

Mc5r Cas9-CKO Strategy

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Overview

Target Gene Name

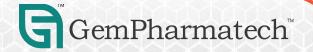
• Mc5r

Project Type

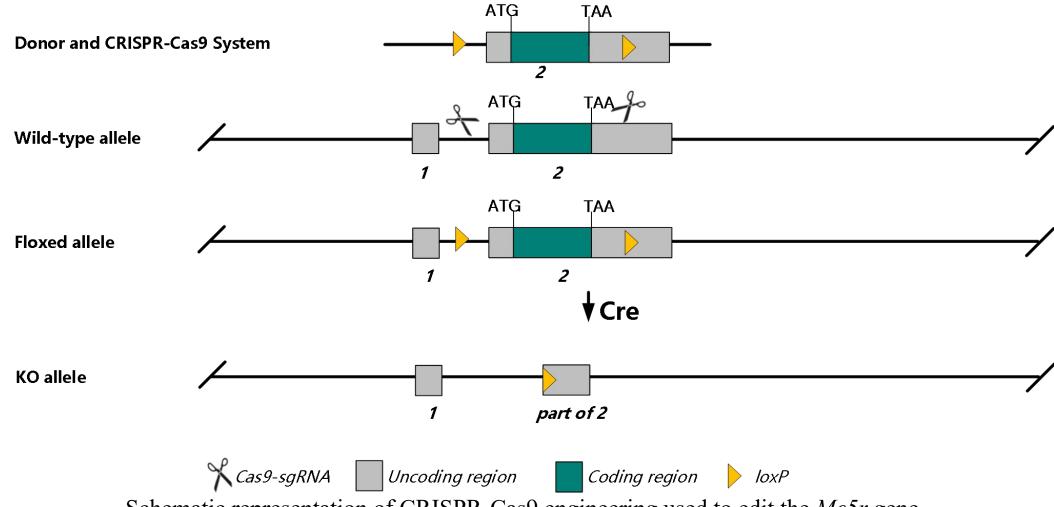
• Cas9-CKO

Genetic Background

• C57BL/6JGpt



Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Mc5r gene.



Technical Information

- The *Mc5r* gene has 1 transcript. According to the structure of *Mc5r* gene, exon2 of *Mc5r*-201 (ENSMUST00000172148.5) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Mc5r* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.



Gene Information

Mc5r melanocortin 5 receptor [Mus musculus (house mouse)]

Gene ID: 17203, updated on 13-Mar-2020



Source: https://www.ncbi.nlm.nih.gov/

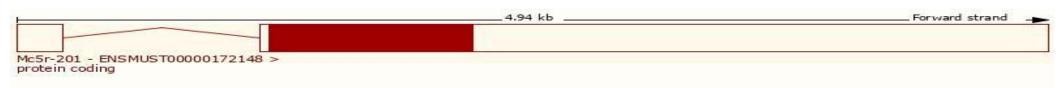


Transcript Information

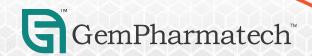
The gene has 1 transcript, and the transcript is shown below:

| Transcript ID 🗼 | Name 4 | bp 🌲 | Protein | Biotype 🍦 | CCDS 🍦 | UniProt Match 🍦 | Flags | | | \$ |
|----------------------|----------|------|--------------|----------------|--------|-------------------|-------------------|---------------|-----------|-------|
| ENSMUST00000172148.5 | Mc5r-201 | 3996 | <u>325aa</u> | Protein coding | | G3UWB6 & P41149 ₽ | Ensembl Canonical | GENCODE basic | APPRIS P1 | TSL:1 |

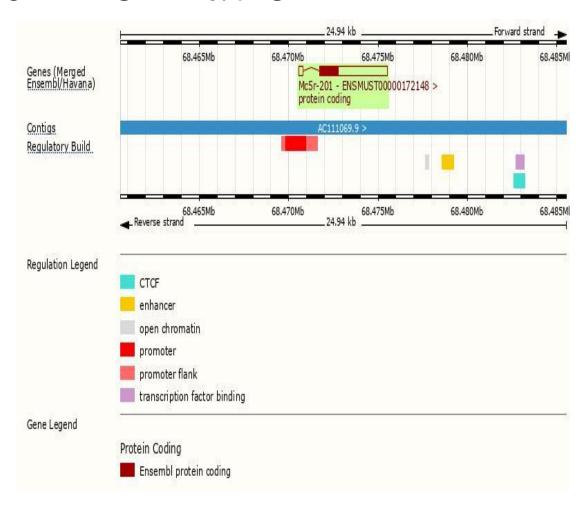
The strategy is based on the design of Mc5r-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org



Genomic Information





Source: : https://www.ensembl.org

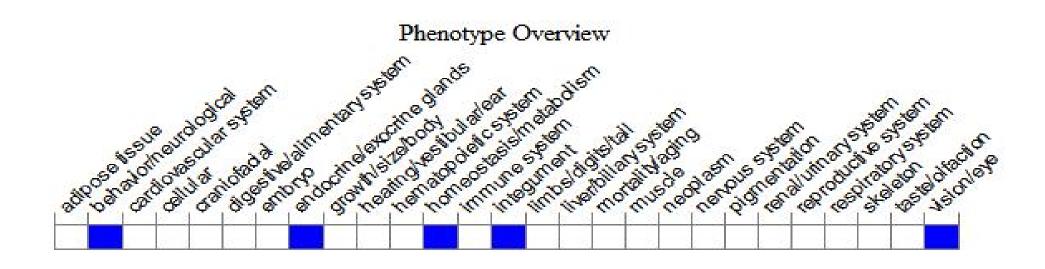
Protein Information





Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



• Homozygous mutation of this gene results in a severe defect in water repulsion and thermoregulation due to decreased production of sebaceous lipids. Males exhibit less aggressive and more defensive behavior when placed with wildtype males.



Source: https://www.informatics.jax.org

Important Information

- *Mc5r* is located on Chr18. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.
- The 3-terminal loxp is directly inserted into the 3'UTR, hich may affect the regulation of this gene.

