Cx3cr1-IRES-EGFP Mouse Model Strategy -CRISPR/Cas9 technology

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Project Overview

Project Name	***Cx3cr1*-IRES-EGFP**
Project type	**Cas9-KI**
Strain background	**C57BL/6JGpt**

Technical Description

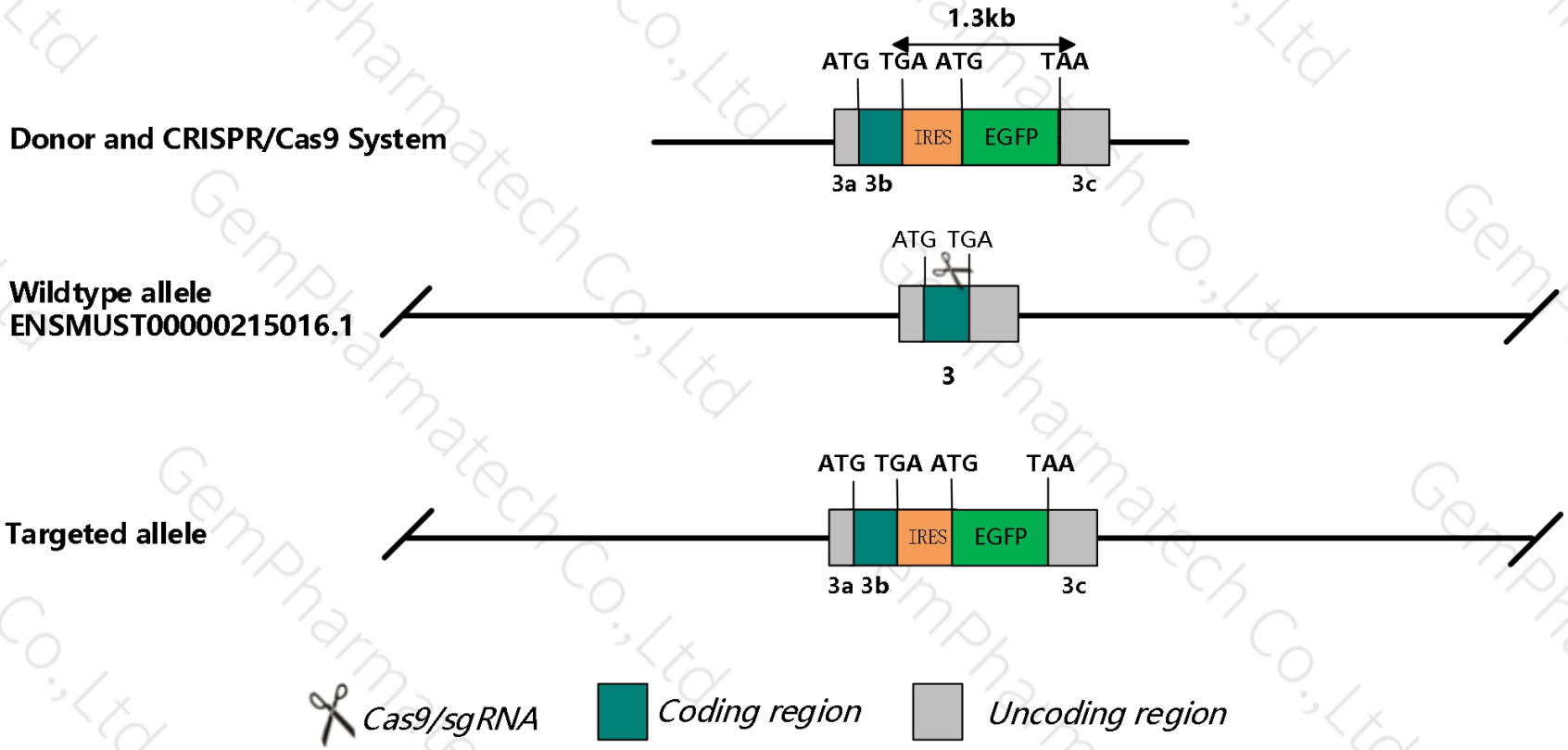
➢ The *Cx3cr1* gene has 3 transcripts.

➢ According to the structure of *Cx3cr1* gene, the element IRES-EGFP will be inserted at the translation stop codon of *Cx3cr1-203*(ENSMUST00000215016.1) , the length of inserted fragment is about 1.3kb.

➢ In this project we use CRISPR/Cas9 technology to modify *Cx3cr1* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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.

Strategy

This model will use CRISPR/Cas9 technology to edit the *Cx3cr1* gene. The schematic diagram is as follows:



Notice

➢ According to the existing MGI data,age related retinal degeneration with abnormal subretinal microglial cell accumulation in one homozygous null mice. Other null mice shows impaired monocyte recruitment after vascular injury, kidney ischemia and reperfusion, and bacterial infection of the instestine.

➢ It is necessary to introduce 1-2 synonymous mutation in exon3.

➢ The IERS-linked genes will be tarnscripted together and then be translated two protein separately, but the downstream protein is lower than the upstream protein.

➢ The *Cx3cr1* 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.

➢ The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene between the 3'UTR and the gene coding region may affect the expression of endogenous and foreign genes. Due to the complexity of biological processes, it cannot be predicted completely at the present technology level.

Gene information （NCBI）

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Cx3cr1 chemokine (C-X3-C motif) receptor 1 [Mus musculus (house mouse)]

Gene ID: 13051, updated on 13-Mar-2020

Summary

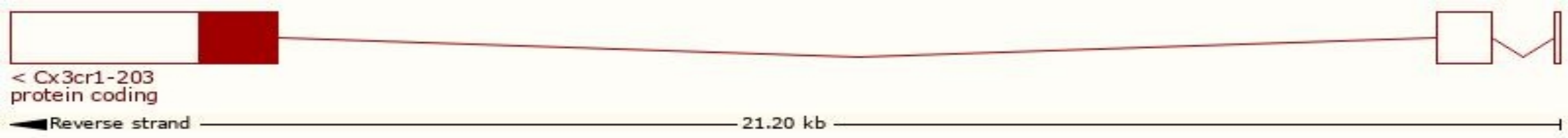
Official Symbol	Cx3cr1 provided by MGI
Official Full Name	chemokine (C-X3-C motif) receptor 1 provided byMGI
Primary source	MGI:MGI:1333815
See related	Ensembl:ENSMUSG00000052336
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Ubiquitous expression in cortex adult (RPKM 8.3), frontal lobe adult (RPKM 7.0) and 27 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

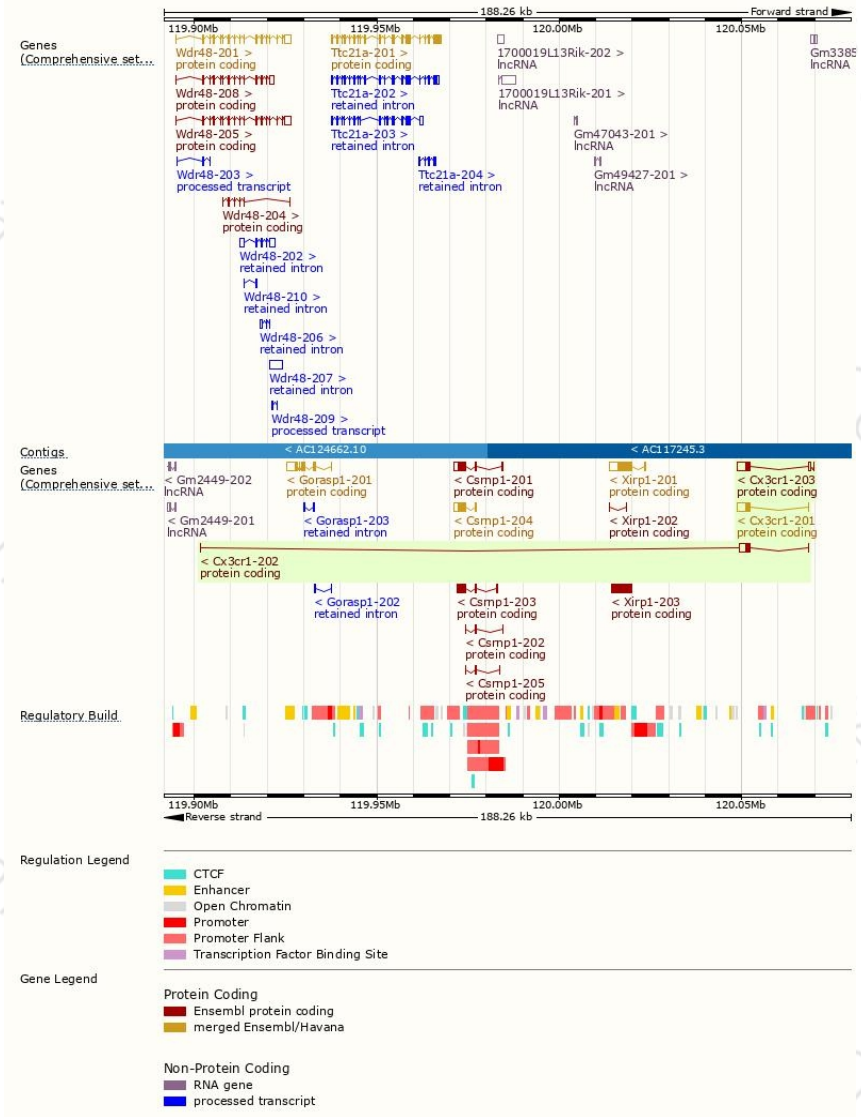
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cx3cr1-203	ENSMUST00000215016.1	4493	354aa	Protein coding	CCDS40806	Q543X3 Q9Z0D9	TSL:5 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
Cx3cr1-201	ENSMUST00000064165.4	3753	354aa	Protein coding	CCDS40806	Q543X3 Q9Z0D9	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
Cx3cr1-202	ENSMUST00000177637.1	3156	354aa	Protein coding	CCDS40806	Q543X3 Q9Z0D9	TSL:5 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

The strategy is based on the design of *Cx3cr1-203* transcript,the transcription is shown below:

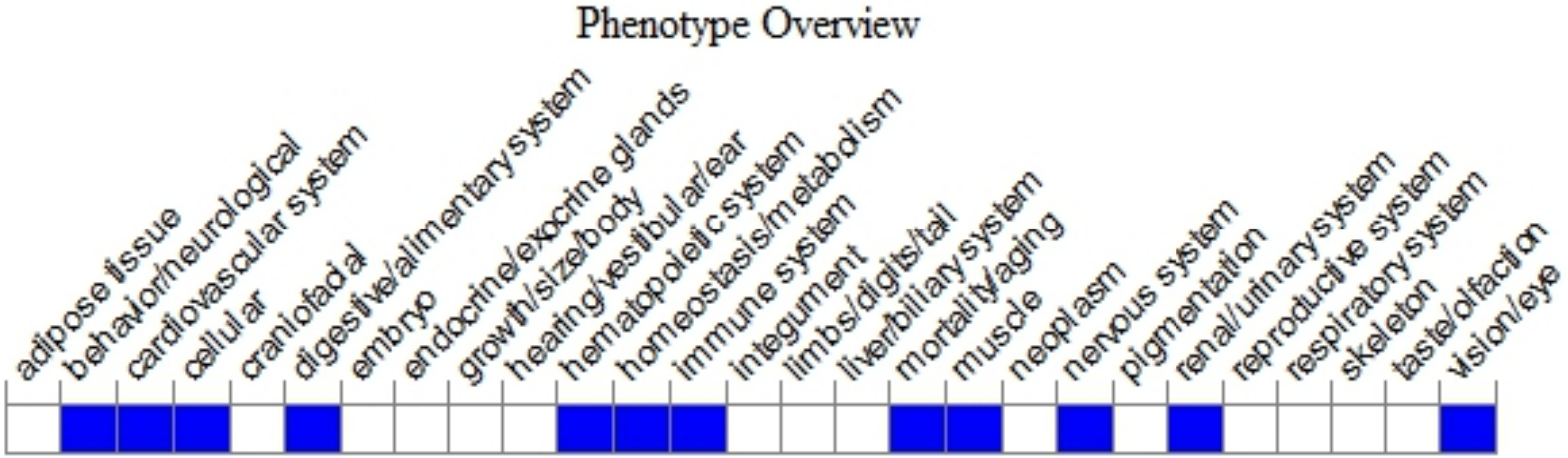


Genomic location distribution



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.

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