

Ache Cas9-KO Strategy

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Project Overview

Project Name

Ache

Project type

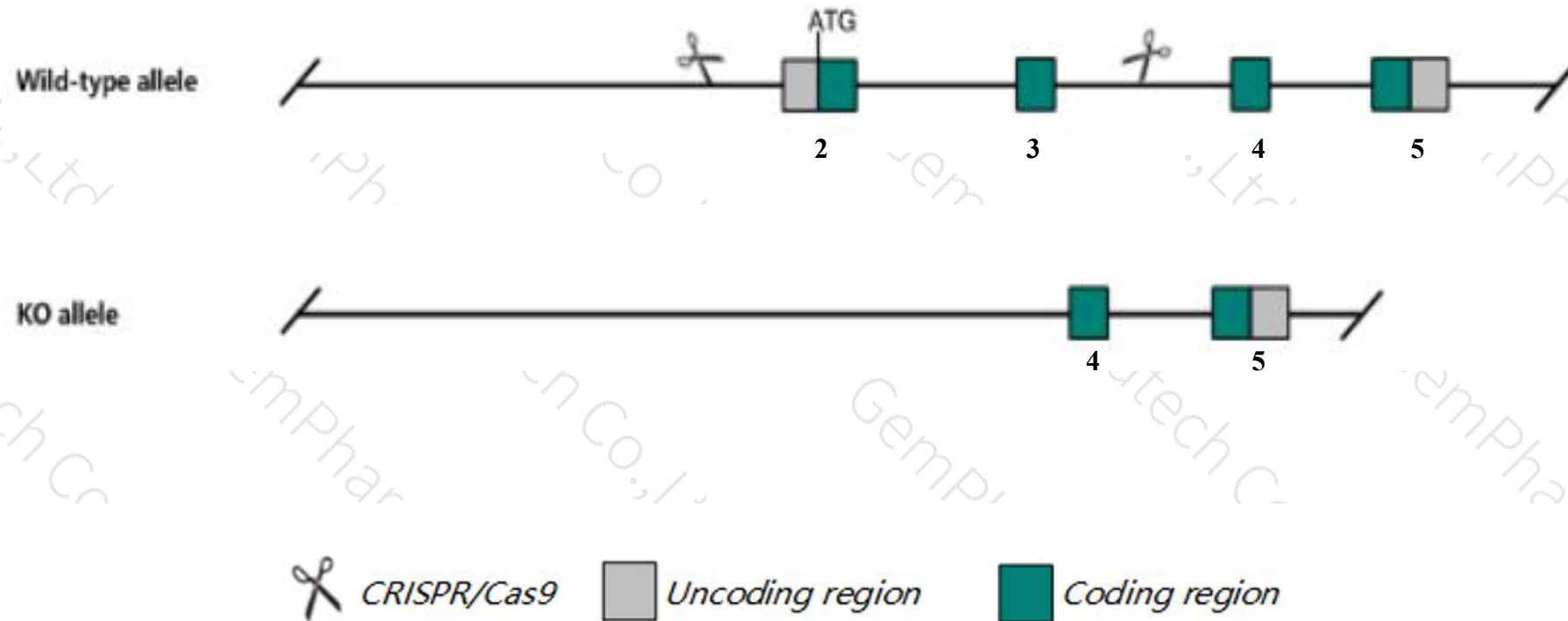
Cas9-KO

Strain background

C57BL/6JGpt

Knockout strategy

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



Technical routes

- The *Ache* gene has 9 transcripts. According to the structure of *Ache* gene, exon2-exon3 of *Ache*-201(ENSMUST00000024099.10) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ache* gene. The brief process is as follows: CRISPR/Cas9 system 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.

- According to the existing MGI data, homozygous mutants show retarded postnatal development, tremors, impaired righting response, delayed maturation of external ear, failure of eyelids to open, and die by 3-wk. of age. Mutants are highly sensitive to butyrylcholinesterase inhibitor toxicity.
- The KO region contains functional region of the *Ufsp1*, *Mir8116* and *Gm42456* gene. Knockout the region may affect the function of *Ufsp1*, *Mir8116* and *Gm42456* gene.
- The *Ache* gene is located on the Chr5. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Ache acetylcholinesterase [Mus musculus (house mouse)]

Gene ID: 11423, updated on 13-Mar-2020

Summary

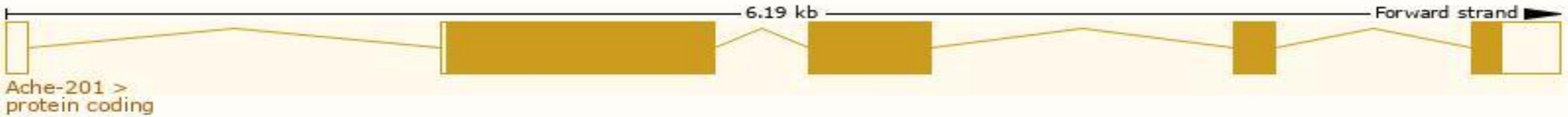
Official Symbol	Ache provided by MGI
Official Full Name	acetylcholinesterase provided by MGI
Primary source	MGI:MGI:87876
See related	Ensembl:ENSMUSG00000023328
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Broad expression in cerebellum adult (RPKM 25.6), thymus adult (RPKM 23.5) and 19 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

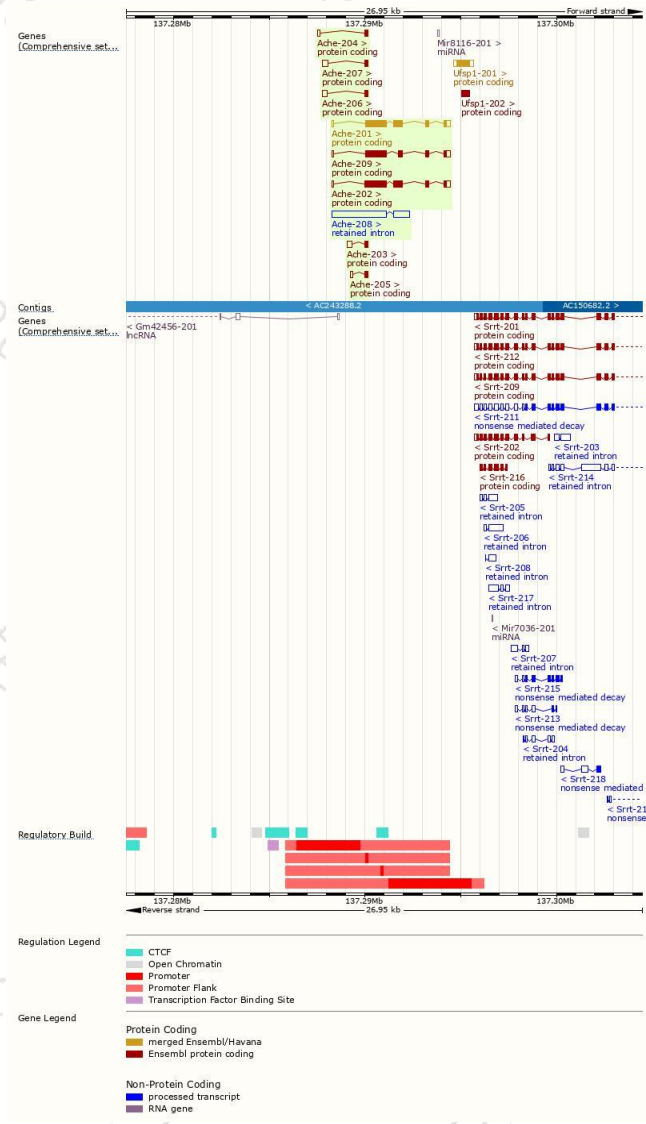
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ache-201	ENSMUST00000024099.10	2186	614aa	Protein coding	CCDS19763	P21836 Q543Z1	TSL:1 GENCODE basic APPRIS P1
Ache-202	ENSMUST00000085934.3	2176	614aa	Protein coding	CCDS19763	P21836 Q543Z1	TSL:1 GENCODE basic APPRIS P1
Ache-209	ENSMUST00000196208.4	1911	526aa	Protein coding	-	A0A0G2JDM6	TSL:5 GENCODE basic
Ache-207	ENSMUST00000141123.7	438	40aa	Protein coding	-	D3Z064	CDS 3' incomplete TSL:1
Ache-206	ENSMUST00000138591.7	409	40aa	Protein coding	-	D3Z064	CDS 3' incomplete TSL:1
Ache-203	ENSMUST00000125195.7	360	40aa	Protein coding	-	D3Z064	CDS 3' incomplete TSL:1
Ache-204	ENSMUST00000132191.7	262	40aa	Protein coding	-	D3Z064	CDS 3' incomplete TSL:1
Ache-205	ENSMUST00000137126.1	254	40aa	Protein coding	-	D3Z064	CDS 3' incomplete TSL:1
Ache-208	ENSMUST00000150983.1	3687	No protein	Retained intron	-	-	TSL:1

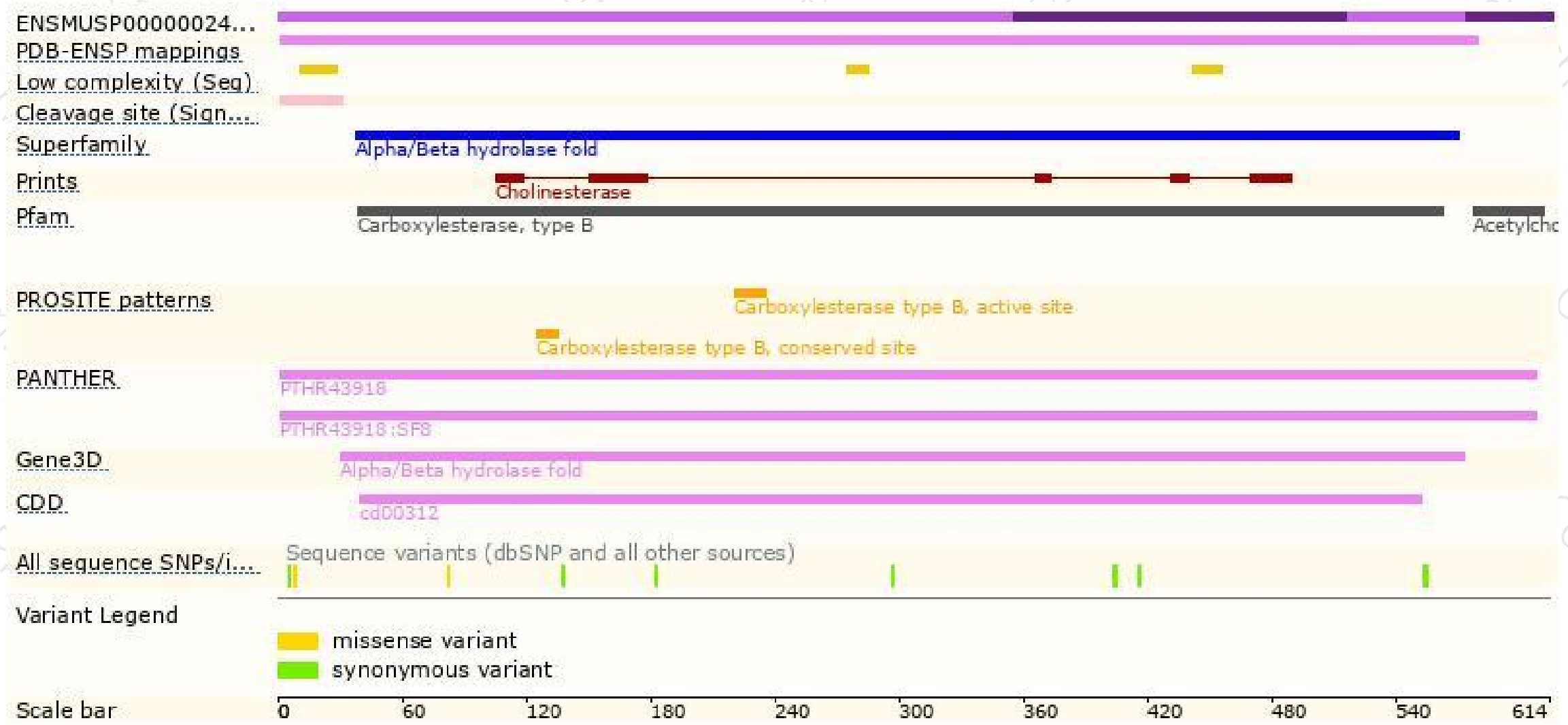
The strategy is based on the design of *Ache-201* transcript,the transcription is shown below:



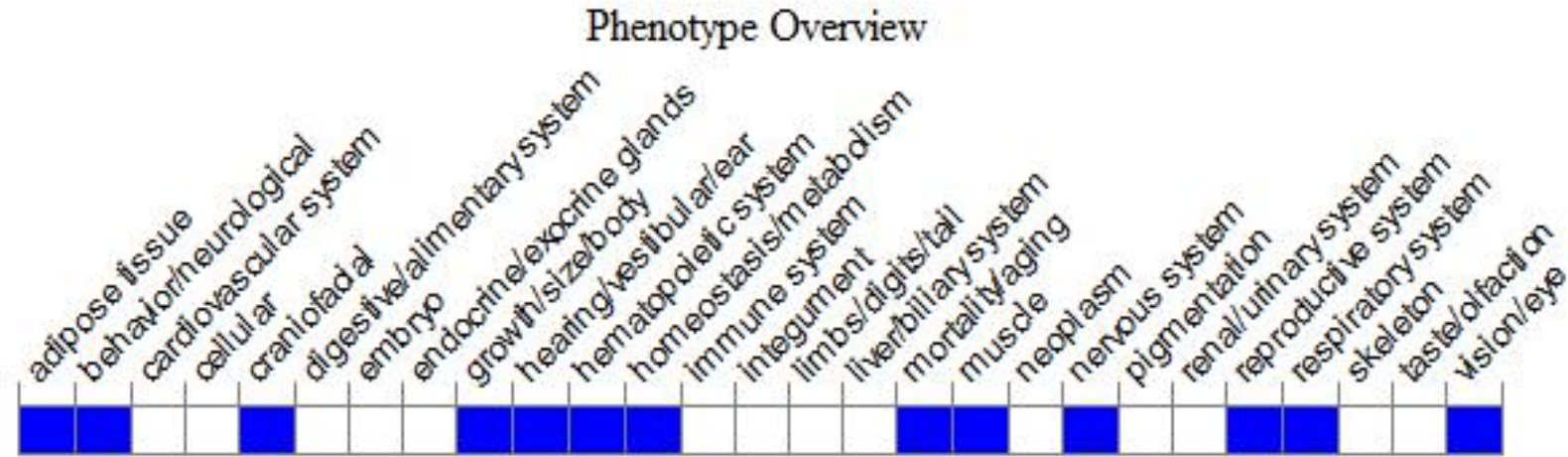
Genomic location distribution



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/>).

According to the existing MGI data, homozygous mutants show retarded postnatal development, tremors, impaired righting response, delayed maturation of external ear, failure of eyelids to open, and die by 3-wk. of age. Mutants are highly sensitive to butyrylcholinesterase inhibitor toxicity.

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

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