

***Traf6-C70A* Mouse Model Strategy**

-CRISPR/Cas9 technology

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Project Overview

Project Name

Traf6-C70A

Project type

cas9-ki

Strain background

C57BL/6JGpt

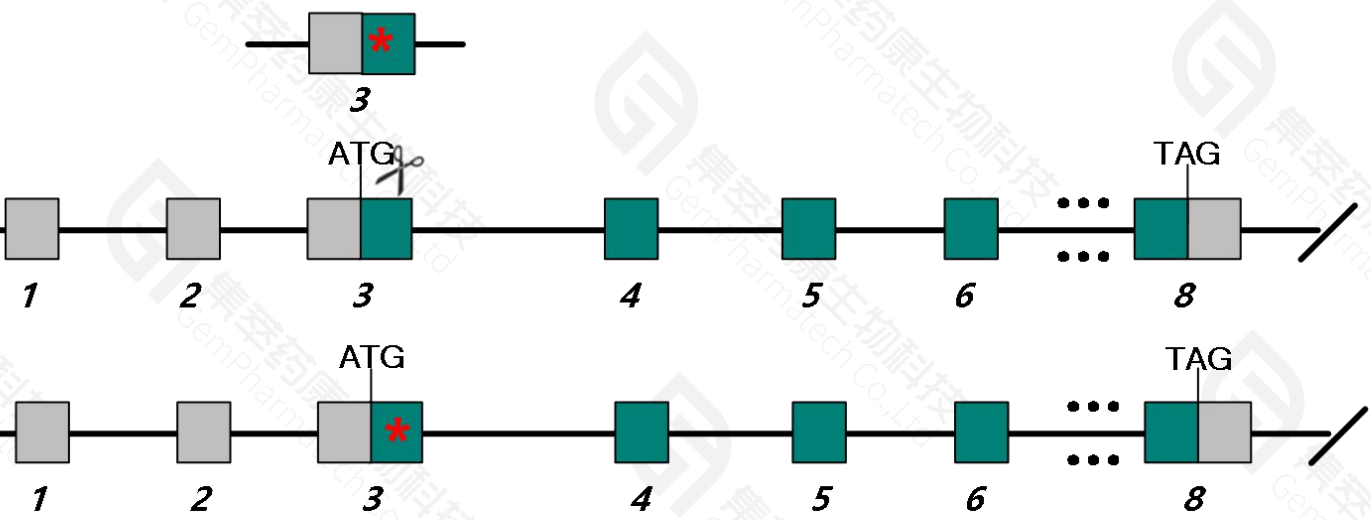
This model uses CRISPR/Cas9 technology to edit the *Traf6* gene and the schematic diagram is as follow:

Donor and CRISPR/Cas9 System

ENSMUST0000004949.8

Wild-type allele

Targeted allele



CRISPR/Cas9 Uncoding region Coding region * Mutant site:C70A

Technical Description

- The mouse *Traf6* gene has 3 transcripts.
- This project produced *Traf6*-C70A point mutation on exon3 of the transcript of *Traf6*-201(ENSMUST00000004949.8). The 70th amino acid will be mutated from C to A, and the corresponding nuclearinic acid will be mutated to GCG from the TGT.
- In this project, *Traf6* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: CRISPR/Cas9 system and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR and sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.

- According to the existing MGI data, viability is reduced in mice lacking both functional copies of this gene, with death occurring just before birth or around weaning. Mutants exhibit osteopetrosis and immune defects including abnormal immune cell development and function.
- One or two synonymous mutations of amino acids will be introduced on exon3 of *Traf6*.
- Mouse *Traf6* gene is located on Chr2. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr2, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Analysis of Homology

	51	60	70	80	90	100	110	120	130	140	150	
mTraf6-P	51	EEIQGYDVEFDPPLESKYECPICLMALREAVQTPCGHRFCACIIKSIRDAGHKCPVDNEILLENQLFPDNFAKREILSLTVKCPNKGCLQKMELRHLEDHQVI										
hTraf6-P	51	EEIQGYDVEFDPPLESKYECPICLMALREAVQTPCGHRFCACIIKSIRDAGHKCPVDNEILLENQLFPDNFAKREILSLMVKCPNEGCLHKMELRHLEDHQA										
Consensus	51	EEIQGYDVEFDPPLESKYECPICLMALREAVQTPCGHRFCACIIKSIRDAGHKCPVDNEILLENQLFPDNFAKREILSL VKCPN GCL KMELRHLEDHQ										
Ready												
consensus positions: 91.5% identity positions: 87.9% gr: 344												

hTRAF6-P-70C-*mTraf6*-P-70C

Identity positions: 87.9%

Mutation Site

Before mutation

```
+3 ?C G S S Q S S S D C C A A M A A S C S A A V K D D S V S G S A S T G?  
26101 GCGGGTCCAG CCAGTCGTCC AGTGACTGCT GCGCTGCCAT GGCCGCCTCC TGCAGCGCTG CAGTGAAAGA TGACAGCGTG AGTGGCTCTG CCAGCACC GG  
CGCCCAGGTC GGTCAGCAGG TCACTGACGA CGCGACGGTA CCGGCGGAGG ACGTCGCGAC GTCAC TTTCT ACTGTGCGCAC TCACCGAGAC GGTCGTGGCC  
+3 ?G N L S S S F M E E I Q G Y D V E F D P P L E S K Y E C P I C L M A  
26201 GAACCTCTCC AGCTCCTTCA TGGAGGAGAT CCAGGGCTAC GATGTGGAGT TTGACCCACC TCTGGAGAGC AAGTATGAGT GTCCCATCTG CTTGATGGCT  
CTTGGAGAGG TCGAGGAAGT ACCTCCTCTA GGTCCCGATG CTACACCTCA AACTGGGTGG AGACCTCTCG TTCATACTCA CAGGGTAGAC GAACTACCGA
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After mutation

```
+3 ?C G S S Q S S S D C C A A M A A S C S A A V K D D S V S G S A S T G?  
26101 GCGGGTCCAG CCAGTCGTCC AGTGACTGCT GCGCTGCCAT GGCCGCCTCC TGCAGCGCTG CAGTGAAAGA TGACAGCGTG AGTGGCTCTG CCAGCACC GG  
CGCCCAGGTC GGTCAGCAGG TCACTGACGA CGCGACGGTA CCGGCGGAGG ACGTCGCGAC GTCAC TTTCT ACTGTGCGCAC TCACCGAGAC GGTCGTGGCC  
+3 ?G N L S S S F M E E I Q G Y D V E F D P P L E S K Y E A P I C L M A  
26201 GAACCTCTCC AGCTCCTTCA TGGAGGAGAT CCAGGGCTAC GATGTGGAGT TTGACCCACC TCTGGAGAGC AAGTATGAGG CGCCCATCTG CTTGATGGCT  
CTTGGAGAGG TCGAGGAAGT ACCTCCTCTA GGTCCCGATG CTACACCTCA AACTGGGTGG AGACCTCTCG TTCATACTCC GCGGGTAGAC GAACTACCGA
```

The green region is exon3 of *Traf6-201*, and the red region represents the C70A mutation site.

Gene name and location (NCBI)

Traf6 TNF receptor-associated factor 6 [*Mus musculus* (house mouse)]

Gene ID: 22034, updated on 22-Aug-2021

[Download Datasets](#)

Summary

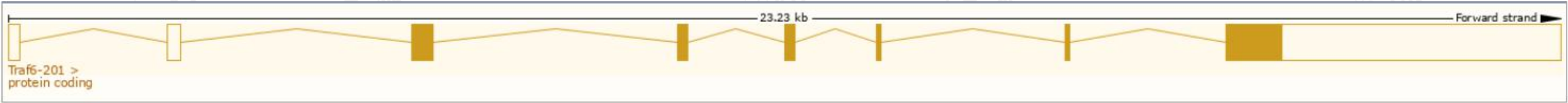
Official Symbol	Traf6 provided by MGI
Official Full Name	TNF receptor-associated factor 6 provided by MGI
Primary source	MGI:MGI:108072
See related	Ensembl:ENSMUSG00000027164
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI851288; 2310003F17Rik; C630032O20Rik
Summary	This gene encodes a member of the TNF receptor associated factor (TRAF) family of adaptor proteins that mediate signaling events from members of the TNF receptor and Toll/IL-1 receptor families to activate transcription factors such as NF-kappa-B and AP-1. The product of this gene is essential for perinatal and postnatal survival. Mice deficient in this protein exhibit osteopetrosis and defective in development of epidermal appendices, normal B cell differentiation, lymph node organogenesis, interleukin-1 signaling, lipopolysaccharide signaling and neural tube closure. This protein possesses ubiquitin ligase activity. Alternate splicing of this gene results in multiple transcript variants. [provided by RefSeq, Dec 2014]
Expression	Ubiquitous expression in CNS E11.5 (RPKM 2.0), thymus adult (RPKM 2.0) and 28 other tissues See more
Orthologs	human all
NEW	Try the new Gene table Try the new Transcript table

Transcript information (Ensembl)

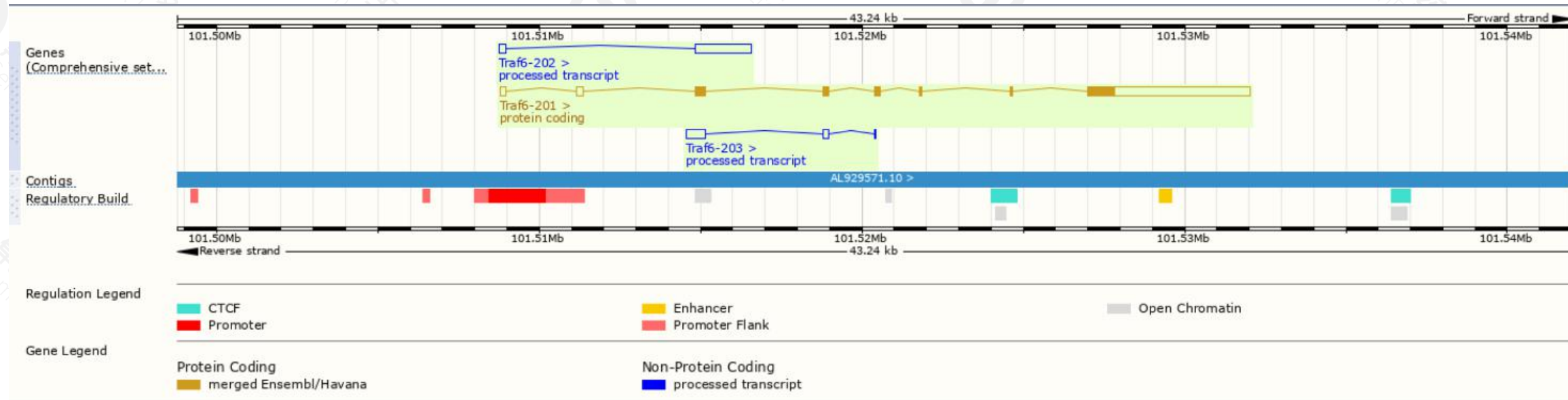
The gene has 3 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Traf6-201	ENSMUST00000004949.8	6169	530aa	Protein coding	CCDS16464	P70196-1	GENCODE basic APPRIS P1 TSL:1
Traf6-202	ENSMUST00000143341.2	1949	No protein	Processed transcript	-	-	TSL:2
Traf6-203	ENSMUST00000144063.2	763	No protein	Processed transcript	-	-	TSL:3

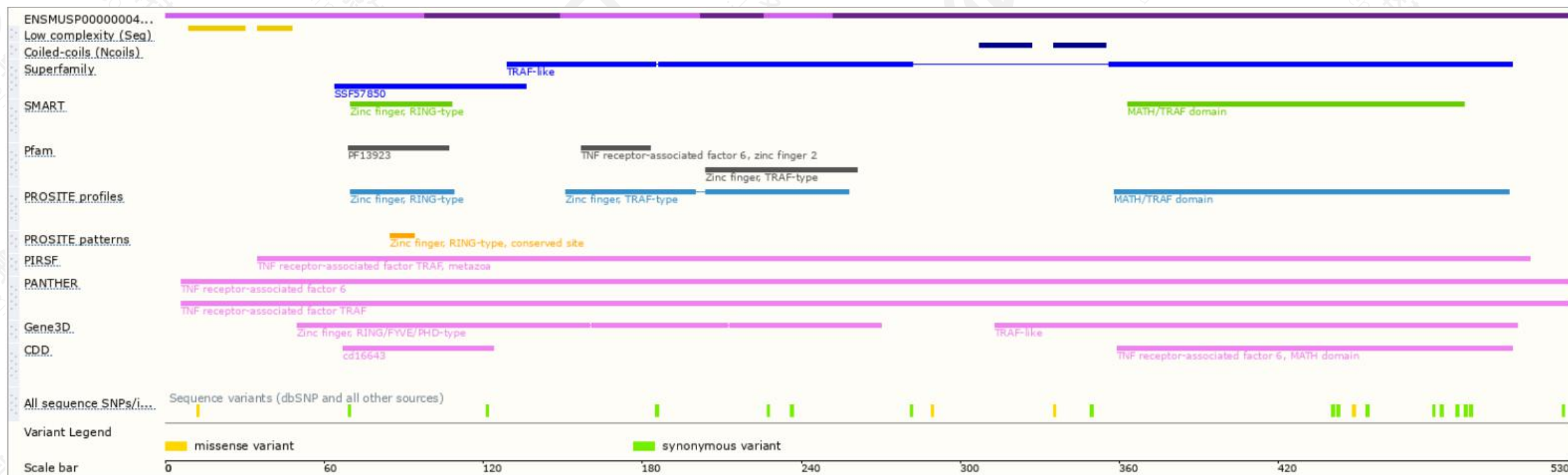
The strategy is based on the design of *Traf6-201* transcript, the transcription is shown below:



Genomic location distribution



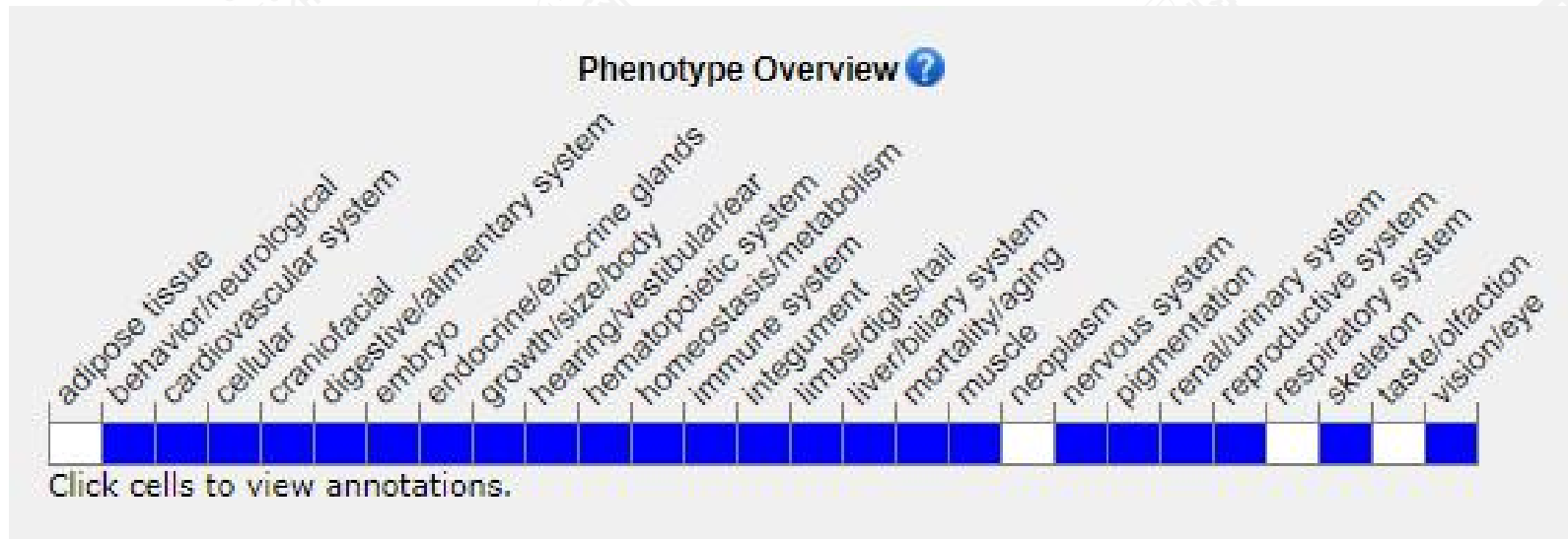
Protein domain



Mouse phenotype description(MGI)

URL link is as follows:

<http://www.informatics.jax.org/marker/MGI:108072>



Viability is reduced in mice lacking both functional copies of this gene, with death occurring just before birth or around weaning. Mutants exhibit osteopetrosis and immune defects including abnormal immune cell development and function.

If you have any questions, please feel free to contact us.
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