

Oprk1 Cas9-CKO Strategy

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Project Overview

Project Name

Oprk1

Project type

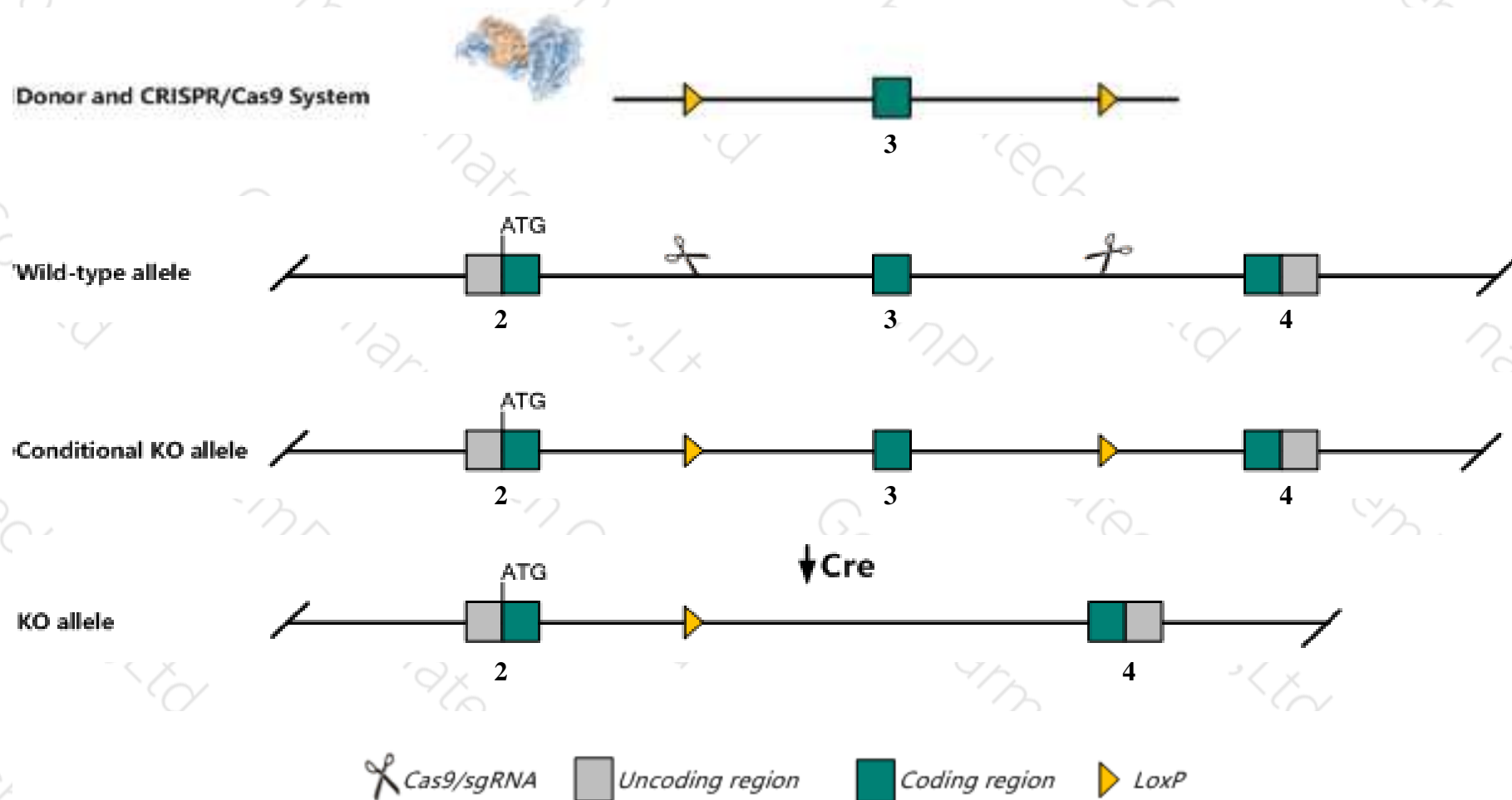
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Oprk1* gene. The schematic diagram is as follows:



- The *Oprk1* gene has 5 transcripts. According to the structure of *Oprk1* gene, exon3 of *Oprk1*-204 (ENSMUST00000160777.7) transcript is recommended as the knockout region. The region contains 353bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Oprk1* 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.
- The flox mice was 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.

- According to the existing MGI data, mice homozygous for a knock-out allele exhibit impaired response to morphine and an opioid agonist, abnormal pain threshold, and increased litter size.
- The *Oprk1* gene is located on the Chr1. 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)

Oprk1 opioid receptor, kappa 1 [*Mus musculus* (house mouse)]

Gene ID: 18387, updated on 12-Aug-2019

Summary



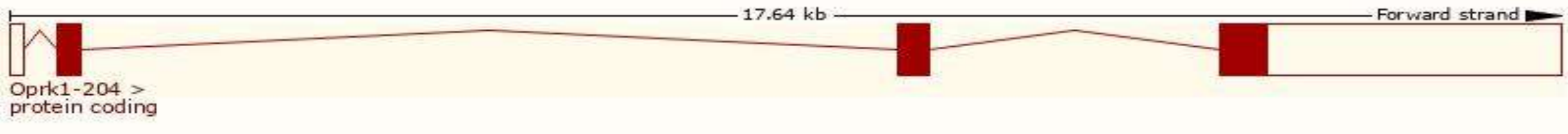
Official Symbol	Oprk1 provided by MGI
Official Full Name	opioid receptor, kappa 1 provided by MGI
Primary source	MGI:MGI:97439
See related	Ensembl:ENSMUSG000000025905
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	KOR; R21; KOR-1; MSL-1; Oprk2; K-OR-1
Summary	This gene encodes an opioid receptor, which is a member of the 7 transmembrane-spanning G protein-coupled receptor family. It functions as a receptor for endogenous ligands, as well as a receptor for various synthetic opioids. Ligand binding results in inhibition of adenylate cyclase activity and neurotransmitter release. This opioid receptor plays a role in the perception of pain and mediating the hypolocomotor, analgesic and aversive actions of synthetic opioids. Variations in this gene have also been associated with alcohol dependence and opiate addiction. Alternatively spliced transcript variants have been found for this gene. A recent study provided evidence for translational readthrough in this gene, and expression of an additional C-terminally extended isoform via the use of an alternative in-frame translation termination codon. [provided by RefSeq, Dec 2017]
Expression	Biased expression in frontal lobe adult (RPKM 1.1), cortex adult (RPKM 0.9) and 8 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

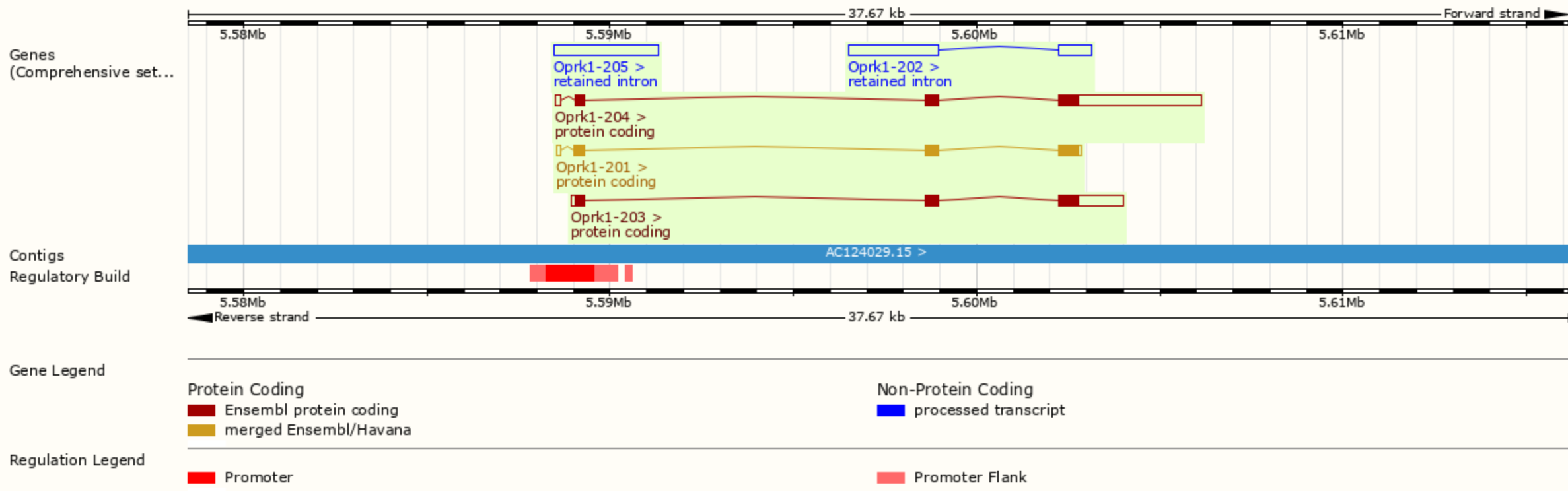
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Oprk1-201	ENSMUST00000027038.10	1376	380aa	Protein coding	CCDS14809	P33534 Q14AL5	TSL:1 GENCODE basic APPRIS P1
Oprk1-202	ENSMUST00000159083.1	3327	No protein	Retained intron	-	-	TSL:1
Oprk1-203	ENSMUST00000160339.1	2481	380aa	Protein coding	CCDS14809	P33534 Q14AL5	TSL:5 GENCODE basic APPRIS P1
Oprk1-204	ENSMUST00000160777.7	4675	380aa	Protein coding	CCDS14809	P33534 Q14AL5	TSL:1 GENCODE basic APPRIS P1
Oprk1-205	ENSMUST00000192685.1	2846	No protein	Retained intron	-	-	TSL:NA

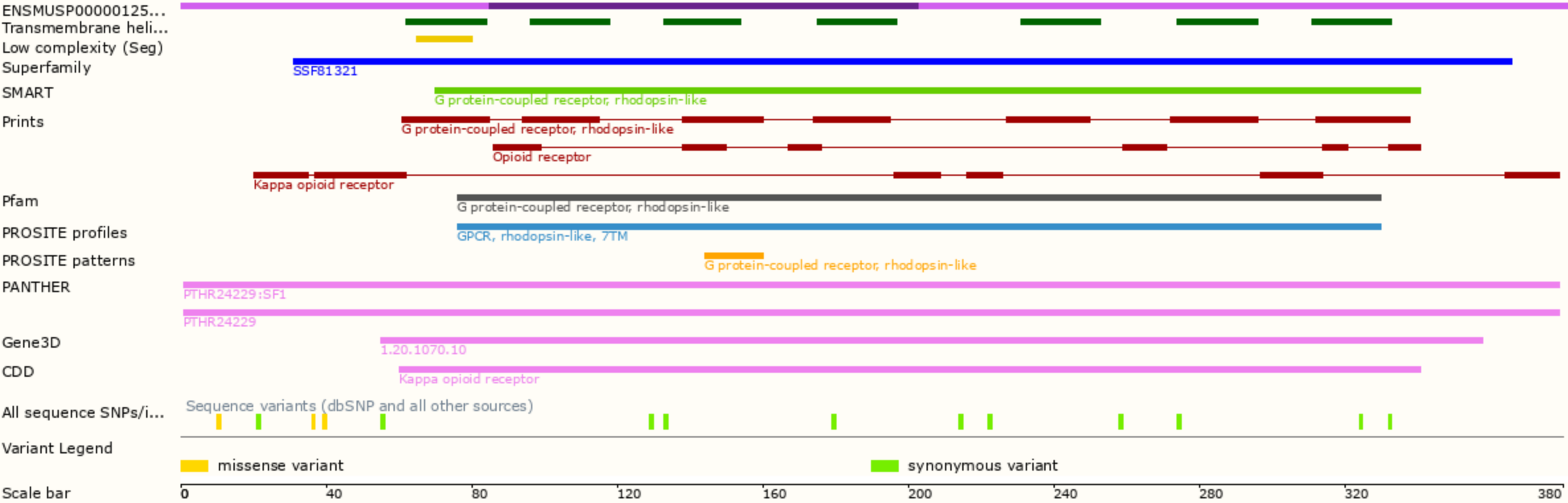
The strategy is based on the design of *Oprk1-204* transcript,The transcription is shown below



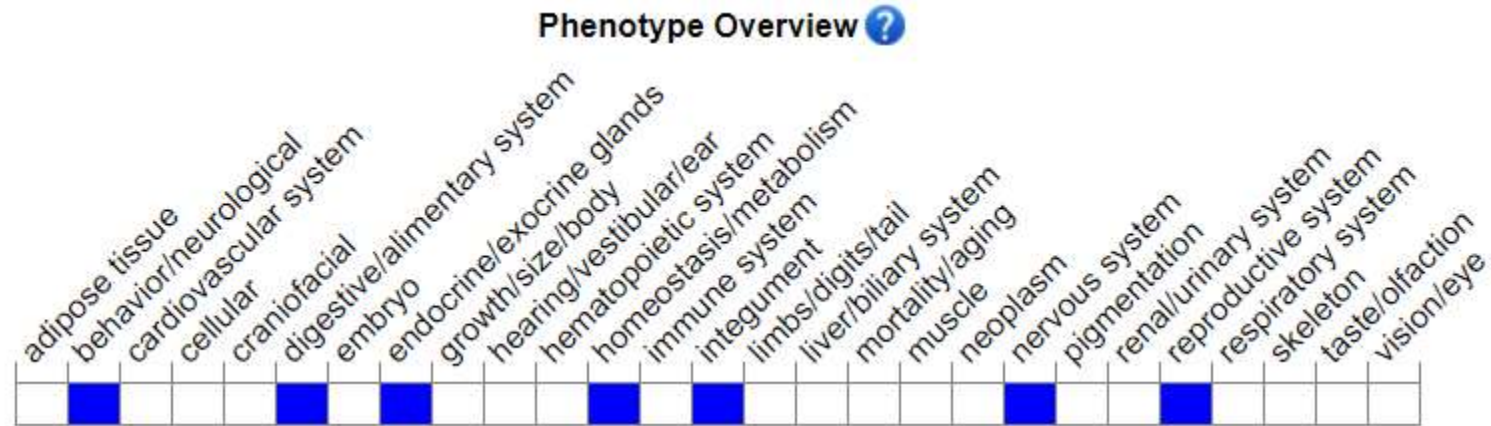
Genomic location distribution



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, Mice homozygous for a knock-out allele exhibit impaired response to morphine and an opioid agonist, abnormal pain threshold, and increased litter size.

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

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