Tcf12 Cas9-CKO Strategy

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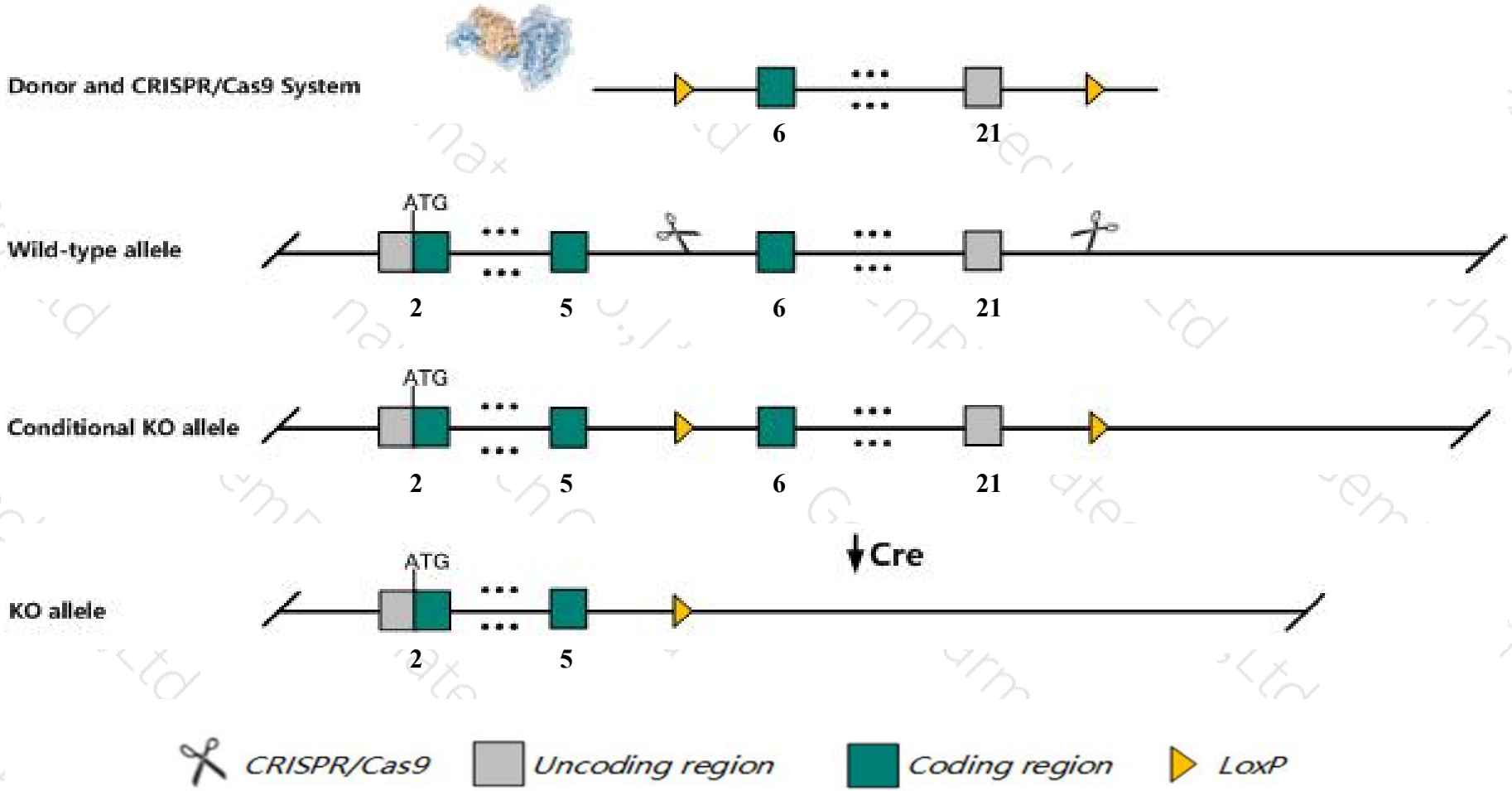
Project Overview

Project Name	***Tcf12***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tcf12* gene. The schematic diagram is as follows:



Technical routes

➢ The *Tcf12* gene has 21 transcripts. According to the structure of *Tcf12* gene, exon6-exon21 of *Tcf12-202*(ENSMUST00000183404.7) transcript is recommended as the knockout region.The region contains most coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Tcf12* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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

➢ According to the existing MGI data,mice homozygous for a targeted null mutation exhibit postnatal lethality within two weeks of birth and a 50% reduction in the number of pro-B cells.

➢ Gene *Gm37879,Gm18821,Gm38111,Gm38057* will be deleted.

➢ The *Tcf12* gene is located on the Chr9. 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.

➢ 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）

集萃药康 GemPharmatech

Tcf12 transcription factor 12 [Mus musculus (house mouse)]

Gene ID: 21406, updated on 13-Mar-2020

Summary

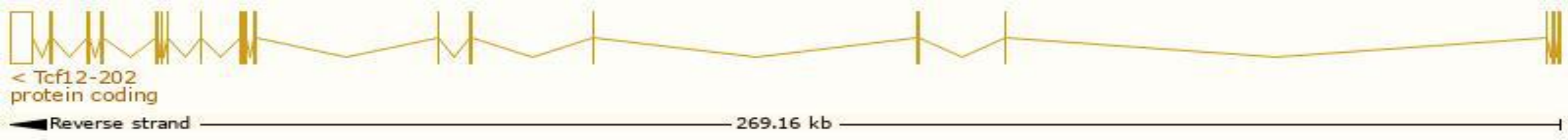
Official Symbol	Tcf12 provided by MGI
Official Full Name	transcription factor 12 provided by MGI
Primary source	MGI:MGI:101877
See related	Ensembl:ENSMUSG00000032228
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A130037E08Rik, ALF1, HEB, HEBAlt, HTF-4, HTF4, ME1, REB, bHLHb20
Expression	Ubiquitous expression in CNS E11.5 (RPKM 41.6), limb E14.5 (RPKM 34.8) and 27 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

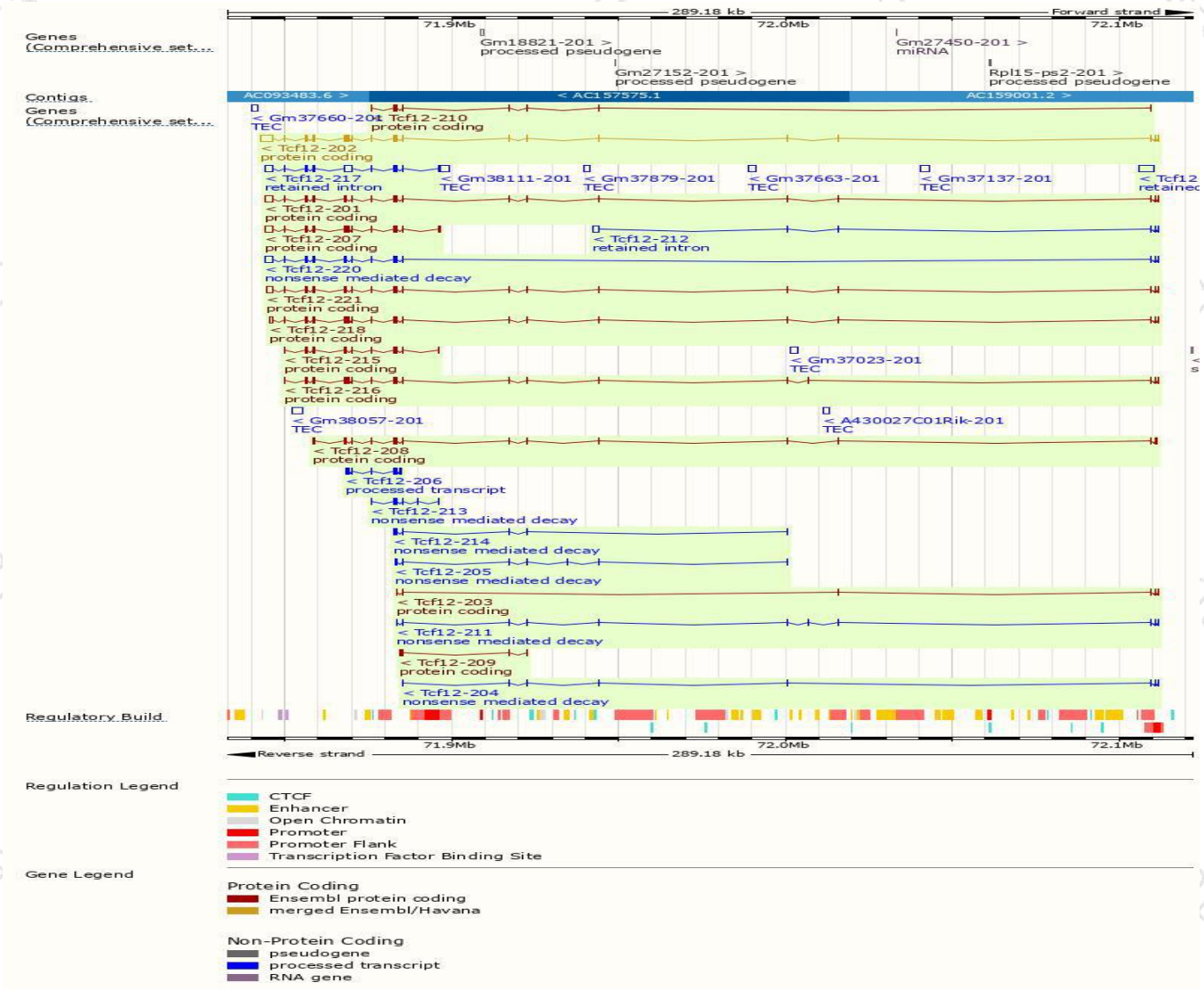
The gene has 21 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tcf12-202	ENSMUST00000183404.7	6299	706aa	Protein coding	CCDS23329	Q61286	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Tcf12-201	ENSMUST00000034755.12	4621	682aa	Protein coding	CCDS72272	Q61286	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1
Tcf12-207	ENSMUST00000183918.7	4308	536aa	Protein coding	CCDS72271	V9GX46	TSL:1 GENCODE basic
Tcf12-221	ENSMUST00000185117.7	3986	682aa	Protein coding	CCDS72272	Q61286	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1
Tcf12-218	ENSMUST00000184783.7	3489	706aa	Protein coding	CCDS23329	Q61286	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Tcf12-216	ENSMUST00000184523.7	2257	666aa	Protein coding	-	V9GWU2	CDS 3' incomplete TSL:5
Tcf12-208	ENSMUST00000183992.7	1780	523aa	Protein coding	-	V9GXC3	CDS 3' incomplete TSL:1
Tcf12-215	ENSMUST00000184448.7	1522	477aa	Protein coding	-	Q3UXQ3	CDS 3' incomplete TSL:1
Tcf12-209	ENSMUST00000184029.1	964	108aa	Protein coding	-	V9GWS4	CDS 5' incomplete TSL:2
Tcf12-210	ENSMUST00000184072.7	838	279aa	Protein coding	-	V9GXR6	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Tcf12-203	ENSMUST00000183492.7	600	127aa	Protein coding	-	V9GX18	CDS 3' incomplete TSL:3
Tcf12-220	ENSMUST00000184867.7	4167	57aa	Nonsense mediated decay	-	V9GXM8	TSL:1
Tcf12-211	ENSMUST00000184107.7	826	79aa	Nonsense mediated decay	-	V9GXP0	TSL:5
Tcf12-204	ENSMUST00000183594.1	818	58aa	Nonsense mediated decay	-	V9GX29	TSL:5
Tcf12-205	ENSMUST00000183647.7	797	57aa	Nonsense mediated decay	-	V9GXG5	CDS 5' incomplete TSL:5
Tcf12-213	ENSMUST00000184378.7	697	27aa	Nonsense mediated decay	-	V9GXQ6	CDS 5' incomplete TSL:3
Tcf12-214	ENSMUST00000184416.7	691	21aa	Nonsense mediated decay	-	V9GX66	CDS 5' incomplete TSL:3
Tcf12-206	ENSMUST00000183784.7	932	No protein	Processed transcript	-	-	TSL:3
Tcf12-217	ENSMUST00000184770.7	5742	No protein	Retained intron	-	-	TSL:5
Tcf12-219	ENSMUST00000184806.1	4343	No protein	Retained intron	-	-	TSL:NA
Tcf12-212	ENSMUST00000184196.1	2193	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Tcf12-202* transcript,the transcription is shown below:

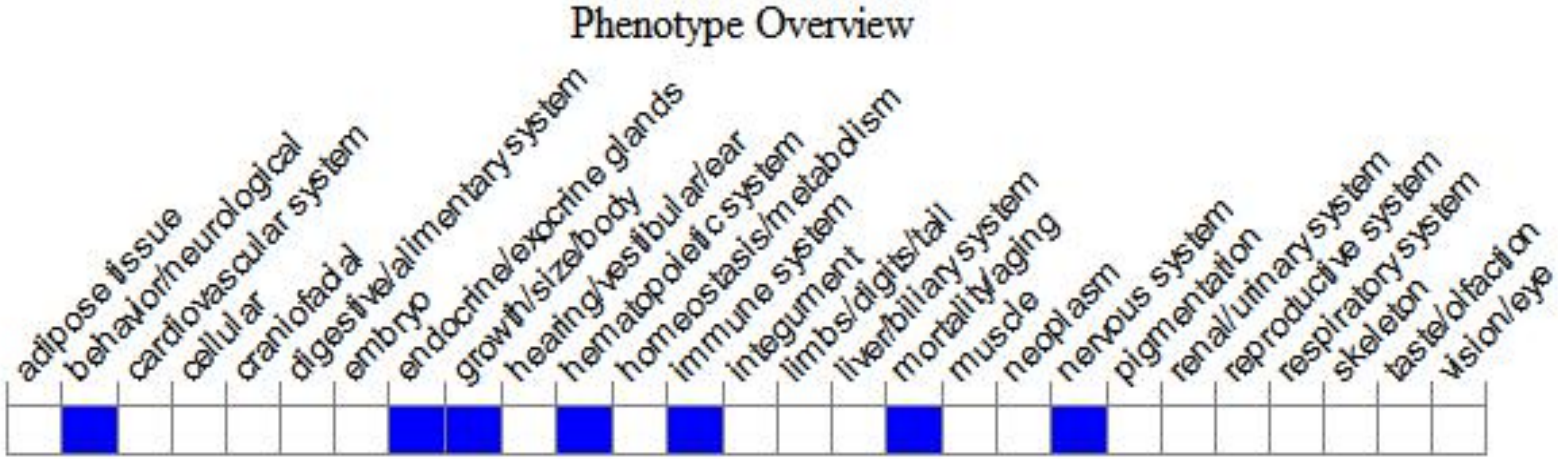


Genomic location distribution



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/).

According to the existing MGI data,mice homozygous for a targeted null mutation exhibit postnatal lethality within two weeks of birth and a 50% reduction in the number of pro-B cells.

If you have any questions, you are welcome to inquire.

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