Lhx8 Cas9-KO Strategy

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Reviewer: Daohua Xu

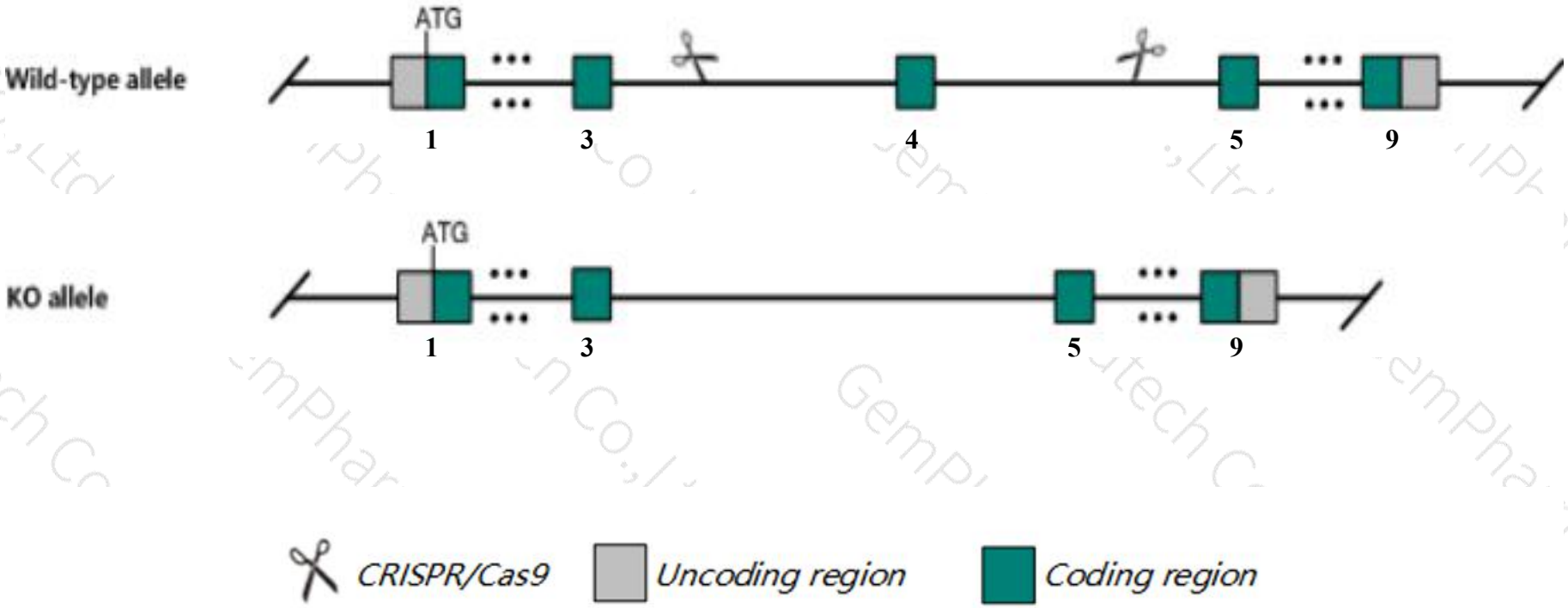
Design Date: 2020-7-20

Project Overview

Project Name	***Lhx8***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Lhx8* gene. The schematic diagram is as follows:



Technical routes

➢ The *Lhx8* gene has 5 transcripts. According to the structure of *Lhx8* gene, exon4 of *Lhx8-201*(ENSMUST00000177846.7) transcript is recommended as the knockout region.The region contains 122bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Lhx8* 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 null mice exhibit partial penetrance of a cleft secondary palate and neonatal lethality; those without cleft palate survive to adulthood. All homozygous null mice have decreased or absent forebrain cholinergic neurons.

➢ The *Lhx8* 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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Lhx8 LIM homeobox protein 8 [Mus musculus (house mouse)]

Gene ID: 16875, updated on 13-Mar-2020

Summary

Official Symbol	Lhx8 provided by MGI
Official Full Name	LIM homeobox protein 8 provided byMGI
Primary source	MGI:MGI:1096343
See related	Ensembl:ENSMUSG00000096225
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	L3, Lhx7
Expression	Biased expression in whole brain E14.5 (RPKM 4.0), CNS E14 (RPKM 3.2) and 6 other tissuesSee more
Orthologs	human all

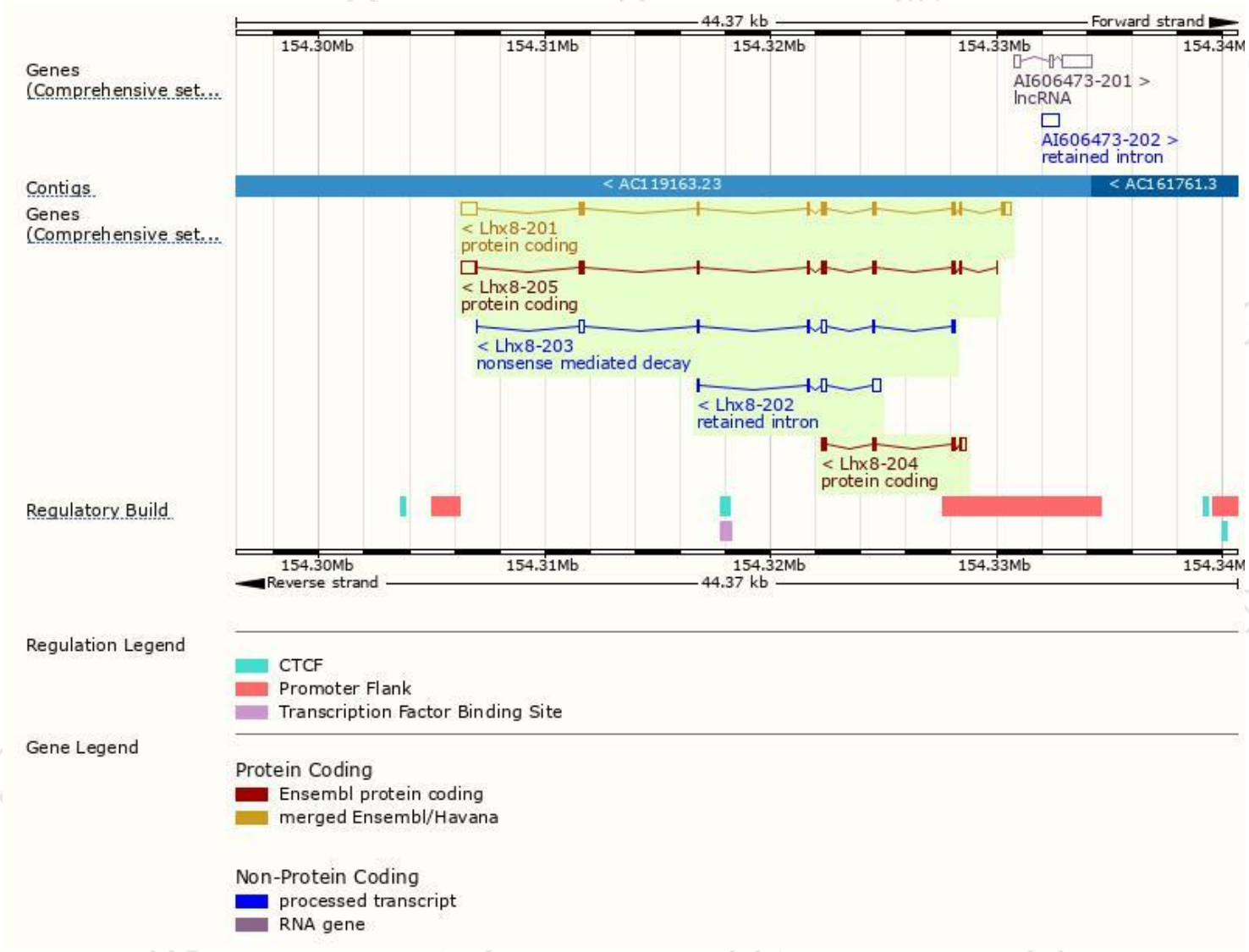
Transcript information（Ensembl）

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

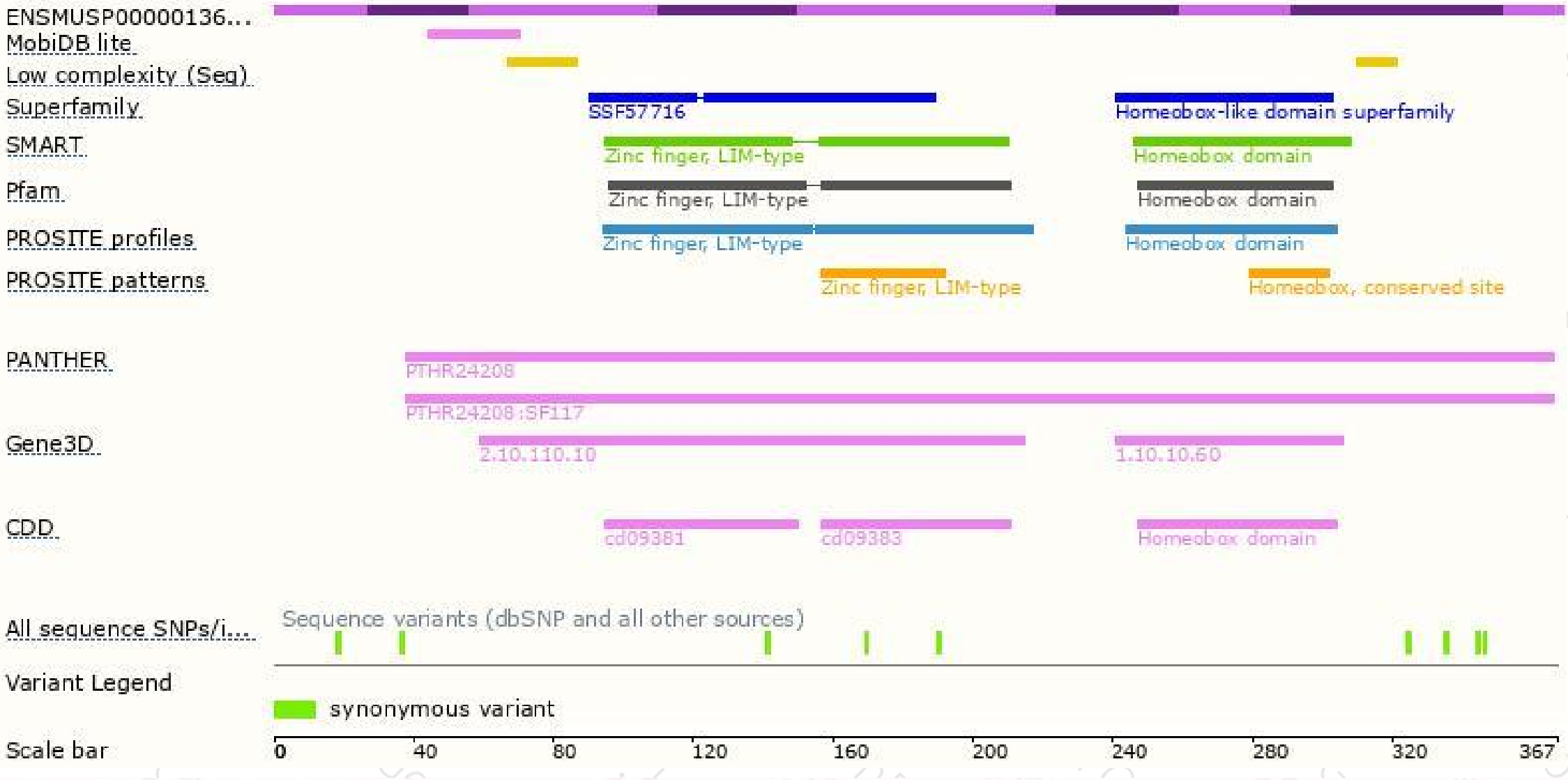
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lhx8-201	ENSMUST00000177846.7	2076	367aa	Protein coding	CCDS17926	O35652	TSL:1 GENCODE basic
Lhx8-205	ENSMUST00000205251.2	1729	346aa	Protein coding	-	H3BJ54	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Lhx8-204	ENSMUST00000204403.1	769	188aa	Protein coding	-	A0A0N4SWH1	CDS 3' incomplete TSL:2
Lhx8-203	ENSMUST00000204171.2	876	68aa	Nonsense mediated decay	-	A0A0N4SUJ5	CDS 5' incomplete TSL:3
Lhx8-202	ENSMUST00000203692.1	763	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Lhx8-201* transcript,the transcription is shown below:

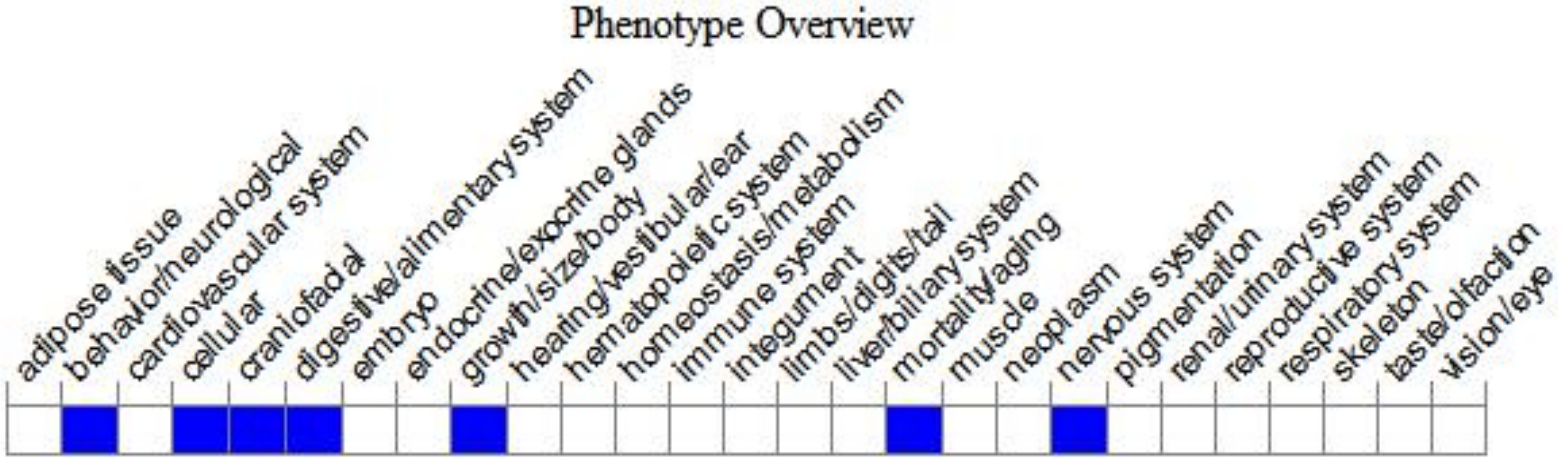
Genomic location distribution



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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