

# Mcf2l Cas9-CKO Strategy

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Reviewer: Xiaojing Li

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## **Project Overview**



Project Name Mcf21

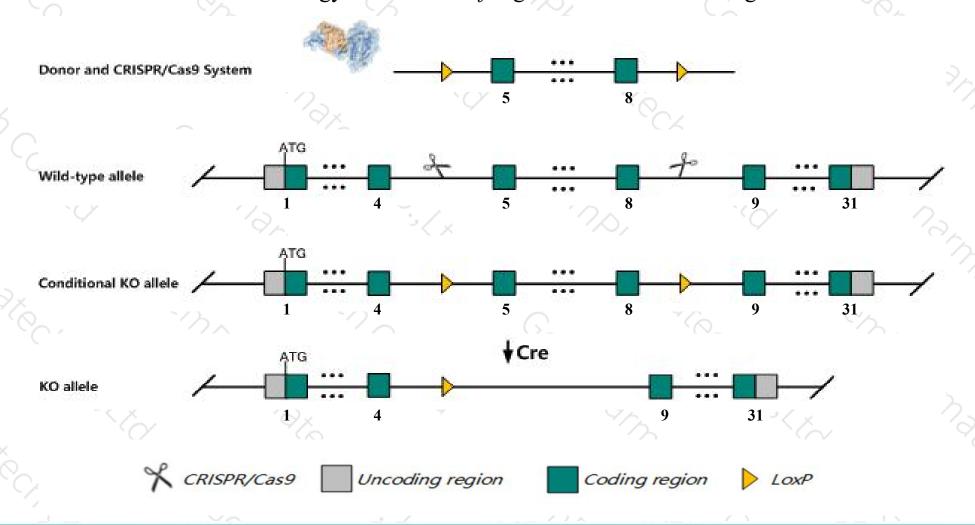
Project type Cas9-CKO

Strain background C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Mcf2l* gene. The schematic diagram is as follows:



### Technical routes



- The *Mcf2l* gene has 23 transcripts. According to the structure of *Mcf2l* gene, exon5-exon8 of *Mcf2l-207*(ENSMUST00000110876.8) transcript is recommended as the knockout region. The region contains 512bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Mcf2l* 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.

### **Notice**



- > The *Mcf2l* gene is located on the Chr8. 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.
- > Some amino acids will remain at the N-terminus and some functions may be retained.
- > Transcript 220,223 CDS 3' incomplete the influences is unknown; Transcript 209,210 CDS 3' incomplete the influences is unknown; Transcript 213,216 CDS 5' and 3' incomplete the influences is unknown.
- ➤ 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)



#### Mcf2l mcf.2 transforming sequence-like [Mus musculus (house mouse)]

Gene ID: 17207, updated on 31-Jan-2019

#### Summary

☆ ?

Official Symbol Mcf2l provided by MGI

Official Full Name mcf.2 transforming sequence-like provided by MGI

Primary source MGI:MGI:103263

See related Ensembl: ENSMUSG00000031442

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 C130040G20Rik, Dbs, Ost, mKIAA0362

Expression Broad expression in CNS E18 (RPKM 14.7), frontal lobe adult (RPKM 13.1) and 22 other tissuesSee more

Orthologs human all

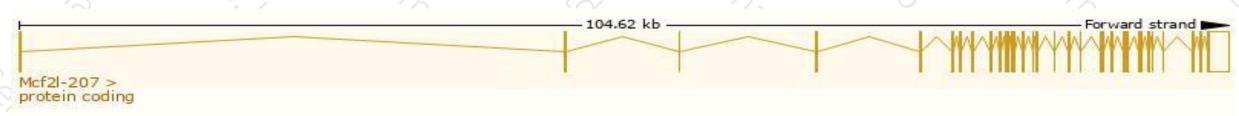
## Transcript information (Ensembl)



#### The gene has 23 transcripts, all transcripts are shown below:

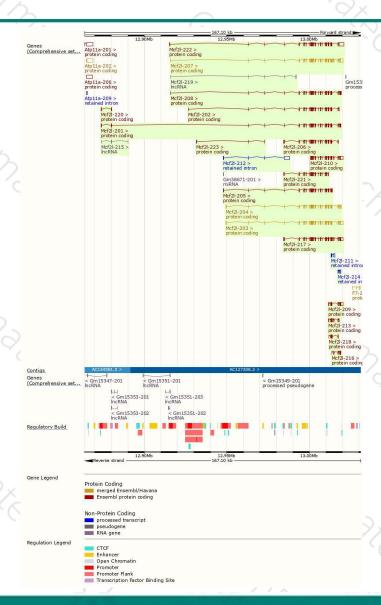
| Name      | Transcript ID        | bp   | Protein       | Biotype         | CCDS      | UniProt       | Flags  |
|-----------|----------------------|------|---------------|-----------------|-----------|---------------|--|
| Mcf2I-207 | ENSMUST00000110876.8 | 5253 | 1175aa        | Protein coding  | CCDS40225 | E9PXE2        | TSL:1 GENCODE basic APPRIS P3  |
| Mcf2I-203 | ENSMUST00000110866.8 | 5108 | 1097aa        | Protein coding  | CCDS52483 | E9PY13        | TSL:1 GENCODE basic APPRIS ALT2  |
| Mcf2I-204 | ENSMUST00000110867.8 | 3864 | 1101aa        | Protein coding  | CCDS52482 | E9PY12        | TSL:1 GENCODE basic APPRIS ALT2  |
| Mcf2I-222 | ENSMUST00000173099.7 | 6081 | 1059aa        | Protein coding  | - 0.      | G3UXP9        | TSL:5 GENCODE basic  |
| Mcf2I-217 | ENSMUST00000145067.7 | 5313 | 1118aa        | Protein coding  |           | <u>G3UX72</u> | TSL:1 GENCODE basic APPRIS ALT2  |
| Mcf2I-210 | ENSMUST00000126905.7 | 4061 | 807aa         | Protein coding  | 15        | F6VR53        | CDS 5' incomplete TSL:1  |
| Mcf2I-201 | ENSMUST00000095456.9 | 3926 | 1149aa        | Protein coding  |           | E9QPM7        | TSL:1 GENCODE basic APPRIS ALT2  |
| Mcf2I-208 | ENSMUST00000110879.8 | 3664 | 1125aa        | Protein coding  | - 0       | E9PXE1        | TSL:5 GENCODE basic APPRIS ALT2  |
| Mcf2I-205 | ENSMUST00000110871.7 | 3401 | 1046aa        | Protein coding  |           | D3YUF4        | TSL:5 GENCODE basic  |
| Mcf2I-202 | ENSMUST00000098927.9 | 3215 | <u>1067aa</u> | Protein coding  | 15        | E9Q863        | TSL:5 GENCODE basic  |
| Mcf2I-206 | ENSMUST00000110873.9 | 3169 | 866aa         | Protein coding  |           | D3YUF3        | CDS 3' incomplete TSL:5  |
| Mcf2I-221 | ENSMUST00000173006.7 | 2500 | <u>757aa</u>  | Protein coding  | - 0       | G3UYM8        | CDS 3' incomplete TSL:5  |
| Mcf2I-209 | ENSMUST00000123811.8 | 2390 | <u>295aa</u>  | Protein coding  |           | F6WPM5        | CDS 5' incomplete TSL:5  |
| Mcf2I-213 | ENSMUST00000134227.7 | 761  | 254aa         | Protein coding  | 15        | F7BZ65        | 5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL: |
| Mcf2I-216 | ENSMUST00000139776.3 | 565  | 188aa         | Protein coding  |           | F6UKC6        | 5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL: |
| Mcf2I-220 | ENSMUST00000156560.1 | 521  | <u>55aa</u>   | Protein coding  | - 0       | D3Z3S7        | CDS 3' incomplete TSL:2  |
| Mcf2I-218 | ENSMUST00000145892.2 | 452  | <u>151aa</u>  | Protein coding  |           | F6ZFQ8        | 5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL: |
| Mcf2I-223 | ENSMUST00000238472.1 | 419  | <u>97aa</u>   | Protein coding  | 15        | -             | CDS 3' incomplete  |
| Mcf2I-212 | ENSMUST00000130302.1 | 3760 | No protein    | Retained intron | 8-        | -             | TSL:1  |
| Mcf2I-214 | ENSMUST00000134989.2 | 718  | No protein    | Retained intron | - 0       |               | TSL:2  |
| Mcf2I-211 | ENSMUST00000127229.1 | 666  | No protein    | Retained intron |           |               | TSL:5  |
| Mcf2I-219 | ENSMUST00000156405.7 | 951  | No protein    | IncRNA          |           |               | TSL:1  |
| Mcf2I-215 | ENSMUST00000138387.1 | 385  | No protein    | IncRNA          | -         | 9             | TSL:3  |
|           |                      |      | 77 JF 15      |                 |           |               |  |

The strategy is based on the design of Mcf2l-207 transcript, The transcription is shown below



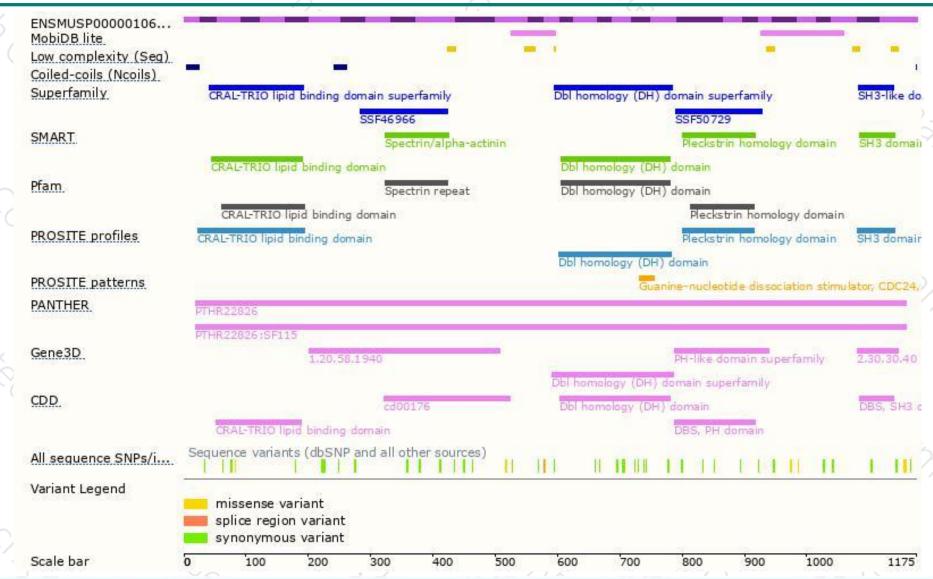
### Genomic location distribution





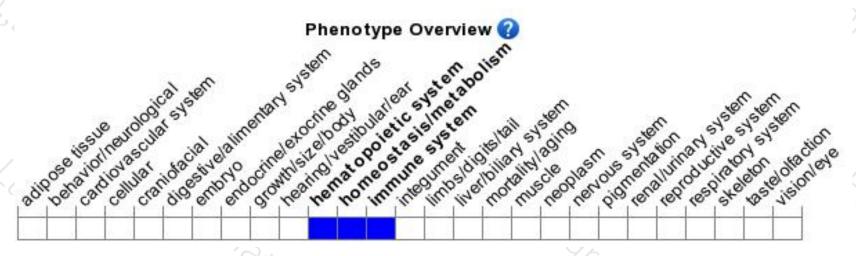
### Protein domain





## Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).



If you have any questions, you are welcome to inquire. Tel: 400-9660890





