Bhlhe40 Cas9-KO Strategy

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Reviewer: Huimin Su

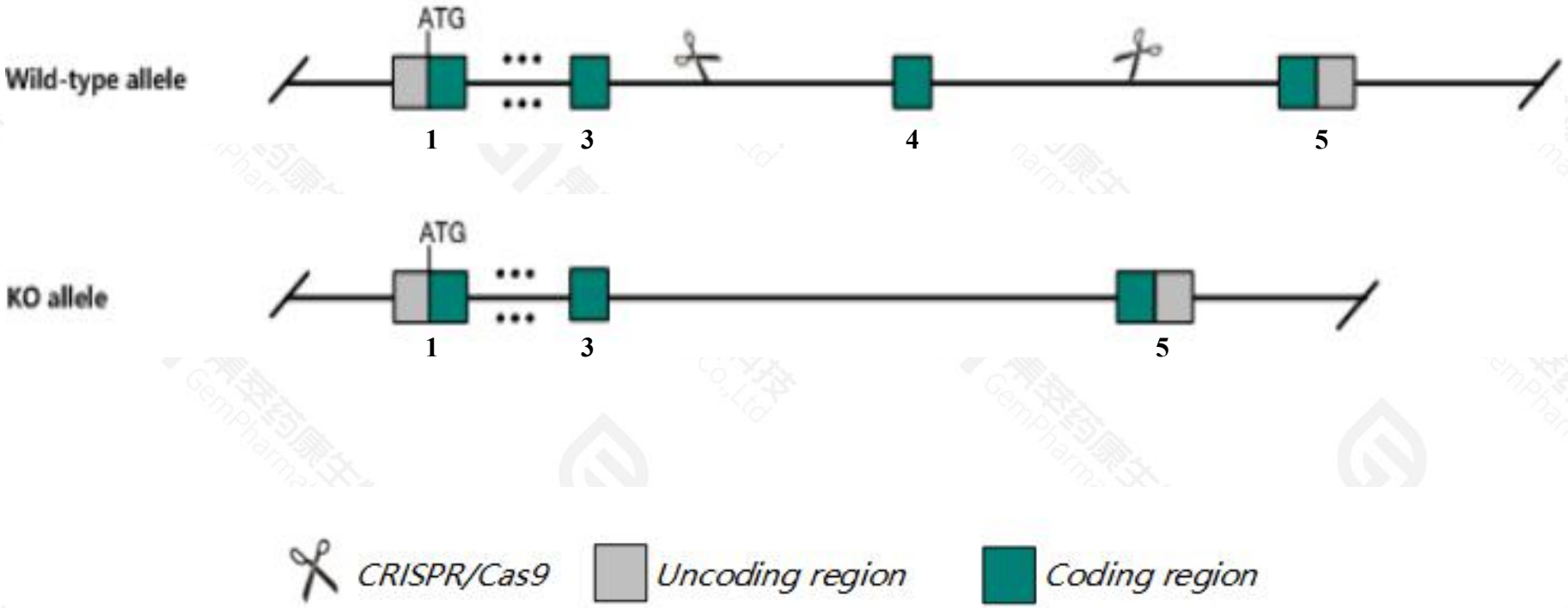
Design Date: 2019-9-26

Project Overview

Project Name	***Bhlhe40***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Bhlhe40* gene. The schematic diagram is as follows:



Technical routes

➢ The *Bhlhe40* gene has 4 transcripts. According to the structure of *Bhlhe40* gene, exon4 of *Bhlhe40-201*(ENSMUST00000032194.11) transcript is recommended as the knockout region.The region contains 124bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Bhlhe40* 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.

Notice

➢ According to the existing MGI data,homozygous mutation of this gene results in impaired immune function and hyperplasia of the lymphoid organs. Aging mutant animals exhibit autoimmune disease.

➢ This strategy may affect the 5-terminal regulation of the *0610040F04Rik* gene.

➢ The *Bhlhe40* gene is located on the Chr6. 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）

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Bhlhe40 basic helix-loop-helix family, member e40 [Mus musculus (house mouse)]

Gene ID: 20893, updated on 13-Mar-2020

Summary

Official Symbol	Bhlhe40 provided by MGI
Official Full Name	basic helix-loop-helix family, member e40 provided by MGI
Primary source	MGI:MGI:1097714
See related	Ensembl:ENSMUSG00000030103
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	Bhlhb2, C130042M06Rik, CR8, Clast5, Dec1, Sharp2, Stra13, Stra14
Summary	This gene encodes a basic helix-loop-helix protein expressed in various tissues. The encoded protein can interact with Arntl or compete for E-box binding sites in the promoter of Per1 and repress Clock/Arntl's transactivation of Per1. This gene is believed to be involved in the control of circadian rhythm and cell differentiation. [provided by RefSeq, Feb 2014]
Expression	Ubiquitous expression in adrenal adult (RPKM 15.2), kidney adult (RPKM 15.1) and 27 other tissues See more
Orthologs	human all

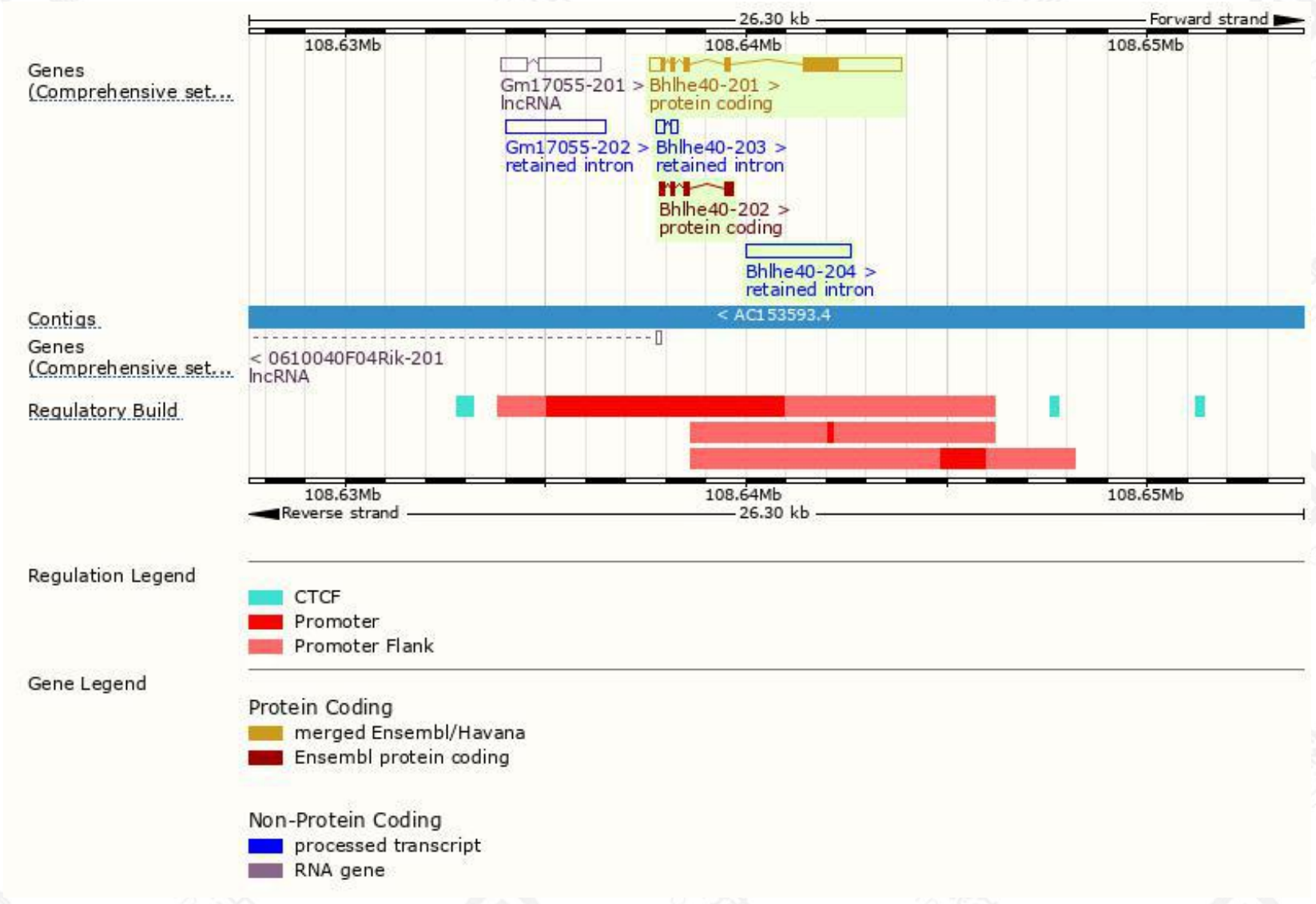
Transcript information（Ensembl）

The gene has 4 transcripts,all transcripts are shown below:

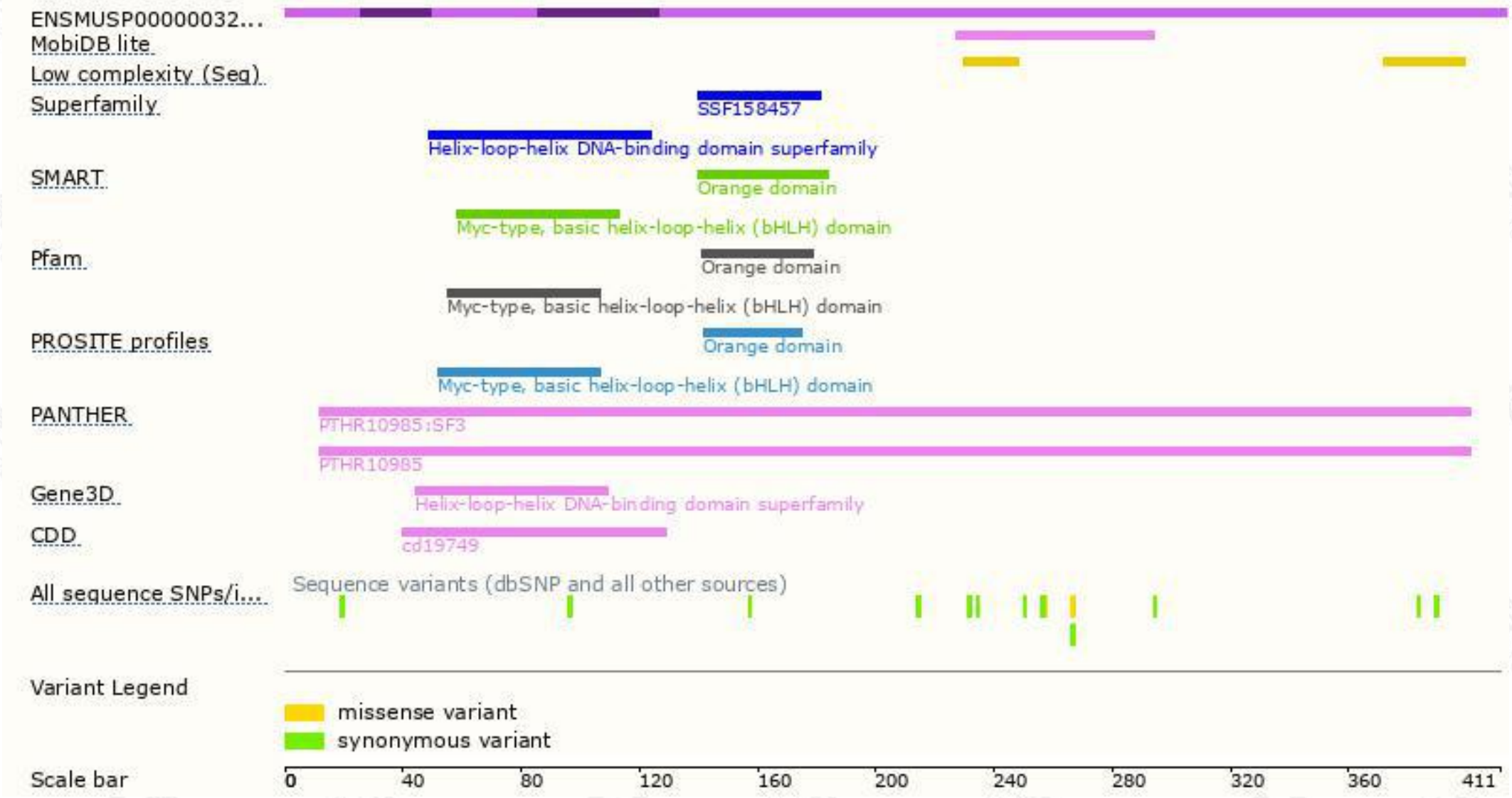
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Bhlhe40-201	ENSMUST00000032194.10	3113	411aa	Protein coding	CCDS20400	O35185 Q542A5	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Bhlhe40-202	ENSMUST00000163617.1	476	132aa	Protein coding	-	E9Q2C3	TSL:2 GENCODE basic
Bhlhe40-204	ENSMUST00000204550.1	2620	No protein	Retained intron	-	-	TSL:NA
Bhlhe40-203	ENSMUST00000166346.1	368	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Bhlhe40-201* transcript,the transcription is shown below:

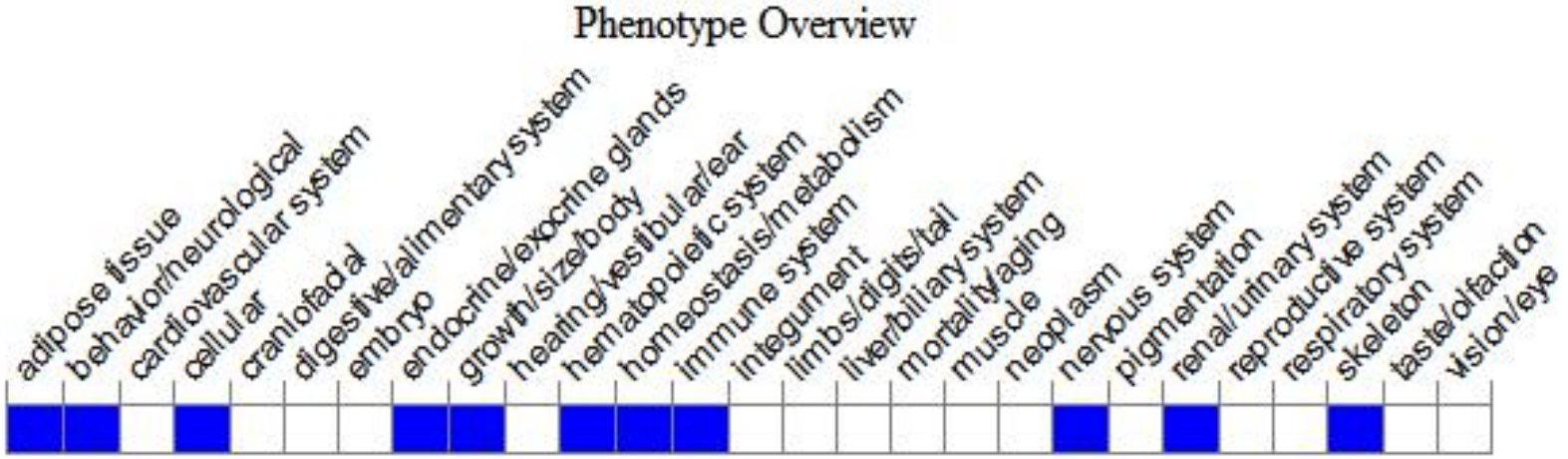
Genomic location distribution



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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