

Flot1 Cas9-KO Strategy

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Project Overview



Project Name Flot1

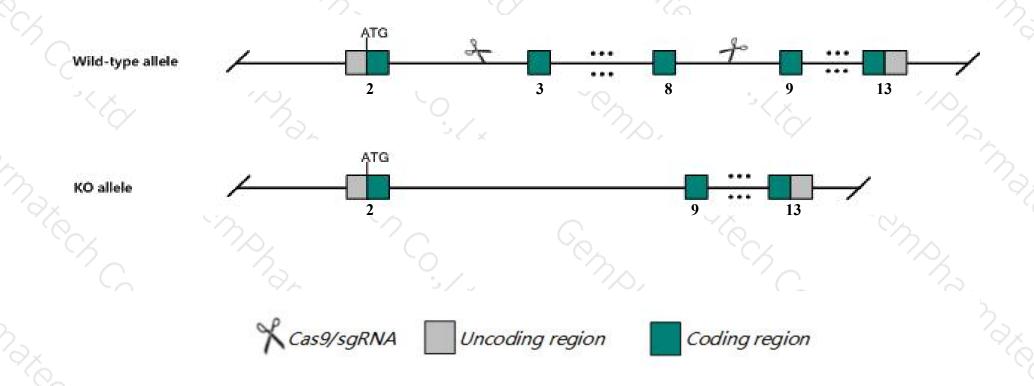
Project type Cas9-KO

Strain background C57BL/6J

Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Flot1* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Flot1* gene has 11 transcripts. According to the structure of *Flot1* gene, exon3-exon8 of *Flot1-201* (ENSMUST0000001569.14) transcript is recommended as the knockout region. The region contains 680bp coding sequence Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Flot1* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

Notice



- > According to the existing MGI data, Mice homozygous for a knock-out allele exhibit impaired neutrophil recruitment.
- ➤ Transcript *Flot1*-204&206&211 may not be affected.
- The knockout region is near to the C-terminal of *Ier3* gene, this strategy may influence the regulatory function of the C-terminal of *Ier3* gene.
- The *Flot1* gene is located on the Chr17. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)



Flot1 flotillin 1 [Mus musculus (house mouse)]

Gene ID: 14251, updated on 8-Dec-2019

Summary

☆ ?

Official Symbol Flot1 provided by MGI

Official Full Name flotillin 1 provided by MGI

Primary source MGI:MGI:1100500

See related Ensembl: ENSMUSG00000059714

Gene type protein coding
RefSeq status PROVISIONAL
Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;

Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

Also known as reggie-2

Expression Ubiquitous expression in bladder adult (RPKM 47.3), heart adult (RPKM 42.6) and 28 other tissues See more

Orthologs human all

Genomic context



Location: 17; 17 B1

See Flot1 in Genome Data Viewer

Exon count: 14

| Annotation release | Status | Assembly | Chr | Location |
|--------------------|-------------------|------------------------------|-----|--------------------------------|
| 108 | current | GRCm38.p6 (GCF_000001635.26) | 17 | NC_000083.6 (3582332035832789) |
| Build 37.2 | previous assembly | MGSCv37 (GCF_000001635.18) | 17 | NC_000083.5 (3596030235969732) |

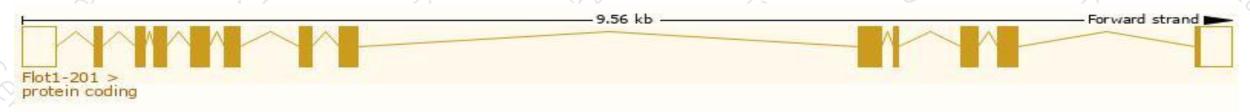
Transcript information (Ensembl)



The gene has 11 transcripts, all transcripts are shown below:

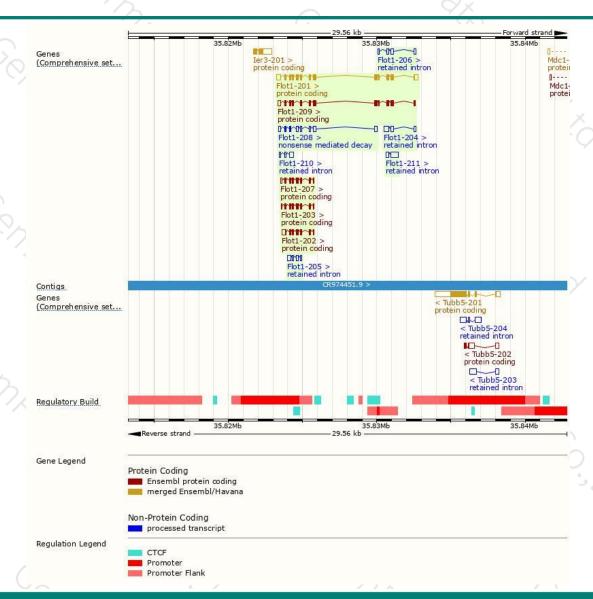
| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|-----------|-----------------------|------|--------------|-------------------------|----------------|---------------|-------------------------------|
| Flot1-201 | ENSMUST00000001569.14 | 1825 | 428aa | Protein coding | CCDS28705 | O08917 Q54014 | TSL:1 GENCODE basic APPRIS P1 |
| Flot1-209 | ENSMUST00000174080.7 | 1383 | 380aa | Protein coding | 5 5 | G3UYU4 | TSL:5 GENCODE basic |
| Flot1-202 | ENSMUST00000172846.1 | 805 | <u>192aa</u> | Protein coding | 49 | G3UZZ5 | CDS 3' incomplete TSL:2 |
| Flot1-207 | ENSMUST00000173493.7 | 697 | <u>192aa</u> | Protein coding | 29 | <u>G3UZZ5</u> | CDS 3' incomplete TSL:5 |
| Flot1-203 | ENSMUST00000173147.7 | 651 | <u>196aa</u> | Protein coding | 56 | G3UWW8 | CDS 3' incomplete TSL:5 |
| Flot1-208 | ENSMUST00000173628.7 | 948 | <u>40aa</u> | Nonsense mediated decay | +8 | G3XA73 | TSL:5 |
| Flot1-211 | ENSMUST00000174297.1 | 682 | No protein | Retained intron | 10 | - | TSL:1 |
| Flot1-204 | ENSMUST00000173273.1 | 592 | No protein | Retained intron | 29 | 120 | TSL:2 |
| Flot1-205 | ENSMUST00000173337.1 | 538 | No protein | Retained intron | 56 | 7 | TSL:3 |
| Flot1-206 | ENSMUST00000173381.7 | 502 | No protein | Retained intron | #8 | 2- | TSL:2 |
| Flot1-210 | ENSMUST00000174227.1 | 405 | No protein | Retained intron | 4 8 | 4- | TSL:3 |
| | 7/ 1/ | | - / / / | | | N 27 | V v |

The strategy is based on the design of Flot1-201 transcript, The transcription is shown below



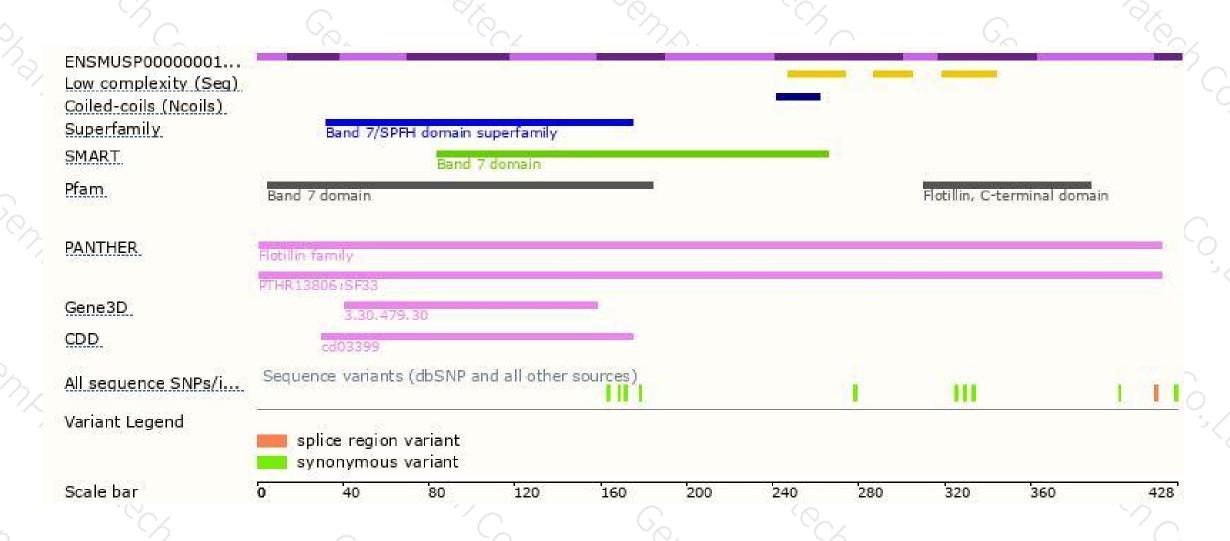
Genomic location distribution





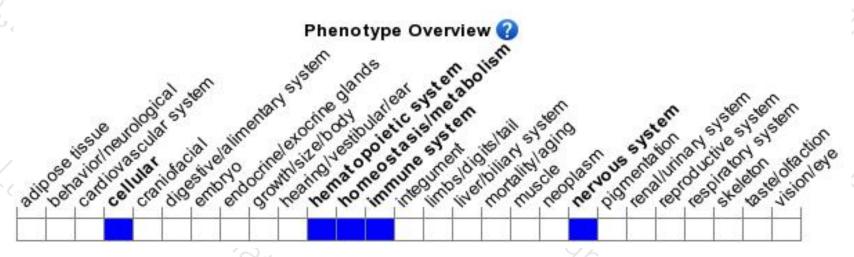
Protein domain





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 impaired neutrophil recruitment.



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

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