Grk3 Cas9-CKO Strategy

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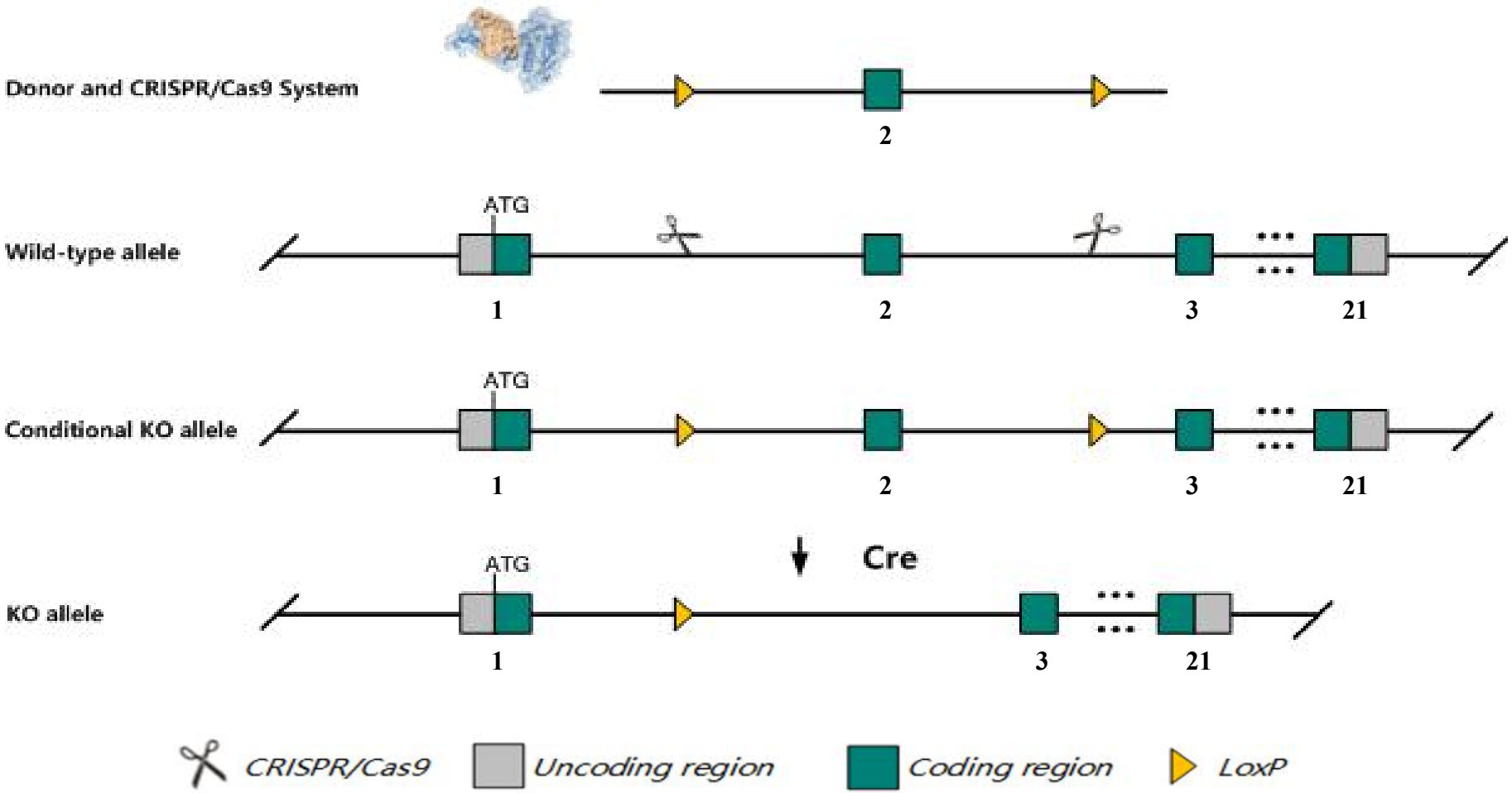
Project Overview

Project Name	***Grk3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Grk3* gene. The schematic diagram is as follows:



Technical routes

The *Grk3* gene has 6 transcripts. According to the structure of *Grk3* gene, exon2 of *Grk3-201* (ENSMUST00000065167.8) transcript is recommended as the knockout region.The region contains 77bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Grk3* gene. The brief process is as follows:CRISPR/Cas9 system 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,animals homozygous for a mutation of this gene appear normal and are fertile, but exhibit a lack of odorant receptor-mediated desensitization in the olfactory epithelium.

The *Grk3* gene is located on the Chr5. 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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Grk3 G protein-coupled receptor kinase 3 [Mus musculus (house mouse)]

Gene ID: 320129, updated on 13-Mar-2020

Summary

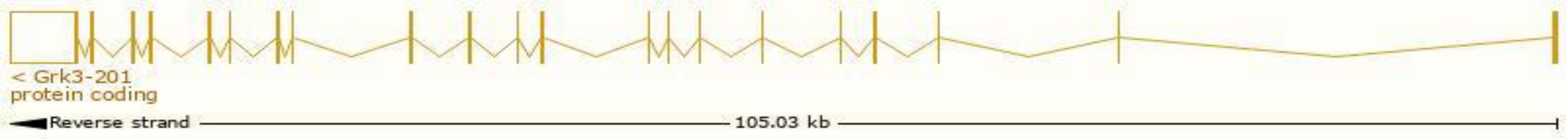
Official Symbol	Grk3 provided by MGI
Official Full Name	G protein-coupled receptor kinase 3 provided by MGI
Primary source	MGI:MGI:87941
See related	Ensembl:ENSMUSG00000042249
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4833444A01Rik, AI851927, AW551196, Adrbk-2, Adrbk2, Bark-2
Expression	Ubiquitous expression in frontal lobe adult (RPKM 7.8), cerebellum adult (RPKM 7.5) and 26 other tissues See more
Orthologs	human all

Transcript information Ensembl

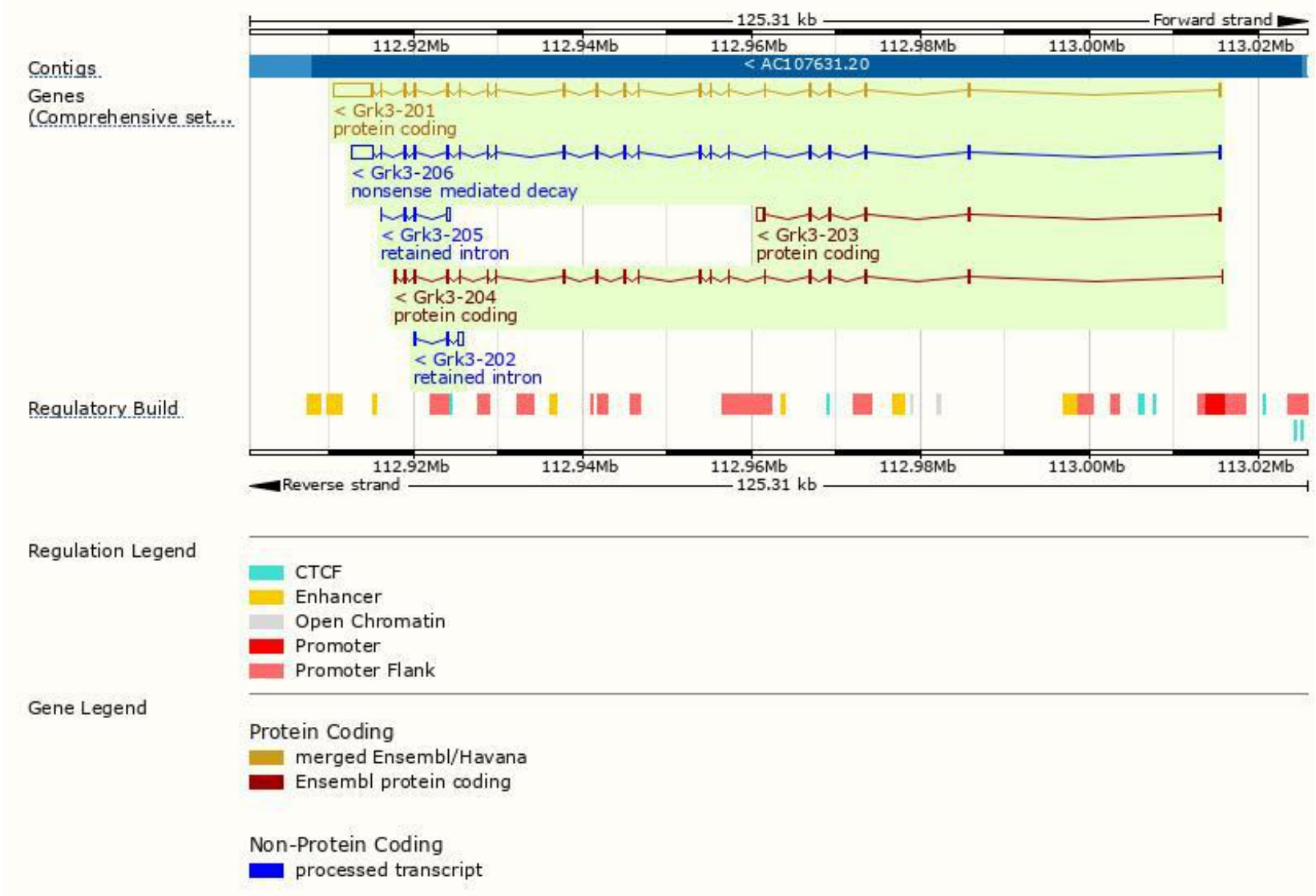
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Grk3-201	ENSMUST00000065167.8	6533	688aa	Protein coding	CCDS19545	Q3UYH7	TSL:1 GENCODE basic APPRIS P1
Grk3-204	ENSMUST00000197888.4	1938	556aa	Protein coding	-	Q8BVT9	TSL:1 GENCODE basic
Grk3-203	ENSMUST00000197776.1	1570	217aa	Protein coding	-	Q8BX71	TSL:1 GENCODE basic
Grk3-206	ENSMUST00000200332.4	4455	92aa	Nonsense mediated decay	-	A0A0G2JEW1	TSL:1
Grk3-205	ENSMUST00000199337.1	781	No protein	Retained intron	-	-	TSL:3
Grk3-202	ENSMUST00000195928.1	759	No protein	Retained intron	-	-	TSL:3

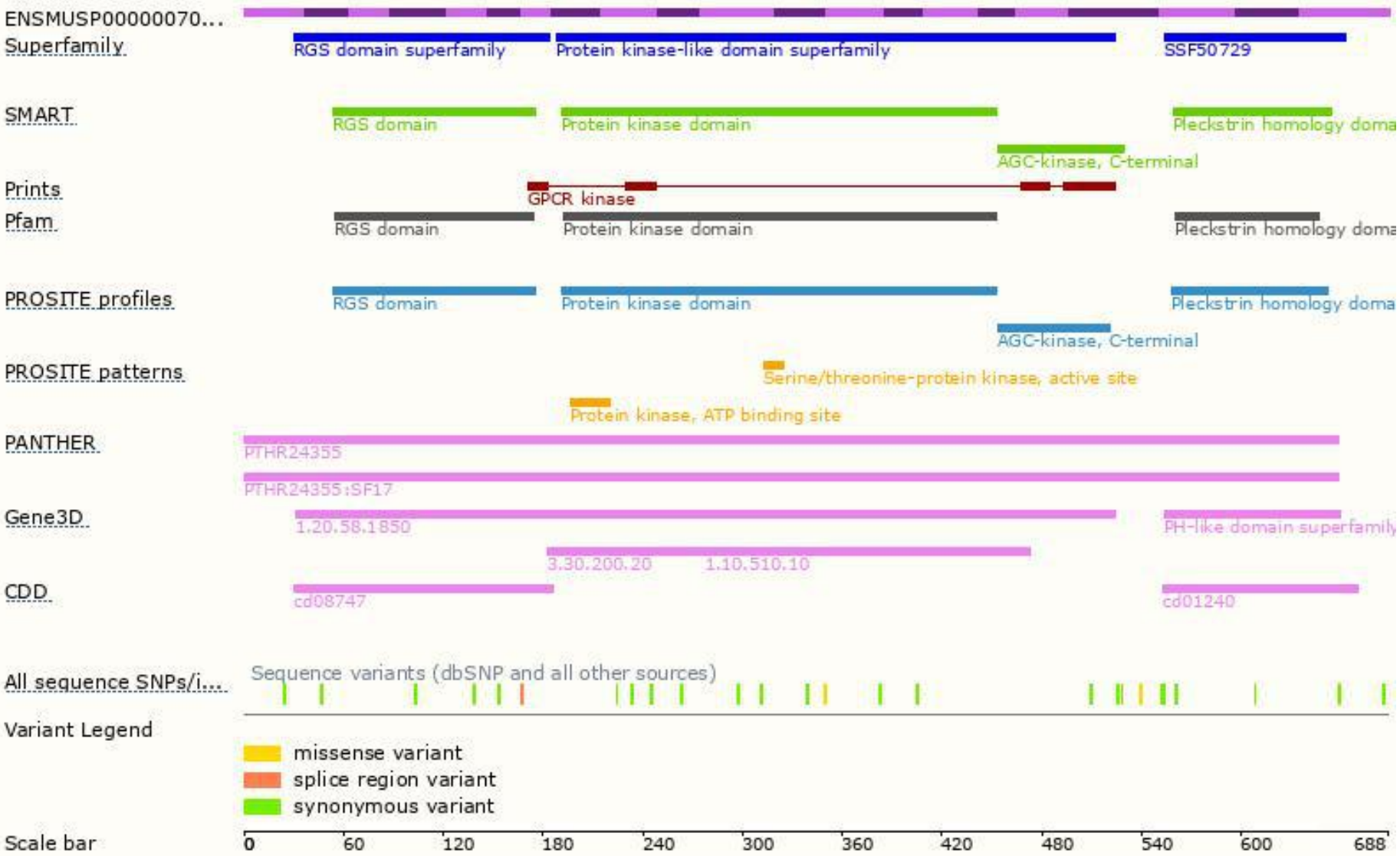
The strategy is based on the design of *Grk3-201* transcript,the transcription is shown below:



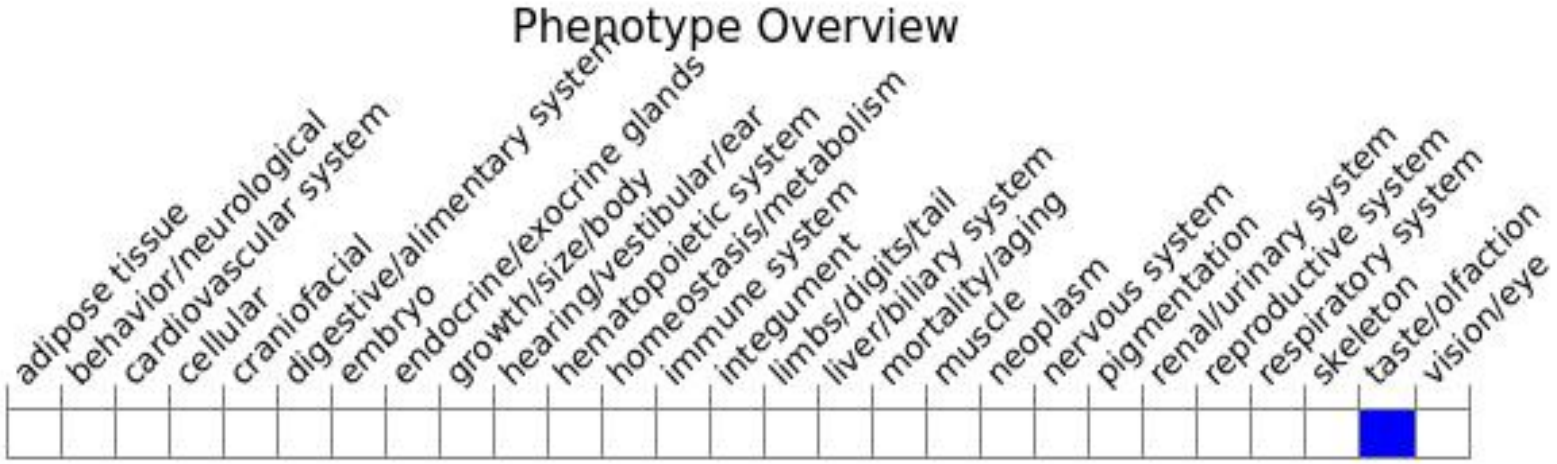
Genomic location distribution



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/).

According to the existing MGI data,animals homozygous for a mutation of this gene appear normal and are fertile, but exhibit a lack of odorant receptor-mediated desensitization in the olfactory epithelium.

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