

# Mc5r Cas9-KO Strategy

Designer: Jia Yu

Reviewer: Yanhua Shen

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#### Overview

#### Target Gene Name

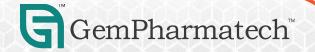
• Mc5r

#### Project Type

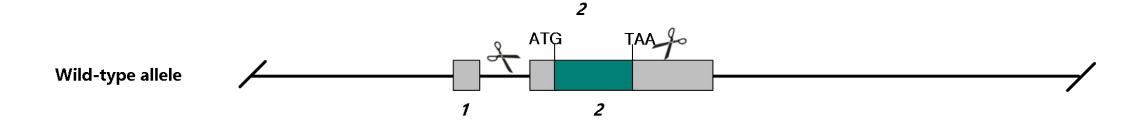
• Cas9-KO

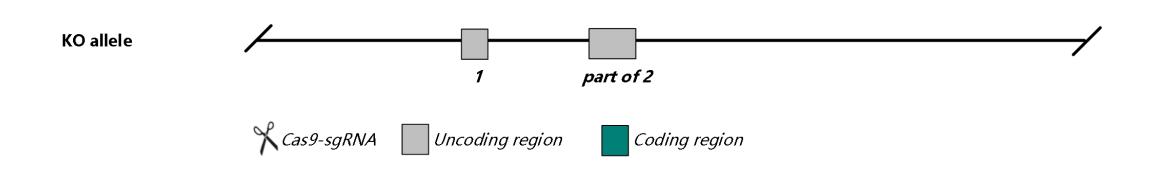
#### Genetic Background

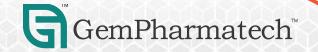
• C57BL/6JGpt



## Strain Strategy







#### **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: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 ontarget 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.



#### 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/

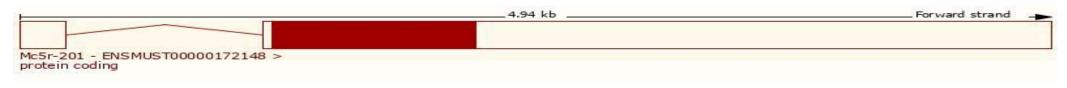


### Transcript Information

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

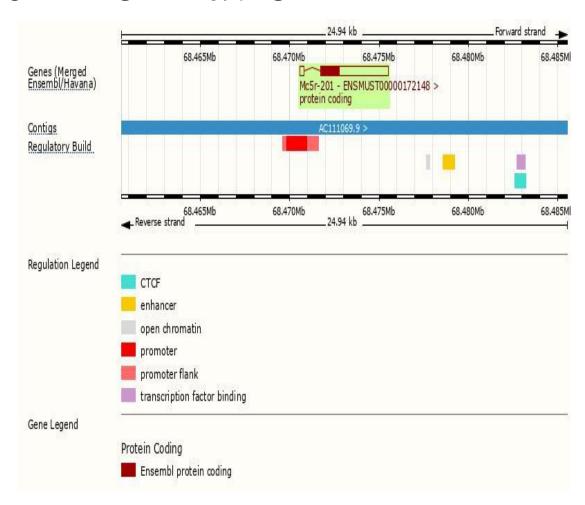
The strategy is based on the design of Mc5r-201 transcript, the transcription is shown below:

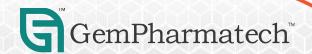


Source: https://www.ensembl.org



### Genomic Information





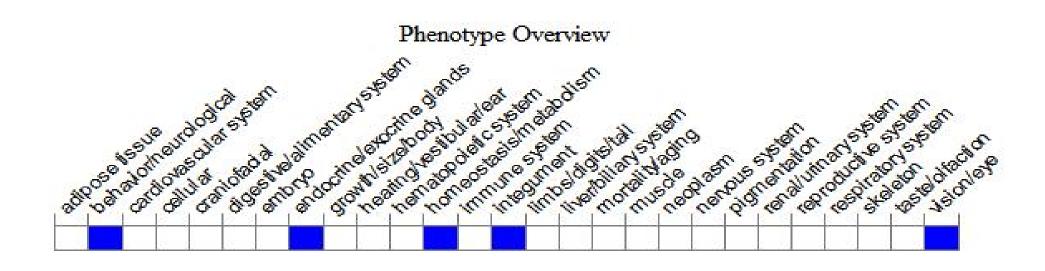
Source: : https://www.ensembl.org

#### Protein Information





### 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 risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

