

Rlf Cas9-CKO Strategy

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Design Date: 2020-8-10

Project Overview

Project Name

Rlf

Project type

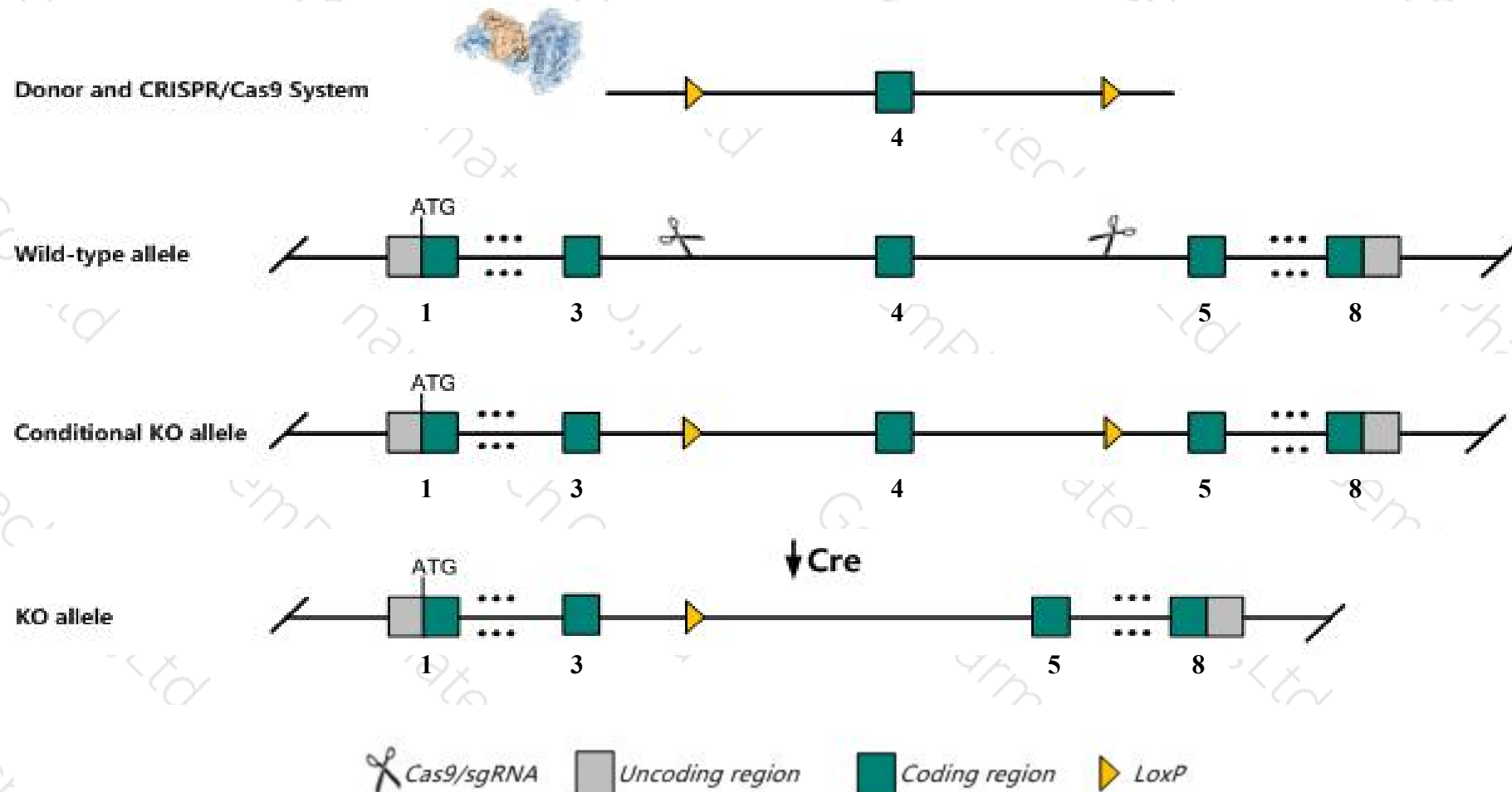
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Rlf* gene has 4 transcripts. According to the structure of *Rlf* gene, exon4 of *Rlf-201*(ENSMUST00000056635.12) transcript is recommended as the knockout region. The region contains 133bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rlf* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA and Donor 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.
- The flox mice was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

- According to the existing MGI data, mice homozygous for a hypomorphic ENU-induced allele exhibit postnatal lethality. Only a few mice survive to weaning age exhibiting a decreased body size.
- Transcript *Rlf*-204 may not be affected.
- The *Rlf* gene is located on the Chr4. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)

Rlf rearranged L-myc fusion sequence [Mus musculus (house mouse)]

Gene ID: 109263, updated on 13-Mar-2020

Summary



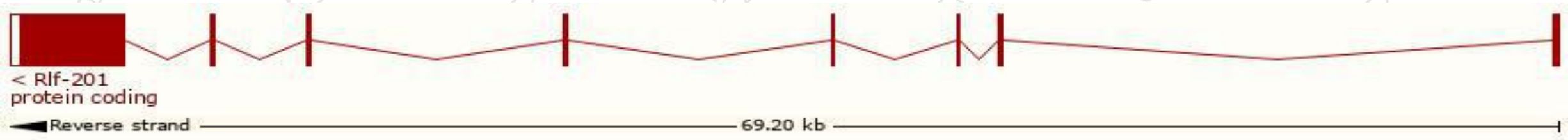
Official Symbol	Rlf provided by MGI
Official Full Name	rearranged L-myc fusion sequence provided by MGI
Primary source	MGI:MGI:1924705
See related	Ensembl:ENSMUSG00000049878
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	9230110M18Rik, AI195322, MommeD8
Expression	Ubiquitous expression in CNS E11.5 (RPKM 5.5), CNS E18 (RPKM 5.5) and 25 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

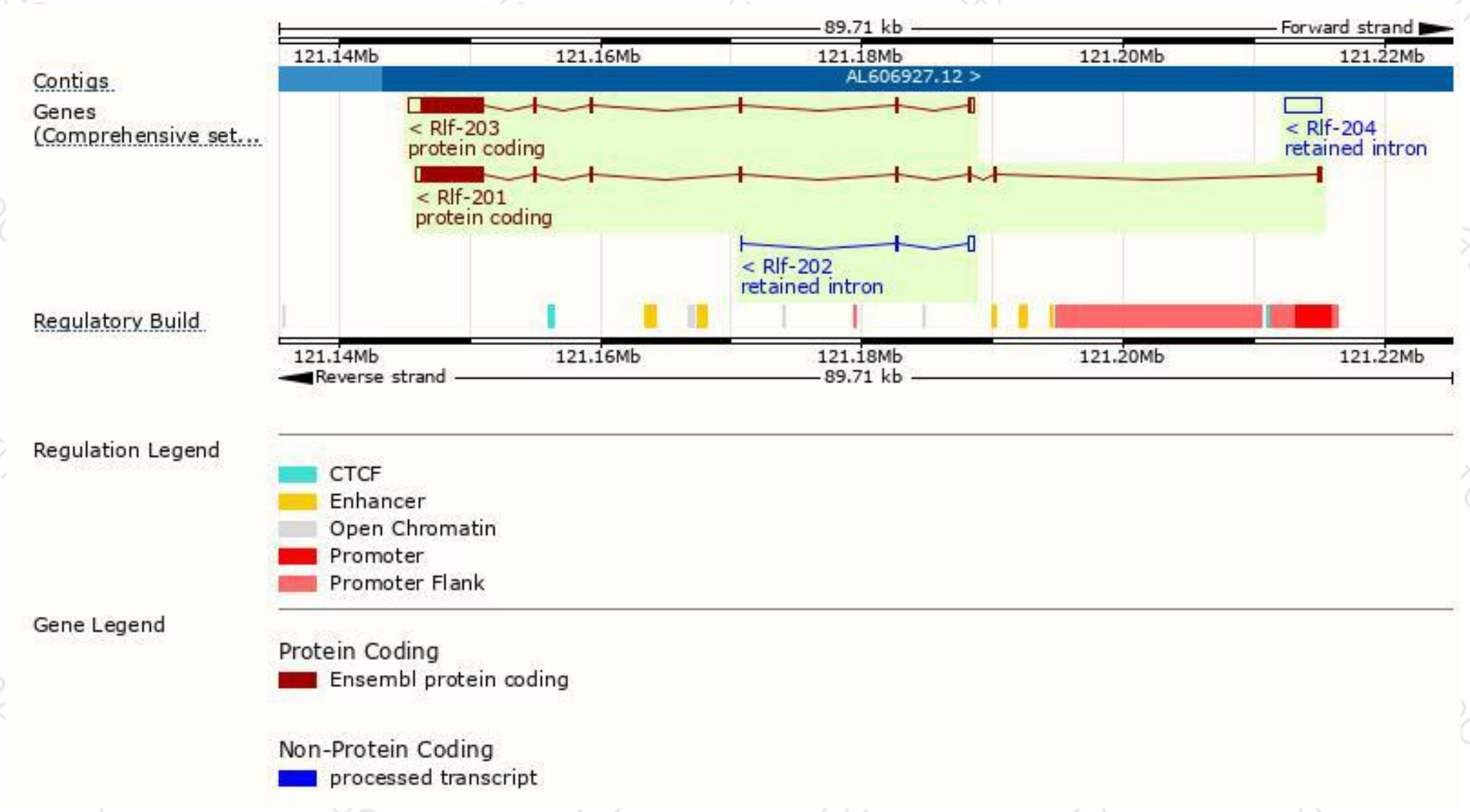
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rlf-201	ENSMUST00000056635.12	6242	1918aa	Protein coding	CCDS84789	A2A7F4	TSL:5 GENCODE basic APPRIS P2
Rlf-203	ENSMUST00000168615.7	6585	1808aa	Protein coding	-	E9Q532	TSL:5 GENCODE basic APPRIS ALT2
Rlf-204	ENSMUST00000222814.1	2752	No protein	Retained intron	-	-	TSL:NA
Rlf-202	ENSMUST00000142647.1	497	No protein	Retained intron	-	-	TSL:3

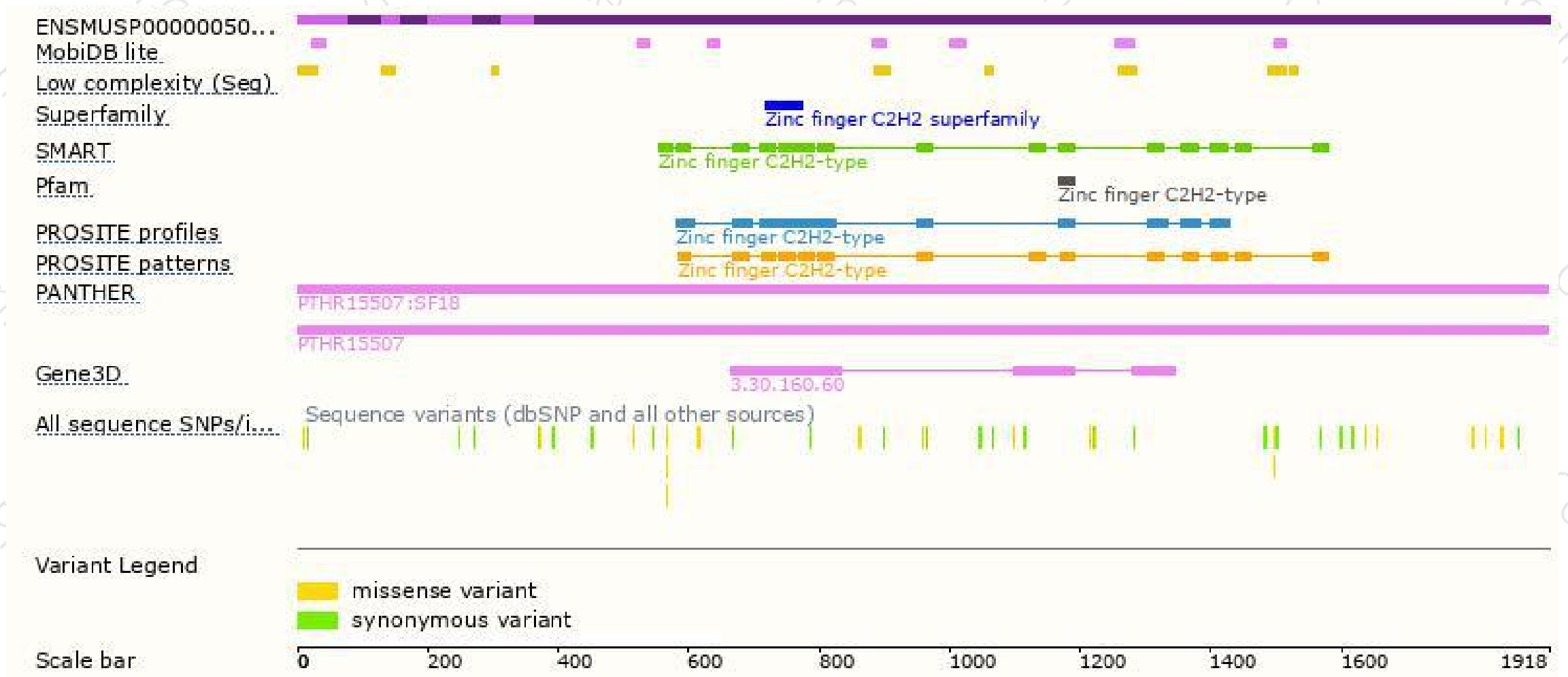
The strategy is based on the design of *Rlf-201* transcript,the transcription is shown below:



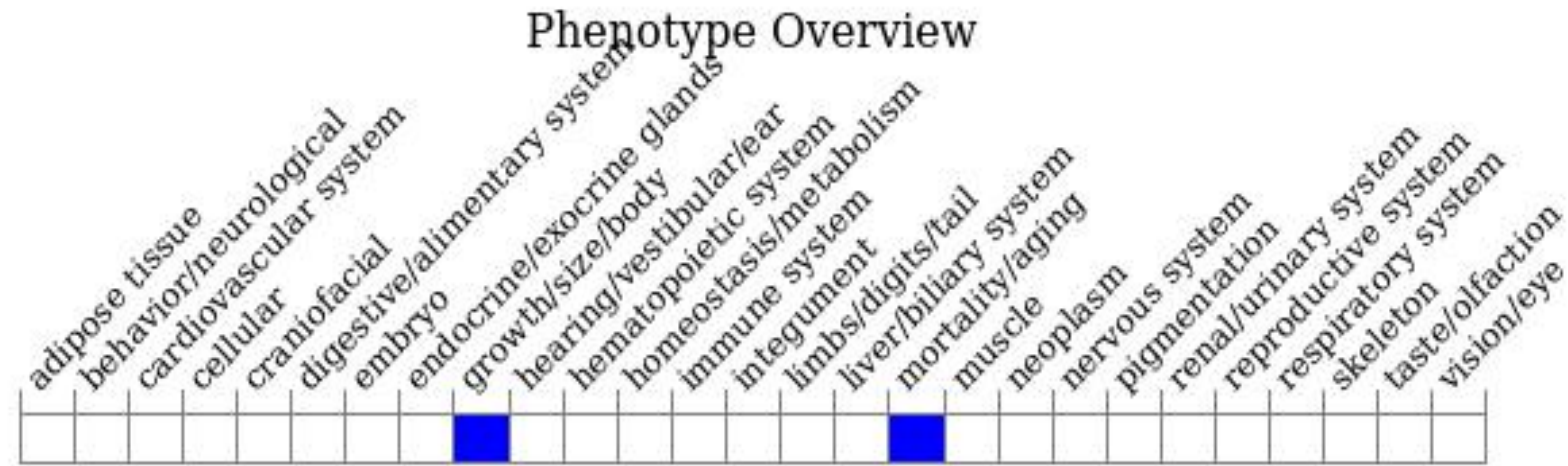
Genomic location distribution



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/>).

According to the existing MGI data, mice homozygous for a hypomorphic ENU-induced allele exhibit postnatal lethality. Only a few mice survive to weaning age exhibiting a decreased body size.

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

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