Drosha Cas9-KO Strategy

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Project Overview

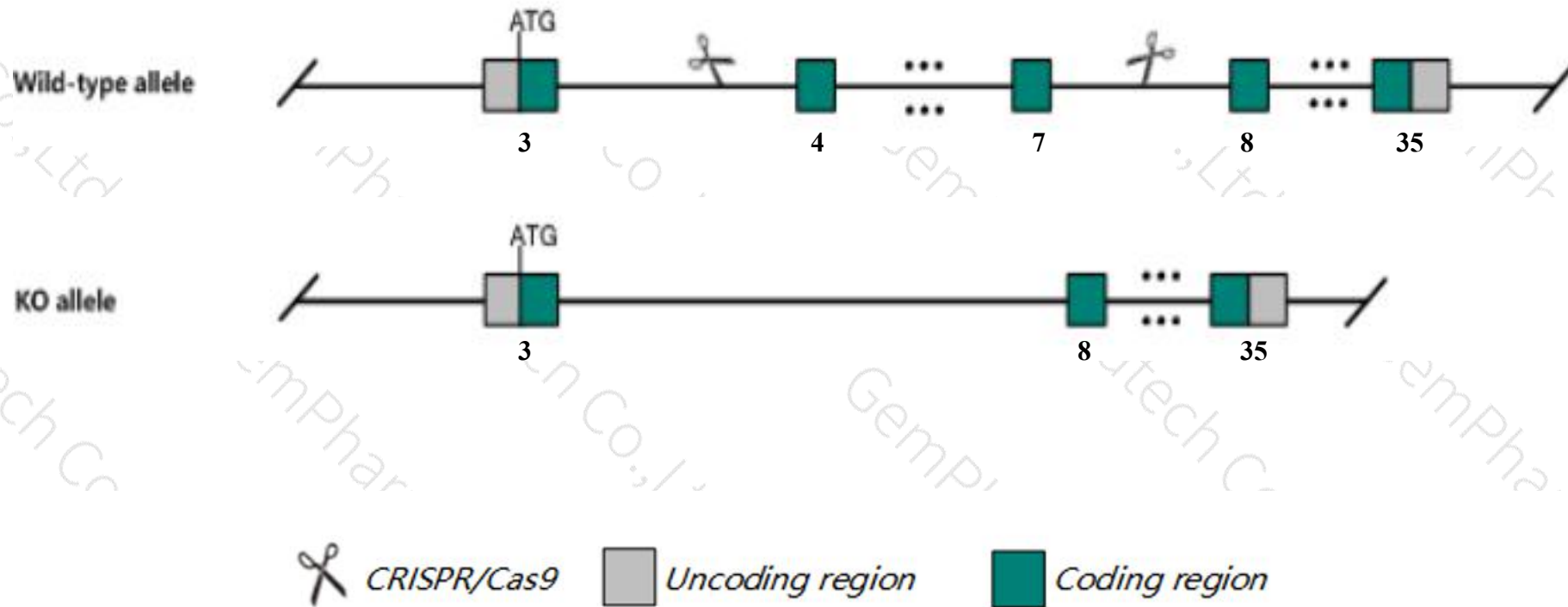|	
Project Name	*Drosha*
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

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

Technical routes

➢ The *Drosha* gene has 7 transcripts. According to the structure of *Drosha* gene, exon4-exon7 of *Drosha-207*(ENSMUST00000169061.7) transcript is recommended as the knockout region.The region contains 1270bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Drosha* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

➢ According to the existing MGI data,mice heterozygous for a knock-out allele and a conditional allele activated in the immune system exhibit increased inflammation in multiple systems, cachexia and premature death.

➢ Gene *Gm47288* will be deleted .

➢ The *Drosha* gene is located on the Chr15. 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）

Drosha drosha, ribonuclease type III [Mus musculus (house mouse)]

Gene ID: 14000, updated on 13-Mar-2020

▲ Summary

|	
Official Symbol	Drosha provided by MGI
Official Full Name	drosha, ribonuclease type III provided by MGI
Primary source	MGI:MGI:1261425
See related	Ensembl:ENSMUSG00000022191
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	1110013A17Rik, AI874853, Etohi2, Rn3, Rnasen
Expression	Ubiquitous expression in CNS E11.5 (RPKM 18.2), CNS E14 (RPKM 14.7) and 27 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Drosha-207	ENSMUST00000169061.7	4570	1373aa	Protein coding	CCDS49583	Q5HZJ0	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
Drosha-201	ENSMUST00000090292.12	4519	1373aa	Protein coding	CCDS49583	Q5HZJ0	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
Drosha-203	ENSMUST00000141706.7	881	No protein	Processed transcript	-	-	TSL:3
Drosha-204	ENSMUST00000147647.7	881	No protein	Processed transcript	-	-	TSL:5
Drosha-206	ENSMUST00000156594.1	699	No protein	Processed transcript	-	-	TSL:5
Drosha-202	ENSMUST00000141010.1	588	No protein	Processed transcript	-	-	TSL:3
Drosha-205	ENSMUST00000150211.7	3248	No protein	Retained intron	-	-	TSL:5

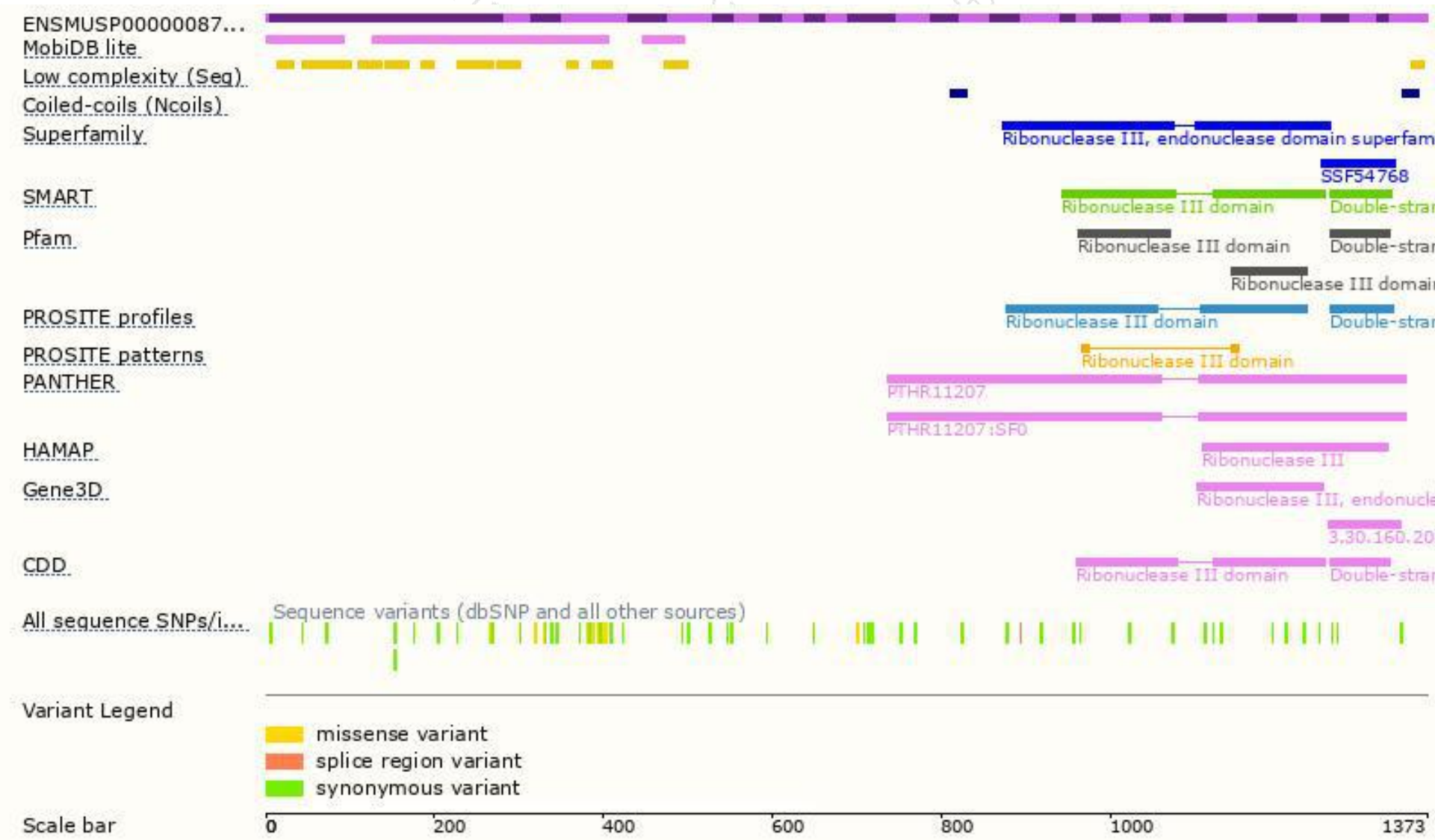The strategy is based on the design of *Drosha-207* transcript,the transcription is shown below:
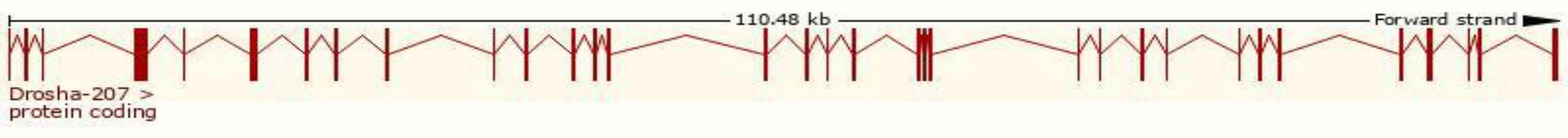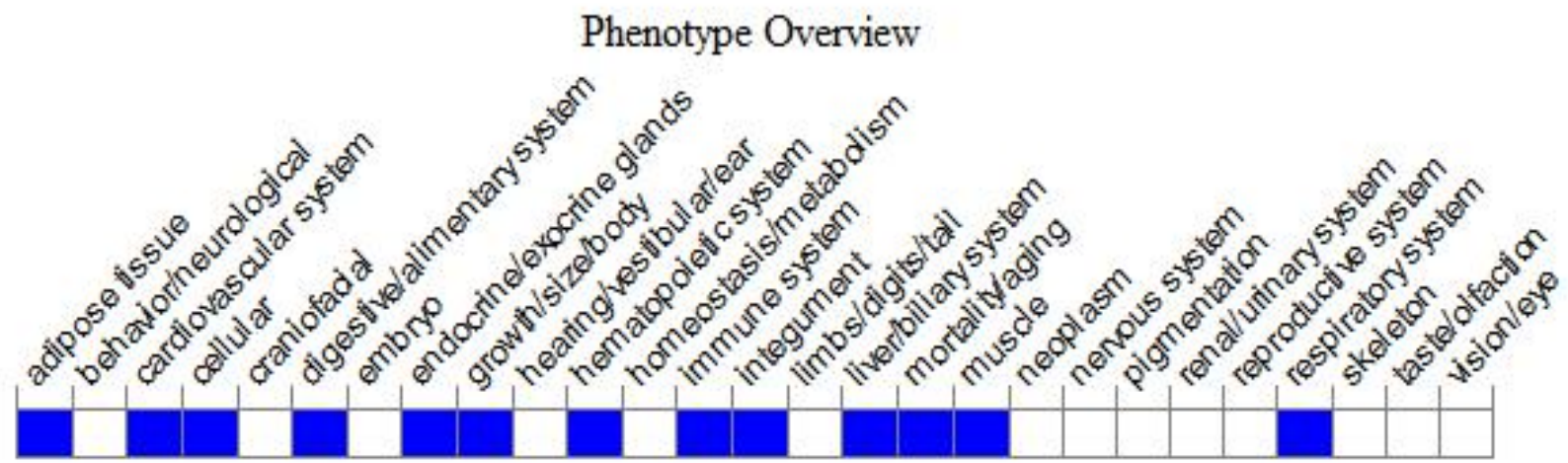
Genomic location distribution
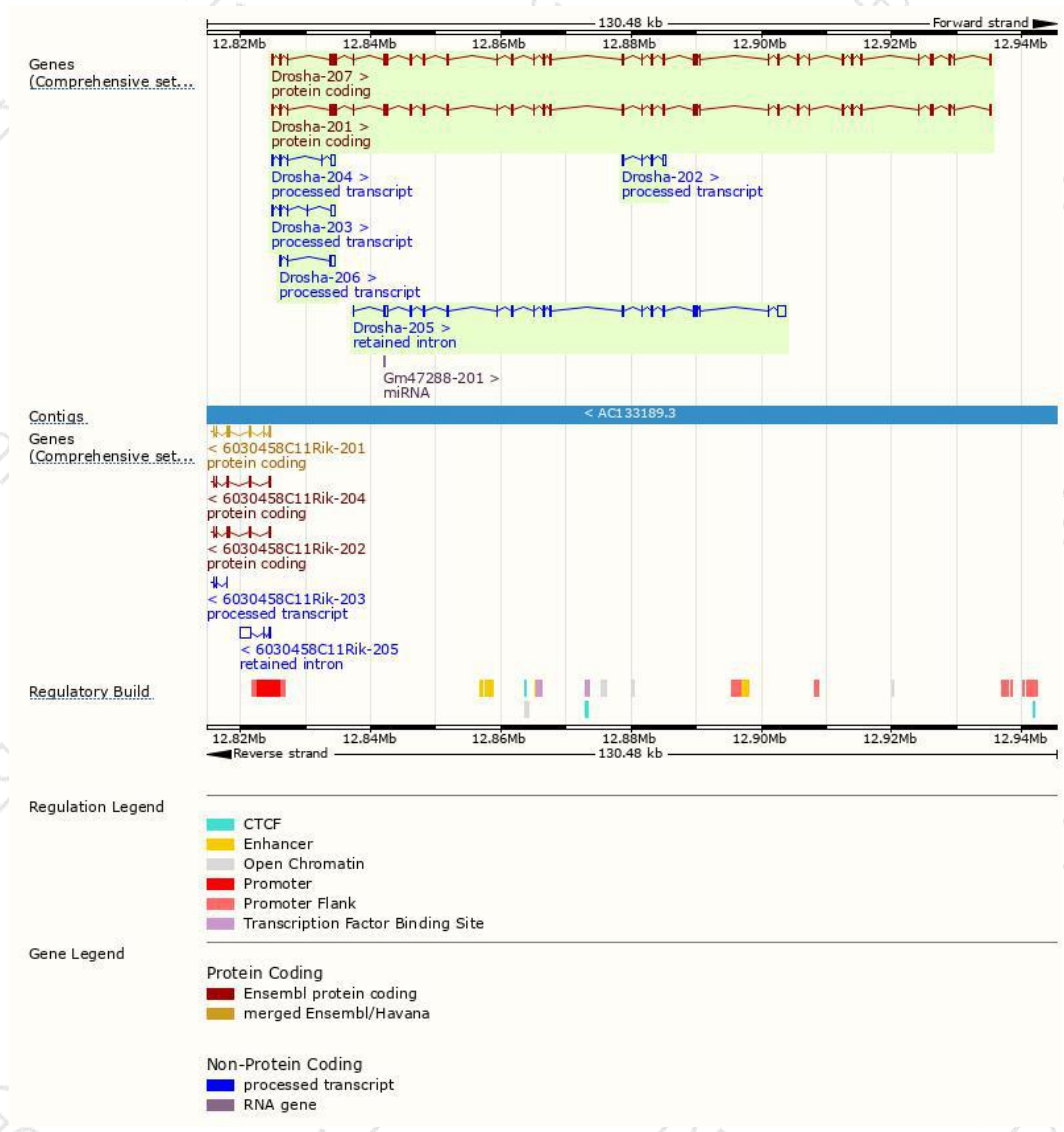
Protein domain

Mouse phenotype description(MGI)

Phenotype Overview

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 heterozygous for a knock-out allele and a conditional allele activated in the immune system exhibit increased inflammation in multiple systems, cachexia and premature death.

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