

# ***En1 Cas9-CKO Strategy***

**Designer:**

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**Design Date:**

**2019-7-26**

# Project Overview

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**Project Name**

***En1***

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**Project type**

**Cas9-CKO**

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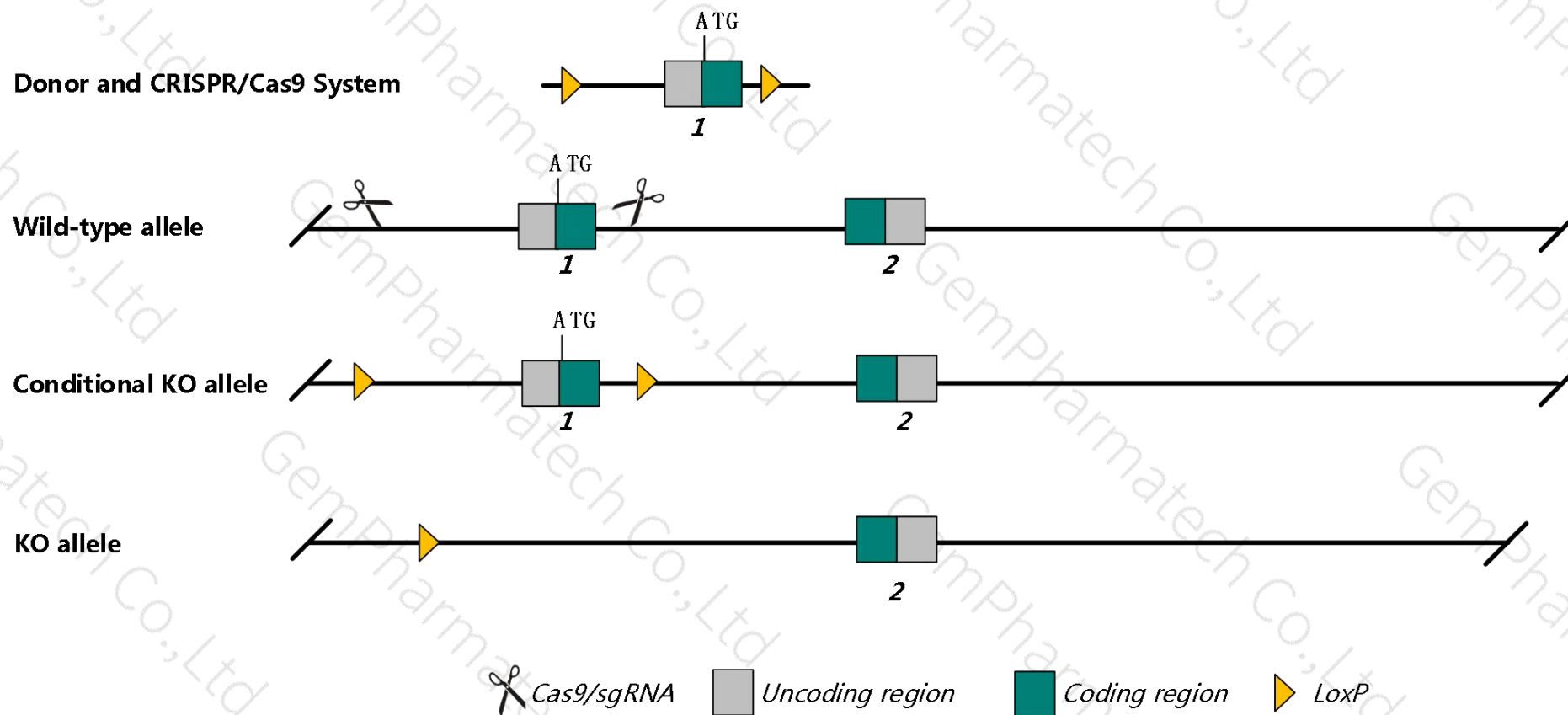
**Strain background**

**C57BL/6JGpt**

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# Conditional Knockout strategy

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



- The *En1* gene has 1 transcript. According to the structure of *En1* gene, the predicted promoter and exon1 of *En1*-201 (ENSMUST00000079721.8) transcript is recommended as the knockout region. The region contains the promoter region sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *En1* 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 or cell types.

- According to the existing MGI data: Mutant homozygotes usually die within 24 hours of birth. Mutants exhibit nervous system defects, including a lack of most of the colliculi, cerebellum, and the third and fourth cranial nerves in some lines. Skeletal anomalies have also been described.
- The *En1* gene is located on the Chr1. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.



# Gene information ( NCBI )

## En1 engrailed 1 [ *Mus musculus* (house mouse) ]

Gene ID: 13798, updated on 11-Dec-2018

### Summary



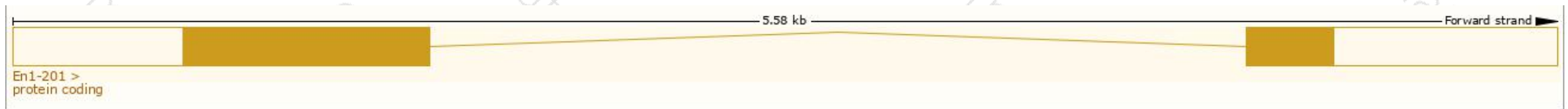
Official Symbol	En1 provided by <a href="#">MGI</a>
Official Full Name	engrailed 1 provided by <a href="#">MGI</a>
Primary source	<a href="#">MGI:MGI:95389</a>
See related	<a href="#">Ensembl:ENSMUSG000000058665</a>
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<a href="#">Mus musculus</a>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	En-1; Mo-en.1; engrailed-1
Expression	Biased expression in CNS E11.5 (RPKM 5.5), whole brain E14.5 (RPKM 3.8) and 5 other tissues <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>

# Transcript information ( Ensembl )

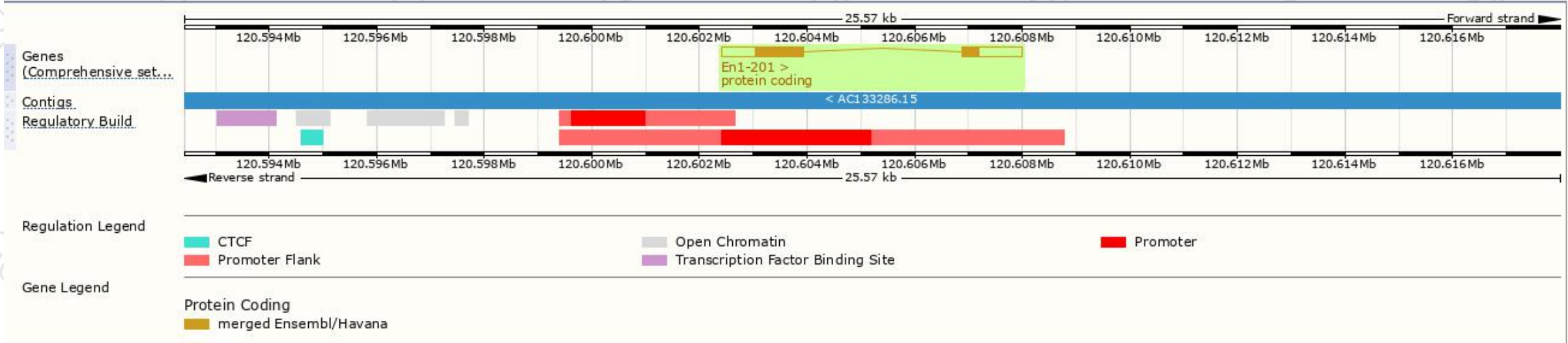
The gene has 1 transcript, and all transcripts are shown below:

Name ▲	Transcript ID ▲	bp ▲	Protein ▲	Biotype ▲	CCDS ▲	UniProt ▲	Flags
En1-201	<a href="#">ENSMUST00000079721.8</a>	2624	<a href="#">401aa</a>	Protein coding	<a href="#">CCDS15235</a>	<a href="#">P09065</a>	TSL:1 Gencode basic APPRIS P1

The strategy is based on the design of *En1* -201 transcript, The transcription is shown below

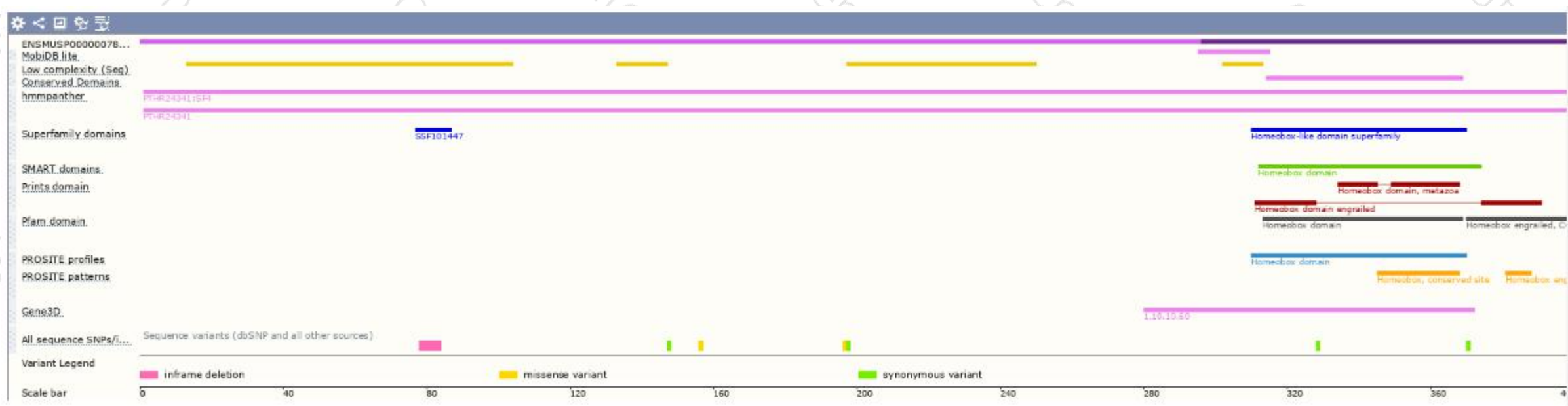


# Genomic location distribution

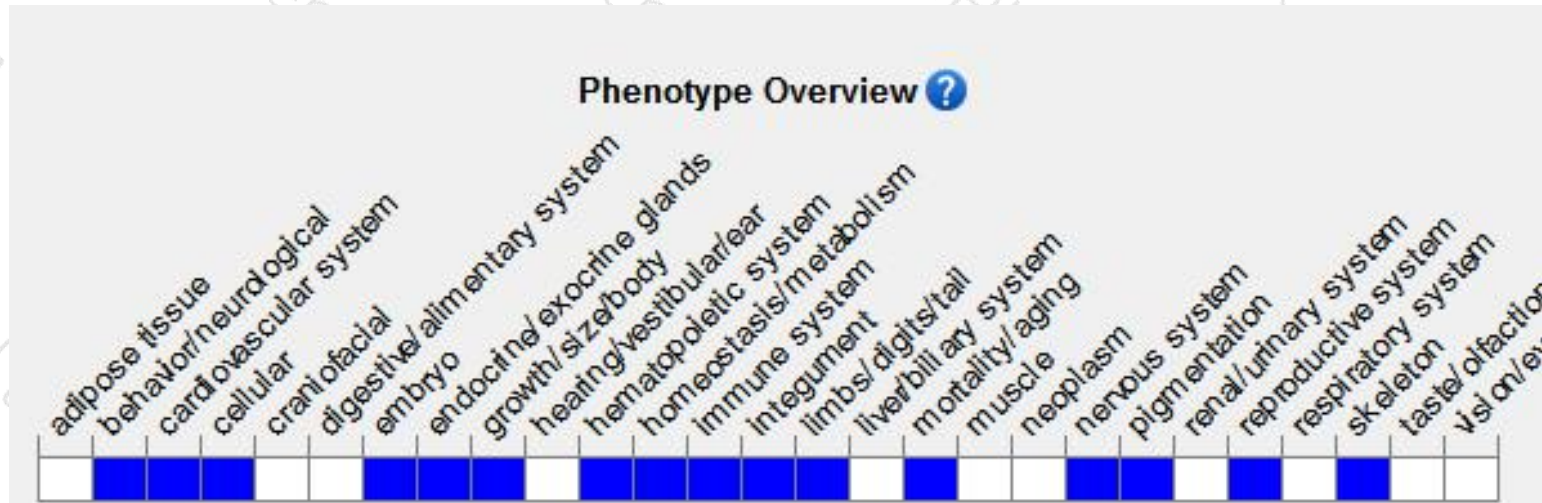




# Protein domain



# Mouse phenotype description(MGI)



*Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/>) .*

Mutant homozygotes usually die within 24 hours of birth. Mutants exhibit nervous system defects, including a lack of most of the colliculi, cerebellum, and the third and fourth cranial nerves in some lines. Skeletal anomalies have also been described.

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