Hcrtr2 Cas9-CKO Strategy

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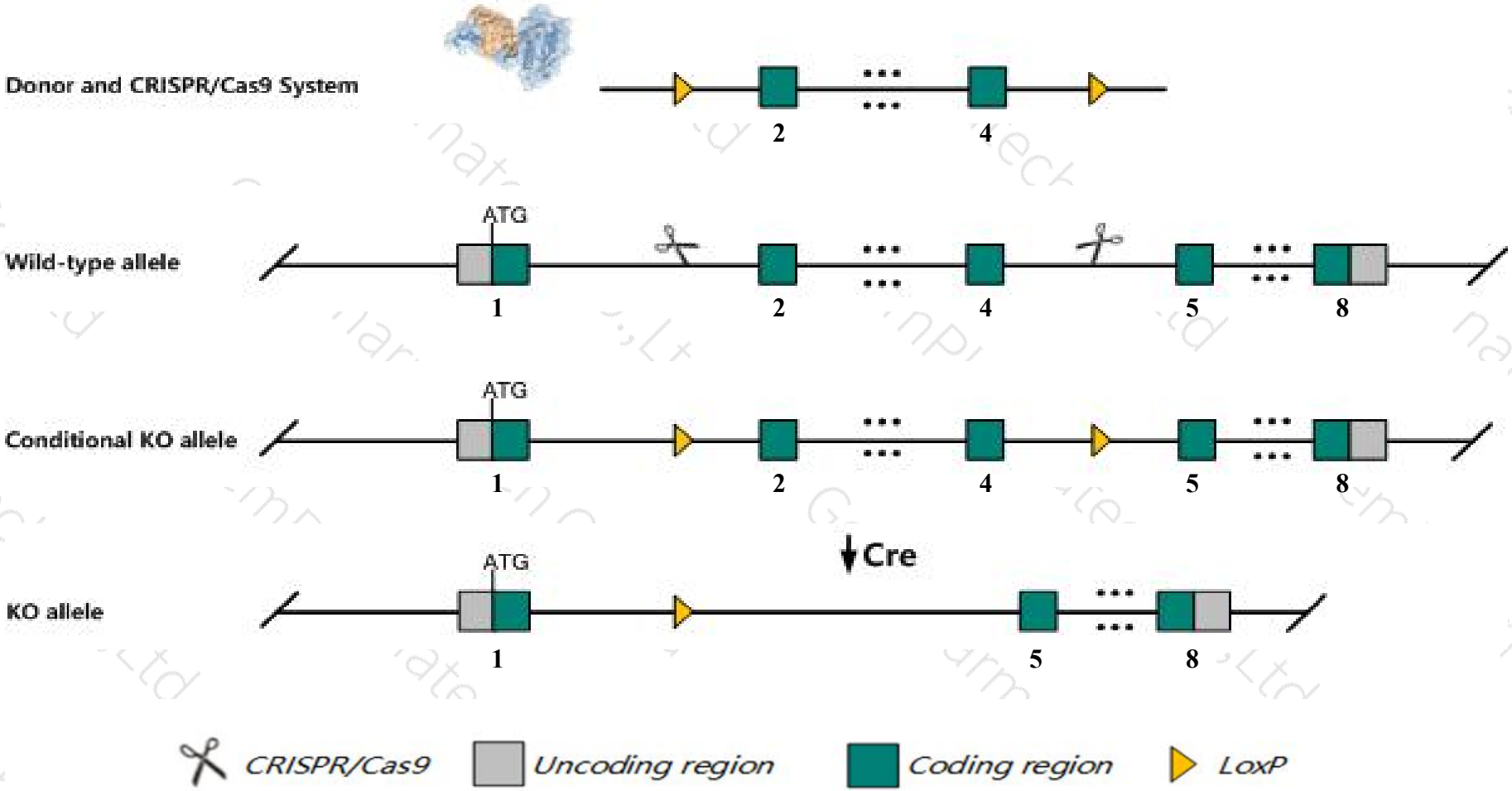
Design Date: 2019-9-2

Project Overview

Project Name	***Hcrtr2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Hcrtr2* gene. The schematic diagram is as follows:



Technical routes

- The *Hcrtr2* gene has 3 transcripts. According to the structure of *Hcrtr2* gene, exon2-exon4 of *Hcrtr2-201* (ENSMUST00000063140.14) transcript is recommended as the knockout region.The region contains 539bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Hcrtr2* 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,mice bearing targeted mutations in this gene exhibit fragmentation of sleep/wake states with similarity to narcolepsy and rare or very rare episodes of cataplexy. In addition, mice homozygous for a funtionally null allele display enhanced depression-likebehavior.

➢ The *Hcrtr2* 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.

➢ 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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Hcrtr2 hypocretin (orexin) receptor 2 [*Mus musculus* (house mouse)]

Gene ID: 387285, updated on 12-Aug-2019

Summary

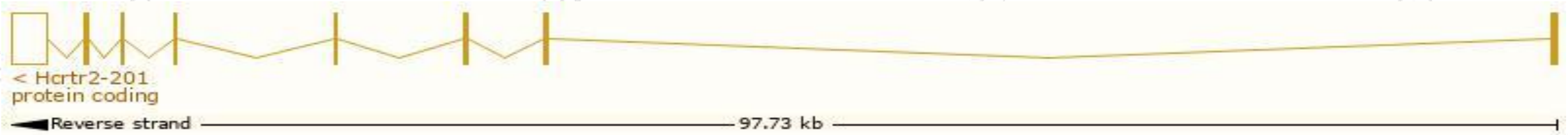
Official Symbol	Hcrtr2 provided by MGI
Official Full Name	hypocretin (orexin) receptor 2 provided by MGI
Primary source	MGI:MGI:2680765
See related	Ensembl:ENSMUSG00000032360
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	OX2R; OXR2; OX2aR; OX2bR; mOXR2; mOX2aR; mOX2bR
Expression	Low expression observed in reference dataset See more
Orthologs	human all

Transcript information（Ensembl）

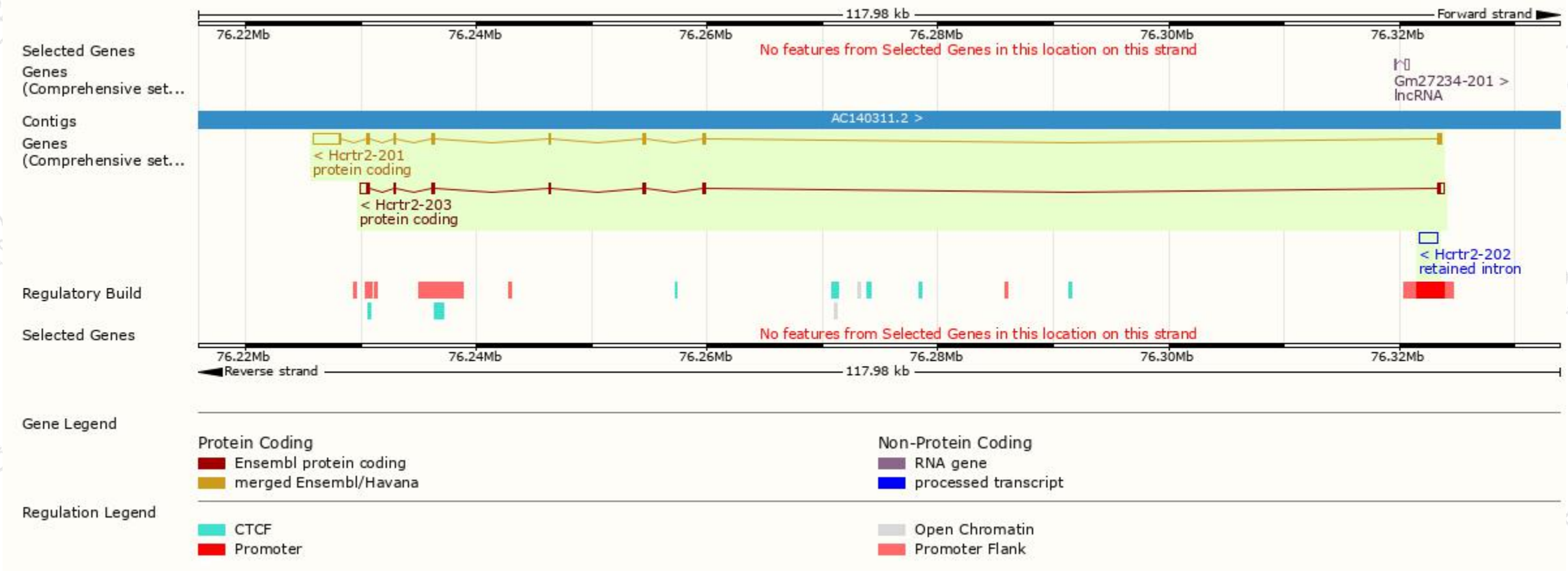
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hcrtr2-201	ENSMUST00000063140.14	3761	460aa	Protein coding	CCDS23350	P58308	TSL:1 GENCODE basic APPRIS P2
Hcrtr2-203	ENSMUST00000184757.1	2230	443aa	Protein coding	-	P58308	TSL:1 GENCODE basic APPRIS ALT1
Hcrtr2-202	ENSMUST00000184200.1	1647	No protein	Retained intron	-	-	TSL:NA

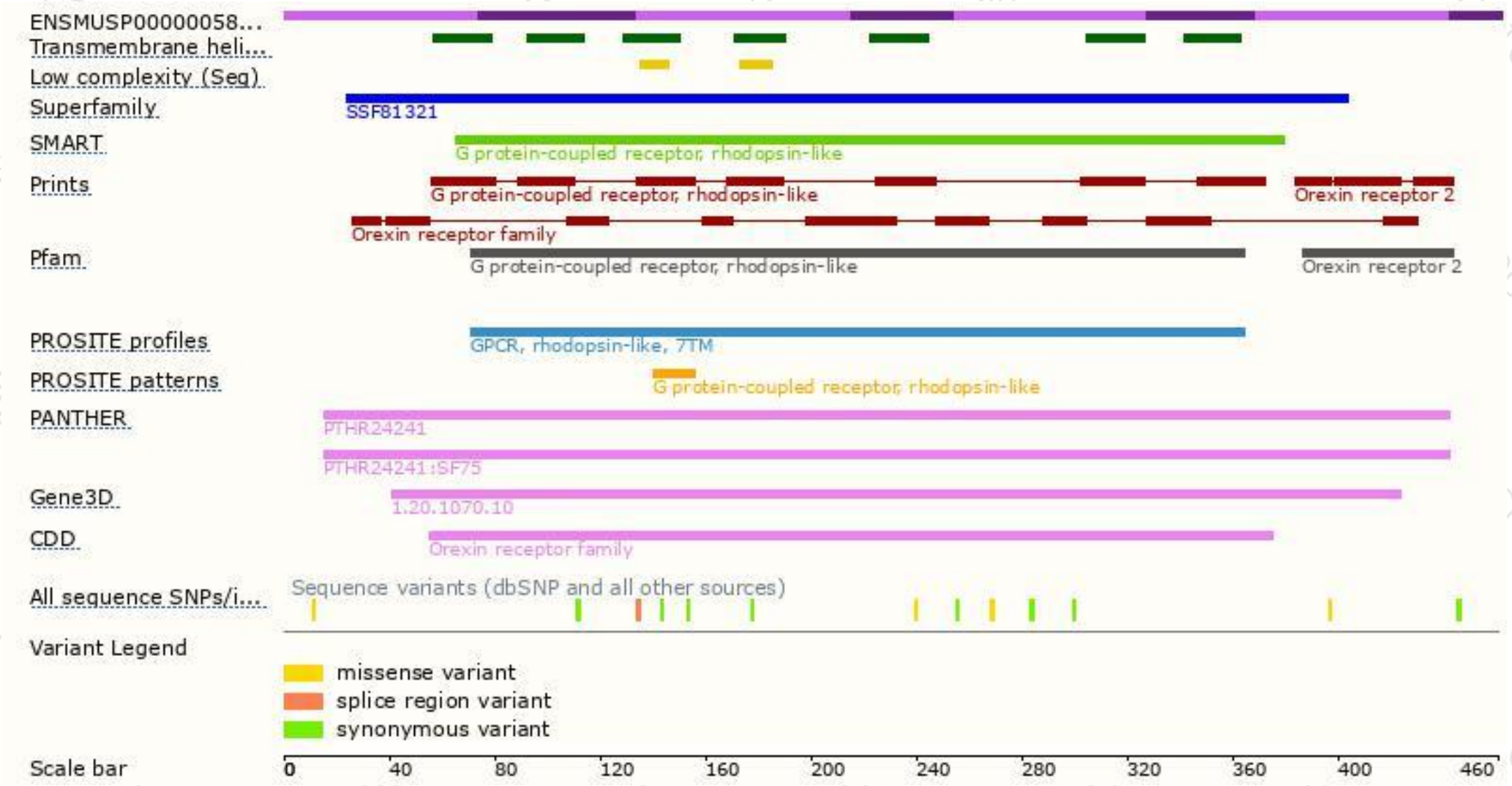
The strategy is based on the design of *Hcrtr2-201* transcript,The transcription is shown below



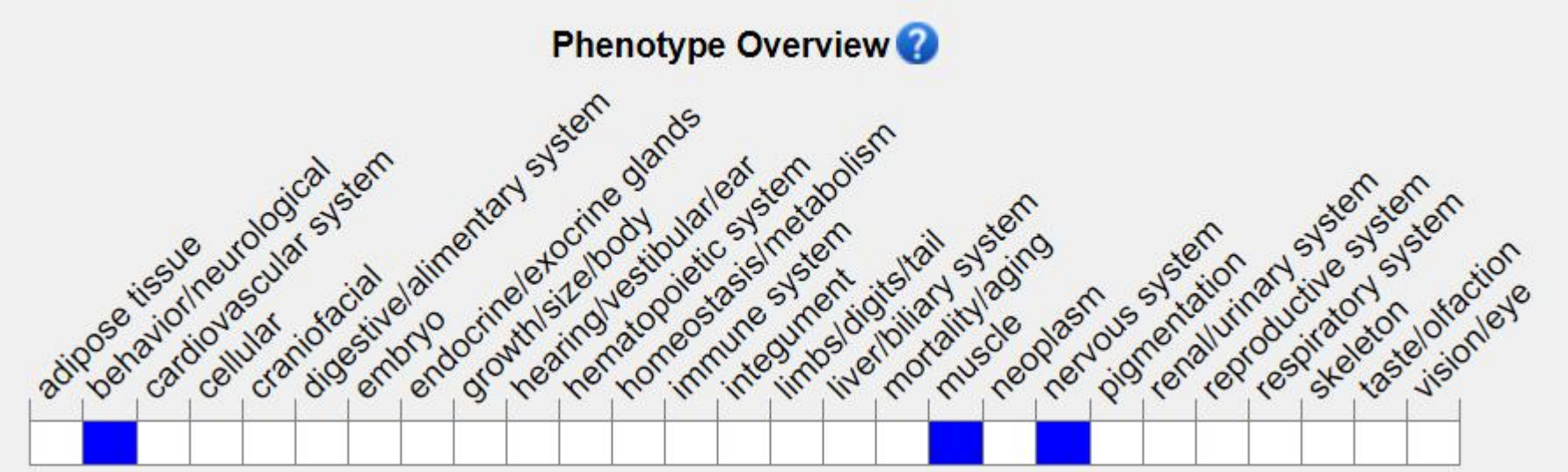
Genomic location distribution



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/).

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

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