

Runx3 Cas9-KO Strategy

Designer: Xueting Zhang

Reviewer: Ruirui Zhang

Design Date: 2022-12-12

Overview

Target Gene Name

- Runx3

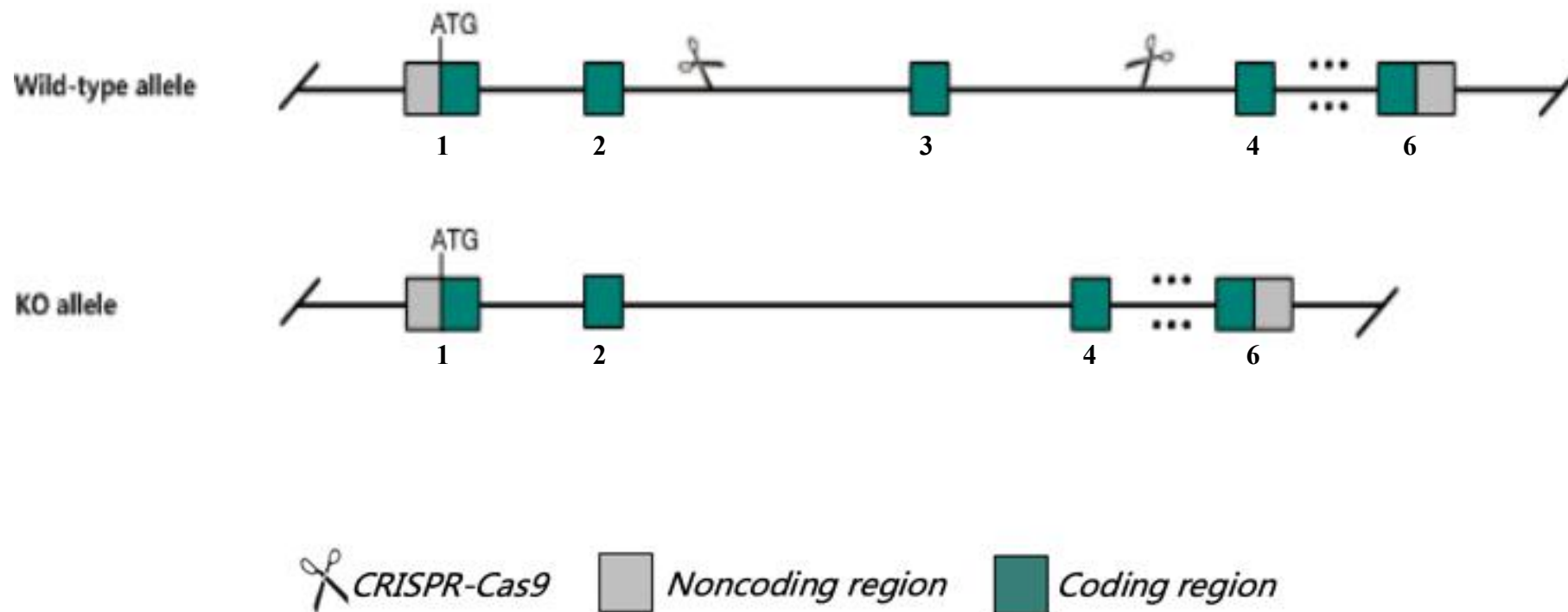
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Runx3* gene.

Technical Information

- The *Runx3* gene has 6 transcripts. According to the structure of *Runx3* gene, exon3 of *Runx3-201*(ENSMUST00000056977.14) transcript is recommended as the knockout region. The region contains 157bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Runx3* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.

Gene Information

Runx3 runt related transcription factor 3 [Mus musculus (house mouse)]

Gene ID: 12399, updated on 13-Mar-2020

Summary	
Official Symbol	Runx3 provided by MGI
Official Full Name	runt related transcription factor 3 provided by MGI
Primary source	MGI:MGI:102672
See related	Ensembl:ENSMUSG00000070691
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	AML2, Cbfa3, Pebp2a3, Rx3
Expression	Biased expression in spleen adult (RPKM 11.1), thymus adult (RPKM 6.0) and 11 other tissues See more
Orthologs	human all

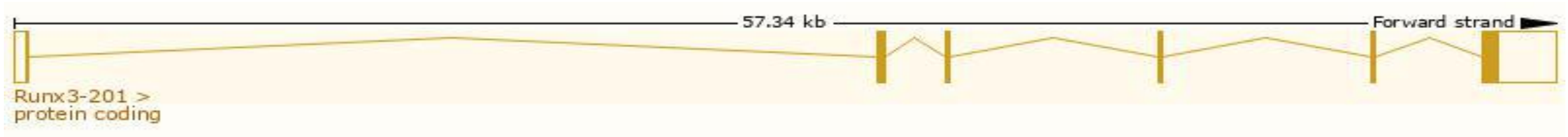
Source: <https://www.ncbi.nlm.nih.gov/>

Transcript Information

The gene has 6 transcripts, all transcripts are shown below:

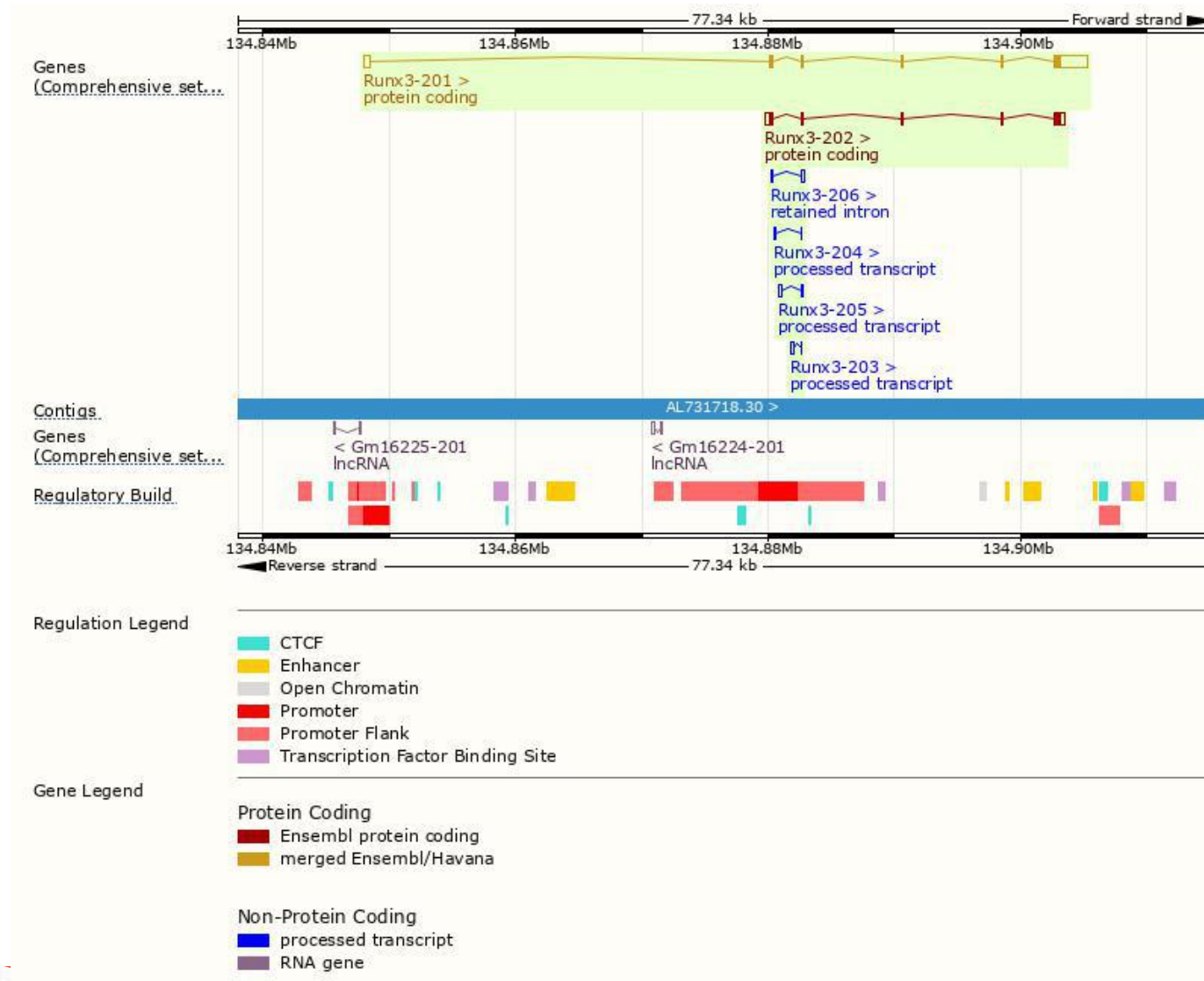
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Runx3-201	ENSMUST00000056977.13	3884	423aa	Protein coding	CCDS18782	Q3U1Q3	TSL:1 GENCODE basic APPRIS P2
Runx3-202	ENSMUST00000119564.1	1855	409aa	Protein coding	-	Q64131	TSL:1 GENCODE basic APPRIS ALT2
Runx3-205	ENSMUST00000140642.1	372	No protein	Processed transcript	-	-	TSL:5
Runx3-203	ENSMUST00000127109.1	353	No protein	Processed transcript	-	-	TSL:3
Runx3-204	ENSMUST00000137027.1	227	No protein	Processed transcript	-	-	TSL:3
Runx3-206	ENSMUST00000156478.1	447	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Runx3-201* transcript, the transcription is shown below:

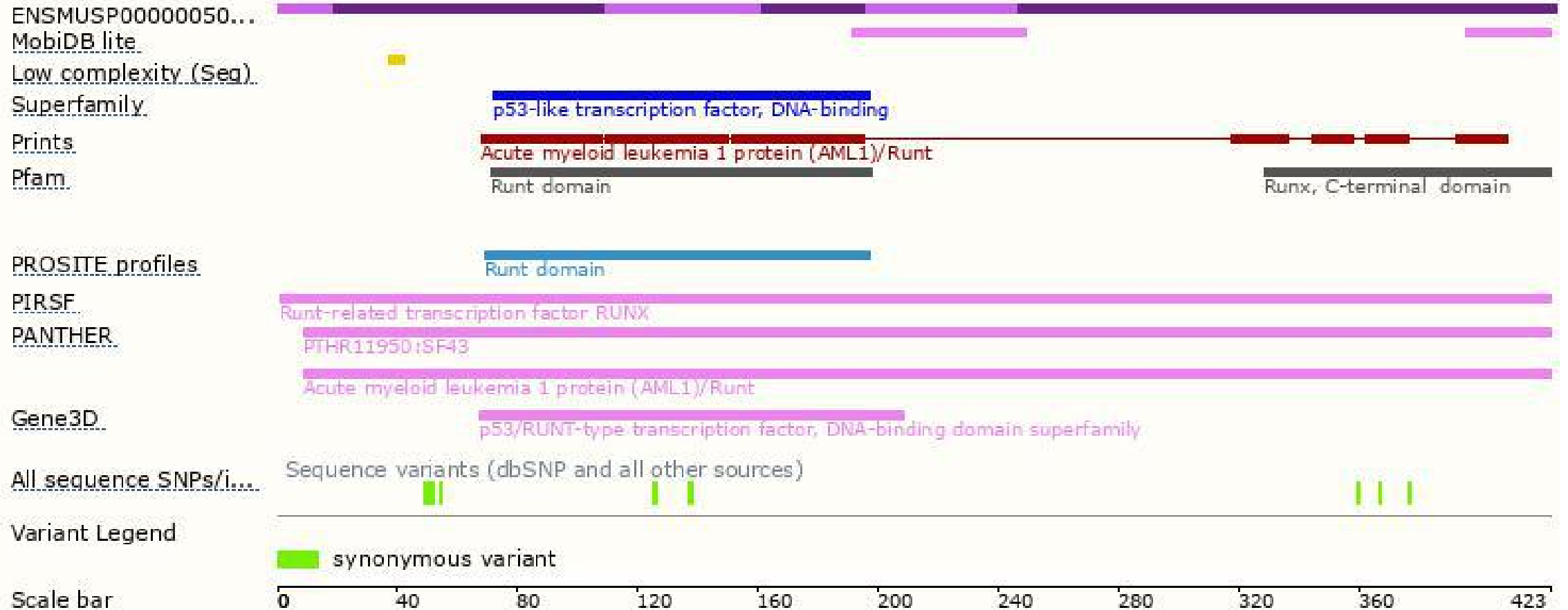


Source: <https://www.ensembl.org>

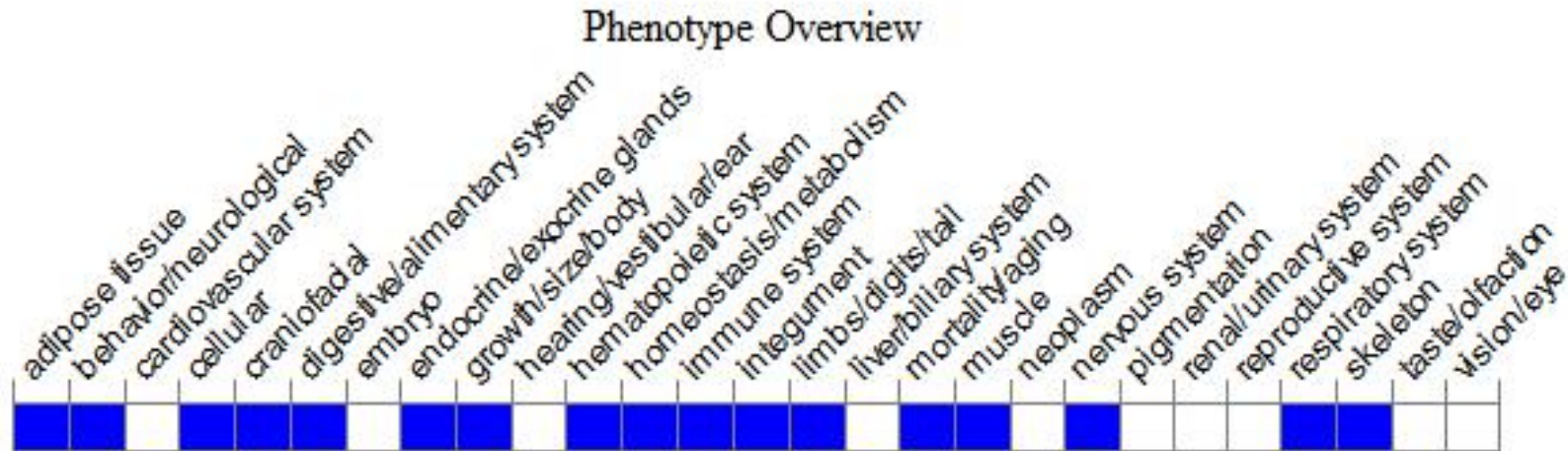
Genomic Information



Protein Information



Mouse Phenotype Information (MGI)



- Nullizygous mutations can lead to variable phenotypes, including postnatal lethality, ataxia, skeletal and behavioral defects, altered differentiation and function of T cells and dendritic cells, gastric hyperplasia, intestinal and lung inflammation, hair shape changes, and absent Langerhans cells.

Important Information

- According to the existing MGI data, nullizygous mutations can lead to variable phenotypes, including postnatal lethality, ataxia, skeletal and behavioral defects, altered differentiation and function of T cells and dendritic cells, gastric hyperplasia, intestinal and lung inflammation, hair shape changes, and absent Langerhans cells.
- According to the breeding data, the gene knockout homozygous mice died at the embryonic stage.
- *Runx3* is located on Chr4. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.