

# Rnf220 Cas9-CKO Strategy

Designer: Xueting Zhang

Reviewer: Daohua Xu

Design Date: 2023-7-25

# Overview

## Target Gene Name

- Rnf220

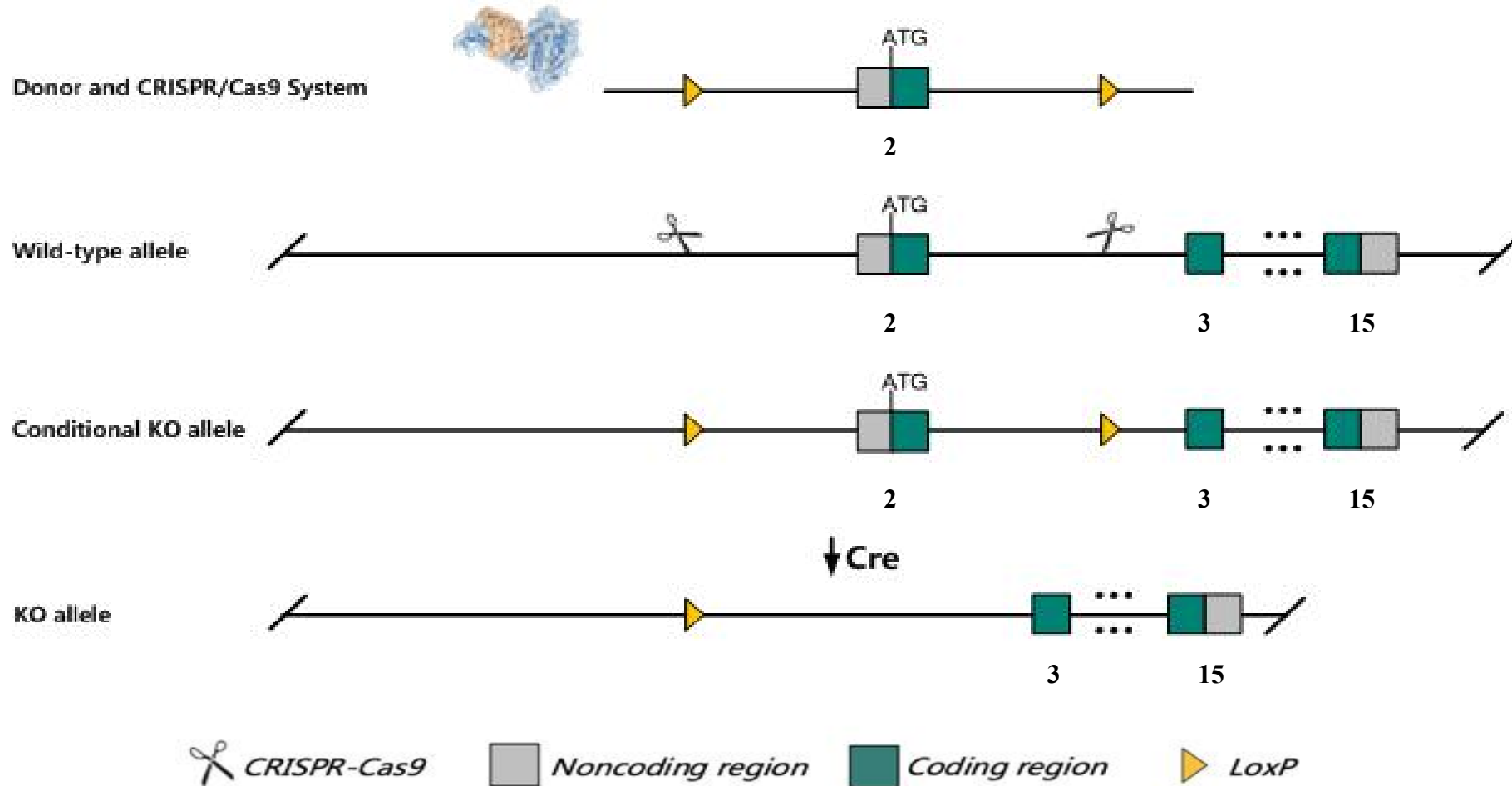
## Project Type

- Cas9-CKO

## Genetic Background

- C57BL/6JGpt

# Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Rnf220* gene.

# Technical Information

- The *Rnf220* gene has 14 transcripts. According to the structure of *Rnf220* gene, exon2 of *Rnf220*-201 (ENSMUST00000030439.15) transcript is recommended as the knockout region. The region contains start codon ATG. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Rnf220* gene. The brief process is as follows: CRISPR-Cas9 system 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 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.
- The flox mice will be 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.

# Gene Information

## Rnf220 ring finger protein 220 [Mus musculus (house mouse)]

Gene ID: 66743, updated on 31-May-2023

### Summary

<b>Official Symbol</b>	Rnf220 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	ring finger protein 220 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1913993</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000028677</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	VALIDATED
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Also known as</b>	4732477A13, 4931406I20Rik, 5730503K05Rik
<b>Summary</b>	Enables ubiquitin protein ligase activity. Involved in protein autoubiquitination. Acts upstream of or within several processes, including nervous system development; positive regulation of DNA-binding transcription factor activity; and protein monoubiquitination. Located in cytoplasm. Is expressed in several structures, including central nervous system; dorsal root ganglion; genitourinary system; retina; and tooth. Orthologous to human RNF220 (ring finger protein 220). [provided by Alliance of Genome Resources, Apr 2022]
<b>Expression</b>	Ubiquitous expression in testis adult (RPKM 15.8), ovary adult (RPKM 14.8) and 28 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

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

# Transcript Information

The gene has 14 transcripts, all transcripts are shown below:

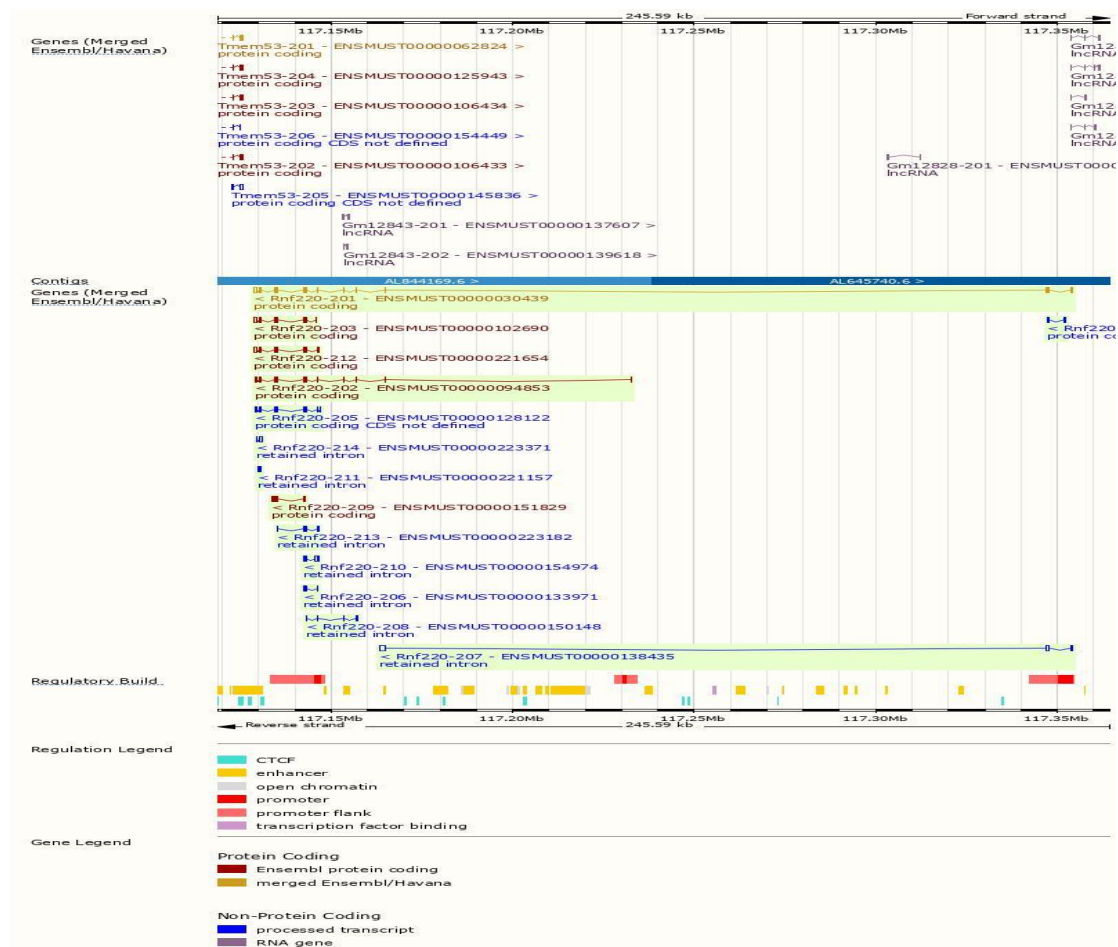
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rnf220-201	<a href="#">ENSMUST00000030439.15</a>	3029	<a href="#">566aa</a>	Protein coding	<a href="#">CCDS18534</a>		A single transcript chosen for a gene which is the most conserved, most highly expressed, has the longest coding sequence and is represented in other key resources, such as NCBI and UniProt. This is defined in detail on <a href="http://www.ensembl.org/info/genome/genebuild/canonical.html">http://www.ensembl.org/info/genome/genebuild/canonical.html</a> Ensembl Canonical, The GENCODE set is the gene set for human and mouse. GENCODE basic, TSL1,
Rnf220-212	<a href="#">ENSMUST000000221654.2</a>	1949	<a href="#">308aa</a>	Protein coding			The GENCODE set is the gene set for human and mouse. GENCODE basic, TSL1,
Rnf220-203	<a href="#">ENSMUST000000102690.9</a>	1842	<a href="#">245aa</a>	Protein coding	<a href="#">CCDS80149</a>		The GENCODE set is the gene set for human and mouse. GENCODE basic, TSL1,
Rnf220-202	<a href="#">ENSMUST000000094953.9</a>	1675	<a href="#">353aa</a>	Protein coding	<a href="#">CCDS80150</a>		The GENCODE set is the gene set for human and mouse. GENCODE basic, APPRIS P1, TSL1,
Rnf220-209	<a href="#">ENSMUST000000151829.2</a>	761	<a href="#">188aa</a>	Protein coding			TSL3, CDS 5' incomplete,
Rnf220-205	<a href="#">ENSMUST000000128122.9</a>	1330	No protein	Protein coding CDS not defined			TSL5,
Rnf220-204	<a href="#">ENSMUST000000123222.2</a>	458	No protein	Protein coding CDS not defined			TSL3,
Rnf220-207	<a href="#">ENSMUST000000138435.2</a>	2630	No protein	Retained intron			TSL1,
Rnf220-210	<a href="#">ENSMUST000000154974.0</a>	1492	No protein	Retained intron			TSL1,
Rnf220-214	<a href="#">ENSMUST000000223371.2</a>	728	No protein	Retained intron			TSL2,
Rnf220-211	<a href="#">ENSMUST000000221157.2</a>	720	No protein	Retained intron			TSL1,
Rnf220-208	<a href="#">ENSMUST000000150148.2</a>	713	No protein	Retained intron			TSL3,
Rnf220-213	<a href="#">ENSMUST000000223182.2</a>	534	No protein	Retained intron			TSL5,
Rnf220-206	<a href="#">ENSMUST000000133971.3</a>	343	No protein	Retained intron			TSL1,

The strategy is based on the design of *Rnf220-201* transcript, the transcription is shown below:



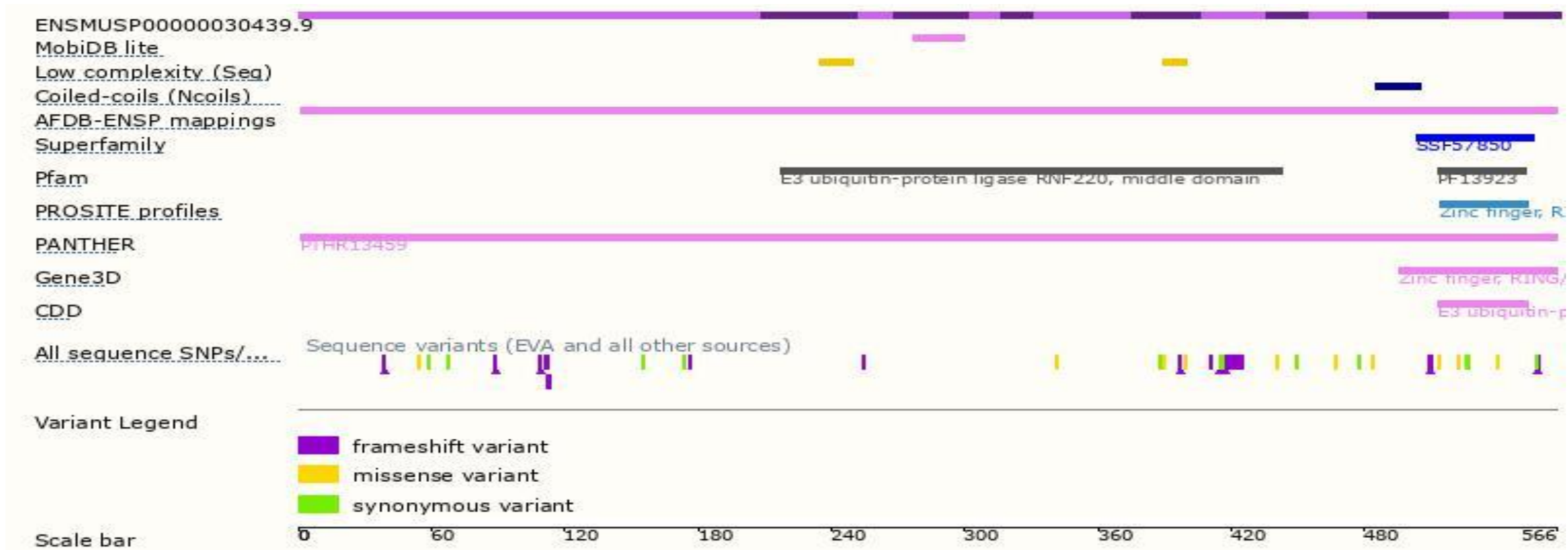
Source: <https://www.ensembl.org>

# Genomic Information



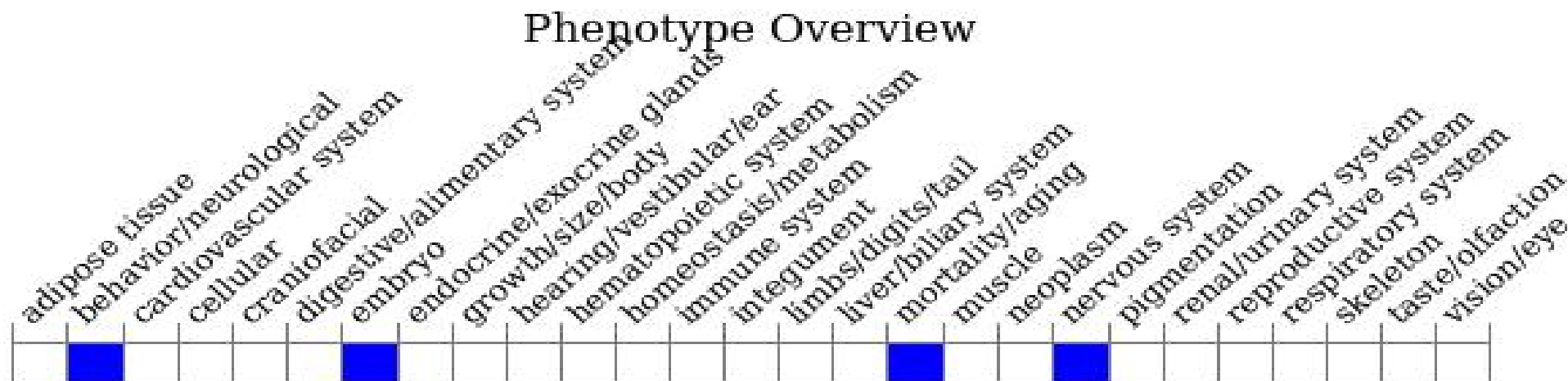


# Protein Information





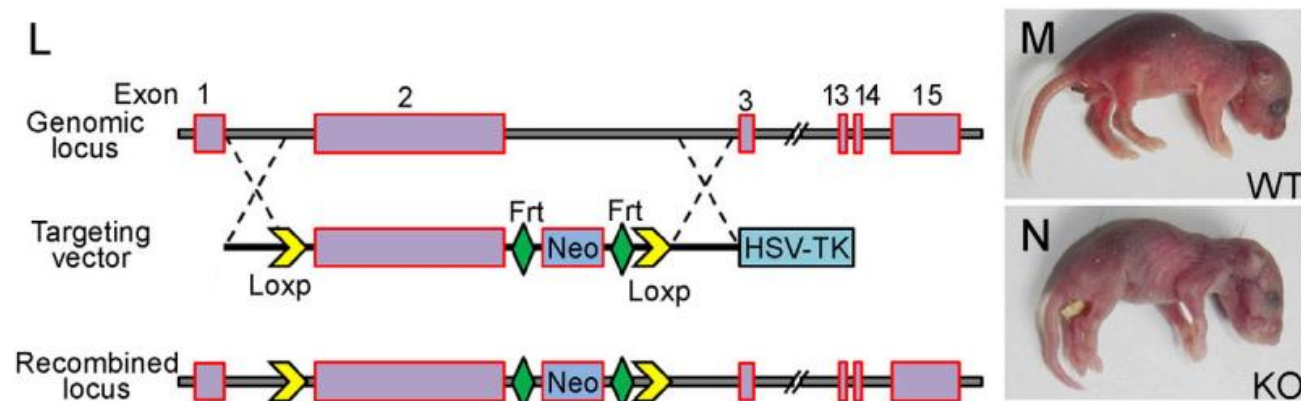
# Mouse Phenotype Information (MGI)



- Homozygous knockout results in the reduction of the number of motor neurons and some types of spinal interneurons and the increase of other types of spinal interneurons in embryos.

# Reference Information

Ma P, et al., Fine-Tuning of Shh/Gli Signaling Gradient by Non-proteolytic Ubiquitination during Neural Patterning. *Cell Rep.* 2019 Jul 9;28(2):541-553.e4



(L) Diagram of the targeting construct and expected recombination events. Exon2 is floxed by two LoxP sites.

(M and N) Photographs showing control (M) and RNF220<sup>-/-</sup> (N) pups on the day of birth. The RNF220<sup>-/-</sup> pups die after birth. WT, wild-type; KO, knockout.

## Animals, Staging and Genotyping

All mice were maintained and handled according to guidelines approved by the Animal Care and Use Committee of the Kunming Institute of Zoology, Chinese Academy of Sciences. All mice were maintained on a C57BL/6 background. Analysis was performed only after lines were crossed to C57BL/6 for at least three generations. The conditional RNF220 knockout allele, RNF220<sup>fl</sup> was generated by insertion of two loxP sites into introns flanking exon 2 through homologous recombination. To obtain RNF220 knockout embryos, Vasa-cre mice were used to generate germ cell RNF220 conditional knockout female mice (RNF220<sup>fl/wt</sup>; Vasa-cre) first and then the female mice were used to mate with the male RNF220 floxed mice (RNF220<sup>fl/fl</sup>).