

# Traf7 Cas9-CKO Strategy

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### Overview

#### Target Gene Name

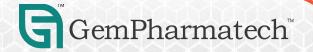
• Traf7

#### Project Type

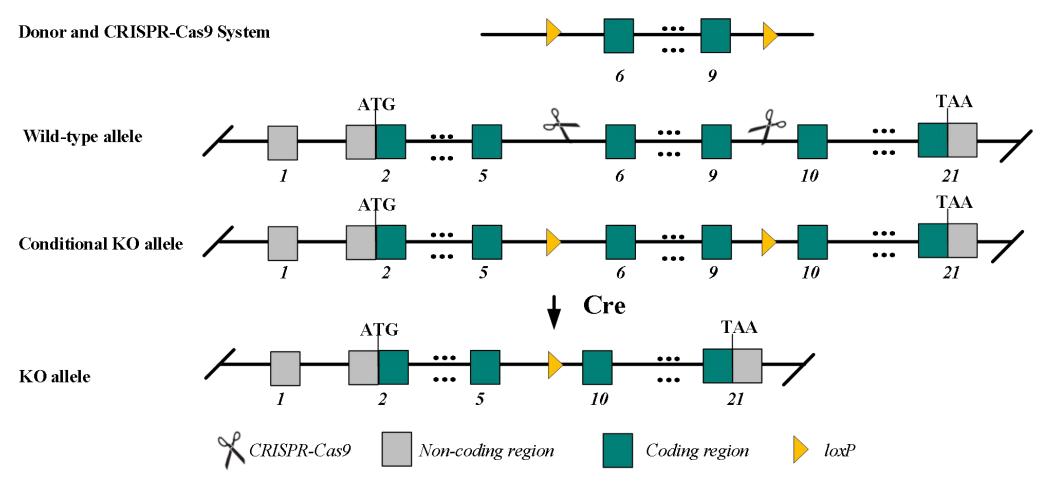
• Cas9-CKO

#### Genetic Background

• C57BL/6JGpt



## Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Traf7* gene.



#### **Technical Information**

- The *Traf7* gene has 26 transcripts. According to the structure of *Traf7* gene, exon 6-exon 9 of *Traf7*-215 (ENSMUST00000176652.8) transcript is recommended as the knockout region. The region contains 446bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Traf7* 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

#### Traf7 TNF receptor-associated factor 7 [ Mus musculus (house mouse) ]

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Gene ID: 224619, updated on 23-Nov-2023



Official Symbol Traf7 provided by MGI

Official Full Name TNF receptor-associated factor 7 provided by MGI

Primary source MGI:MGI:3042141

See related Ensembl: ENSMUSG00000052752 AllianceGenome: MGI: 3042141

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; Muridae; Mus; Mus

Also known as RFWD1

Summary Predicted to enable ubiquitin-protein transferase activity. Acts upstream of or within several processes, including cellular response to interleukin-6; negative regulation of transcription by RNA

polymerase II; and positive regulation of protein sumoylation. Located in cytosol; nucleus; and perinuclear region of cytoplasm. Is expressed in genitourinary system and pancreas epithelium.

Orthologous to human TRAF7 (TNF receptor associated factor 7). [provided by Alliance of Genome Resources, Apr 2022]

Expression Ubiquitous expression in ovary adult (RPKM 69.0), lung adult (RPKM 60.1) and 28 other tissues See more

Orthologs <u>human</u> all

w Try the new Gene table

Try the new Transcript table

Source: https://www.ncbi.nlm.nih.gov/



## Transcript Information

The gene has 26 transcripts, all transcripts are shown below:

| Transcript ID         | Name      | bp 🌲 | Protein ▼    | Biotype                        | CCDS        | UniProt Match | Flags   |
|-----------------------|-----------|------|--------------|--------------------------------|-------------|---------------|---|
| ENSMUST00000176652.8  | Traf7-215 | 3003 | 669aa        | Protein coding                 | CCDS50014 € | F8WJF7₽       | Ensembl Canonical GENCODE basic APPRIS P1 TSL:5 |
| ENSMUST00000088464.12 | Traf7-202 | 2504 | 669aa        | Protein coding                 | CCDS50014 ₺ | F8WJF7®       | GENCODE basic APPRIS P1 TSL:5                   |
| ENSMUST00000176353.8  | Traf7-210 | 2503 | 629aa        | Protein coding                 | CCDS50015 € | Q8C2K8@       | GENCODE basic TSL:1                             |
| ENSMUST00000070777.13 | Traf7-201 | 2384 | 629aa        | Protein coding                 | CCDS50015 € | Q8C2K8@       | GENCODE basic TSL:5                             |
| ENSMUST00000176237.8  | Traf7-208 | 950  | 271aa        | Protein coding                 |             | H3BJD9@       | TSL:5   CDS 3' incomplete                       |
| ENSMUST00000177154.8  | Traf7-222 | 758  | 228aa        | Protein coding                 |             | H3BLQ3₽       | TSL:5   CDS 3' incomplete                       |
| ENSMUST00000177193.8  | Traf7-223 | 644  | 170aa        | Protein coding                 |             | H3BK82 ₪      | TSL:3   CDS 3' incomplete                       |
| ENSMUST00000176086.8  | Traf7-205 | 2687 | <u>132aa</u> | Nonsense mediated decay        |             | H3BLM6@       | TSL:1   |
| ENSMUST00000175698.8  | Traf7-203 | 755  | 84aa         | Nonsense mediated decay        |             | H3BKN1₫       | TSL:3   CDS 5' incomplete                       |
| ENSMUST00000176668.8  | Traf7-216 | 2667 | 81aa         | Nonsense mediated decay        |             | H3BKZ1e₽      | TSL:1   |
| ENSMUST00000177405.8  | Traf7-225 | 606  | 80aa         | Nonsense mediated decay        |             | H3BJU4 €      | TSL:3   |
| ENSMUST00000177502.8  | Traf7-226 | 641  | 76aa         | Nonsense mediated decay        |             | H3BJF9₽       | TSL:3 CDS 5' incomplete                         |
| ENSMUST00000176178.8  | Traf7-207 | 680  | 54aa         | Nonsense mediated decay        |             | H3BJ15 €      | TSL:3   |
| ENSMUST00000177401.8  | Traf7-224 | 699  | 45aa         | Nonsense mediated decay        |             | H3BKC9₽       | TSL:2   |
| ENSMUST00000176324.8  | Traf7-209 | 604  | 44aa         | Nonsense mediated decay        |             | H3BKC7₽       | TSL:3   |
| ENSMUST00000177025.2  | Traf7-220 | 428  | 44aa         | Nonsense mediated decay        |             | H3BKC7@       | TSL:3   |
| ENSMUST00000176092.2  | Traf7-206 | 382  | No protein   | Protein coding CDS not defined |             | 191           | TSL:3   |
| ENSMUST00000177139.8  | Traf7-221 | 332  | No protein   | Protein coding CDS not defined |             | 020           | TSL:3   |
| ENSMUST00000177024.8  | Traf7-219 | 3194 | No protein   | Retained intron                |             | 151           | TSL:1   |
| ENSMUST00000176805.2  | Traf7-217 | 1749 | No protein   | Retained intron                |             | (50)          | TSL:5   |
| ENSMUST00000176530.8  | Traf7-213 | 938  | No protein   | Retained intron                |             | 1-1           | TSL:3   |
| ENSMUST00000176900.8  | Traf7-218 | 861  | No protein   | Retained intron                |             | -             | TSL:3   |
| ENSMUST00000176361.2  | Traf7-211 | 693  | No protein   | Retained intron                |             | 101           | TSL:3   |
| ENSMUST00000176434.8  | Traf7-212 | 559  | No protein   | Retained intron                |             | 1070          | TSL:2   |
| ENSMUST00000176633.2  | Traf7-214 | 500  | No protein   | Retained intron                |             | -             | TSL:2   |
| ENSMUST00000175732.2  | Traf7-204 | 366  | No protein   | Retained intron                |             | (4)           | TSL:1   |

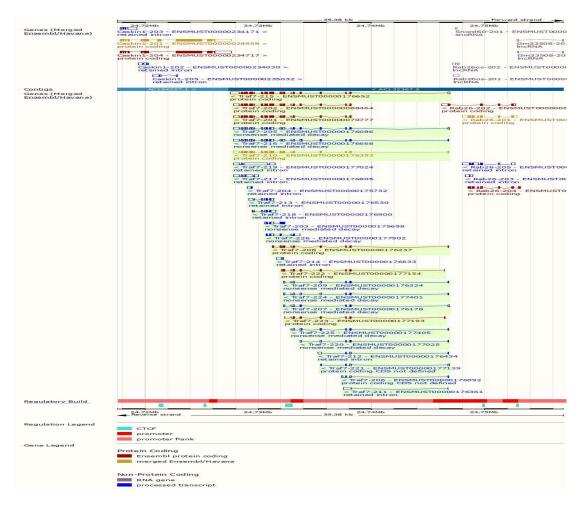
The strategy is based on the design of *Traf7*-215 transcript, the transcription is shown below:



Source: https://www.ensembl.org



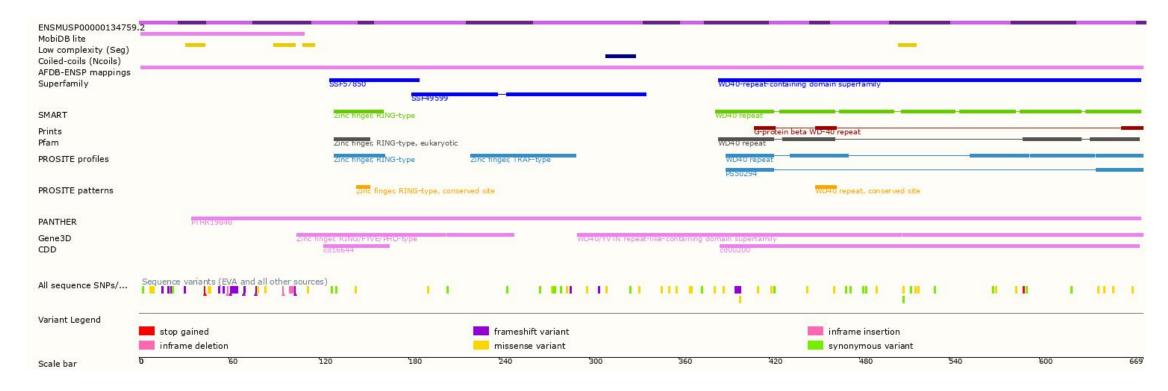
#### Genomic Information





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

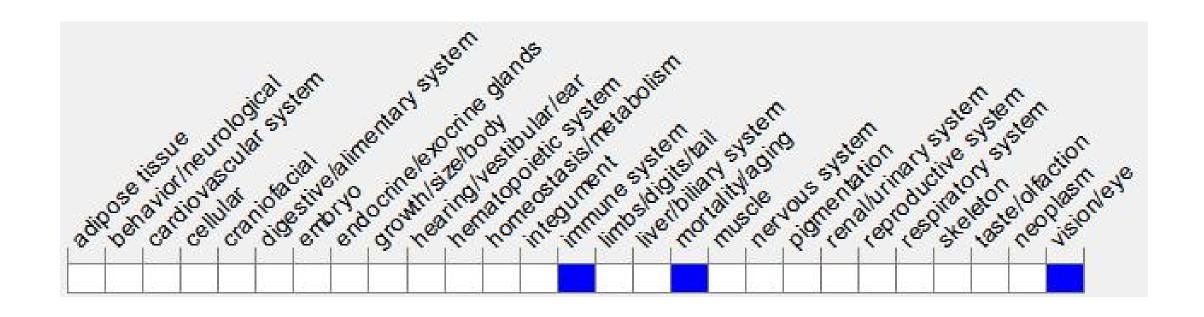
#### **Protein Information**





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

## Mouse Phenotype Information (MGI)





## Important Information

- According to MGI, Traf7 knockout homozygous mice died.
- Both introns 5-6 (495 bp) and 9-10 (563 bp) are short, and loxp insertions may interfere with normal splicing.
- *Traf7* is located on Chr17. 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.

