Eef2-T57M cas9-ki(PM) Mouse Model Strategy -CRISPR/Cas9 technology

Designer: Xiaojing Li

Reviewer: JiaYu

Design Date: 2021-4-27

Project Overview



Project Name

Eef2-T57M

Project type

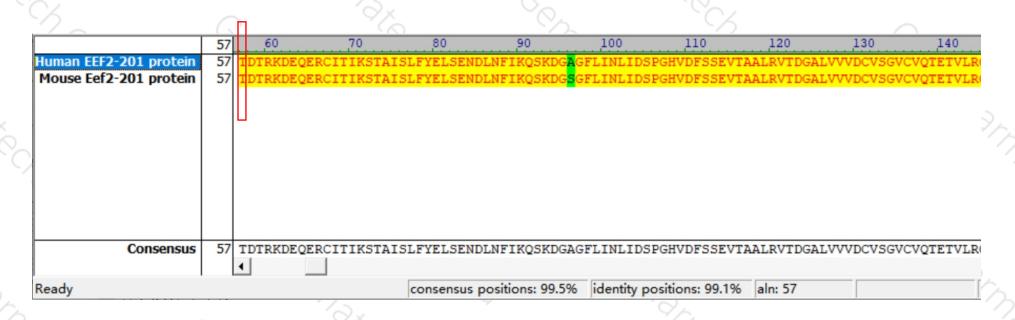
cas9-ki(PM)

Strain background

C57BL/6JGpt



A comparison of the aa homology of human and mouse Eef2 gene



The 57th amino acid(T) of human *EEF2* gene corresponds to the 57th amino acid(T) of mouse *Eef2* gene after comparing homology of mouse *Eef2* gene and human *EEF2* gene.

Technical Description



- The mouse *Eef2* gene has 3 transcripts. The human *EEF2* gene has 7 transcripts.
- According to the structure of *Eef2* gene and requirements of customer, the 57th amino acid(T) of human *EEF2*(NM_001961.4) gene corresponds to the 57th amino acid(T) of mouse *Eef2* gene after comparing homology of mouse *Eef2* gene and human *EEF2* gene. This project produced *Eef2*-T57A point mutation on exon2 of the transcript of *Eef2*-201(ENSMUST00000047864.11, NM_007907.2). The 57th amino acids will be mutated from T to M, and the corresponding codon will be mutated to ATG by the ACT.
- The mouse *Eef2*-201 transcript contains 14 exons. The translation initiation site ATG is located at exon1, and the translation termination site TAG is located at exon14, encoding 858aa.
- In this project, *Eef2* 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.

Mutation Site



Before mutation

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	TCI	TAG	STCG	G A	CCG.	ACG	GGT	AGG	GGG	GGTG	G T	GTC	CACI	TG	AA	GTG:	TCAT	rc :	TAG	CTA	AGGC	AC	GGT	AGTA	СС	TGT	CTT	TC	GGI	TGT	TAGG	C (CTTG	TAC	AGT	
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	CAG	TAG	GCGG	G I	ACA	CCI	GGT	GC	CGT	TCAG	G T	GCGZ	ACTO	GC	TG	AGG	GAAC	CA (CAC	STTO	CGA	CC	GTA	GTAA	C G	GAG	ACGG	GC	TCG	ACC	CCT	C 1	rgcg	CGA	AGT	
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	GA	TGI	rgag	C G	TTC	CTA	CTC	GT(CCT	CGCG	A C	GTA(GTGT	TA	GT:	TTA	GGT	C I	ACT(CACI	CCC	CT	GTC	GGGG	СТ	CCC	CAAC	AC	GAG	ACC	CAC	A (STGA	GCC	CAC	

After mutation

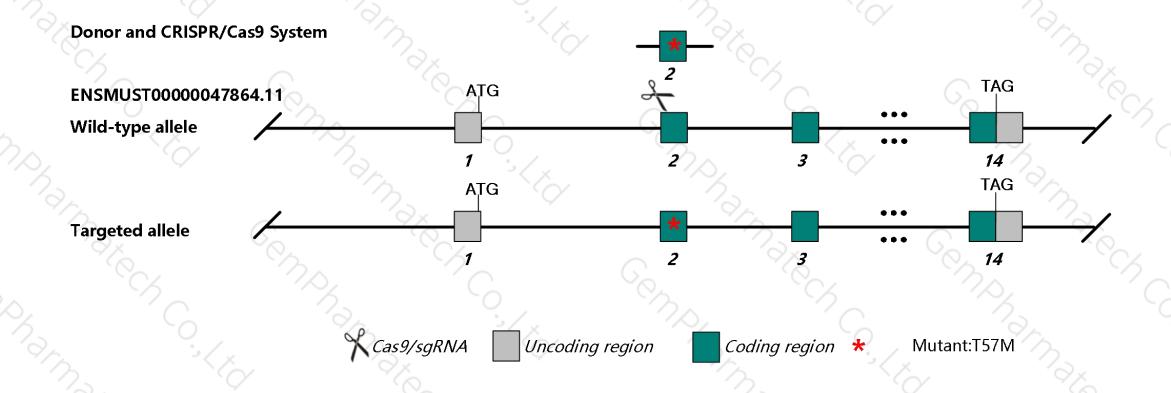
+2		V N F T	V D Q I R	A I M D K K /	A N I R N M S
3001	AGAATCAGCC TGGCTGCCCA TCCCCCCACC	ACAGGTGAAC TTCACAG	TAG ATCAGATCCG	TGCCATCATG GACAAGAAAG	CCAACATCCG GAACATGTCA
	TCTTAGTCGG ACCGACGGGT AGGGGGGTGG	TGTC <mark>CACTTG AAGTGTC</mark>	ATC TAGTCTAGGC A	ACGGTAGTAC CTGTTCTTTC	GGTTGTAGGC CTTGTACAGT
+2	V I A H V D H G K S	T L T D S L	V C K A	G I I A S A R	AGETRFM?
3101	GTCATCGCCC ATGTGGACCA CGGCAAGTCC	ACGCTGACCG ACTCCCT	GT GTGCAAGGCT (GGCATCATTG CCTCTGCCCG	AGCTGGGGAG ACGCGCTTCA
	CAGTAGCGGG TACACCTGGT GCCGTTCAGG	TGCGACTGGC TGAGGGA	ACA CACGTTCCGA (CCGTAGTAAC GGAGACGGGC	TCGACCCCTC TGCGCGAAGT
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3201	TGGACACTCG CAAGGATGAG CAGGAGCGCT	GCATCACAAT CAAATCC	ACG TGAGTGAGGG (GACAGCCCCG AGGGGTTGTG	CTCTGGGTGT CACTCGGGTG
	ACCTGTGAGC GTTCCTACTC GTCCTCGCGA	CGTAGTGTTA GTTTAGG	<mark>rg</mark> c actcactccc (CTGTCGGGGC TCCCCAACAC	GAGACCCACA GTGAGCCCAC
(V .	- /	/ /	11 7	/ >	

The yellow region is exon2 of *Eef2-201*, the red region represents the mutation site.

Strategy



This model uses CRISPR/Cas9 technology to edit the *Eef2* gene and the schematic diagram is as follow:



Notice



- According to the data of MGI, mice homozygous for a mutation removing the diphthamide modification display partial neonatal lethality, fetal growth retardation and abnormal cell physiology. Most of the homozygous mice with *Eef2*-G717R point mutation died shortly after birth.
- > One or Two synonymous mutations of amino acids will be intronduced on exon2 of *Eef2*.
- > The mutation site is about 5.2kb away from the 5-terminal of *Dapk3* gene, which may affect its 5-terminal regulation.
- Mouse *Eef2* gene is located on Chr10. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr10, 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.

Gene name and location (NCBI)



Eef2 eukaryotic translation elongation factor 2 [*Mus musculus* (house mouse)]

Gene ID: 13629, updated on 3-Jan-2021

Summary

☆ ?

Official Symbol Eef2 provided by MGI

Official Full Name eukaryotic translation elongation factor 2 provided by MGI

Primary source MGI:MGI:95288

See related Ensembl: ENSMUSG00000034994

Gene type protein coding
RefSeq status VALIDATED
Organism Mus musculus

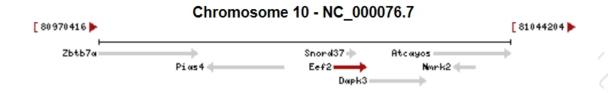
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;

Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

Also known as Ef-2

Expression Ubiquitous expression in ovary adult (RPKM 1248.0), colon adult (RPKM 810.0) and 28 other tissues See more

Orthologs human all



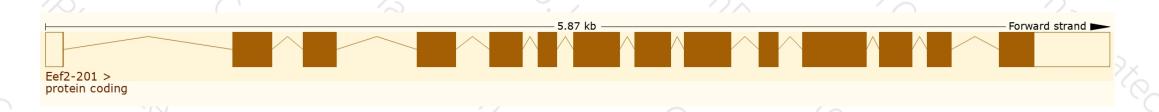
Transcript information (Ensembl)



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

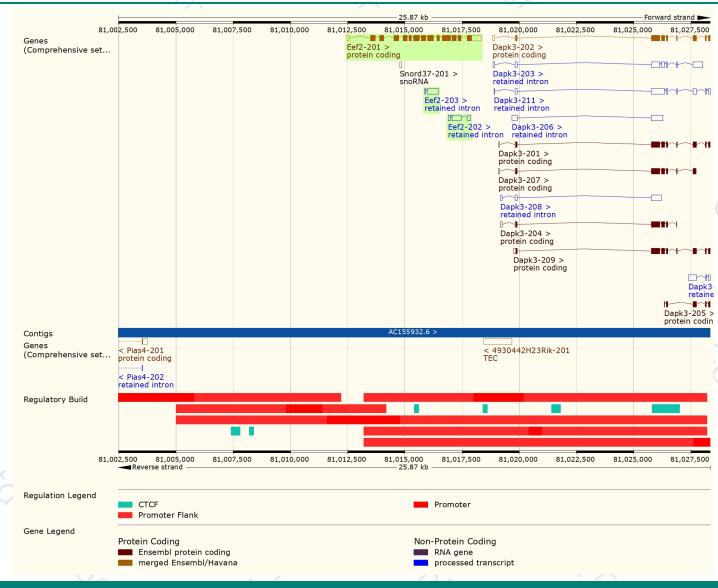
Name 🛦	Transcript ID 🗼	bp 🍦	Protein 🛊	Biotype 🍦	CCDS 🍦	UniProt Match 🍦	Flags
Eef2-201	ENSMUST00000047864.11	3089	<u>858aa</u>	Protein coding	CCDS35993	P58252 &	TSL:1 GENCODE basic APPRIS P1
Eef2-202	ENSMUST00000219497.2	651	No protein	Retained intron	-	-	TSL:3
Eef2-203	ENSMUST00000219943.2	519	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Eef2-201* transcript, the transcription is shown below:



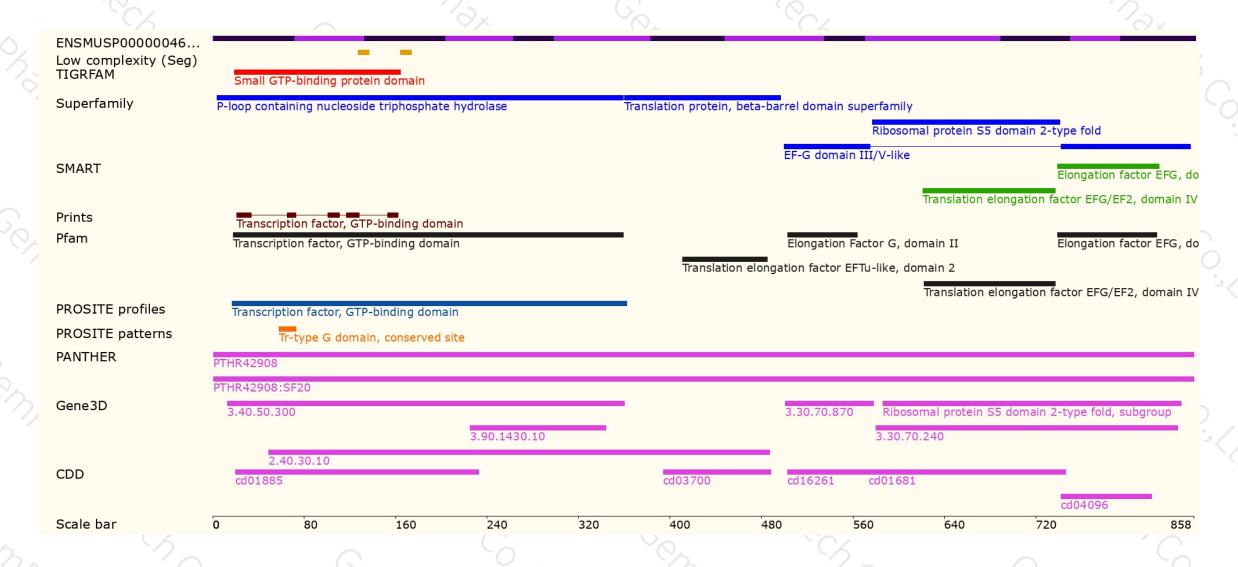






Protein domain



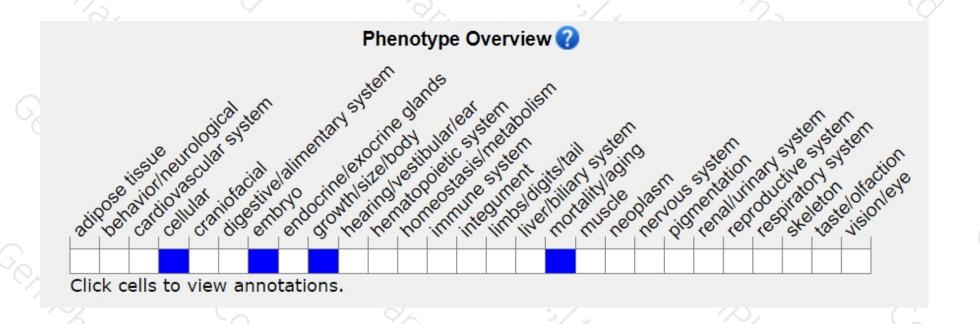


Mouse phenotype description(MGI)



URL link is as follows:

http://www.informatics.jax.org/marker/MGI:95288



Mice homozygous for a mutation removing the diphthamide modification display partial neonatal lethality, fetal growth retardation and abnormal cell physiology.

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





