Cx3cl1 Cas9-KO *Strategy*

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Reviewer: Shilei Zhu

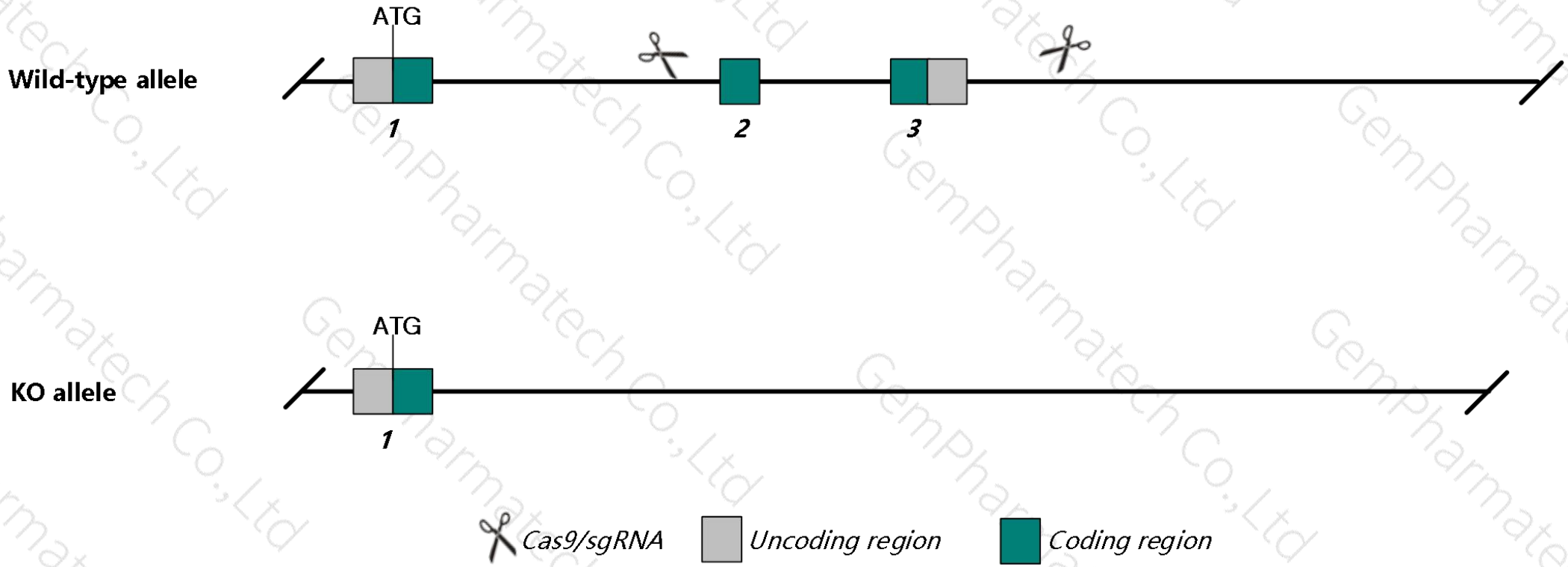
Date: 2018/9/7

Project Overview

Project Name	***Cx3cl1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cx3cl1* gene. The schematic diagram is as follows:



Technical routes

- The *Cx3cl1* gene has 6 transcripts.According to the structure of *Cx3cl1* gene, exon2-3 of *Cx3cl1*-201 (ENSMUST00000034230.6) 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 *Cx3cl1* 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 knock-out allele show a specific reduction in Gr1(low) monocyte levels, and increased neuronal cell loss in a neurotoxin (MPTP)-induced model of Parkinson disease. Mice homozygous for a different knock-out allele are less susceptible to cerebral ischemia-reperfusion injury.
- The *Cx3cl1* 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.
- 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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Cx3cl1 chemokine (C-X3-C motif) ligand 1 [*Mus musculus* (house mouse)]

Gene ID: 20312, updated on 13-Mar-2020

Summary

Official Symbol	Cx3cl1 provided by MGI
Official Full Name	chemokine (C-X3-C motif) ligand 1 provided by MGI
Primary source	MGI:MGI:1097153
See related	Ensembl:ENSMUSG00000031778
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	CX3C; Cxc3; Scyd1; ABCD-3; AB030188; AI848747; D8Bwg0439e
Expression	Biased expression in cortex adult (RPKM 170.7), frontal lobe adult (RPKM 132.2) and 14 other tissues See more
Orthologs	human all

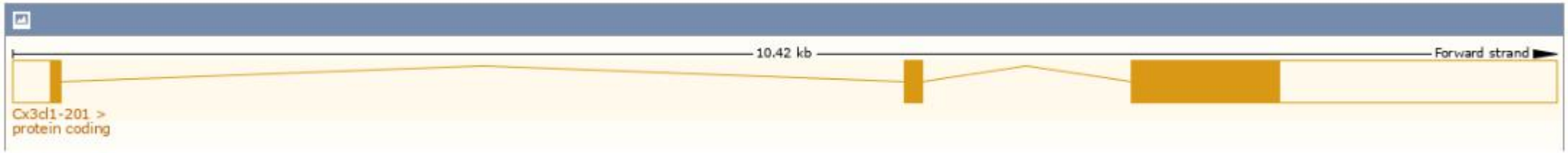
Transcript information （Ensembl）

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

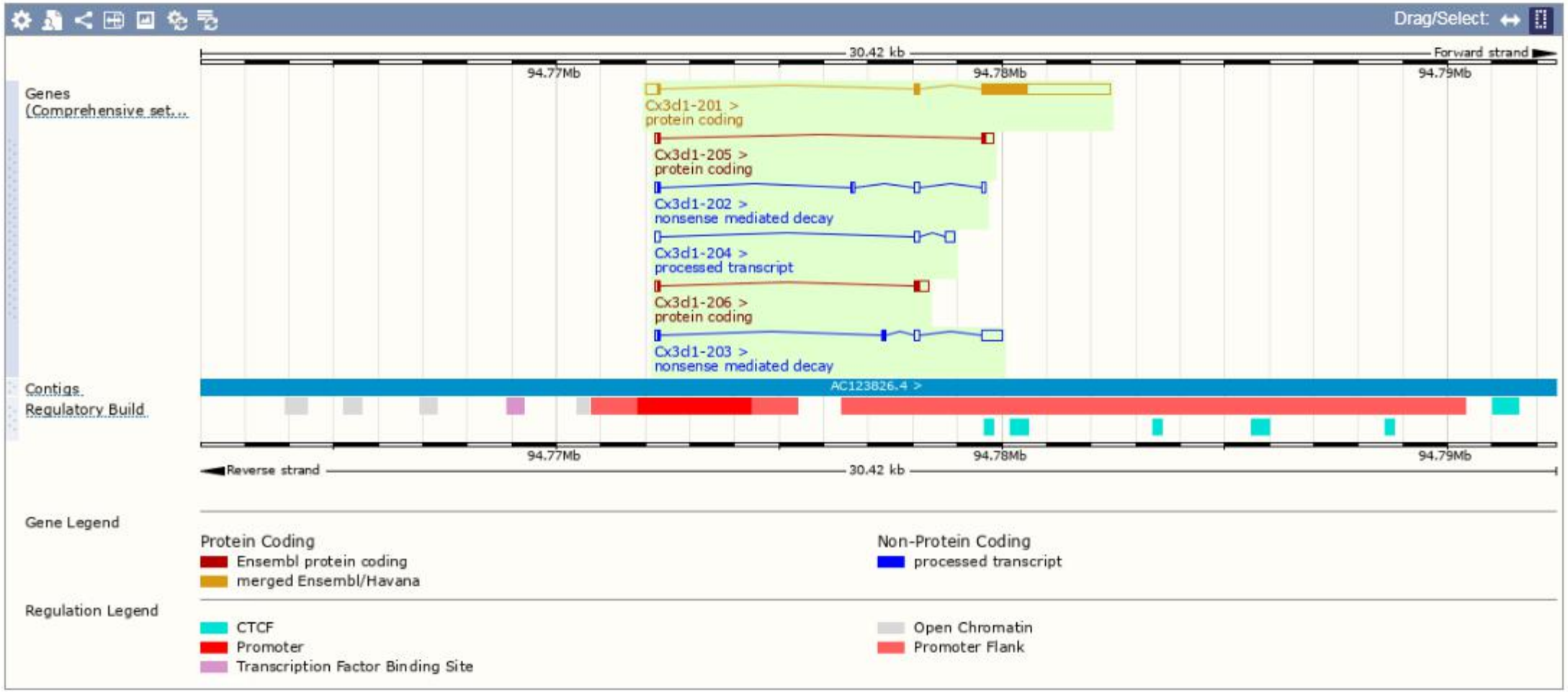
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cx3cl1-201	ENSMUST00000034230.6	3315	395aa	Protein coding	CCDS22548	O35188	TSL:1 GENCODE basic APPRIS P1
Cx3cl1-206	ENSMUST00000211956.1	470	64aa	Protein coding	-	A0A1D5RMK8	TSL:2 GENCODE basic
Cx3cl1-205	ENSMUST00000211947.1	377	45aa	Protein coding	-	A0A1D5RLW9	TSL:5 GENCODE basic
Cx3cl1-203	ENSMUST00000150307.1	780	57aa	Nonsense mediated decay	-	D6RCV0	TSL:3
Cx3cl1-202	ENSMUST00000135970.1	432	37aa	Nonsense mediated decay	-	E0CXC3	TSL:3
Cx3cl1-204	ENSMUST00000151783.8	462	No protein	Processed transcript	-	-	TSL:3

The strategy is based on the design of *Cx3cl1*-201 transcript,The transcription is shown below

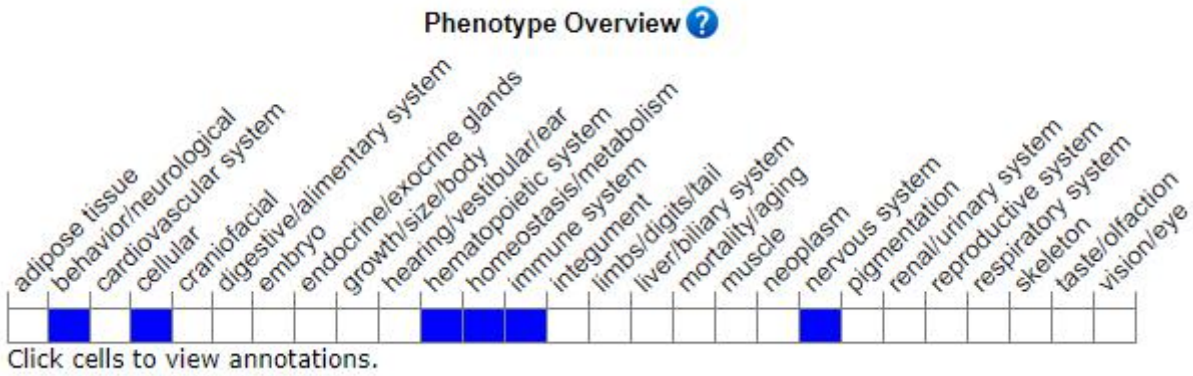


Genomic location distribution



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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Mice homozygous for a different knock-out allele are less susceptible to cerebral ischemia-reperfusion injury.

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

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