

Cysltr1 Cas9-CKO Strategy

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Reviewer:

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Design Date:

2019-12-18

Project Overview

Project Name

Cysltr1

Project type

Cas9-CKO

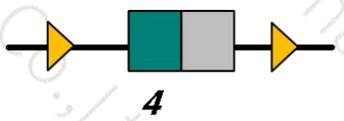
Strain background

C57BL/6JGpt

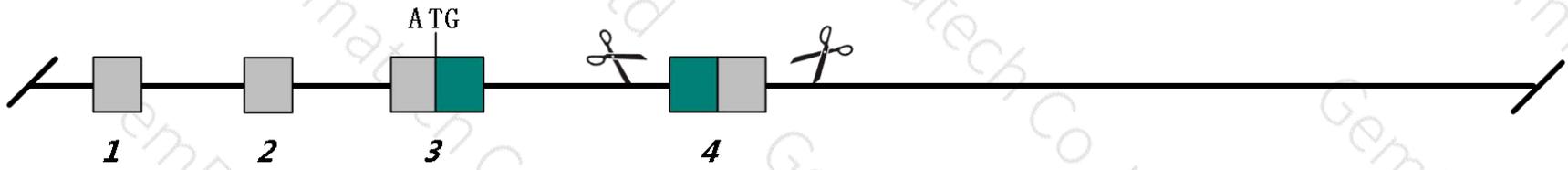
Conditional Knockout strategy

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

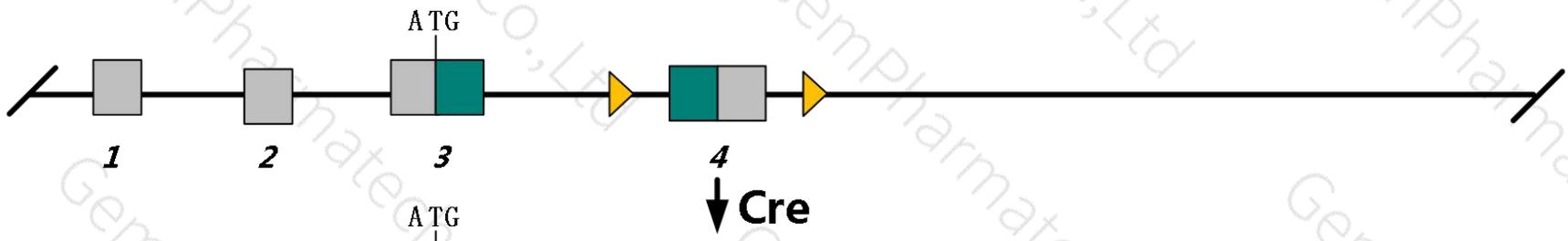
Donor and CRISPR/Cas9 System



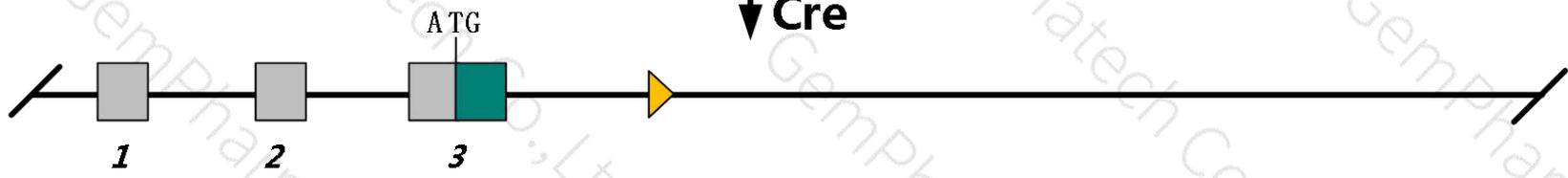
Wild-type allele



Conditional KO allele



KO allele



- The *Cysltr1* gene has 2 transcripts. According to the structure of *Cysltr1* gene, exon 4 of *Cysltr1*-202 (ENSMUST00000113480.1) 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 *Cysltr1* 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 for disruptions in this gen develop normally and both sexes are fertile. However, they display abnormalities in vascular permeability associated with inflammation.
- The *Cysltr1* gene is located on the Chr X. 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 CKO region contains functional region of the Gm5127 gene. Knockout the region may affect the function of Gm5127 gene.
- 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)

Cysltr1 cysteinyl leukotriene receptor 1 [*Mus musculus* (house mouse)]

Gene ID: 58861, updated on 26-Jun-2019

Summary



Official Symbol	Cysltr1 provided by MGI
Official Full Name	cysteinyl leukotriene receptor 1 provided by MGI
Primary source	MGI:MGI:1926218
See related	Ensembl:ENSMUSG00000052821
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	Cysl1; CysLT1R; BB147369
Expression	Broad expression in placenta adult (RPKM 1.9), bladder adult (RPKM 1.0) and 18 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

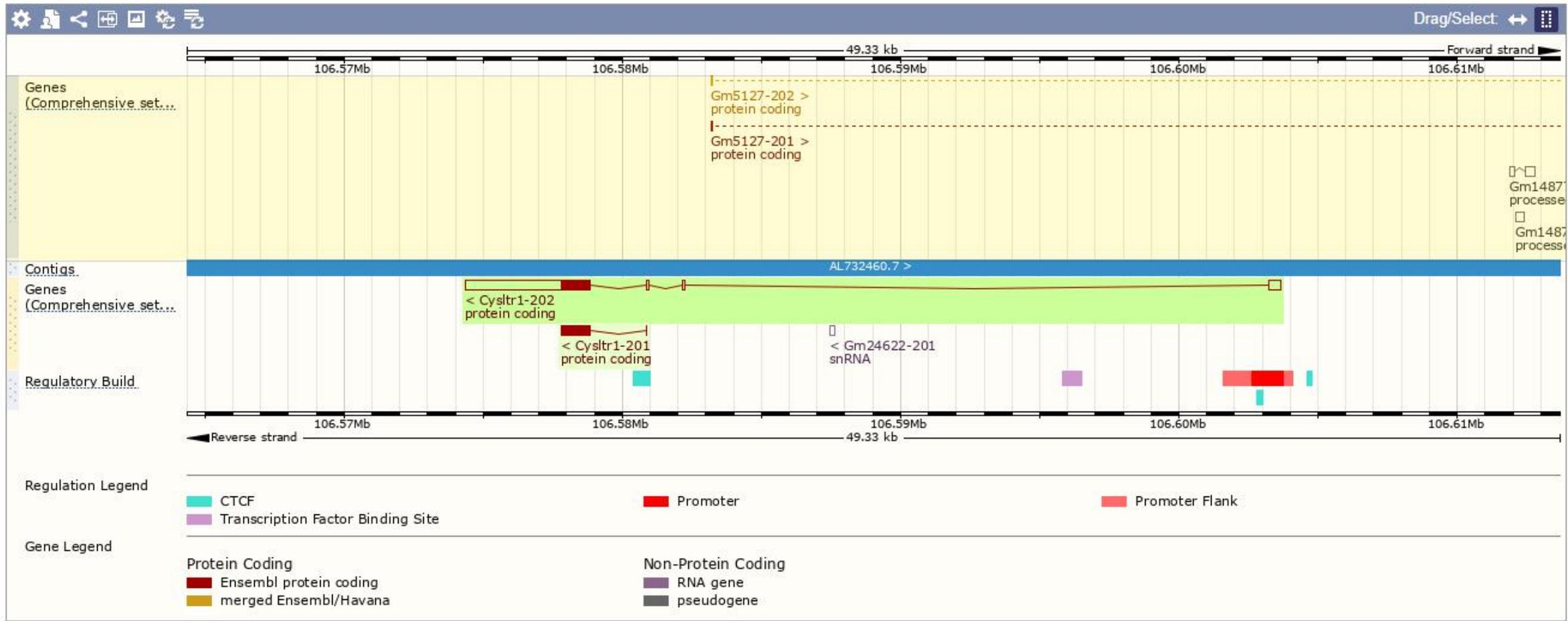
The gene has 2 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cysltr1-202	ENSMUST00000113480.1	5142	352aa	Protein coding	CCDS41098	Q99JA4	TSL:1 Gencode basic APPRIS P1
Cysltr1-201	ENSMUST00000064892.3	1059	352aa	Protein coding	CCDS41098	Q99JA4	TSL:2 Gencode basic APPRIS P1

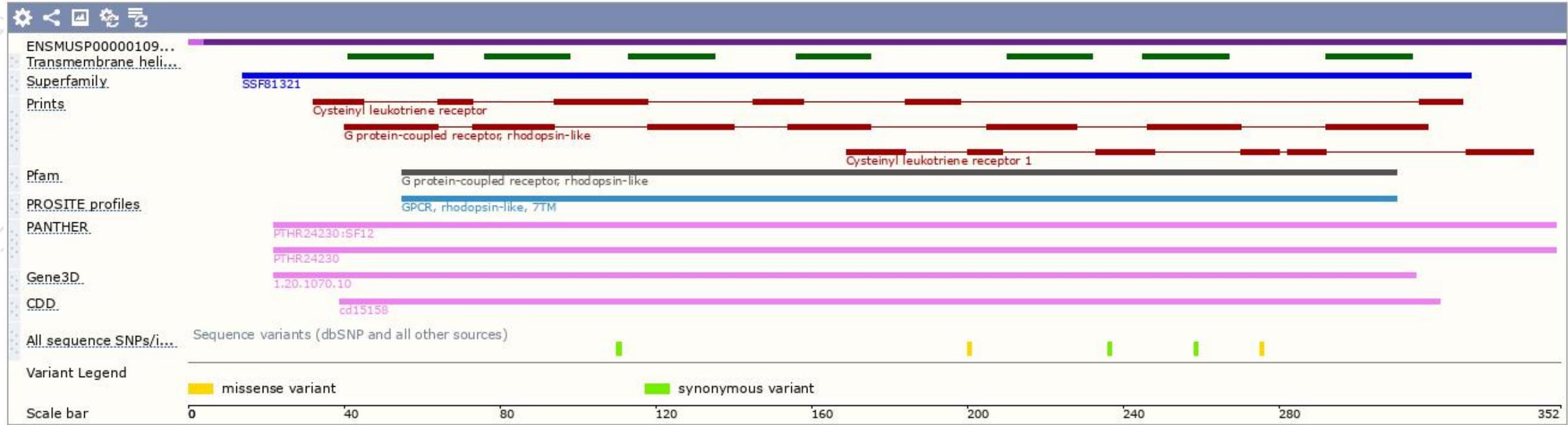
The strategy is based on the design of *Cysltr1*-202 transcript, The transcription is shown below



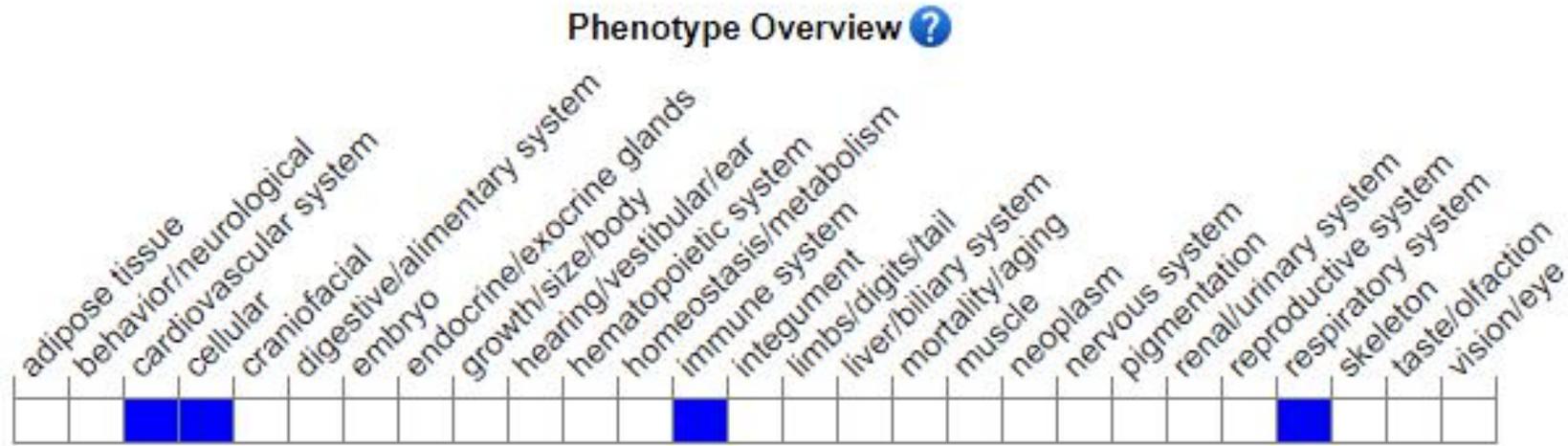
Genomic location distribution



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/>).

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.

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