Ccr8 Cas9-KO Strategy

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Reviewer: Yang Zeng

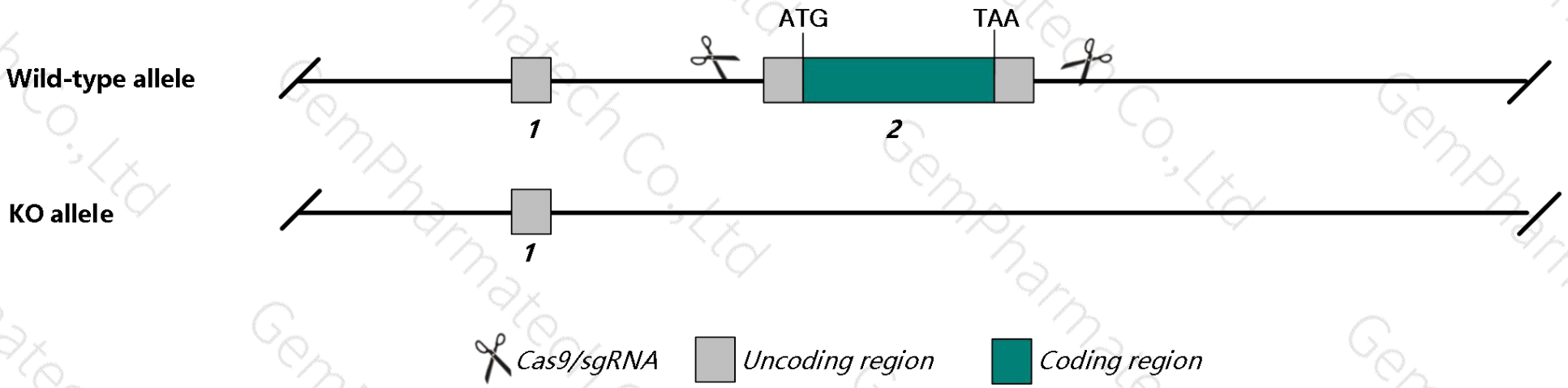
Design Date: 2018-6-28

Project Overview

Project Name	***Ccr8***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ccr8* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ccr8* gene has 1 transcript. According to the structure of *Ccr8* gene, exon2 of *Ccr8-201* (ENSMUST00000048777.3) transcript is recommended as the knockout region.The region contains all 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 *Ccr8* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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 either of two independently generated knock-out alleles show normal lung eosinophilia and Th2 cytokine responses in OVA-elicited asthma models. Mice homozygous for a third knock-out allele show a delay in onset of clinical signs of experimental autoimmune encephalomyelitis.

- The KO region is close to 5'UTR region of the *Gm47050* gene.Knockout the region may affect the regulatory function of *Gm47050* gene.

- The *Ccr8* gene is located on the Chr9. 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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Ccr8 chemokine (C-C motif) receptor 8 [*Mus musculus* (house mouse)]

Gene ID: 12776, updated on 12-Aug-2019

Summary

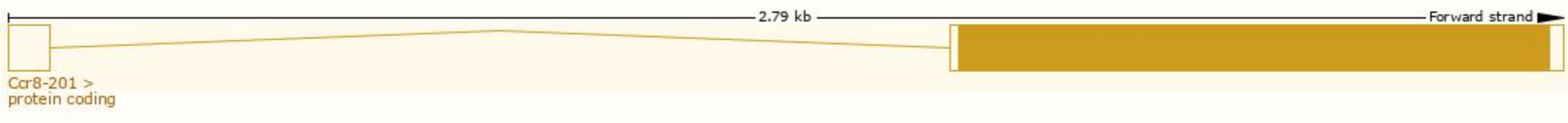
Official Symbol	Ccr8 provided by MGI
Official Full Name	chemokine (C-C motif) receptor 8 provided by MGI
Primary source	MGI:MGI:1201402
See related	Ensembl:ENSMUSG00000042262
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	C-C; CCR-8; CKR-8; mCCR8; Cmkbr8; CC-CKR-8; C-C CKR-8
Expression	Biased expression in thymus adult (RPKM 6.3), mammary gland adult (RPKM 1.1) and 3 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

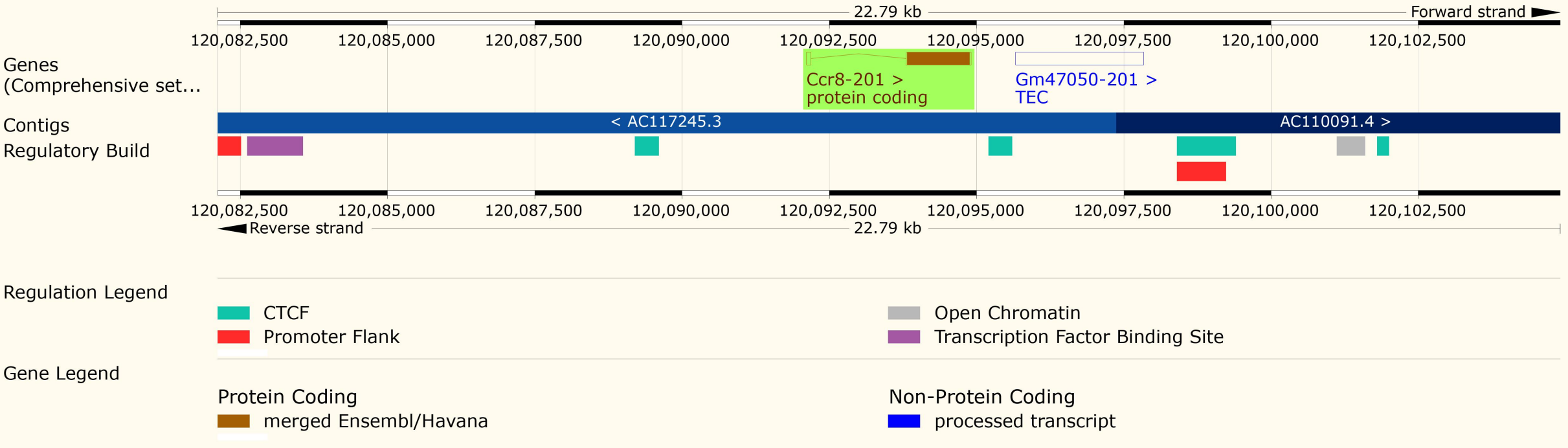
The gene has 1 transcript,and the transcript is shown below:

Name ▲	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Ccr8-201	ENSMUST00000048777.3	1175	353aa	ENSMUSP00000038473.2	Protein coding	CCDS23621	P56484 Q3ZB17	TSL:1 GENCODE basic APPRIS P1

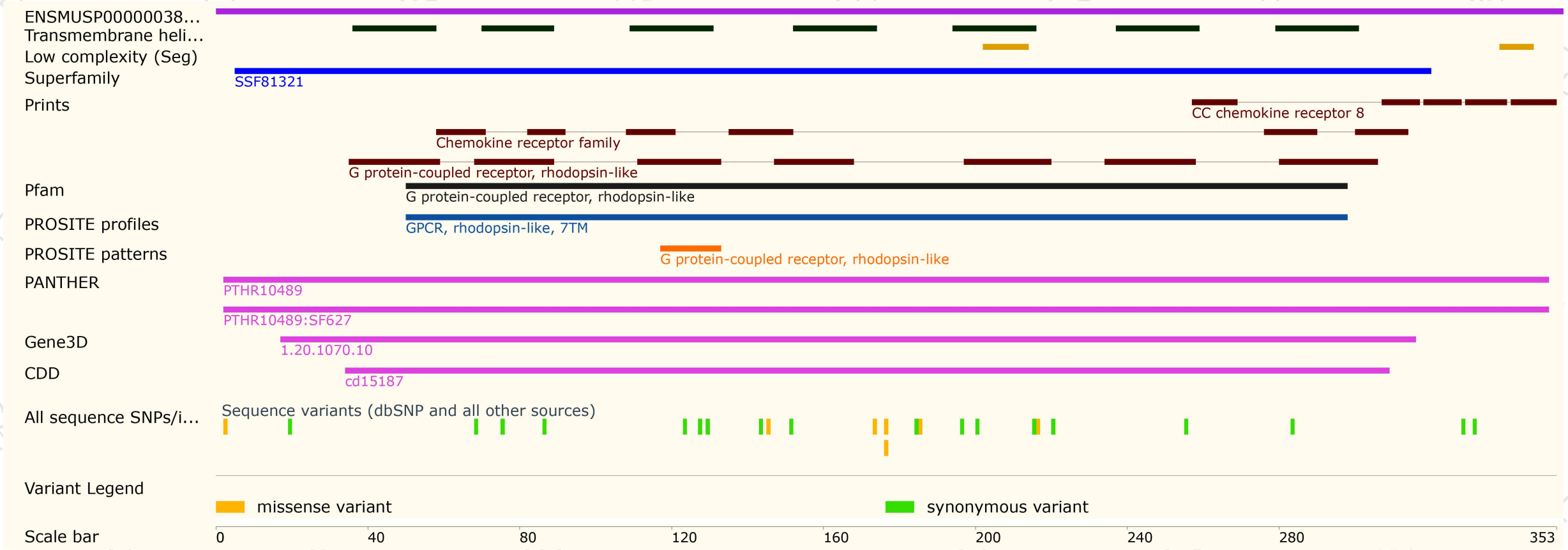
The strategy is based on the design of *Ccr8-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



Mouse phenotype description(MGI)

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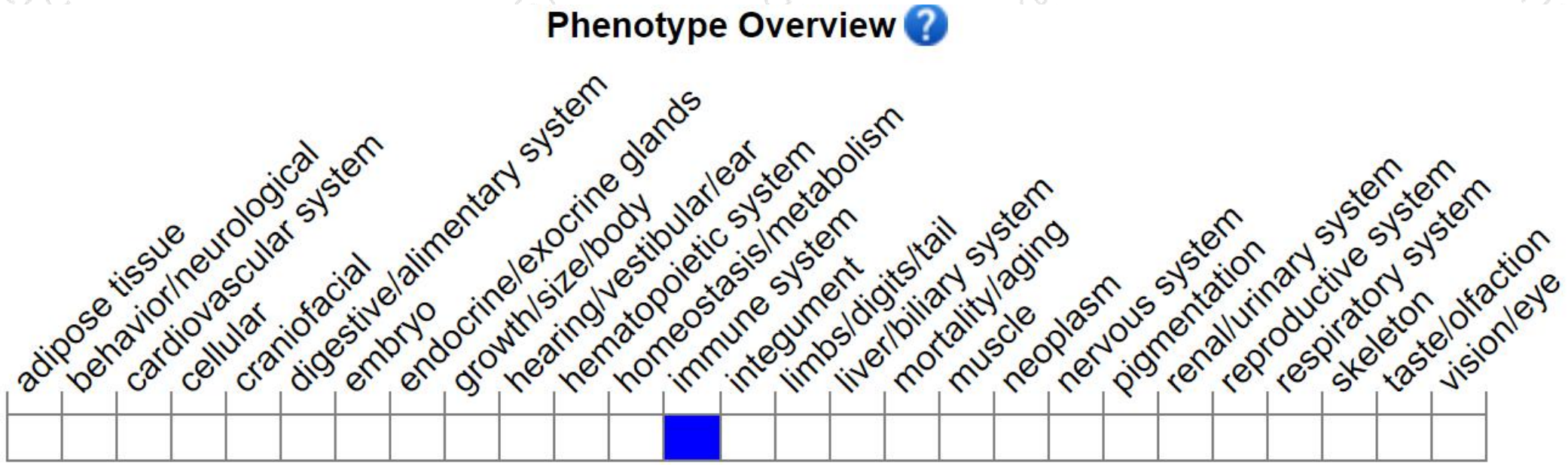

Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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

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