Negr1 Cas9-CKO Strategy

Designer: Huimin Su

Reviewer: Ruirui Zhang

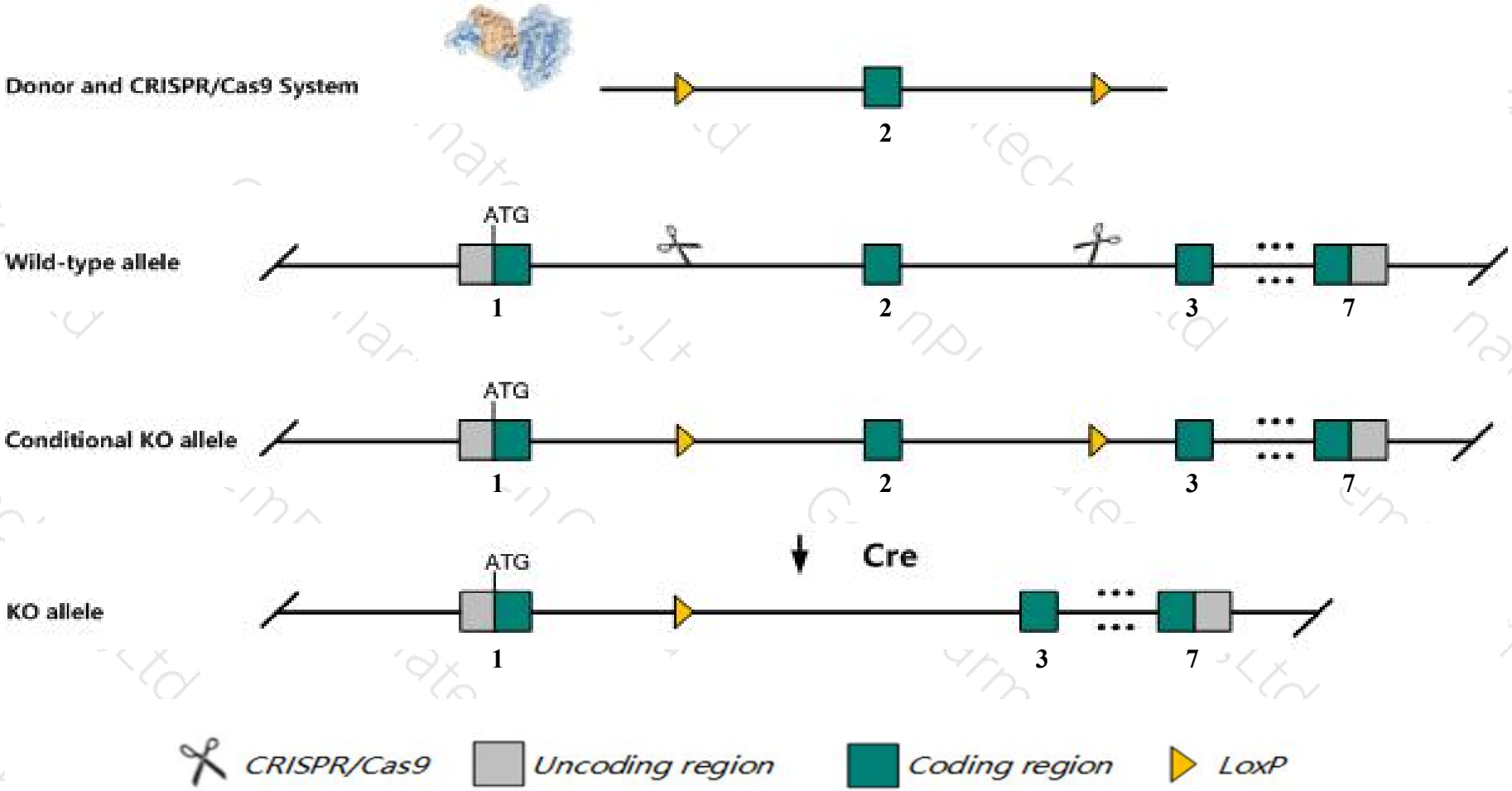
Design Date: 2020-2-20

Project Overview

Project Name	***Negr1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Negr1* gene. The schematic diagram is as follows:



Technical routes

集萃药康 GemPharmatech

- The *Negr1* gene has 5 transcripts. According to the structure of *Negr1* gene, exon2 of *Negr1-202* (ENSMUST00000074015.10) transcript is recommended as the knockout region.The region contains 233bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Negr1* 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,Mice homozygous for a knock-out or ENU-induced allele exhibit reduced body weight.

➢ The KO region deletes exon2 of *Negr1-204* transcript, but does not result in frameshift.

➢ The *Negr1* gene is located on the Chr3. 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）

集萃药康 GemPharmatech

Negr1 neuronal growth regulator 1 [*Mus musculus* (house mouse)]

Gene ID: 320840, updated on 12-Nov-2019

Summary

Official Symbol	Negr1 provided by MGI
Official Full Name	neuronal growth regulator 1 provided by MGI
Primary source	MGI:MGI:2444846
See related	Ensembl:ENSMUSG00000040037
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	Ntra; KILON; 5330422G01Rik
Expression	Biased expression in CNS E18 (RPKM 8.0), frontal lobe adult (RPKM 7.4) and 9 other tissues See more
Orthologs	human all

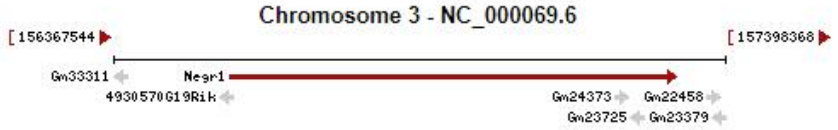
Genomic context

Location: 3; 3 H4

See Negr1 in Genome Data Viewer

Exon count: 11

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	3	NC_000069.6 (156561790..157316464)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	3	NC_000069.5 (156224758..156979411)

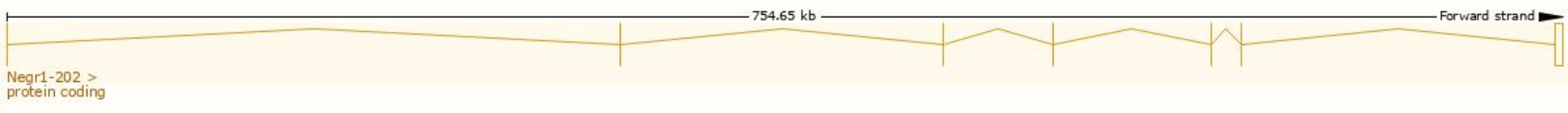


Transcript information（Ensembl）

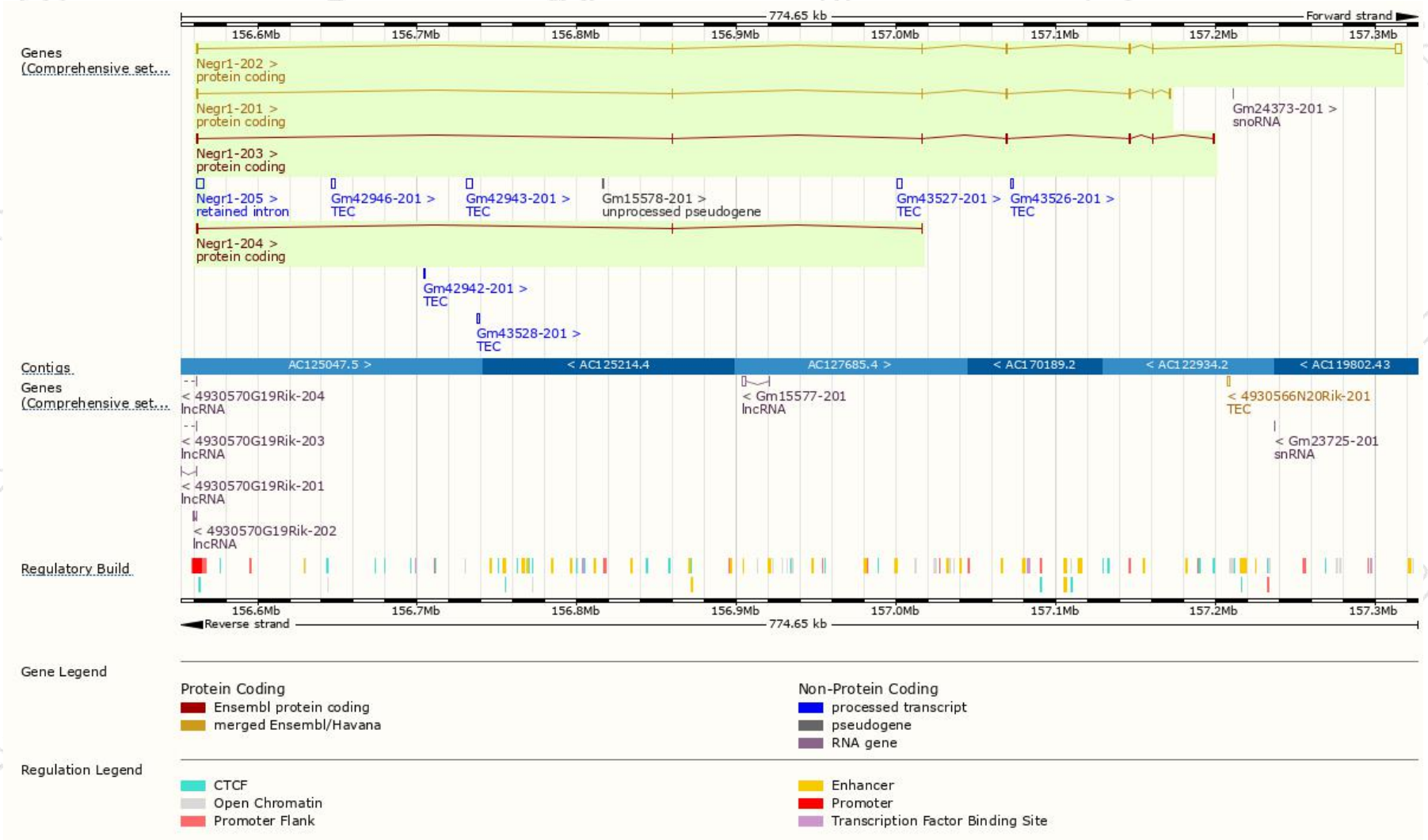
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Negr1-202	ENSMUST00000074015.10	4983	348aa	Protein coding	CCDS17930	Q80Z24	TSL:1 GENCODE basic APPRIS P1
Negr1-201	ENSMUST00000041425.11	2095	329aa	Protein coding	CCDS17931	A0A4W9	TSL:1 GENCODE basic
Negr1-203	ENSMUST00000106065.1	1953	329aa	Protein coding	-	D3Z4T6	TSL:1 GENCODE basic
Negr1-204	ENSMUST00000175773.1	412	122aa	Protein coding	-	H3BKU7	CDS 3' incomplete TSL:3
Negr1-205	ENSMUST00000197246.1	3988	No protein	Retained intron	-	-	TSL:NA

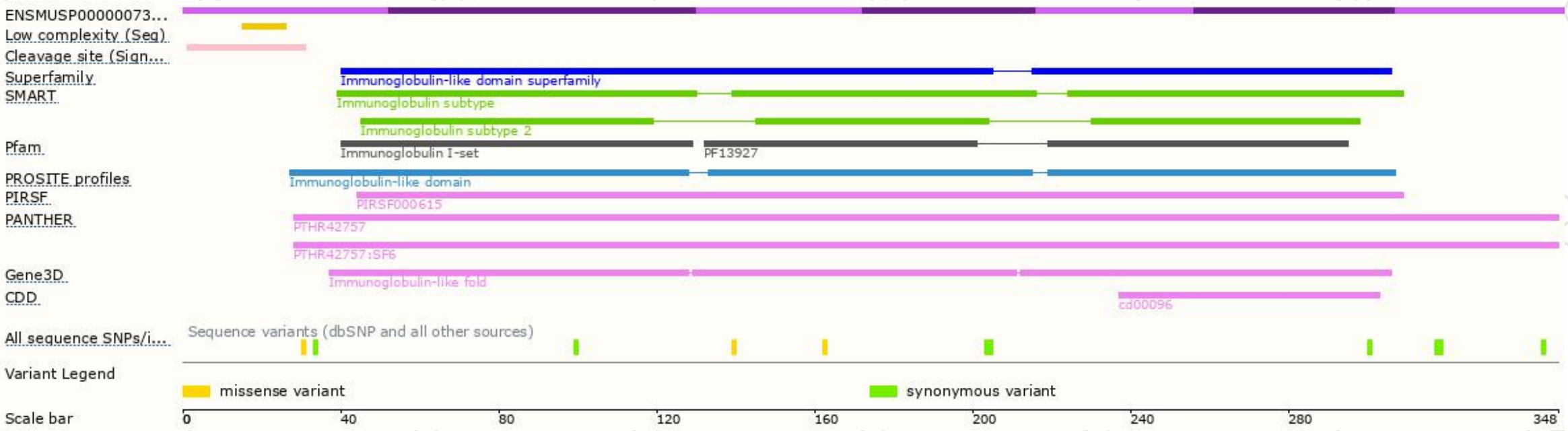
The strategy is based on the design of *Negr1-202* transcript,The transcription is shown below



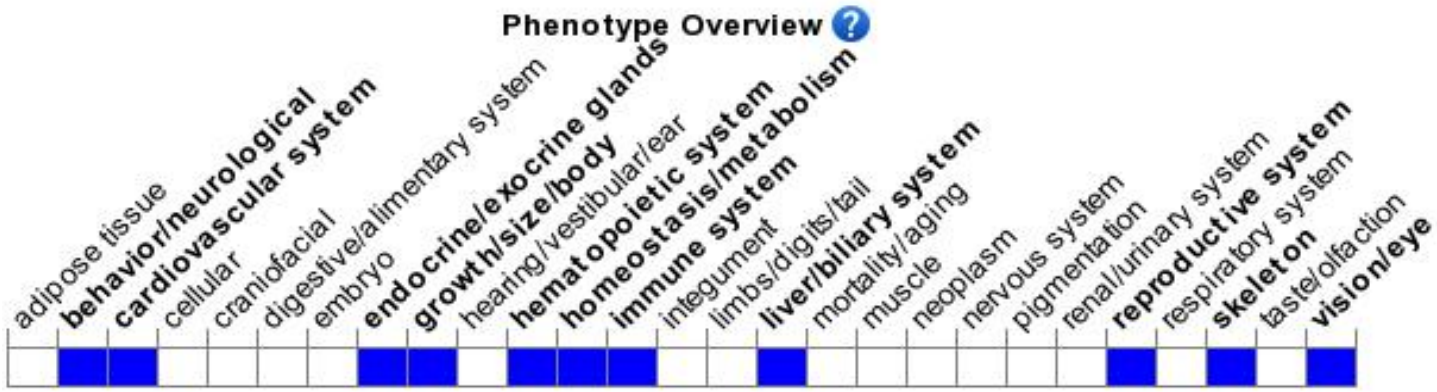
Genomic location distribution



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,Mice homozygous for a knock-out or ENU-induced allele exhibit reduced body weight.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd