En2 Cas9-KO *Strategy*

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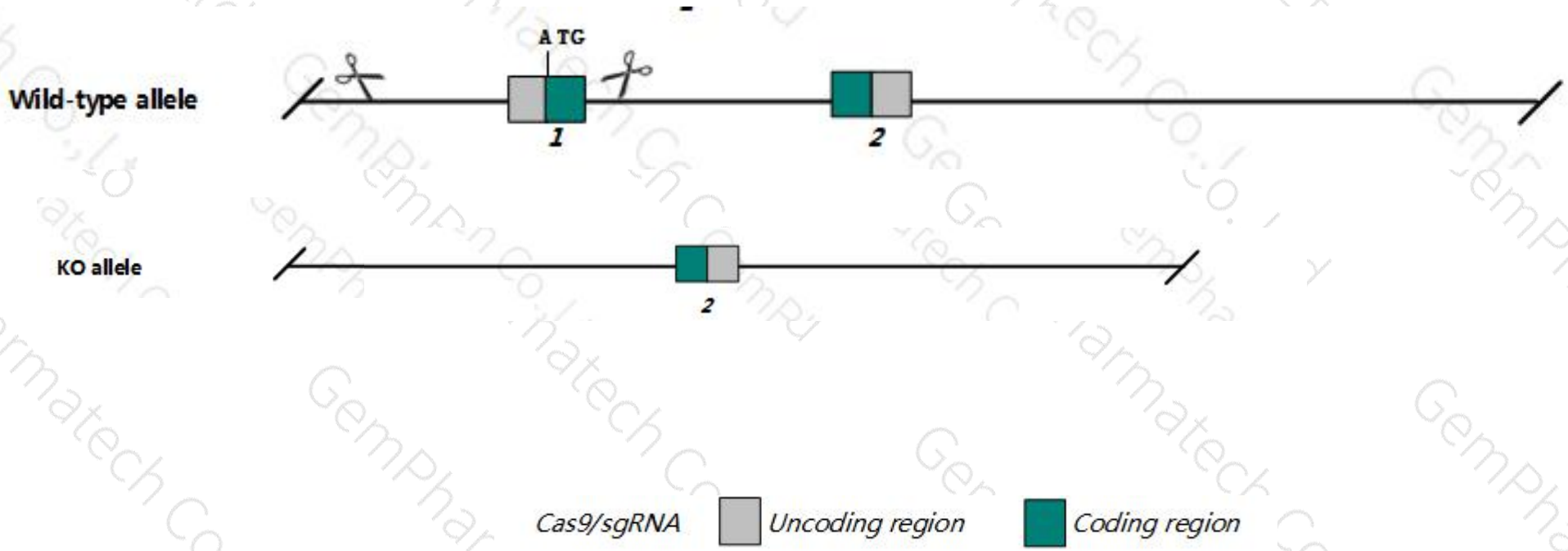
Project Overview

Project Name	***En2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *En2* gene. The schematic diagram is as follows:



Technical routes

- The *En2*gene has 1 transcript. According to the structure of *En2* gene, exon1 of *En2*-201 (ENSMUST00000036177.8) transcript is recommended as the knockout region.The region contains the predicted promoter sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *En2* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

Notice

- According to the existing MGI data: This locus affects anterior-posterior cerebellar patterning. Homozygous null mutants show altered foliation pattern and perform poorly in motor learning (rotarod) tests. Heterozygotes test intermediate on rotarod. Hypomorphs show no phenotypic effects.

- The *En2* 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）

集萃药康 GemPharmatech

En2 engrailed 2 [*Mus musculus* (house mouse)]

Gene ID: 13799, updated on 8-Dec-2018

Summary

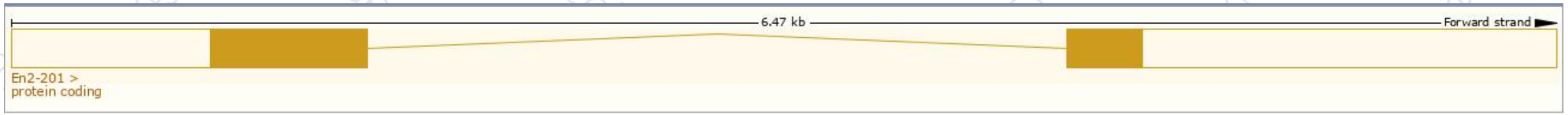
Official Symbol	En2 provided by MGI
Official Full Name	engrailed 2 provided by MGI
Primary source	MGI:MGI:95390
See related	Ensembl:ENSMUSG00000039095
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	En-2; mo-En-2; BB131122
Expression	Biased expression in cerebellum adult (RPKM 21.8), CNS E11.5 (RPKM 13.0) and 3 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

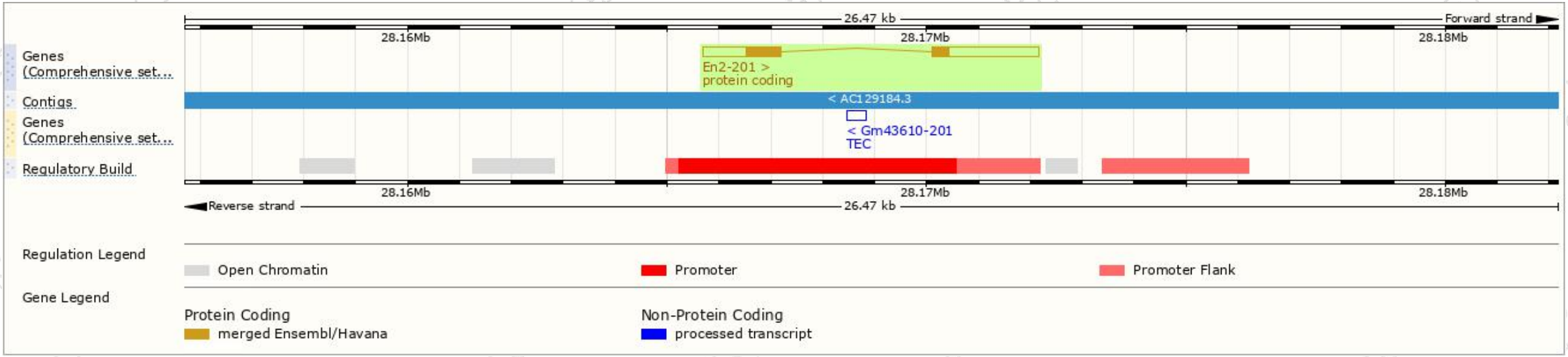
The gene has 1 transcript, and all transcripts are shown below:

Name ▲	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
En2-201	ENSMUST00000036177.8	3539	324aa	Protein coding	CCDS19143	P09066 Q3TZM2	TSL:1 GENCODE basic APPRIS P1

The strategy is based on the design of *En2* -201 transcript,The transcription is shown below

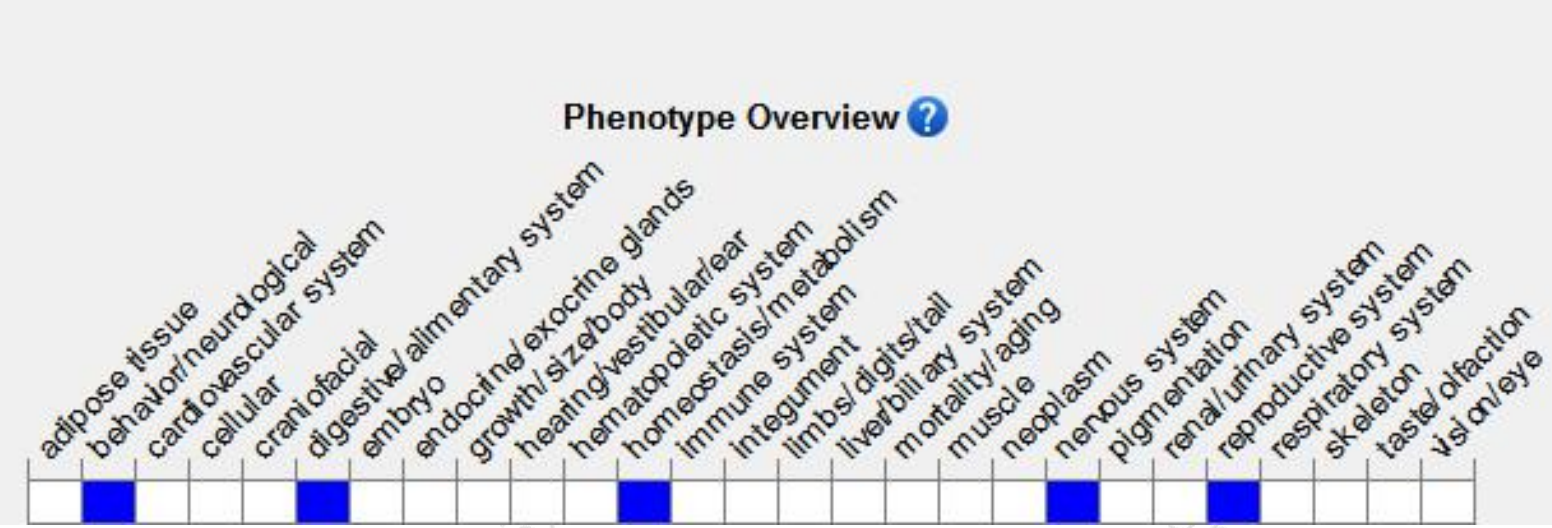


Genomic location distribution



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