

Cxcr3 Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Cxcr3

Project type

Cas9-CKO

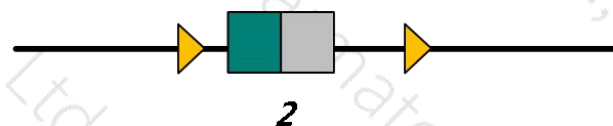
Strain background

C57BL/6JGpt

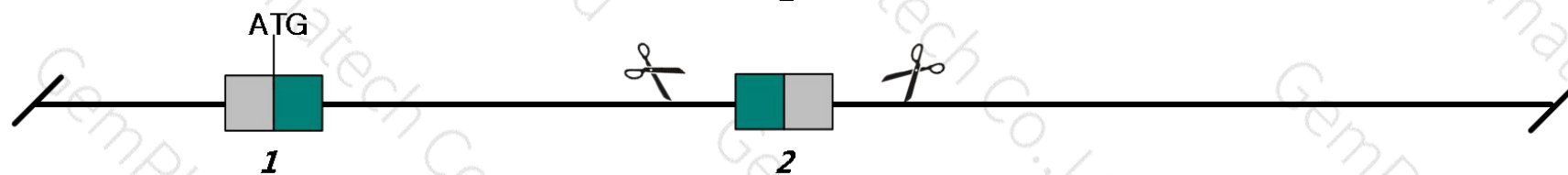
Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cxcr3* gene. The schematic diagram is as follows:

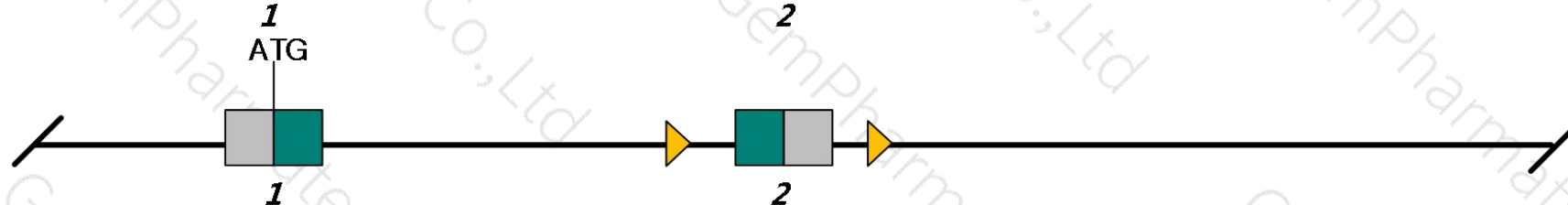
Donor and CRISPR/Cas9 System



Wild-type allele



Conditional KO allele



KO allele



Cas9/sgRNA



Uncoding region



Coding region



LoxP

- The *Cxcr3* gene has 1 transcript. According to the structure of *Cxcr3* gene, exon2 of *Cxcr3*-201 (ENSMUST00000056614.6) 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 *Cxcr3* 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 or cell types.

- According to the existing MGI data , Mice homozygous or hemizygous for disruptions in this gene display immune system abnormalities. Hemizygous male mice exhibit elevated serum glucose levels.
- The KO region deletes most of the coding sequence, but does not result in frameshift.
- The *Cxcr3* gene is located on the ChrX. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Cxcr3 chemokine (C-X-C motif) receptor 3 [*Mus musculus* (house mouse)]

Gene ID: 12766, updated on 8-Jun-2019

Summary

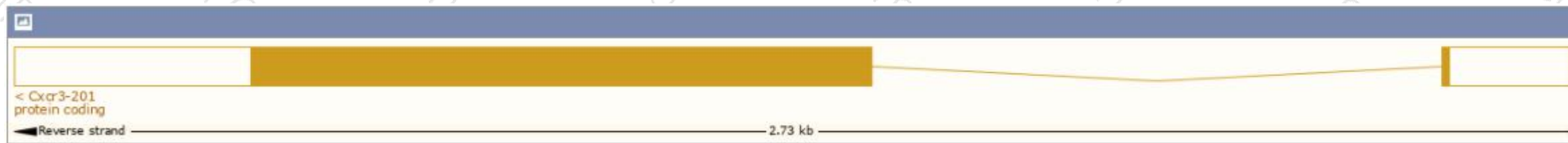
Official Symbol	Cxcr3 provided by MGI
Official Full Name	chemokine (C-X-C motif) receptor 3 provided by MGI
Primary source	MGI:MGI:1277207
See related	Ensembl:ENSMUSG00000050232
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Cd183; Cmkar3
Summary	This gene encodes a transmembrane protein that functions as a receptor for C-X-R chemokines. Signalling through this protein regulates a variety of biological processes, including inflammation, immunity, and wound healing. This protein also plays a role in tumor growth and metastasis. [provided by RefSeq, May 2015]
Expression	Biased expression in spleen adult (RPKM 18.9), mammary gland adult (RPKM 10.3) and 11 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

The gene has 1 transcript, and all transcripts is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
Cxcr3-201	ENSMUST00000056614.6	1731	367aa	Protein coding	CCDS30319	O88410	NM_009910 NP_034040	TSL:1 GENCODE basic APPRIS P1

The strategy is based on the design of *Cxcr3-201* transcript, The transcription is shown below



Genomic location distribution

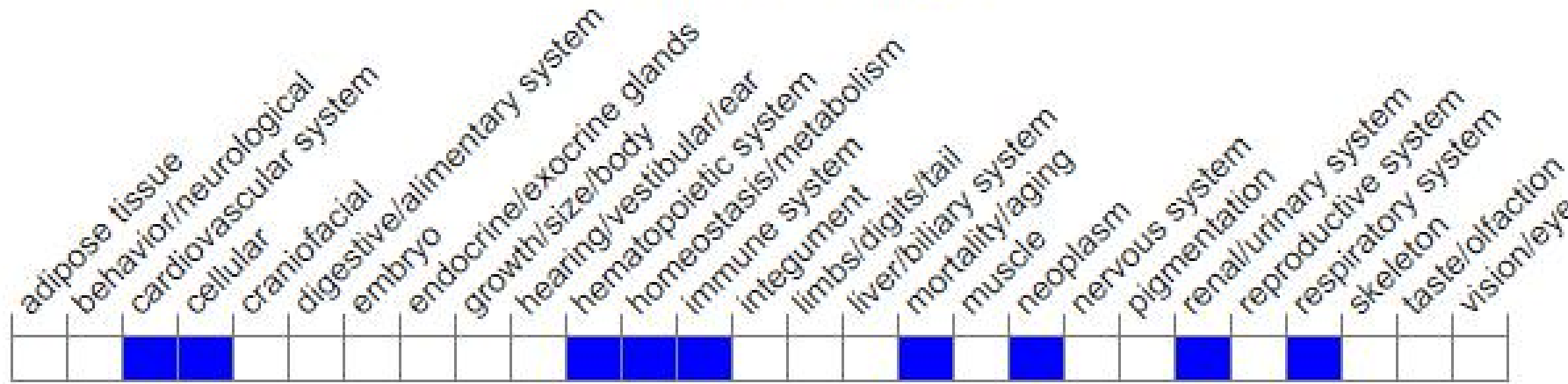


Protein domain



Mouse phenotype description(MGI)

Phenotype Overview ?



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

According to the existing MGI data, Mutations in this locus affect cell-cycle regulation and apoptosis. Null homozygotes show high, early-onset tumor incidence; some have persistent hyaloid vasculature and cataracts. Truncated or temperature-sensitive alleles cause early aging phenotypes.