

Amph Cas9-CKO Strategy

Designer: Huimin Su

Reviewer: Ruirui Zhang

Design Date: 2020-8-3

Project Overview

Project Name

Amph

Project type

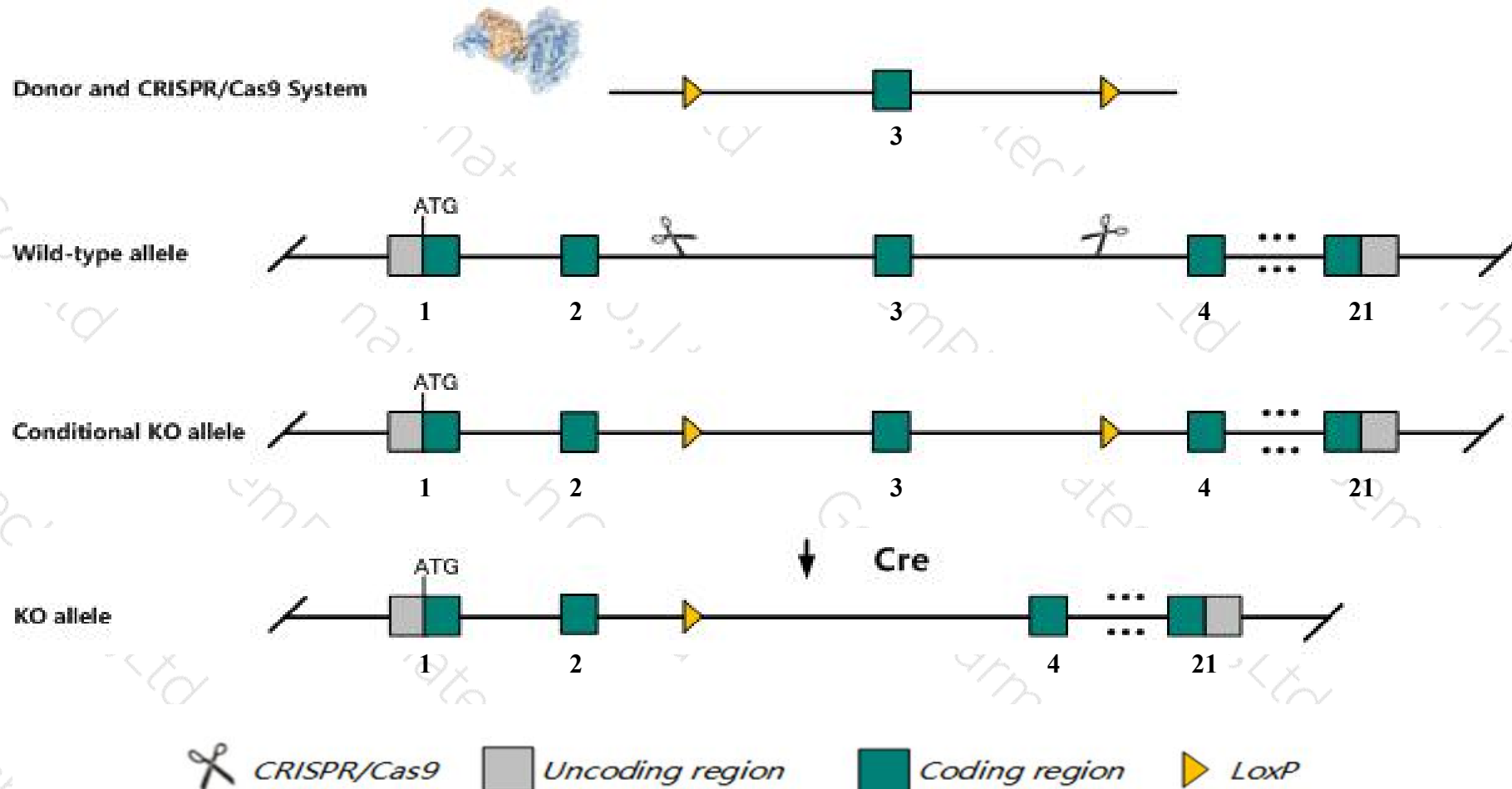
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Amph* gene has 6 transcripts. According to the structure of *Amph* gene, exon3 of *Amph-204*(ENSMUST00000200466.4) transcript is recommended as the knockout region. The region contains 55bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Amph* 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.

- According to the existing MGI data, mice homozygous for a targeted mutation of this gene exhibit learning deficits and synaptic vesicle recycling defects, and die between 2 to 5 months of age from rare irreversible seizures.
- The *Amph* gene is located on the Chr13. 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)

Amph amphiphysin [*Mus musculus* (house mouse)]

Gene ID: 218038, updated on 26-Jun-2020

Summary

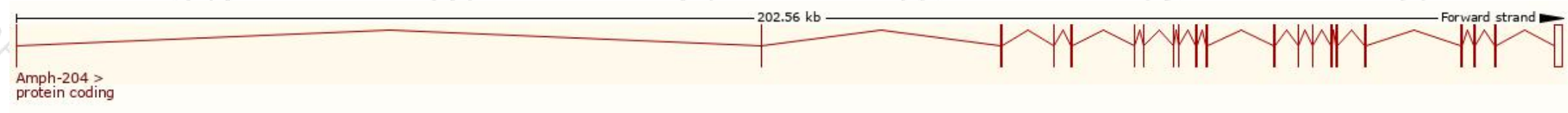
Official Symbol	Amph provided by MGI
Official Full Name	amphiphysin provided by MGI
Primary source	MGI:MGI:103574
See related	Ensembl:ENSMUSG00000021314
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<i>Mus musculus</i>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Biased expression in cerebellum adult (RPKM 23.7), frontal lobe adult (RPKM 22.9) and 7 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

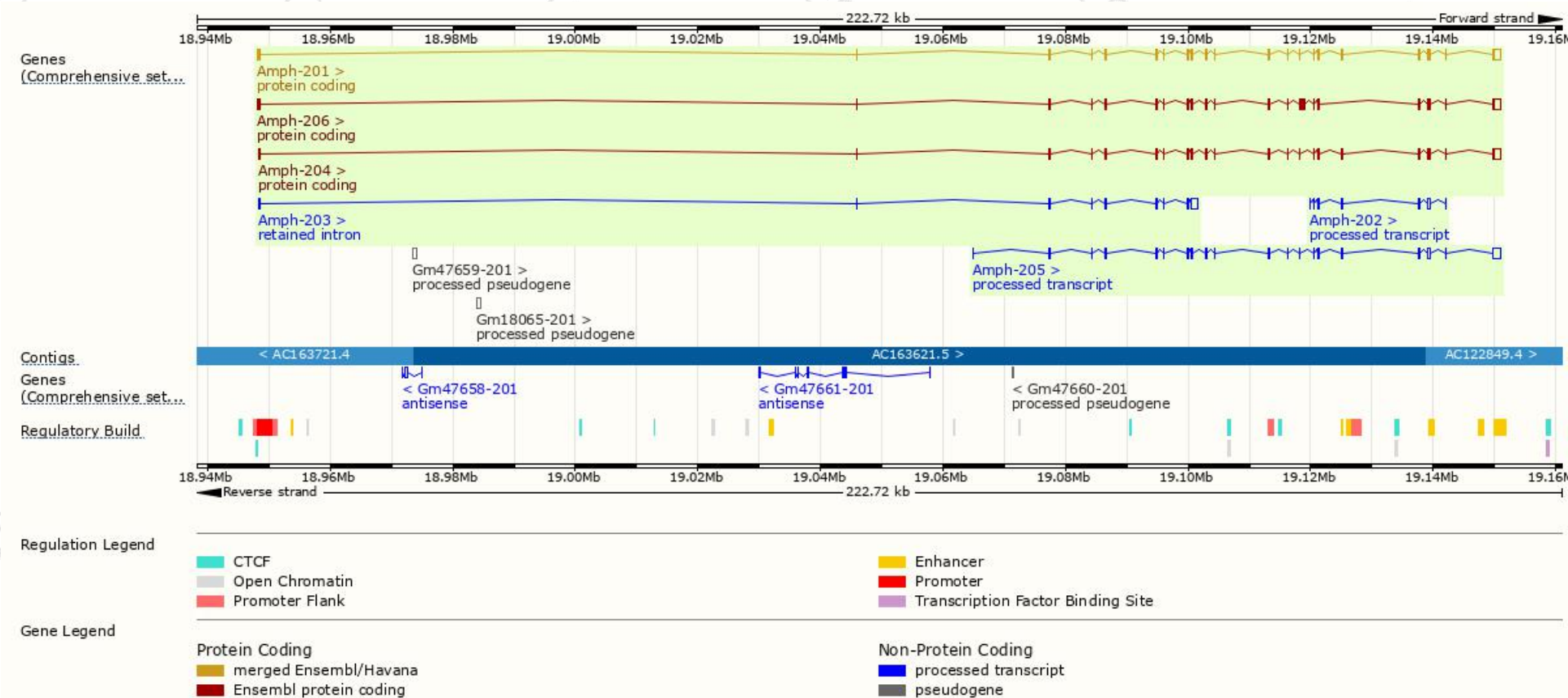
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Amph-201	ENSMUST00000003345.9	3367	686aa	Protein coding	CCDS26258	Q7TQF7	TSL:1 GENCODE basic APPRIS P3
Amph-204	ENSMUST00000200466.4	3224	690aa	Protein coding	CCDS79165	A0A0G2JEG8	TSL:1 GENCODE basic APPRIS ALT2
Amph-206	ENSMUST00000239207.1	3760	820aa	Protein coding	-	-	GENCODE basic APPRIS ALT2
Amph-205	ENSMUST00000222698.1	3082	No protein	Processed transcript	-	-	TSL:1
Amph-202	ENSMUST00000197545.1	804	No protein	Processed transcript	-	-	TSL:3
Amph-203	ENSMUST00000200273.1	1756	No protein	Retained intron	-	-	TSL:1

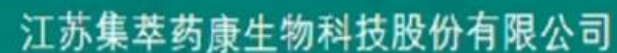
The strategy is based on the design of *Amph-204* transcript,the transcription is shown below:



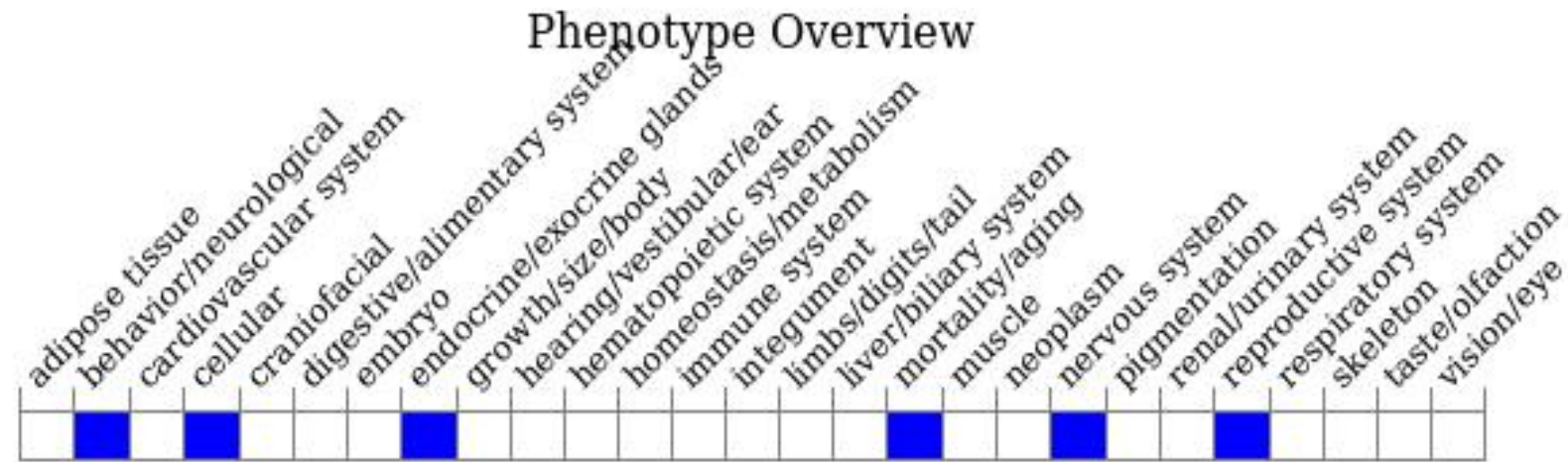
Genomic location distribution



集萃药康
GemPharmatech



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 mutation of this gene exhibit learning deficits and synaptic vesicle recycling defects, and die between 2 to 5 months of age from rare irreversible seizures.

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

Tel: 400-9660890

