Cxcr6 Cas9-CKO Strategy

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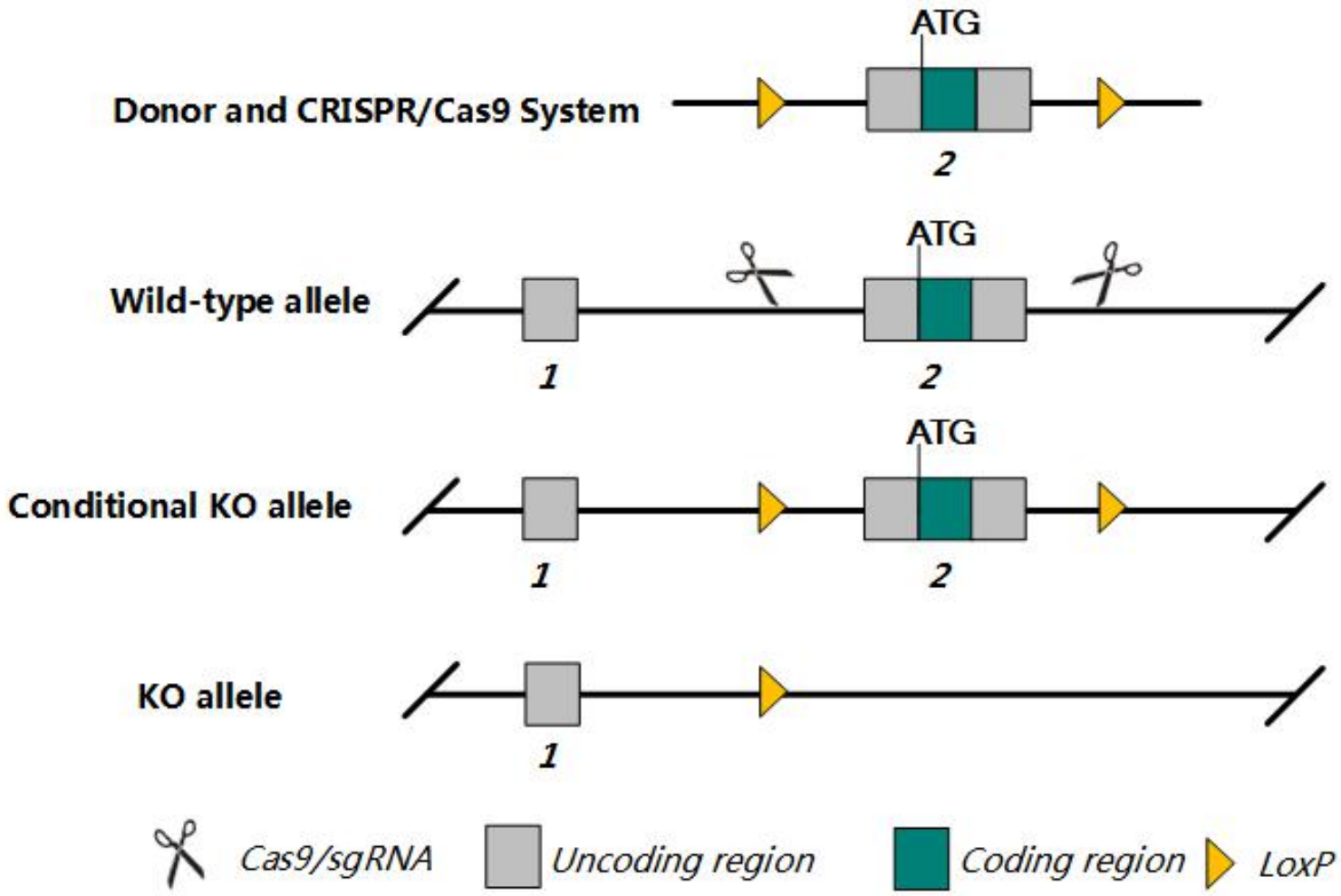
Project Overview

Project Name	***Cxcr6***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cxcr6* gene. The schematic diagram is as follows:



Technical routes

- The *Cxcr6* gene has 2 transcripts. According to the structure of *Cxcr6* gene, exon2 of *Cxcr6-201* (ENSMUST00000049810.8) transcript is recommended as the knockout region.The region contains all the coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Cxcr6* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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

➢ According to the existing MGI data, A small percentage of mice that are heterozygous or homozygous for a knock-out allele develop medulloblastomas in the cerebellum after 12 months of age.

➢The partial intron of *Fyco1* gene will be deleted together in this strategy.

➢ The *Cxcr6* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Cxcr6 chemokine (C-X-C motif) receptor 6 [*Mus musculus* (house mouse)]

Gene ID: 80901, updated on 21-May-2019

Summary

Official Symbol	Cxcr6 provided by MGI
Official Full Name	chemokine (C-X-C motif) receptor 6 provided by MGI
Primary source	MGI:MGI:1934582
See related	Ensembl:ENSMUSG00000048521
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	BONZO; STRL33; BB217514
Expression	Biased expression in mammary gland adult (RPKM 2.4), spleen adult (RPKM 2.2) and 14 other tissues See more
Orthologs	human all

Genomic context

Location: 9 F4; 9 74.57 cM

See Cxcr6 in Genome Data Viewer

Exon count: 2

Annotation release	Status	Assembly	Chr	Location
106	current	GRCm38.p4 (GCF_000001635.24)	9	NC_000075.6 (123806477..123811754)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	9	NC_000075.5 (123715595..123720872)

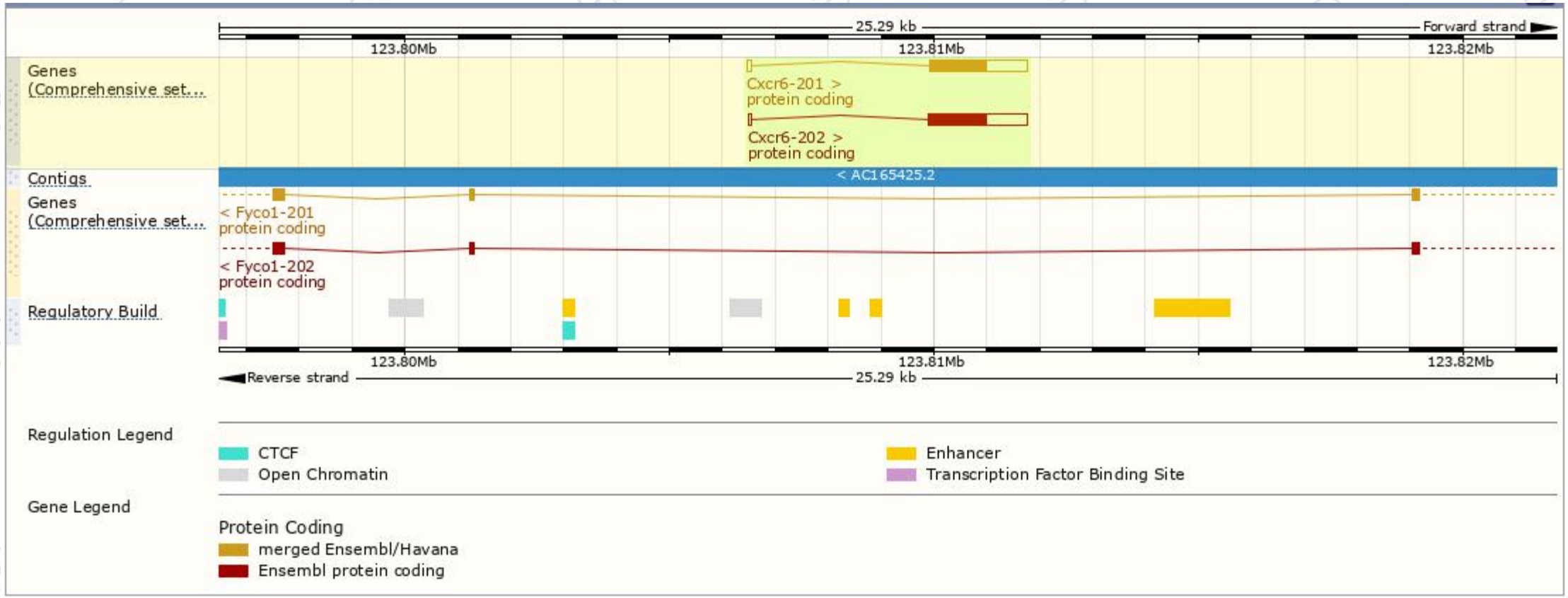
Transcript information（Ensembl）

The gene has 2 transcripts,all transcripts are shown below:

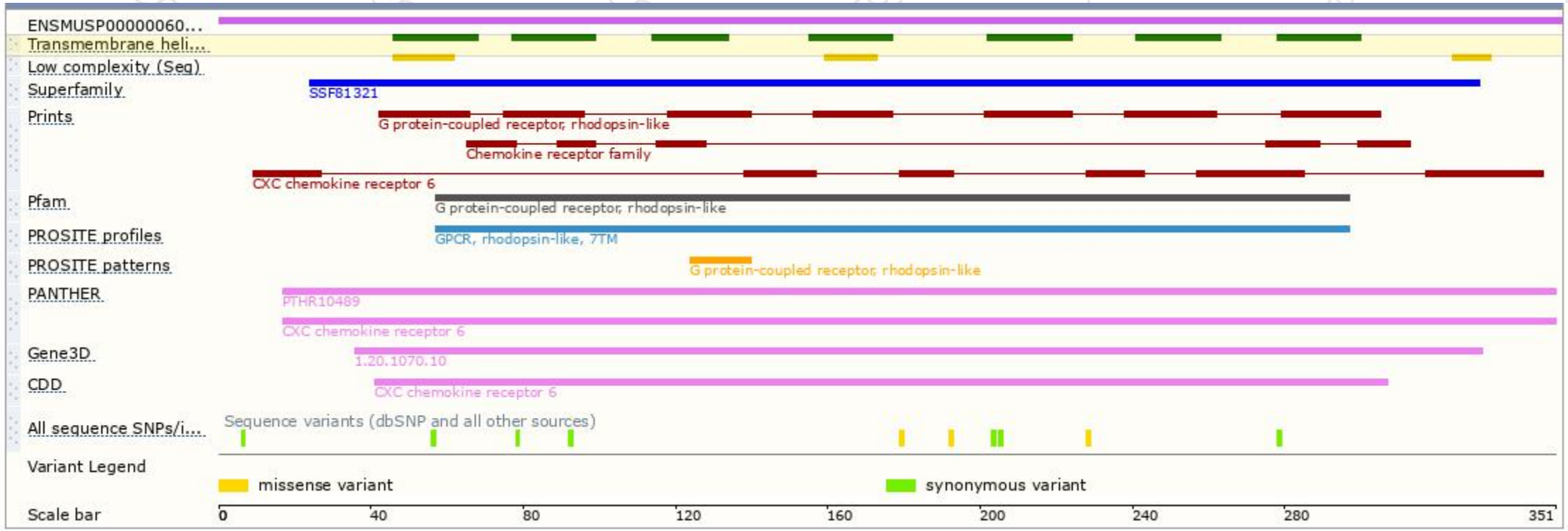
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cxcr6-201	ENSMUST00000049810.8	1897	351aa	Protein coding	CCDS23664	Q9EQ16	TSL:1 GENCODE basic APPRIS P2
Cxcr6-202	ENSMUST00000216072.1	1907	358aa	Protein coding	-	A0A1L1SVJ3	TSL:1 GENCODE basic APPRIS ALT2

The strategy is based on the design of *Cxcr6-201* transcript,The transcription is shown below

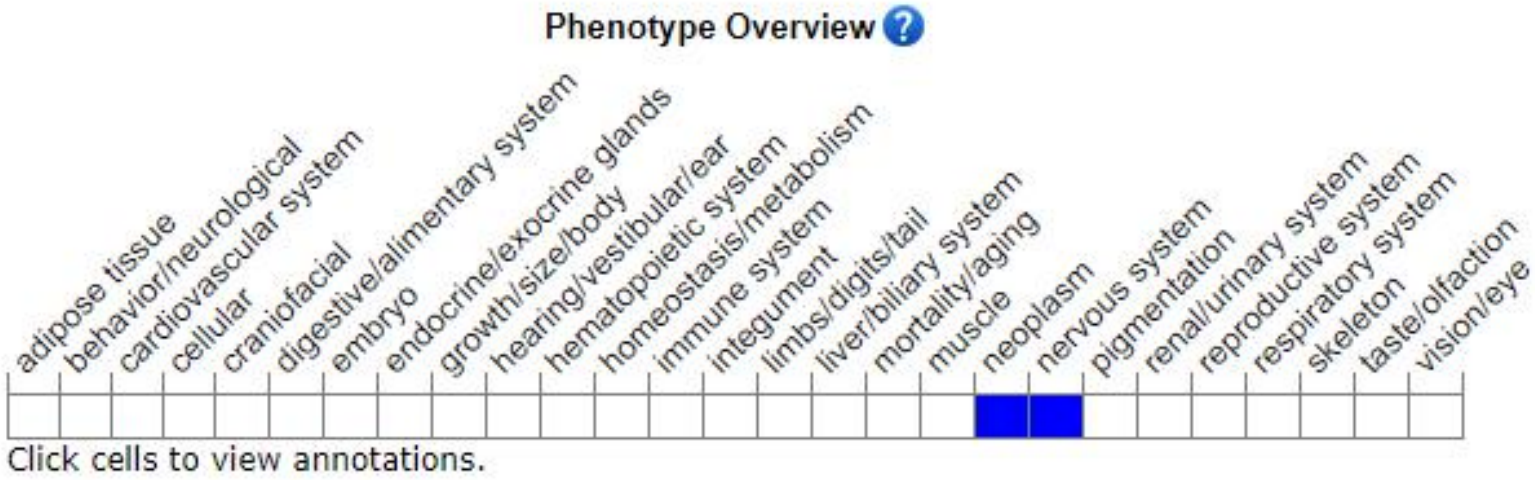
Genomic location distribution



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/).

A small percentage of mice that are heterozygous or homozygous for a knock-out allele develop medulloblastomas in the cerebellum after 12 months of age.

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

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