

# ***Ctcf-c.1699 C>T* Mouse Model Strategy**

## **-CRISPR/Cas9 technology**

**Designer: Daohua Xu**

**Reviewer: Yanhua Shen**

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# Project Overview

**Project Name**

**Ctcf-c.1699 C>T**

**Project type**

**cas9-ki(PM)**

**Strain background**

**C57BL/6JGpt**

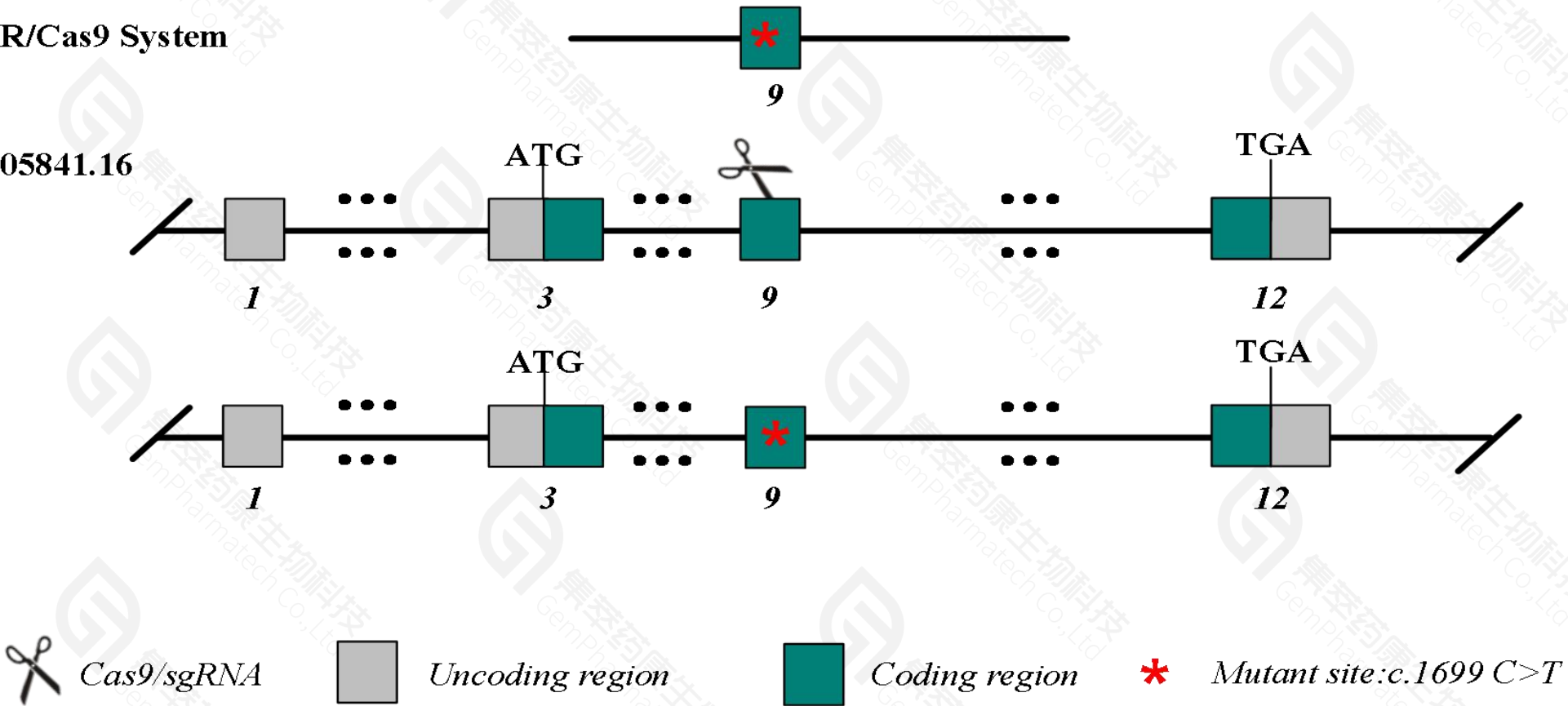
This model uses CRISPR/Cas9 technology to edit the *Ctcf* gene and the schematic diagram is as follow:

Donor and CRISPR/Cas9 System

ENSMUST00000005841.16

Wild-type allele

Targeted allele



# Technical Description

- The mouse *Ctcf* gene has 6 transcripts.
- This project produced *Ctcf-c.1699 C>T* point mutation on exon9 of the transcript of *Ctcf*-201(ENSMUST00000005841.16). The 1699th nucleotide of *Ctcf* CDS is mutated from C to T, The 567th amino acid will be mutated from R(Arg) to W(Trp).
- The mouse *Ctcf*-201 transcript contains 12 exons. The translation initiation site ATG is located at exon3, and the translation termination site TGA is located at exon12, encoding 736aa.
- In this project, *Ctcf* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, sgRNA and donor vectors were constructed. Cas9, sgRNA 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 data of MGI, mice homozygous for a null allele die prior at implantation. Mice homozygous for a conditional allele activated in T cells exhibit a defect in the transition from immature single positive T cells to double positive T cells.
- One or two synonymous mutations of amino acids will be introduced on exon9 of *Ctcf*.
- The strategy may affect the normal splicing of the target gene.
- Mouse *Ctcf* gene is located on Chr8. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr8, 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

	541	540	550	560	570	580	590	600	610	620	630	640
CTCF-p-human	541	HFKRYHDPNFVPAAAFVCSKCGKTFTRRNTMARHADNCAGPDGVEGENGGETKKSKRGRKRKMRSKKEDSSDS-ENAEPLDDNEDEEPPAVEIEPEPEPQI										
Ctcf-p-mouse	541	HFKRYHDPNFVPAAAFVCSKCGKTFTRRNTMARHADNCAGPDGVEGENGGETKKSKRGRKRKMRSKKEDSSDSEENAEPLDDNEDEEPPAVEIEPEPEPQI										
Consensus	541	HFKRYHDPNFVPAAAFVCSKCGKTFTRRNTMARHADNCAGPDGVEGENGGETKKSKRGRKRKMRSKKEDSSDS ENAEPLDDNEDEEPPAVEIEPEPEPQI										
Ready		consensus positions: 98.2% identity positions: 98.0% aln: 595										

hCTCF-P-567R-mCtcf-P-567R

Identity positions: 98.0%

	1666	1670	1680	1690	1700	1710	1720	1730	1740	1750	1760
CTCF-human-CDS	1666	GTCTGTTC TAAGTGTGGGAAAACATT TACACGTCGGAA TACCATGGCAAGACATGCTGATAA TTGTGCTGGCCAGATGGCGTAGAGGGGGAAAATGC									
Ctcf-mouse-CDS	1666	GTCTGTTC TAAGTGTGGGAAAACATT CACCCGCGGAA CACAATGGCAAGACATGCAGATAA CTGTGCTGGTCCAGATGGCGTAGAGGGGGAAAATGC									
Consensus	1666	GTCTGTTC TAAGTGTGGGAAAACATT AC CG CGGAA AC ATGGCAAGACATGC GATAA TGTGCTGG CCAGATGGCGTAGAGGGGGAAAATGC									
Ready		consensus positions: 91.5% identity positions: 91.5% gr: 1									

hCTCF-CCDS-1699C-mCtcf-CCDS-1699C

# Mutation Site

## Before mutation

	+1	T	F	R	Q	K	Q	L	L	D	M	H	F	K	R	Y	H	D	P	N	F	V	P	A	A	F	V	C	S	K	C	G	K	T	F?	
49501		ACCTTCCGCC	AGAAACAGCT	CCTCGACATG	CATTTC AAGC	GCTATCATGA	TCCCAACTTT	GTCCCTGCTG	CCTTTGTCTG	TTCCAAGTGT	GGGAAAACAT	TGGAAGGCGG	TCTTTGTCGA	GGAGCTGTAC	GTAAAGTTCG	CGATAGTACT	AGGGTTGAAA	CAGGGACGAC	GGAAACAGAC	AAGG TTCACA	CCCTTTTGTA															
	+1	?F	T	R	R																															
49601		TCACCCGCGG	GGTAAGGCTC	AGGCTCCTGT	TATGGCTCTT	AATAGCACAC	ACTTGATCTT	CAGTTACAGA	ATGGAGTTGT	GGCCACTGGA	GTAATAAATG	AGTGGGCGGC	CCATTCCGAG	TCCGAGGACA	ATACCGAGAA	TTATCGTGTG	TGAACTAGAA	GTCAATGTCT	TACCTCAACA	CCGGTGACCT	CATTATTTAC															

## After mutation

+1	T	F	R	Q	K	Q	L	L	D	M	H	F	K	R	Y	H	D	P	N	F	V	P	A	A	F	V	C	S	K	C	G	K	T	F?	
49501	ACCTTCCGCC	AGAAACAGCT	CCTCGACATG	CATTTC AAGC	GCTATCATGA	TCCCAACTTT	GTCCCTGCTG	CCTTTGTCTG	TTCCAAGTGT	GGGAAAACAT	TGGAAGGCGG	TCTTTGTCGA	GGAGCTGTAC	GTAAAGTTCG	CGATAGTACT	AGGGTTGAAA	CAGGGACGAC	GGAAACAGAC	AAGG TTCACA	CCCTTTTGTA															
+1	?F	T	R	W																															
49601	TCACCCGCTG	GGTAAGGCTC	AGGCTCCTGT	TATGGCTCTT	AATAGCACAC	ACTTGATCTT	CAGTTACAGA	ATGGAGTTGT	GGCCACTGGA	GTAATAAATG	AGTGGGCGAC	CCATTCCGAG	TCCGAGGACA	ATACCGAGAA	TTATCGTGTG	TGAACTAGAA	GTCAATGTCT	TACCTCAACA	CCGGTGACCT	CATTATTTAC															

The green region is exon9 of *Ctcf-201*, and the red region represents the c.1699C>T mutation site.

# Gene name and location (NCBI)

## Ctcf CCCTC-binding factor [ *Mus musculus* (house mouse) ]

Gene ID: 13018, updated on 24-Apr-2021

[Download Datasets](#)

### Summary

**Official Symbol** Ctcf provided by [MGI](#)  
**Official Full Name** CCCTC-binding factor provided by [MGI](#)  
**Primary source** [MGI:MGI:109447](#)  
**See related** [Ensembl:ENSMUSG00000005698](#)  
**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** AW108038  
**Expression** Ubiquitous expression in CNS E11.5 (RPKM 18.2), thymus adult (RPKM 13.4) and 27 other tissues [See more](#)  
**Orthologs** [human](#) [all](#)

**NEW**

Try the new [Gene table](#)

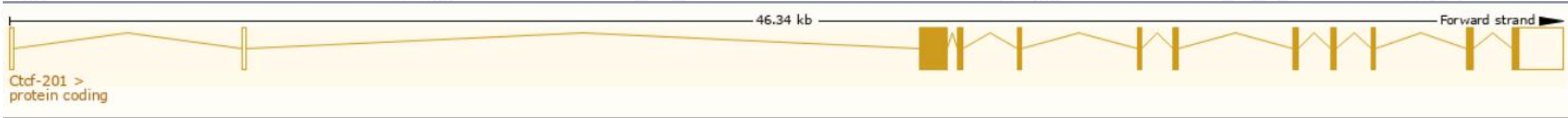
Try the new [Transcript table](#)

# Transcript information (Ensembl)

The gene has 6 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Ctcf-201	<a href="#">ENSMUST00000005841.16</a>	3782	<a href="#">736aa</a>	Protein coding	<a href="#">CCDS22606</a>	<a href="#">Q61164</a>	GENCODE basic APPRIS P1 TSL:1
Ctcf-204	<a href="#">ENSMUST00000132679.8</a>	1554	No protein	Processed transcript	-	-	TSL:5
Ctcf-203	<a href="#">ENSMUST00000129388.2</a>	729	No protein	Processed transcript	-	-	TSL:3
Ctcf-202	<a href="#">ENSMUST00000128510.8</a>	528	No protein	Processed transcript	-	-	TSL:3
Ctcf-205	<a href="#">ENSMUST00000137735.8</a>	469	No protein	Processed transcript	-	-	TSL:3
Ctcf-206	<a href="#">ENSMUST00000156436.2</a>	851	No protein	Retained intron	-	-	TSL:3

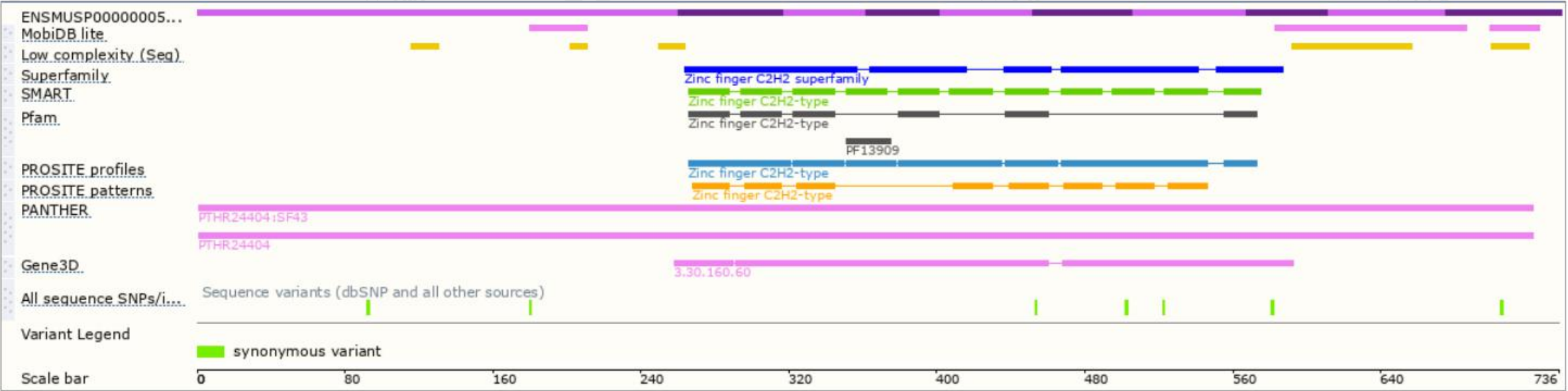
The strategy is based on the design of *Ctcf-201* transcript, the transcription is shown below:



# Genomic location distribution



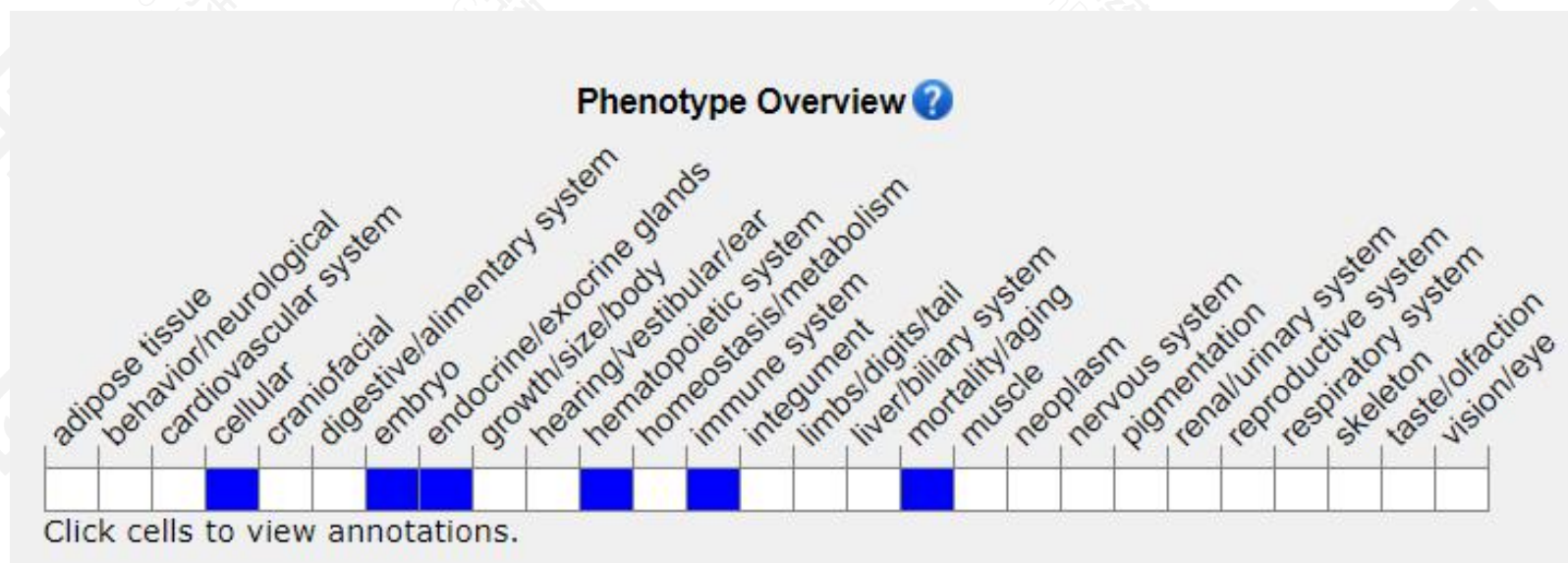
# Protein domain



# Mouse phenotype description(MGI)

URL link is as follows:

<http://www.informatics.jax.org/marker/MGI:109447>



Mice homozygous for a null allele die prior at implantation. Mice homozygous for a conditional allele activated in T cells exhibit a defect in the transition from immature single positive T cells to double positive T cells.

If you have any questions, please feel free to contact us.  
Tel: 025-5864 1534



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