

# *Mfsd4a* Cas9-CKO Strategy

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**Reviewer: Miaomiao Cui**

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# Project Overview

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**Project Name**

*Mfsd4a*

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**Project type**

**Cas9-CKO**

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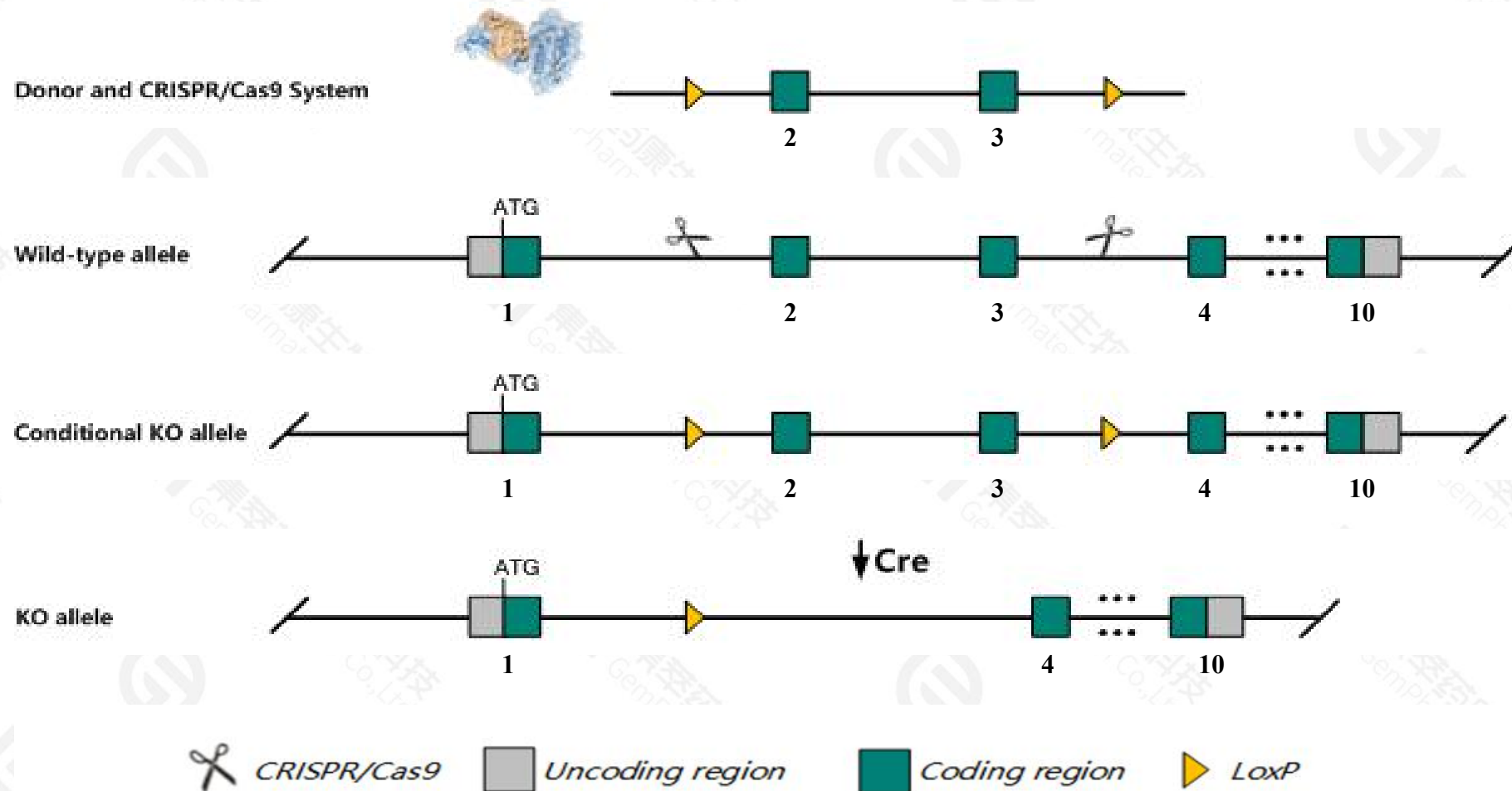
**Strain background**

**C57BL/6JGpt**

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# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mfsd4a* gene. The schematic diagram is as follows:



- The *Mfsd4a* gene has 13 transcripts. According to the structure of *Mfsd4a* gene, exon2-exon3 of *Mfsd4a*-205(ENSMUST00000144548.9) transcript is recommended as the knockout region. The region contains 451bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Mfsd4a* 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 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 *Mfsd4a* gene is located on the Chr1. 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.
- Transcript *Mfsd4a*-211 may not be affected.
- 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)

## Mfsd4a major facilitator superfamily domain containing 4A [Mus musculus (house mouse)]

Gene ID: 213006, updated on 17-Dec-2020

### Summary



**Official Symbol** Mfsd4a provided by [MGI](#)

**Official Full Name** major facilitator superfamily domain containing 4A provided by [MGI](#)

**Primary source** [MGI:MGI:2442786](#)

**See related** [Ensembl:ENSMUSG00000059149](#)

**Gene type** protein coding

**RefSeq status** VALIDATED

**Organism** [Mus musculus](#)

**Lineage** Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

**Also known as** A230072B04, A930031D07Rik, AI850289, Mfsd, Mfsd4

**Expression** Broad expression in colon adult (RPKM 40.5), adrenal adult (RPKM 15.7) and 17 other tissues [See more](#)

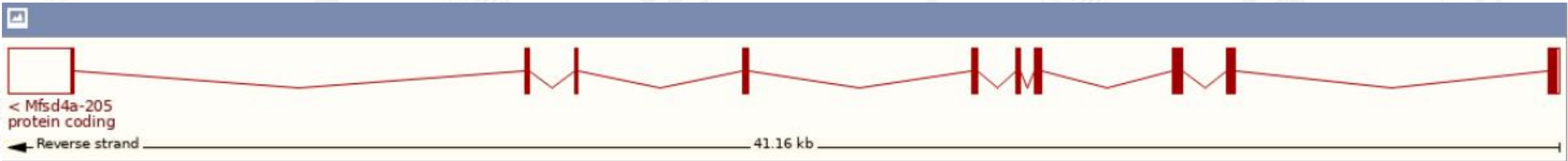
**Orthologs** [human](#) [all](#)

# Transcript information (Ensembl)

The gene has 13 transcripts,all transcripts are shown below:

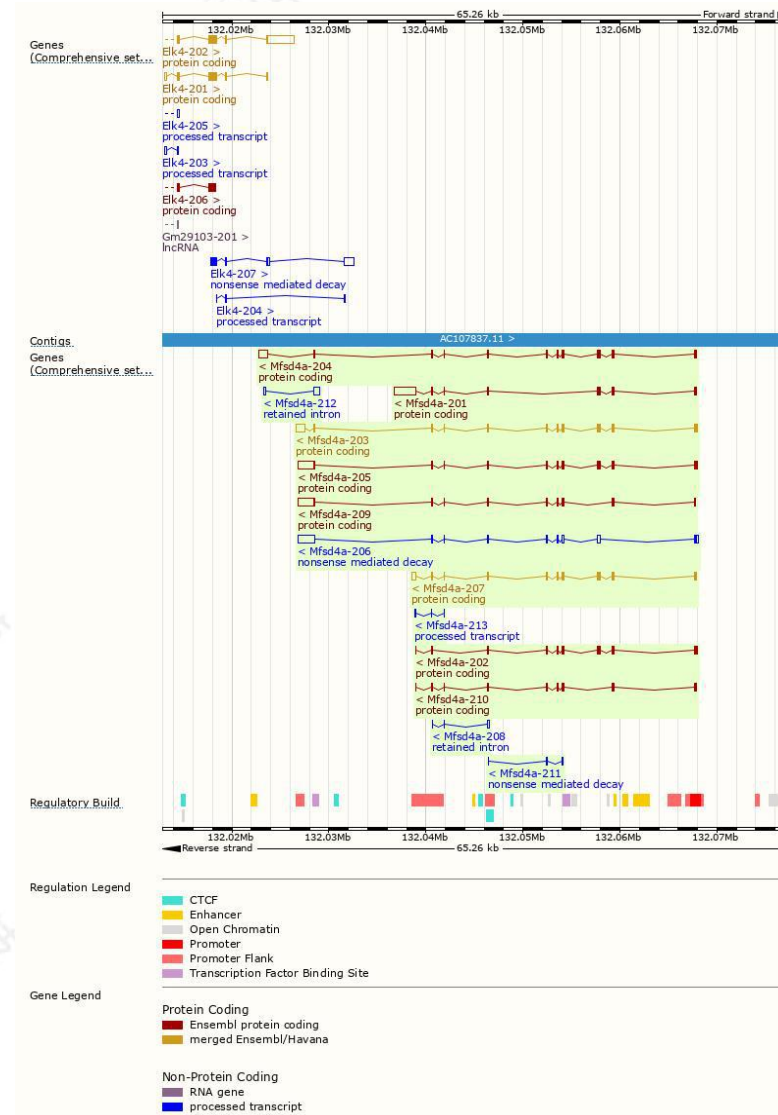
| Name       | Transcript ID                         | bp   | Protein               | Biotype                                 | CCDS                      | UniProt | Flags                               |
|------------|---------------------------------------|------|-----------------------|---|---------------------------|---------|-------------------------------------|
| Mfsd4a-205 | <a href="#">ENSMUST00000144548.9</a>  | 3288 | <a href="#">512aa</a> | <a href="#">Protein coding</a>          | <a href="#">CCDS15280</a> |         | TSL:1 , GENCODE basic ,             |
| Mfsd4a-203 | <a href="#">ENSMUST00000112370.9</a>  | 2565 | <a href="#">512aa</a> | <a href="#">Protein coding</a>          | <a href="#">CCDS15280</a> |         | TSL:1 , GENCODE basic ,             |
| Mfsd4a-204 | <a href="#">ENSMUST00000126927.8</a>  | 2422 | <a href="#">512aa</a> | <a href="#">Protein coding</a>          | <a href="#">CCDS15280</a> |         | TSL:1 , GENCODE basic ,             |
| Mfsd4a-207 | <a href="#">ENSMUST00000159038.8</a>  | 1957 | <a href="#">510aa</a> | <a href="#">Protein coding</a>          | <a href="#">CCDS48357</a> |         | TSL:5 , GENCODE basic ,             |
| Mfsd4a-201 | <a href="#">ENSMUST00000046658.10</a> | 3252 | <a href="#">358aa</a> | <a href="#">Protein coding</a>          | -                         |         | TSL:1 , GENCODE basic ,             |
| Mfsd4a-209 | <a href="#">ENSMUST00000160656.8</a>  | 2899 | <a href="#">406aa</a> | <a href="#">Protein coding</a>          | -                         |         | CDS 5' incomplete , TSL:5 ,         |
| Mfsd4a-202 | <a href="#">ENSMUST00000112365.9</a>  | 1536 | <a href="#">464aa</a> | <a href="#">Protein coding</a>          | -                         |         | TSL:5 , GENCODE basic , APPRIS P1 , |
| Mfsd4a-210 | <a href="#">ENSMUST00000161864.2</a>  | 1285 | <a href="#">425aa</a> | <a href="#">Protein coding</a>          | -                         |         | CDS 3' incomplete , TSL:5 ,         |
| Mfsd4a-206 | <a href="#">ENSMUST00000146267.8</a>  | 3161 | <a href="#">88aa</a>  | <a href="#">Nonsense mediated decay</a> | -                         |         | TSL:1 ,                             |
| Mfsd4a-211 | <a href="#">ENSMUST00000162628.2</a>  | 310  | <a href="#">67aa</a>  | <a href="#">Nonsense mediated decay</a> | -                         |         | CDS 5' incomplete , TSL:5 ,         |
| Mfsd4a-213 | <a href="#">ENSMUST00000189227.2</a>  | 238  | No protein            | <a href="#">Processed transcript</a>    | -                         |         | TSL:1 ,                             |
| Mfsd4a-212 | <a href="#">ENSMUST00000163016.2</a>  | 814  | No protein            | <a href="#">Retained intron</a>         | -                         |         | TSL:3 ,                             |
| Mfsd4a-208 | <a href="#">ENSMUST00000159382.2</a>  | 337  | No protein            | <a href="#">Retained intron</a>         | -                         |         | TSL:5 ,                             |

The strategy is based on the design of *Mfsd4a-205* transcript,the transcription is shown below:



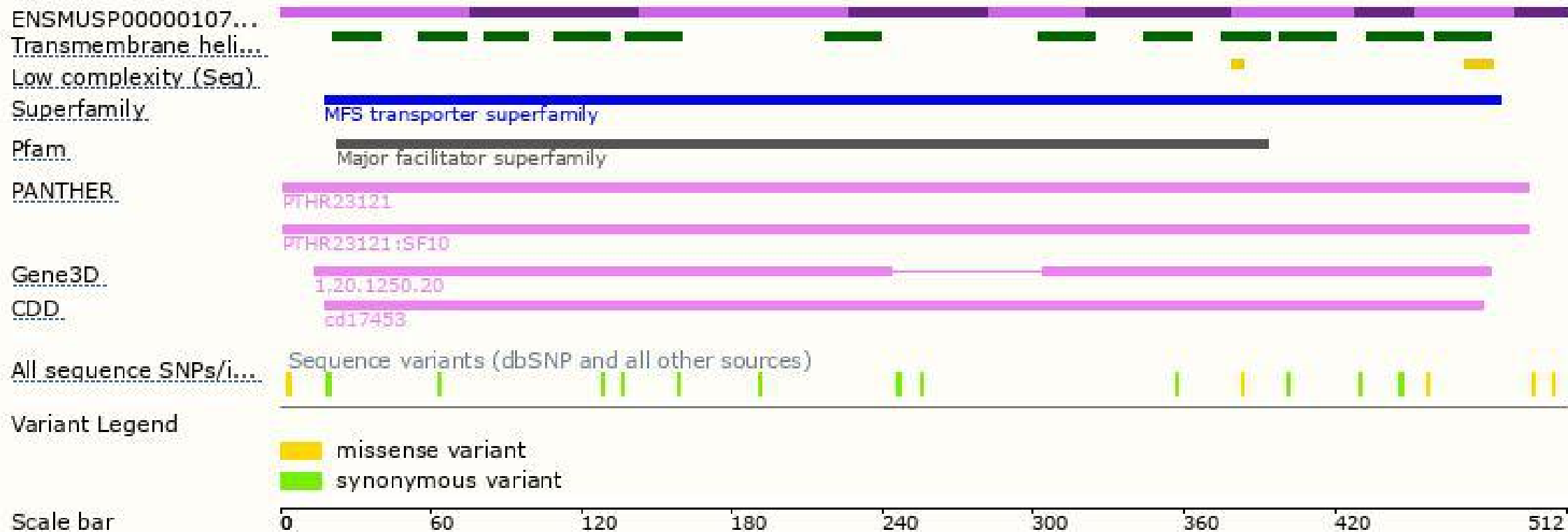


# Genomic location distribution





# Protein domain



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