

Rnf43 Cas9-CKO Strategy

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Overview

Target Gene Name

• Rnf43

Project Type

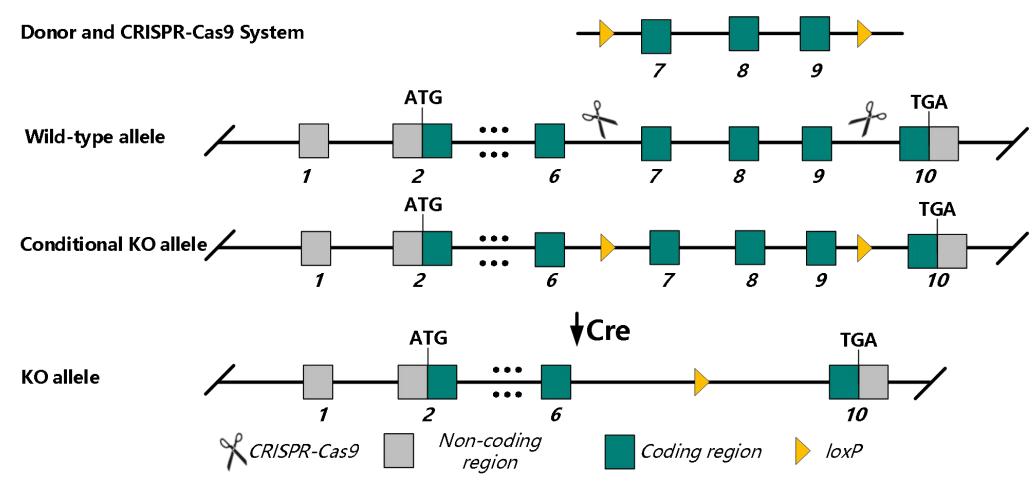
• Cas9-CKO

Genetic Background

• C57BL/6JGpt



Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Rnf43 gene.

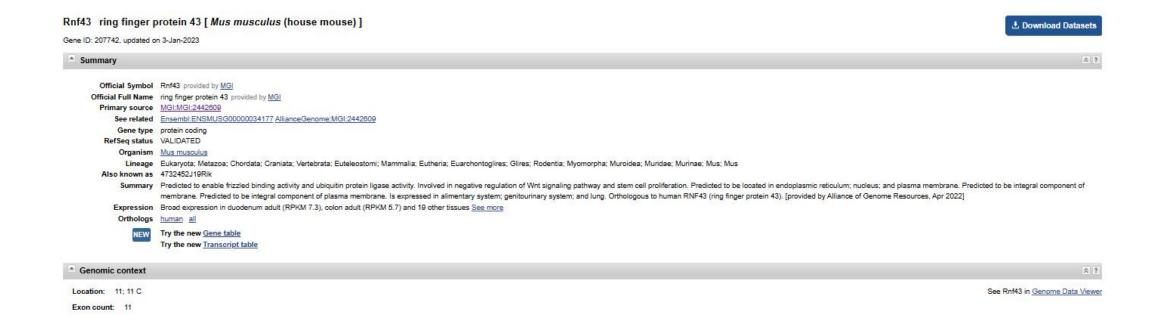


Technical Information

- The *Rnf43* gene has 9 transcripts. According to the structure of *Rnf43* gene, exon 7-9 of *Rnf43*-209 (ENSMUST00000165679.8) transcript is recommended as the knockout region. The region contains 1624 bp of coding sequences. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Rnf43* 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







Transcript Information

The gene has 9 transcripts, all transcripts are shown below:

Transcript ID ▼	Name 🍦	bp 🛊	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000165679.8	Rnf43-209	4310	<u>784aa</u>	Protein coding	CCDS25215 €	Q5NCP0-1 ₽	Ensembl Canonical GENCODE basic APPRIS P1 TSL:5
ENSMUST00000162740.2	Rnf43-208	704	No protein	Retained intron		S-0	TSL:5
ENSMUST00000150866.2	Rnf43-207	364	No protein	Protein coding CDS not defined		121	TSL:3
ENSMUST00000134684.8	Rnf43-206	3116	No protein	Retained intron		154	TSL:1
ENSMUST00000124625.2	Rnf43-205	378	No protein	Retained intron		(*)	TSL:3
ENSMUST00000123658.9	Rnf43-204	950	No protein	Protein coding CDS not defined		9±0	TSL:5
ENSMUST00000121782.9	Rnf43-203	2640	743aa	Protein coding		E9PWJ5@	GENCODE basic TSL:5
ENSMUST00000092800.12	Rnf43-202	4037	784aa	Protein coding	CCDS25215 @	Q5NCP0-1₫	GENCODE basic APPRIS P1 TSL:5
ENSMUST00000040089.5	Rnf43-201	3670	<u>657aa</u>	Protein coding		Q5NCP0-2₽	GENCODE basic TSL:1

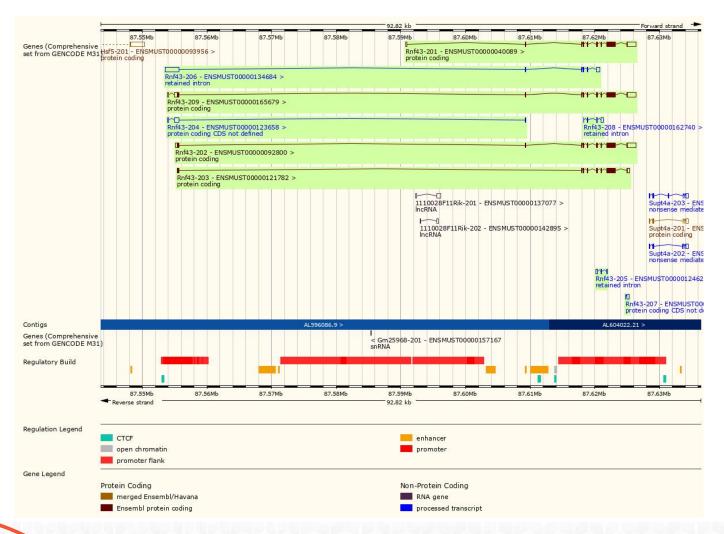
The strategy is based on the design of *Rnf43*-209 transcript, the transcription is shown below:



Source: https://www.ensembl.org



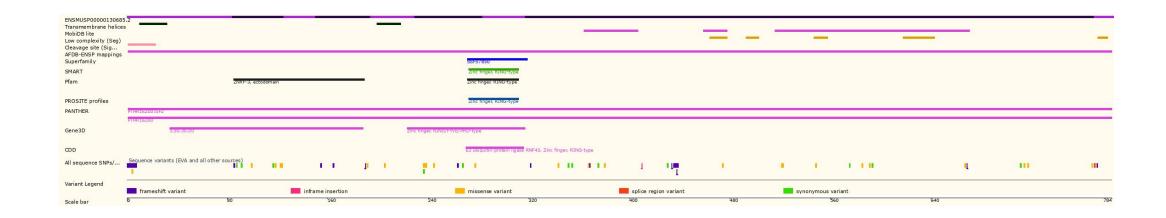
Genomic Information





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

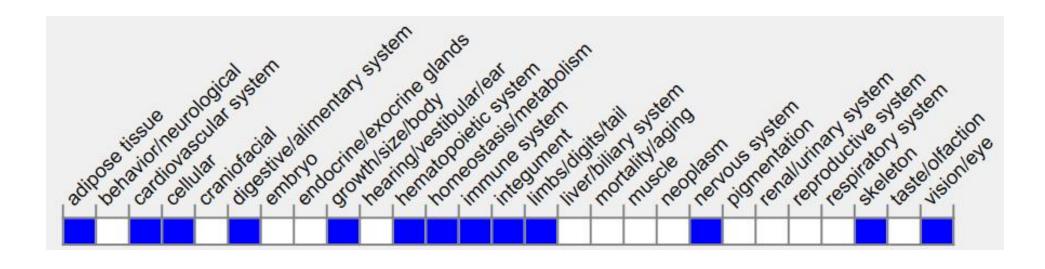
Protein Information





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

Mouse Phenotype Information (MGI)



Homozygous knockout leads to hyperproliferation of stomach epithelium and increases the severity of chronic H. pylori infection pathology.



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

Important Information

- Homozygous knockout leads to hyperproliferation of stomach epithelium and increases the severity of chronic H. pylori infection pathology.
- The effect of this strategy on the uncoded transcript Rnf43-204, Rnf43-207 is unknown.
- A part of amino acid sequence will still remain at the N-terminal of the *Rnf43* gene.
- The knockout region is about 4.5 kb away from the 5' of the *Supt4a* gene, which may affect the regulation of this gene.
- Rnf43 is located on Chr 11. 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.

