

# *Npffr2* Cas9-CKO Strategy

**Designer:**

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**Design Date:**

**2019-8-8**

# Project Overview

**Project Name**

*Npffr2*

**Project type**

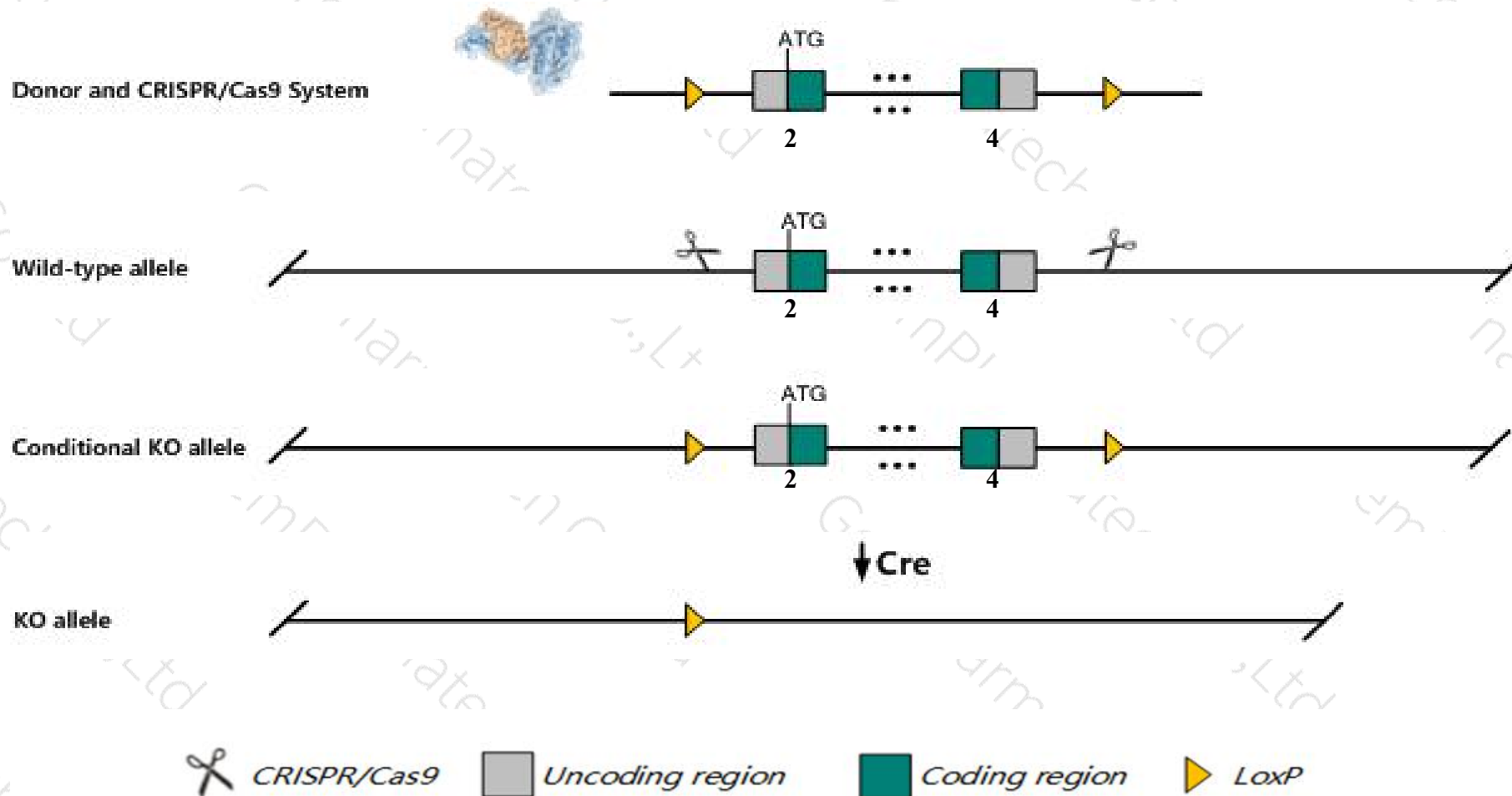
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Npffr2* gene. The schematic diagram is as follows:



- The *Npffr2* gene has 1 transcript. According to the structure of *Npffr2* gene, exon2-exon4 of *Npffr2-201* (ENSMUST00000048557.2) 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 *Npffr2* 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 and cell types.

- The *Npffr2* 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.
- According to the existing MGI data, mice homozygous for a knock-out allele exhibit increased litter size and sex-specific increased susceptibility to diet-induced obesity and adipose accumulation, impaired diet-induced thermogenesis, and altered bone thickness.
- 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)

## Npffr2 neuropeptide FF receptor 2 [ *Mus musculus* (house mouse) ]

Gene ID: 104443, updated on 12-Aug-2019

### Summary

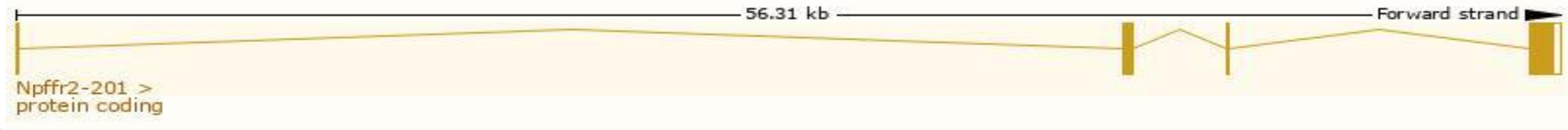
<b>Official Symbol</b>	Npffr2 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	neuropeptide FF receptor 2 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1860130</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000035528</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	PROVISIONAL
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Also known as</b>	HG31; Gpr74; NPFF2
<b>Expression</b>	Low expression observed in reference dataset <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

The gene has 1 transcript, and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Npffr2-201	<a href="#">ENSMUST00000048557.2</a>	1643	<a href="#">417aa</a>	Protein coding	<a href="#">CCDS19408</a>	<a href="#">Q924H0</a>	TSL:1 Gencode basic APPRIS P1

The strategy is based on the design of *Npffr2-201* transcript, The transcription is shown below:

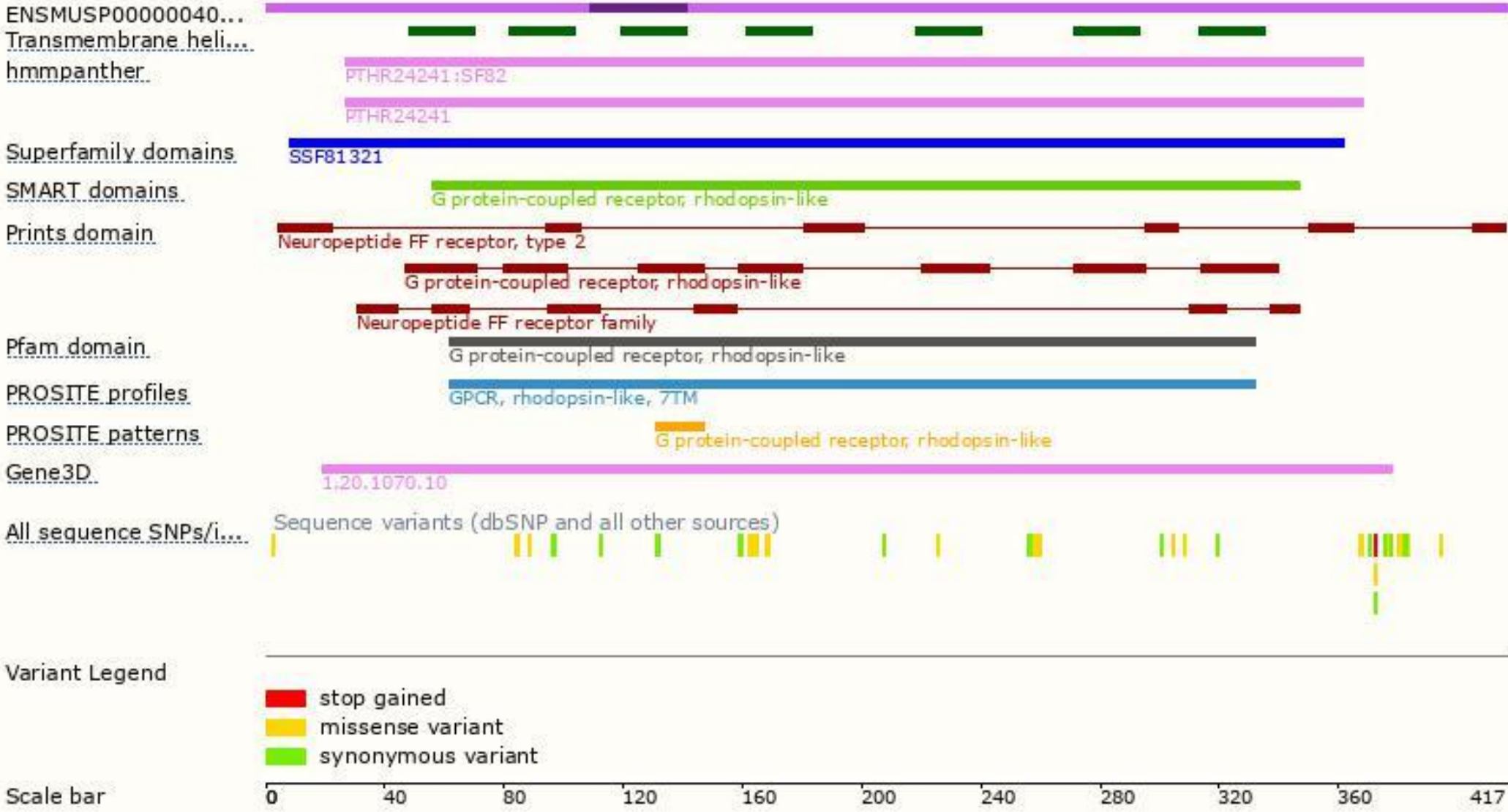


# Genomic location distribution

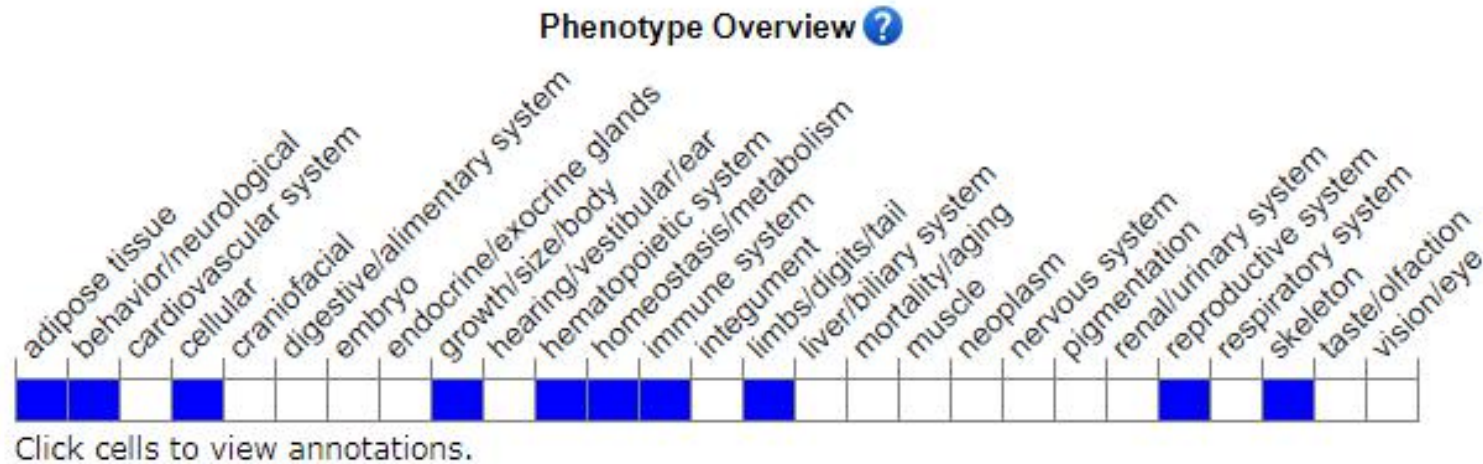




# Protein domain



# 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, Mice homozygous for a knock-out allele exhibit increased litter size and sex-specific increased susceptibility to diet-induced obesity and adipose accumulation, impaired diet-induced thermogenesis, and altered bone thickness.

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

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