Ndrg2 Cas9-CKO *Strategy*

Designer: **Daohua Xu**
Reviewer： **Huimin Su**

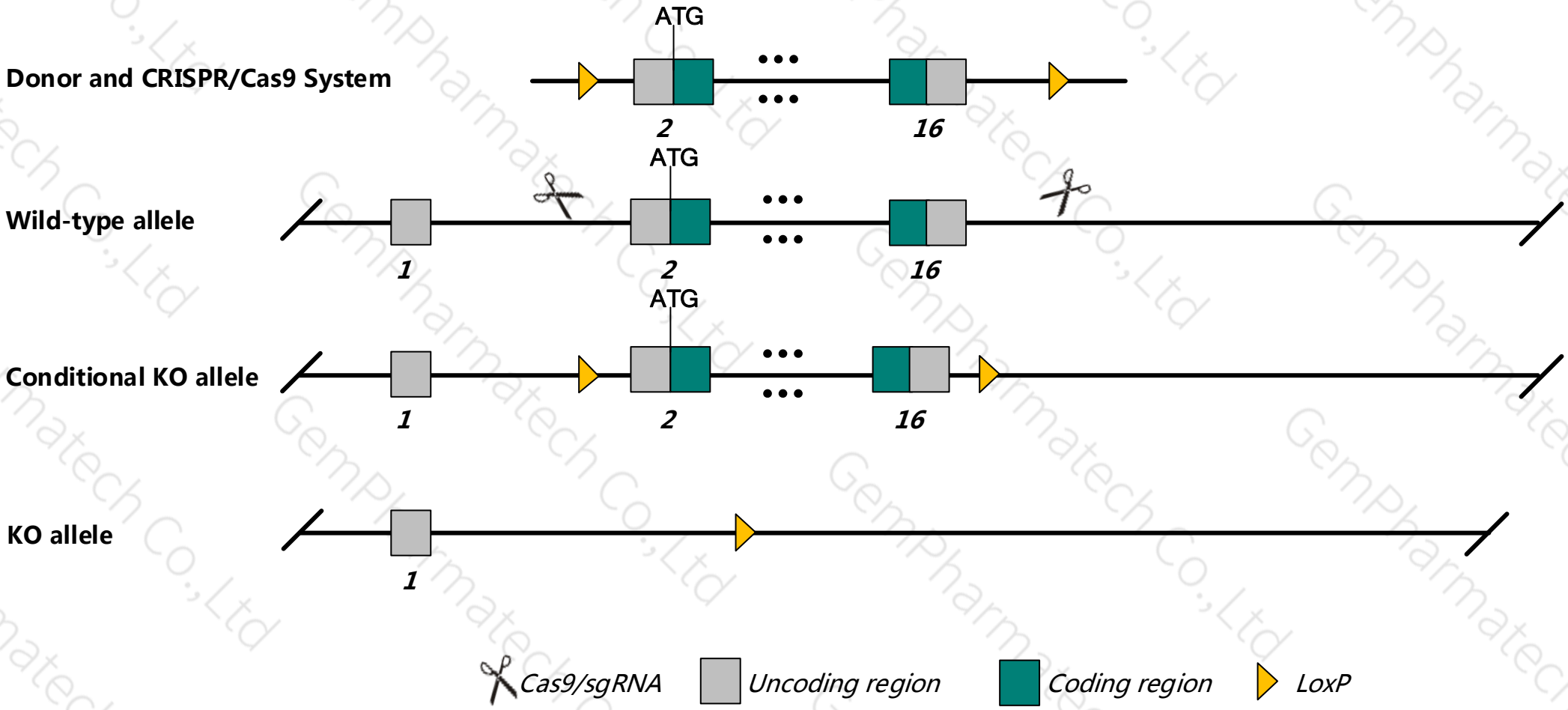
Design Date: **2019-9-28**

Project Overview

Project Name	*Ndrg2*
Project type	Cas9-CKO
Animal background	C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ndrg2* gene. The schematic diagram is as follows:



Technical routes

- The *Ndrg2* gene has 13 transcripts, According to the structure of *Ndrg2* gene, exon2-exon16 of *Ndrg2-201* transcript is recommended as the knockout region.The region contains the all of coding seqence.Knock out the region,result in destruction of protein.

- This project uses CRISPR/Cas9 technology to modify *Ndrg2* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed, Cas9, sgRNA and donor were microinjected into fertilized eggs of C57BL/6JGpt mice and homologous recombination was carried out to obtain F0 mice. A stable and hereditary F1 generation mouse model was obtained by mating F0 generation mice with C57BL/6JGpt mice which were confirmed positive by PCR-sequencing.

- 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.

Notice

- According to the existing MGI data，Mice homozygous for a null allele develop various types of tumors, including T-cell lymphomas, and have a shorter lifespan. Homozygotes for a second null allele show vertebral transformations. Homozygotes for a third null allele show reduced astrogliosis and inflammatory response after brain injury.

- The *Ndrg2* gene is located in the Chr14. If the knockout mice are mixed with other mice, two target genes are avoided on the same chromosome as possible, otherwise the offspring of mice with double gene positive and homozygous gene knockout can not be obtained.

- This Strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information （NCBI）

集萃药康 GemPharmatech

Ndrg2 N-myc downstream regulated gene 2 [*Mus musculus* (house mouse)]

Gene ID: 29811, updated on 31-Jan-2019

Summary

Official Symbol	Ndrg2 provided by MGI
Official Full Name	N-myc downstream regulated gene 2 provided by MGI
Primary source	MGI:MGI:1352498
See related	Ensembl:ENSMUSG00000004558
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	Ndr2; SYLD; AI182517; AU040374
Expression	Broad expression in liver adult (RPKM 416.8), heart adult (RPKM 317.4) and 15 other tissues See more
Orthologs	human all

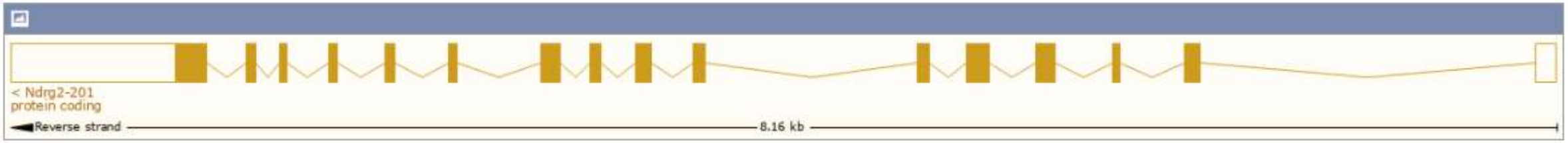
Transcript information （Ensembl）

The gene has 13 transcripts, and all transcripts are shown below ：

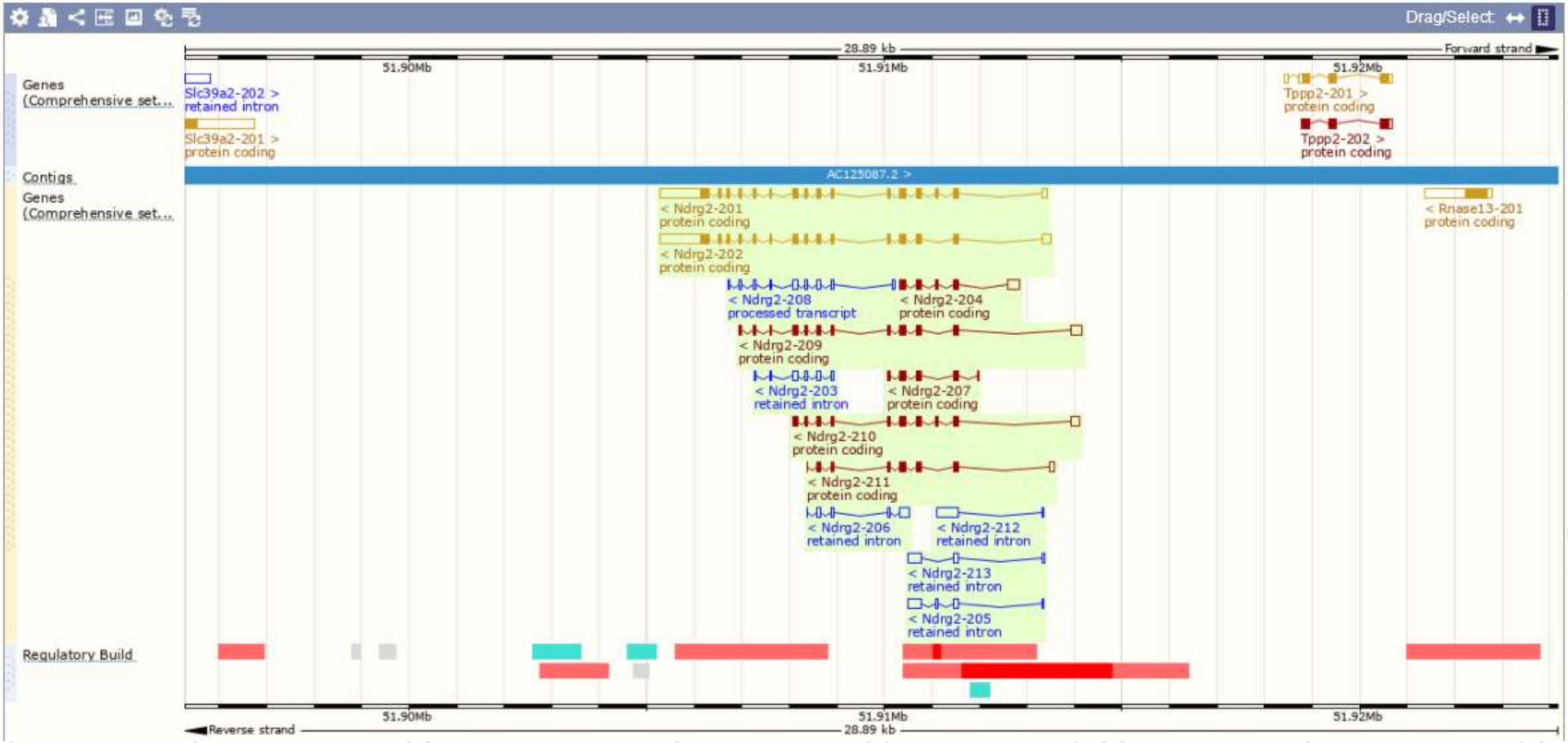
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
Ndrg2-202	ENSMUST00000111632.4	2103	357aa	Protein coding	CCDS49486	Q9QYG0	NM_001145959 NM_001360270 NM_001360271 NM_001360272 NM_001360273 NM_001360276 NP_001139431 NP_001347199 NP_001347200 NP_001347201 NP_001347202 NP_001347203 NP_001347205	TSL:1 GENCODE basic APPRIS P1
Ndrg2-201	ENSMUST00000004673.14	2099	371aa	Protein coding	CCDS27047	Q9QYG0	NM_001360264 NM_001360265 NM_001360266 NM_001360267 NM_001360269 NM_013864 NP_001347193 NP_001347194 NP_001347195 NP_001347196 NP_001347197 NP_001347198 NP_038892	TSL:1 GENCODE basic
Ndrg2-209	ENSMUST00000227237.1	1060	273aa	Protein coding	-	A0A2I3BPW0	-	CDS 3' incomplete
Ndrg2-210	ENSMUST00000227402.1	896	238aa	Protein coding	-	A0A2I3BQR5	-	CDS 3' incomplete
Ndrg2-211	ENSMUST00000228164.1	635	176aa	Protein coding	-	A0A2I3BPL1	-	CDS 3' incomplete
Ndrg2-204	ENSMUST00000226184.1	602	114aa	Protein coding	-	A0A2I3BQM5	-	CDS 3' incomplete
Ndrg2-207	ENSMUST00000226528.1	384	114aa	Protein coding	-	A0A2I3BS20	-	CDS 3' incomplete
Ndrg2-208	ENSMUST00000226698.1	508	No protein	Processed transcript	-	-	-	
Ndrg2-212	ENSMUST00000228173.1	481	No protein	Retained intron	-	-	-	
Ndrg2-213	ENSMUST00000228620.1	439	No protein	Retained intron	-	-	-	
Ndrg2-205	ENSMUST00000226364.1	435	No protein	Retained intron	-	-	-	
Ndrg2-206	ENSMUST00000226366.1	427	No protein	Retained intron	-	-	-	
Ndrg2-203	ENSMUST00000226122.1	384	No protein	Retained intron	-	-	-	

Transcript information （Ensembl）

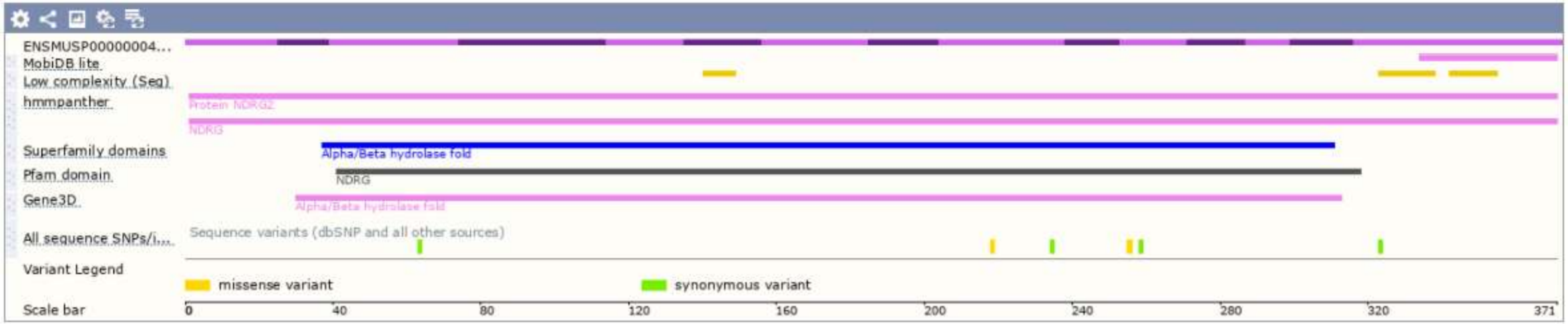
The strategy is based on the design of *Ndrg2-201* transcript,The transcription is shown below：



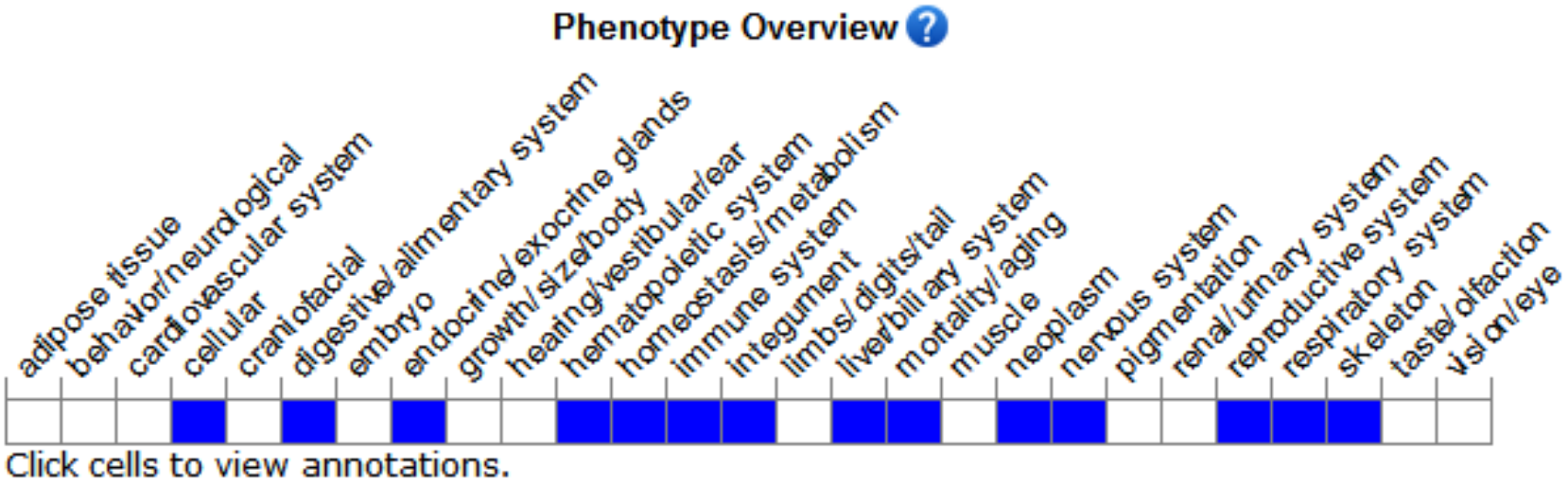
Genomic location distribution



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 null allele develop various types of tumors, including T-cell lymphomas, and have a shorter lifespan. Homozygotes for a second null allele show vertebral transformations. Homozygotes for a third null allele show reduced astrogliosis and inflammatory response after brain injury.

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

Tel: 025-5864 1534

集萃药康生物科技

GemPharmatech Co.,Ltd