

Ntsr2 Cas9-CKO Strategy

Designer:

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Design Date:

2019-8-12

Project Overview

Project Name

Ntsr2

Project type

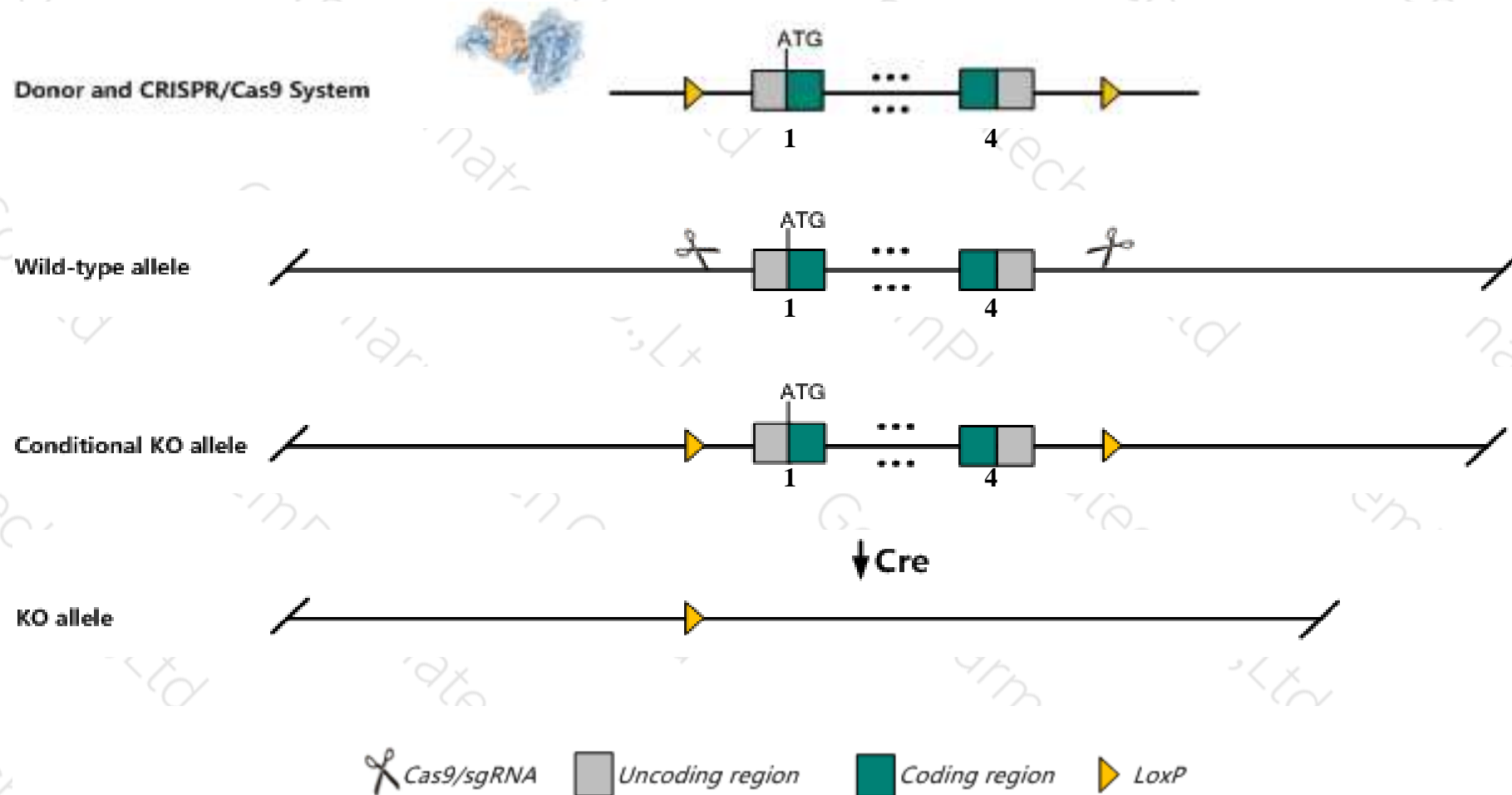
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ntsr2* gene. The schematic diagram is as follows:



- The *Ntsr2* gene has 5 transcripts. According to the structure of *Ntsr2* gene, exon1-exon4 and predicted promoter region of *Ntsr2-201* (ENSMUST00000111064.2) transcript is recommended as the knockout region. The region contains all 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 *Ntsr2* 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, homozygous null mice exhibit abnormal thermal nociception. Mice homozygous for different knock-out allele exhibit increased prepulse inhibition and decreased acoustic startle response.
- The *Ntsr2* gene is located on the Chr12. 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)

Ntsr2 neurotensin receptor 2 [*Mus musculus* (house mouse)]

Gene ID: 18217, updated on 10-Aug-2019

Summary

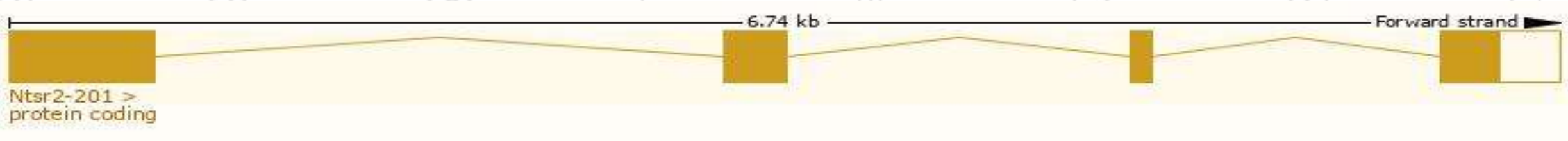
Official Symbol	Ntsr2 provided by MGI
Official Full Name	neurotensin receptor 2 provided by MGI
Primary source	MGI:MGI:108018
See related	Ensembl:ENSMUSG00000020591
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	NT2R; NTR2; NTRL; NT-R-2
Expression	Biased expression in genital fat pad adult (RPKM 70.4), cortex adult (RPKM 64.8) and 5 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

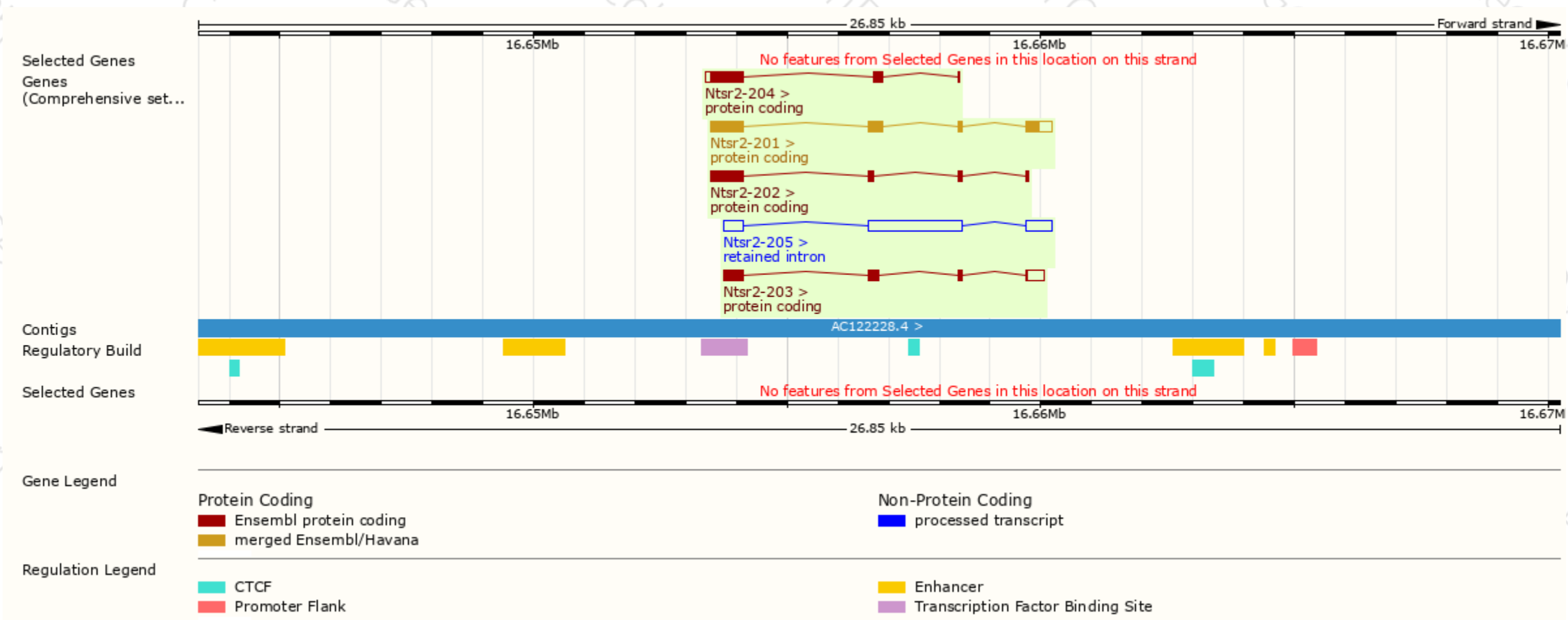
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ntsr2-201	ENSMUST00000111064.2	1524	416aa	Protein coding	CCDS36407	P70310	TSL:1 GENCODE basic APPRIS P1
Ntsr2-203	ENSMUST00000221049.1	1028	236aa	Protein coding	-	A0A1Y7VNS4	CDS 5' incomplete TSL:1
Ntsr2-204	ENSMUST00000221596.1	963	281aa	Protein coding	-	A0A1Y7VIR1	CDS 3' incomplete TSL:5
Ntsr2-202	ENSMUST00000220892.1	846	281aa	Protein coding	-	A0A1Y7VN85	TSL:3 GENCODE basic
Ntsr2-205	ENSMUST00000222957.1	2757	No protein	Retained intron	-	-	TSL:1

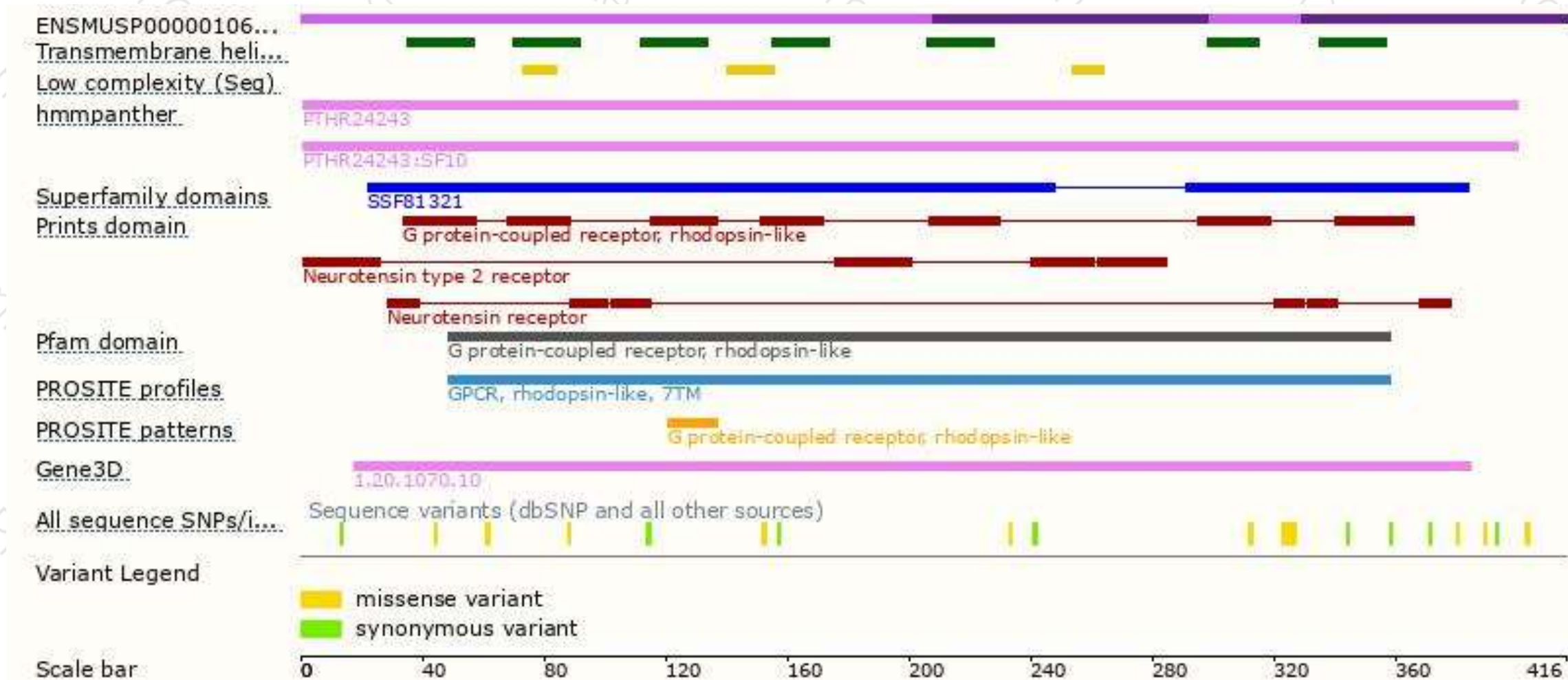
The strategy is based on the design of *Ntsr2-201* transcript,The transcription is shown below



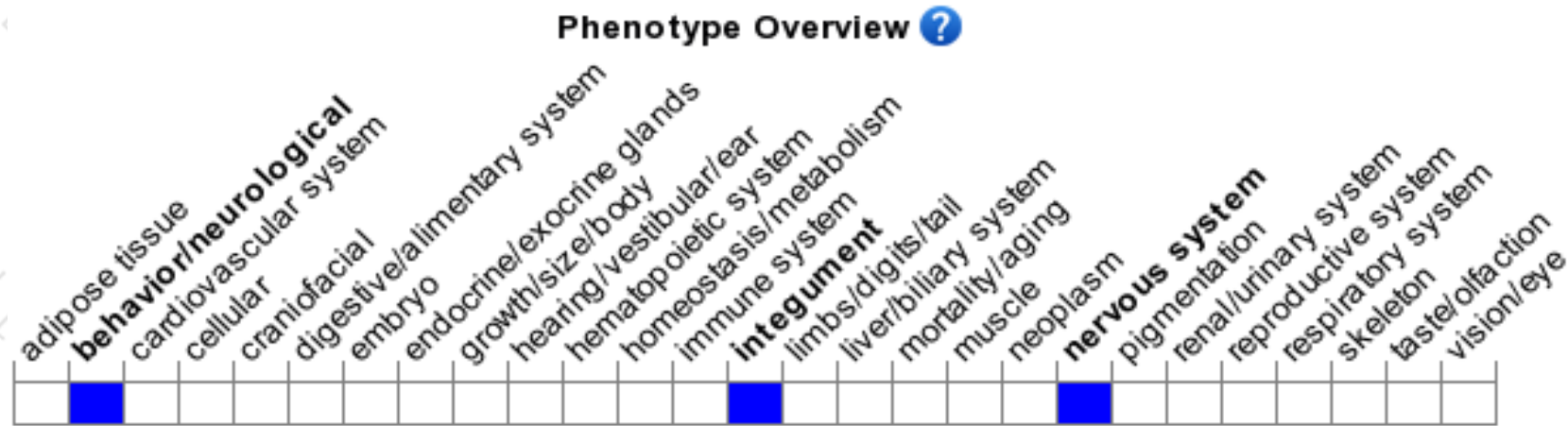
Genomic location distribution



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, Homozygous null mice exhibit abnormal thermal nociception. Mice homozygous for different knock-out allele exhibit increased prepulse inhibition and decreased acoustic startle response.

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

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