Nhlh2 Cas9-KO Strategy

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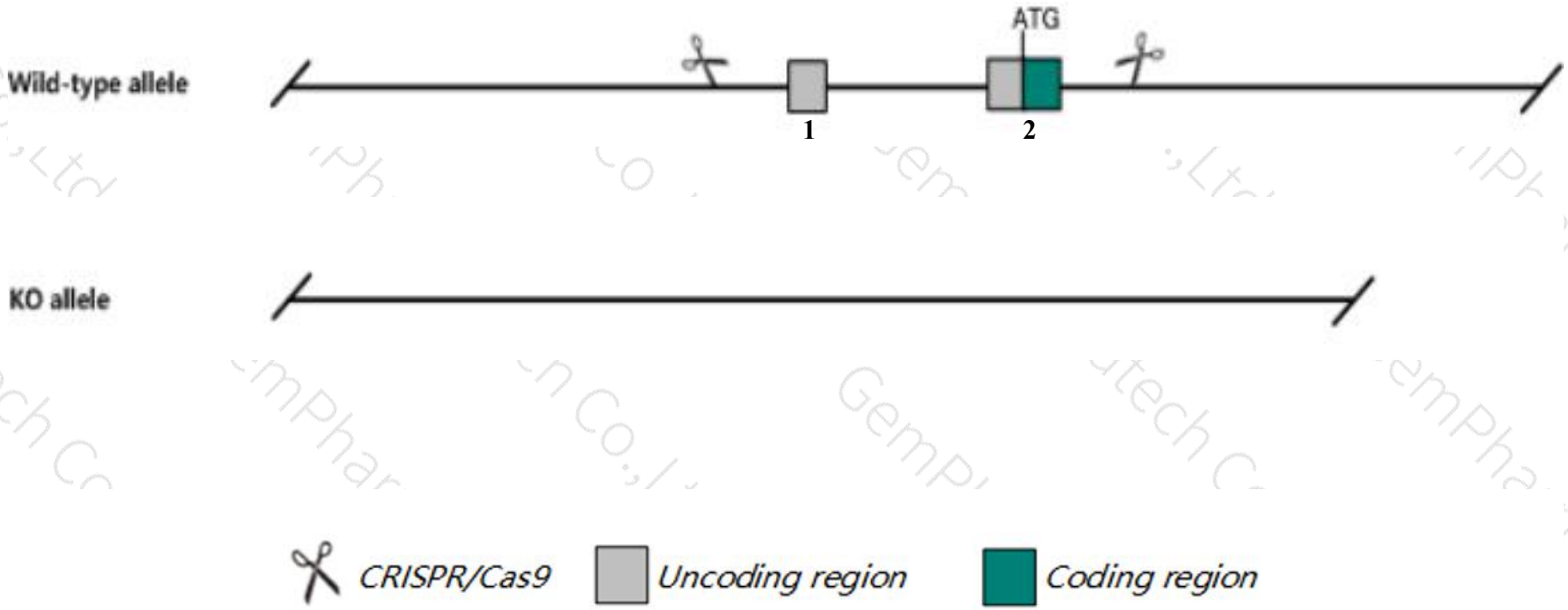
Project Overview

Project Name	***Nhlh2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nhlh2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Nhlh2* gene has 3 transcripts. According to the structure of *Nhlh2* gene, exon1-exon2 of *Nhlh2-203*(ENSMUST00000198675.1) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Nhlh2* 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,loss of function results in adult-onset obesity and reproductive defects, including hypogonadism, due to disruption of the hypothalamic-pituitary axis. Mutant male mice are sterile, whereas female mice show variable fertility dependent on the presence orabsence of male mice.

➢ The *Nhlh2* 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 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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Nhlh2 nescient helix loop helix 2 [*Mus musculus* (house mouse)]

Gene ID: 18072, updated on 26-Jun-2020

Summary

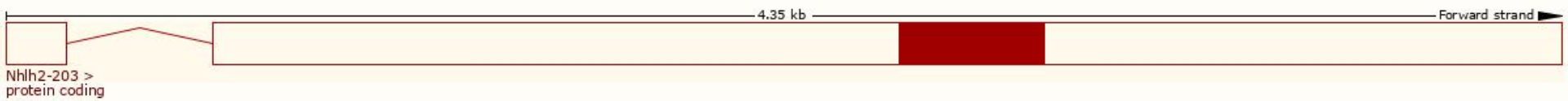
Official Symbol	Nhlh2 provided by MGI
Official Full Name	nescient helix loop helix 2 provided by MGI
Primary source	MGI:MGI:97324
See related	Ensembl:ENSMUSG00000048540
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Hen2; NSCL2; Nscl-2; bHLHa34; 6230401I09Rik
Expression	Biased expression in CNS E11.5 (RPKM 15.9), whole brain E14.5 (RPKM 8.5) and 3 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

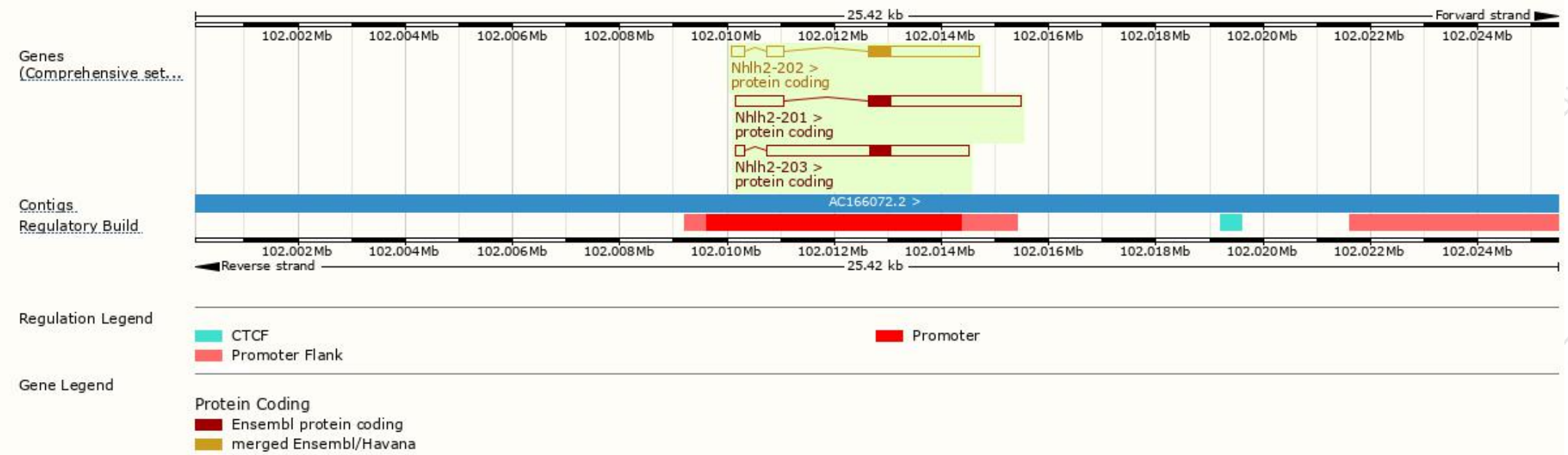
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein ▲	Biotype	CCDS	UniProt	Flags
Nhlh2-203	ENSMUST00000198675.1	3943	135aa	Protein coding	CCDS17685	Q64221	TSL:2 GENCODE basic APPRIS P1
Nhlh2-201	ENSMUST00000066187.5	3761	135aa	Protein coding	CCDS17685	Q64221	TSL:1 GENCODE basic APPRIS P1
Nhlh2-202	ENSMUST00000196324.1	2647	135aa	Protein coding	CCDS17685	Q64221	TSL:1 GENCODE basic APPRIS P1

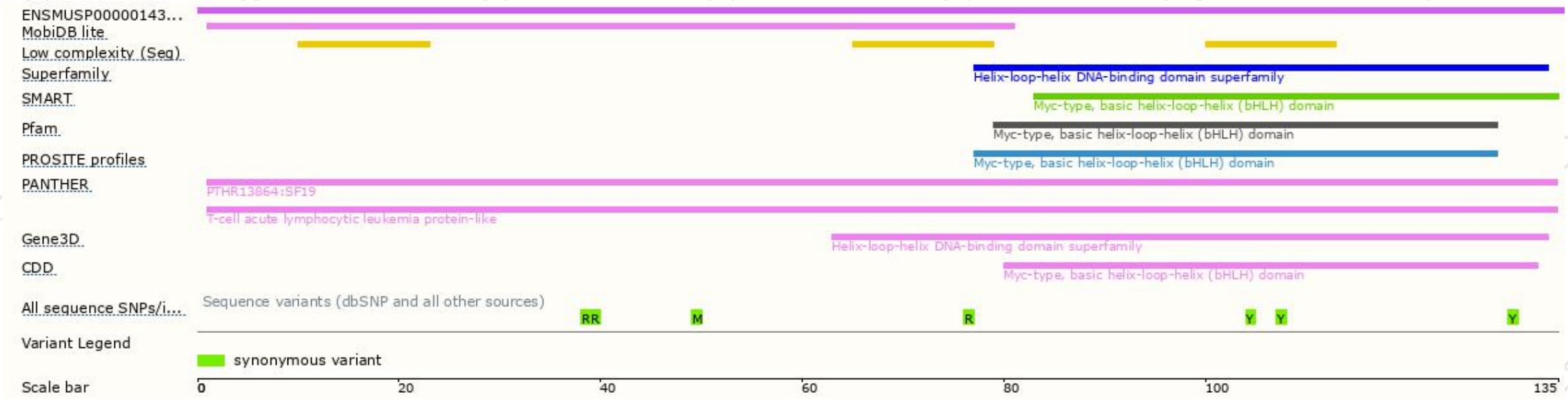
The strategy is based on the design of *Nhlh2-203* transcript,the transcription is shown below:



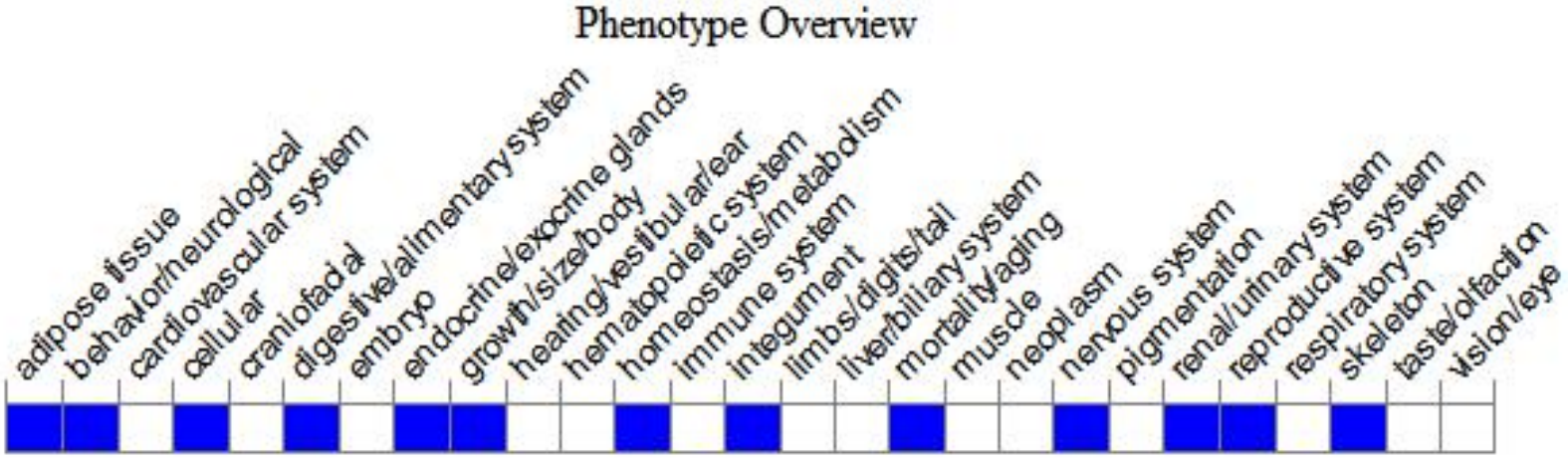
Genomic location distribution



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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