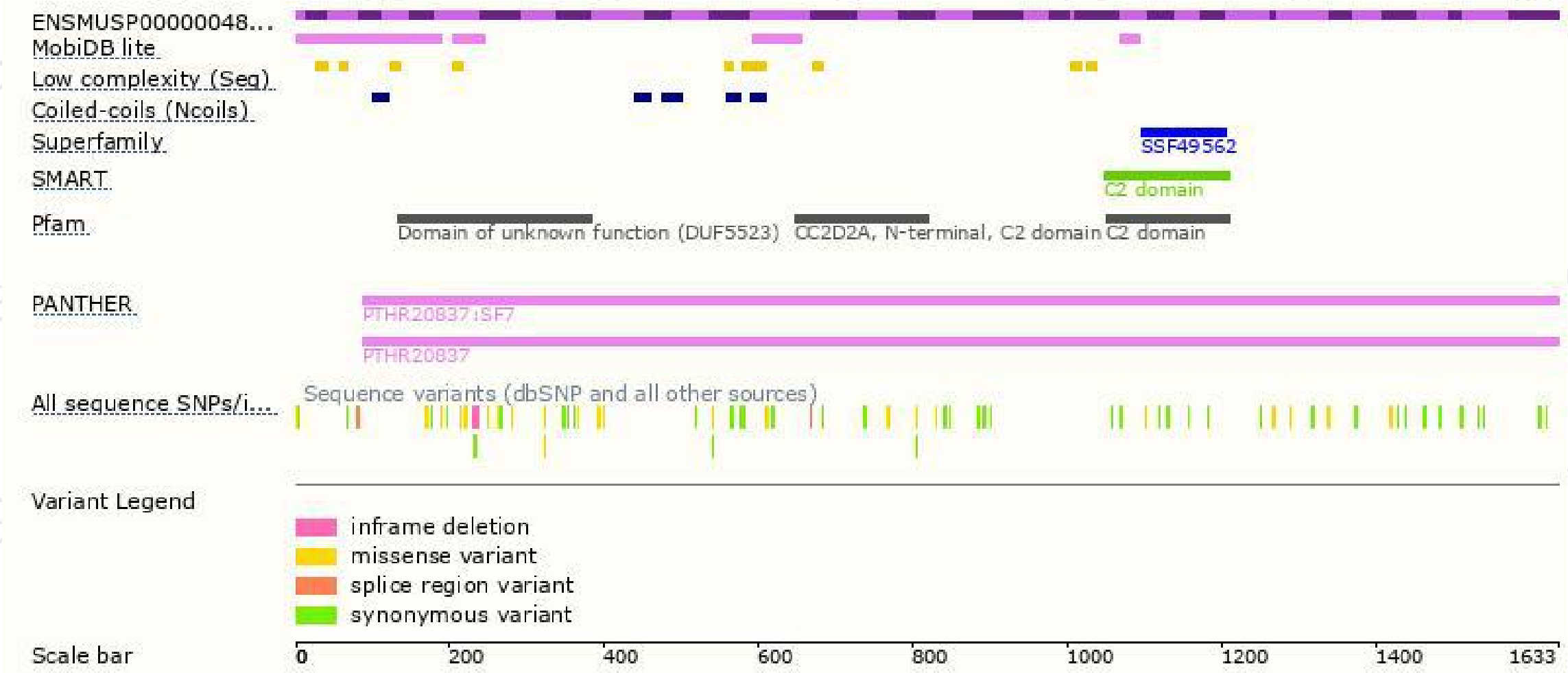
The strategy is based on the design of *Cc2d2a-201* transcript,the transcription is shown below:



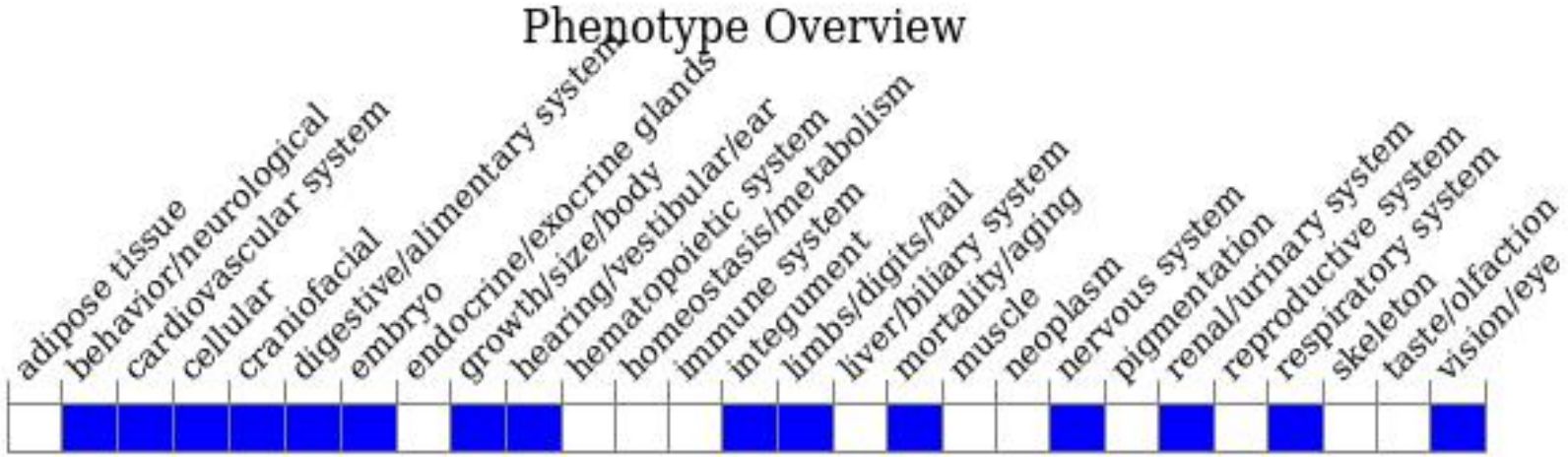
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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

Tel: 025-5864 1534

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