Bcat2 Cas9-CKO Strategy

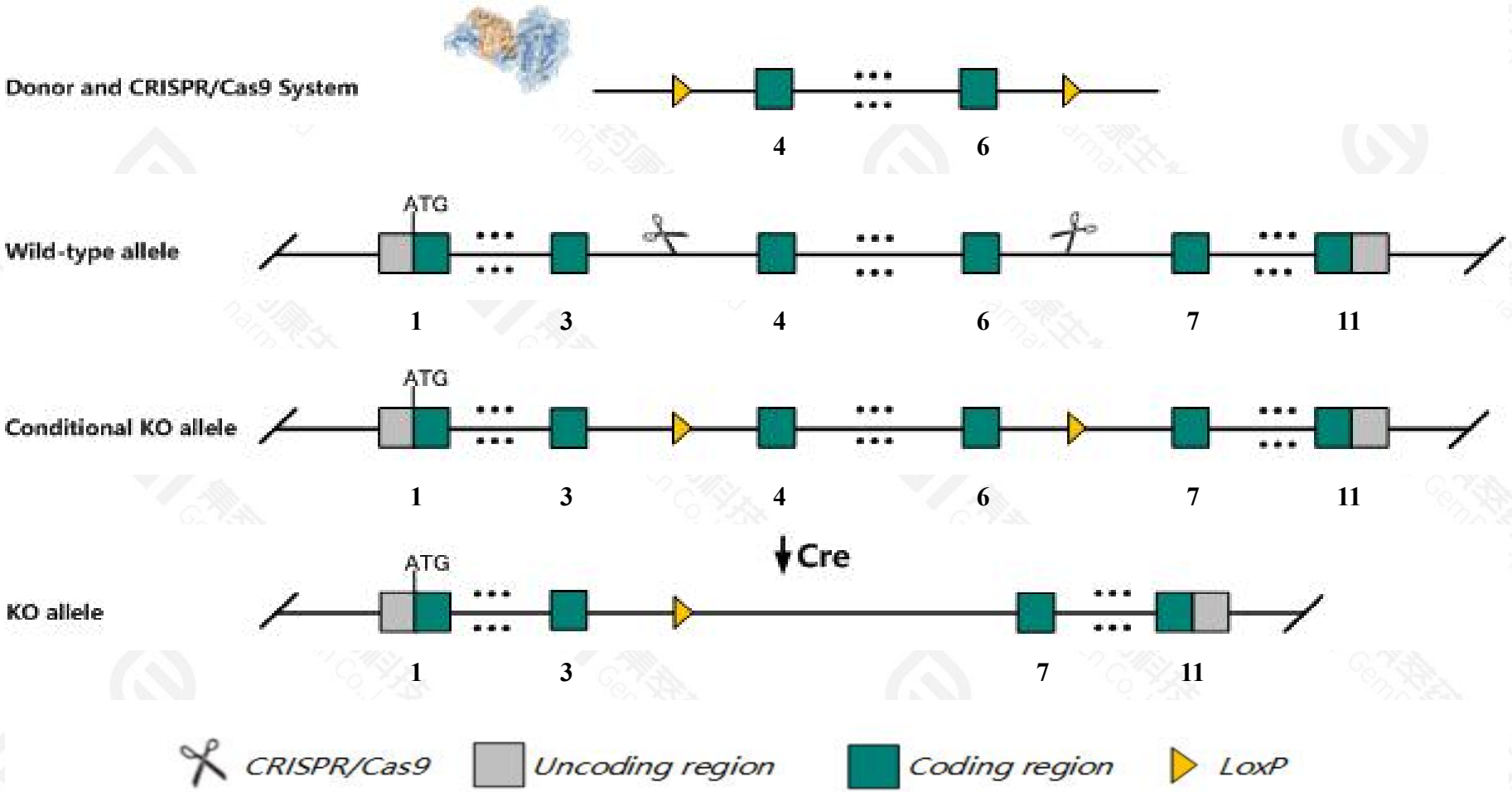
Designer: Daohua Xu

Project Overview

Project Name	***Bcat2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Bcat2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Bcat2* gene has 8 transcripts. According to the structure of *Bcat2* gene, exon4-exon6 of *Bcat2-201*(ENSMUST00000033098.16) transcript is recommended as the knockout region.The region contains 395bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Bcat2* 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 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,the metabolism of branched chain amino acid is impaired in homozygous null mice, resulting in a phenotype similar to human maple syrup urine disease. Mutants exhibit a failure to thrive and die prematurely, though the severity of the symptoms can be ameliorated with a restricted diet.

➢ The *Bcat2* gene is located on the Chr7. 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）

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Bcat2 branched chain aminotransferase 2, mitochondrial [Mus musculus (house mouse)]

Gene ID: 12036, updated on 13-Mar-2020

Summary

Official Symbol	Bcat2 provided by MGI
Official Full Name	branched chain aminotransferase 2, mitochondrial provided by MGI
Primary source	MGI:MGI:1276534
See related	Ensembl:ENSMUSG00000030826
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	Bcat(m), Bcat-2, Eca40
Expression	Ubiquitous expression in adrenal adult (RPKM 36.2), subcutaneous fat pad adult (RPKM 34.2) and 28 other tissues See more
Orthologs	human all

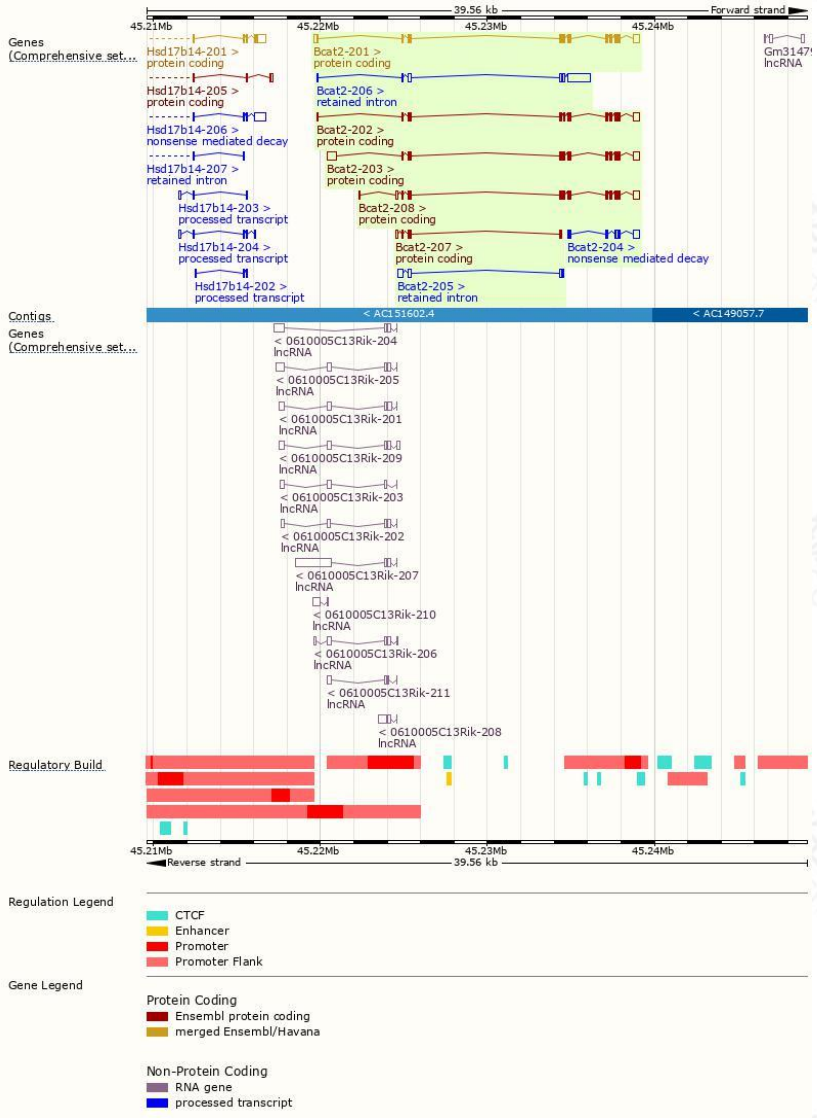
Transcript information（Ensembl）

The gene has 8 transcripts,all transcripts are shown below:

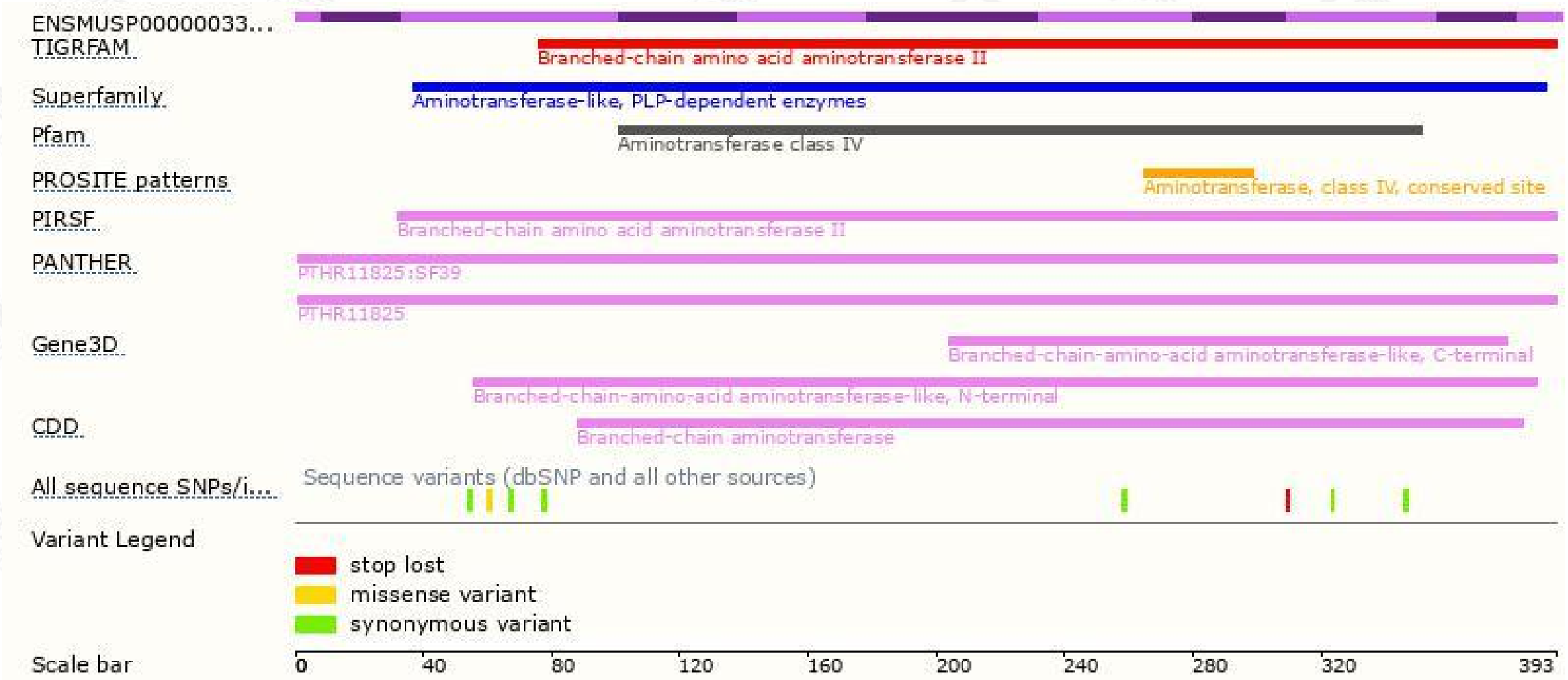
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Bcat2-203	ENSMUST00000209204.1	2081	353aa	Protein coding	CCDS57549	O88374	TSL:1 GENCODE basic
Bcat2-201	ENSMUST00000033098.15	1772	393aa	Protein coding	CCDS21252	O35855 Q3ULU3	TSL:1 GENCODE basic APPRIS P1
Bcat2-208	ENSMUST00000211173.1	1684	353aa	Protein coding	CCDS57549	O88374	TSL:1 GENCODE basic
Bcat2-202	ENSMUST00000120864.9	1536	388aa	Protein coding	-	A0A1B0GX27	TSL:5 GENCODE basic
Bcat2-207	ENSMUST00000210811.1	486	97aa	Protein coding	-	A0A1B0GST1	CDS 3' incomplete TSL:3
Bcat2-204	ENSMUST00000209410.1	917	101aa	Nonsense mediated decay	-	A0A1B0GQY4	CDS 5' incomplete TSL:5
Bcat2-206	ENSMUST00000209569.1	1905	No protein	Retained intron	-	-	TSL:1
Bcat2-205	ENSMUST00000209543.1	632	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Bcat2-201* transcript,the transcription is shown below:

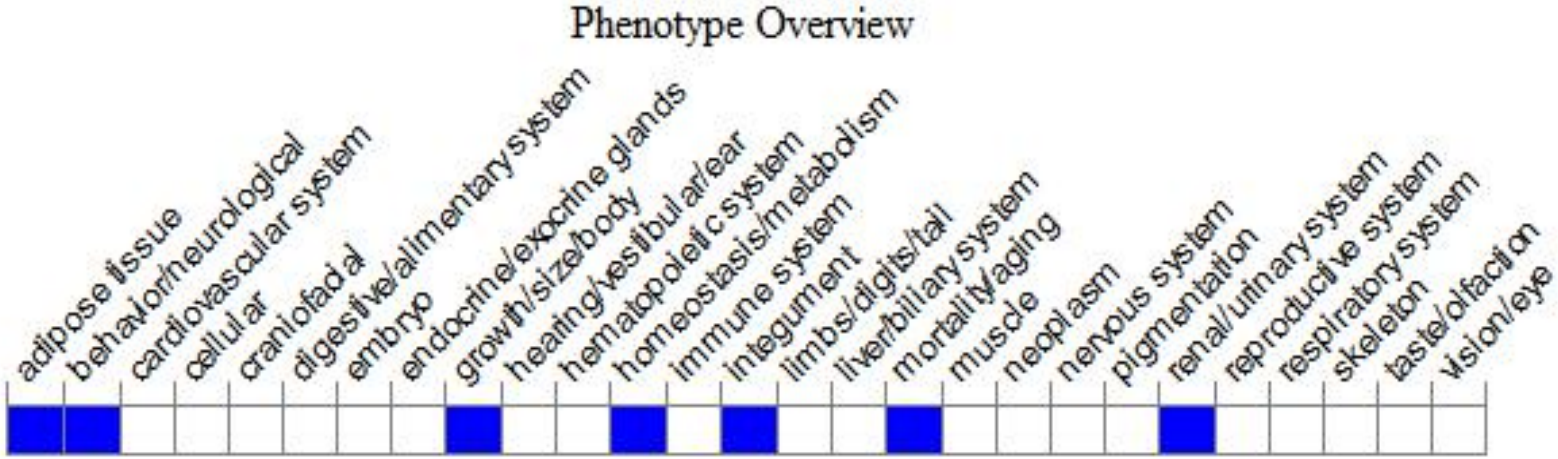
Genomic location distribution



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/).

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集萃药康
GemPharmatech

If you have any questions, you are welcome to inquire.
Tel: 400-9660890

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GemPharmatech Co.,Ltd