

# ***Clu-P2A-CreERT2-P2A-EGFP* cas9-ki Strategy**

**Designer:**

**Xueting Zhang**

**Reviewer**

**Xiaojing Li**

**Design Date:**

**2021-3-23**

# Project Overview

---

<b>Project Name</b>	<b><i>Clu-P2A-CreERT2-P2A-EGFP</i></b>
---------------------	--

---

<b>Project type</b>	<b>cas9-ki</b>
---------------------	----------------

---

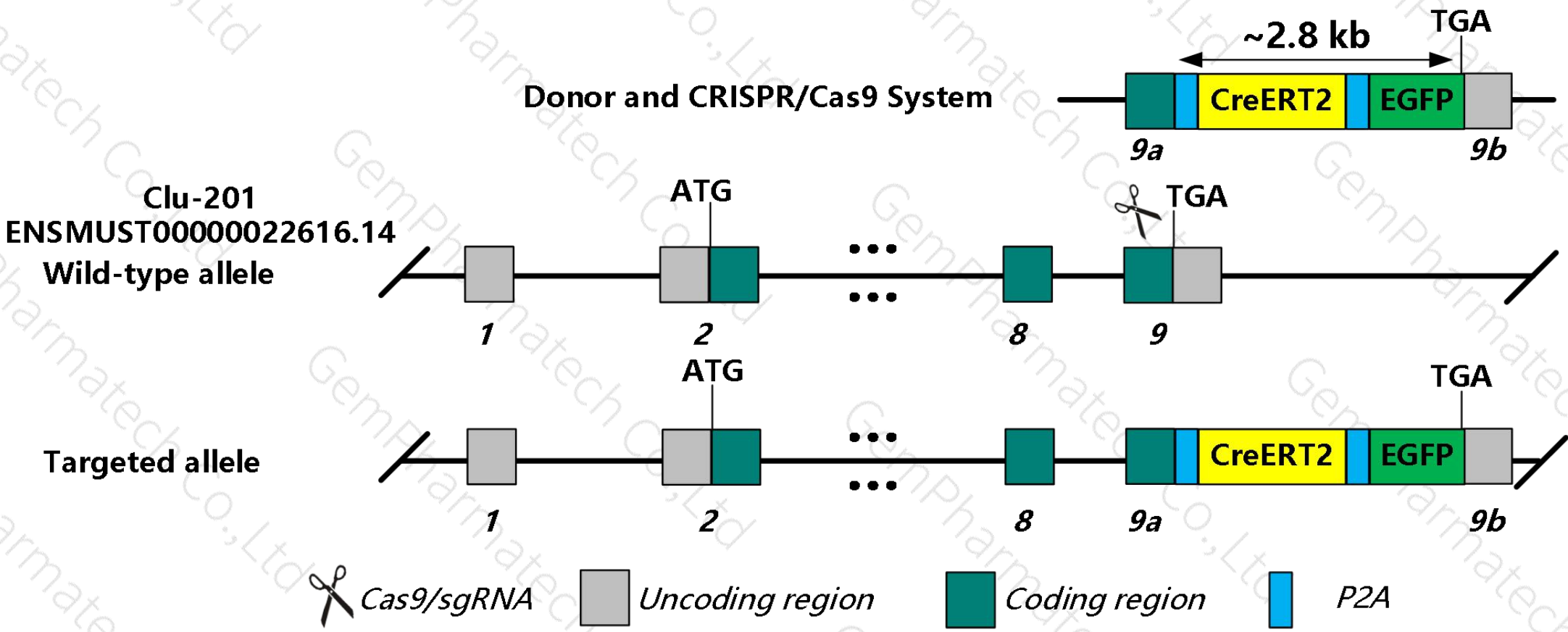
<b>Strain background</b>	<b>C57BL/6JGpt</b>
--------------------------	--------------------

---

- The *Clu* gene has 9 transcripts. According to the structure of *Clu* gene, *Clu-201*(ENSMUST00000022616.14) is selected for presentation of the recommended strategy.
- *Clu-201* gene has 9 exons, with the ATG start codon in exon2 and TGA stop codon in exon9.
- We make *Clu-P2A-CreERT2-P2A-EGFP* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near stop codon(TGA) of *Clu* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in P2A-CreERT2-P2A-EGFP before stop coding(TGA) of *Clu* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

# Knockin strategy

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



- According to the existing MGI data, homozygous inactivation of this gene leads to progressive renal glomerulopathy and increased severity of myosin-induced autoimmune myocarditis.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- Insertion of P2A-CreERT2-P2A-EGFP may affect the regulation of the 3' end of the *Clu* gene.
- The effect on transcript *Clu*-202&203&204&206&209 is unknown.
- There may be 2 to 4 base mutation in 3'UTR or intron8-9 of *Clu* gene in this strategy.
- There may be base mutations in the modeling process because of the repetitive sequences(PolyT) upstream of the insertion site.
- The *Clu* gene is located on the Chr14. If the knockin mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene after the gene coding region may affect the expression of endogenous and foreign genes. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.



# Gene information (NCBI)

## Clu clusterin [ *Mus musculus* (house mouse) ]

Download Datasets

Gene ID: 12759, updated on 13-Mar-2021

### Summary

Official Symbol	Clu provided by MGI
Official Full Name	clusterin provided by MGI
Primary source	MGI:MGI:88423
See related	Ensembl:ENSMUSG00000022037
Gene type	protein coding
RefSeq status	REVIEWED
Organism	<i>Mus musculus</i>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A; C; Sg; Cli; SP-; Sgp; ApoJ; Sgp2; Sugg; SP-40; Sgp-2; D14Ucl; Sugg-2; A1893575; D14Ucla3
Summary	The protein encoded by this gene is a secreted chaperone that can, under some stress conditions, also be found in the cell cytosol. It has been suggested to be involved in several basic biological events such as cell death, tumor progression, and neurodegenerative disorders. The encoded preproprotein undergoes proteolytic processing to generate a disulfide-linked heterodimeric mature protein comprised of alpha and beta subunits. Mice lacking the encoded protein exhibit increased severity of autoimmune myocarditis, faster progression of the acute inflammation to myocardial scarring and decreased brain injury following neonatal hypoxic-ischemic injury. [provided by RefSeq, Nov 2015]
Expression	Biased expression in genital fat pad adult (RPKM 3056.5), ovary adult (RPKM 788.7) and 11 other tissues <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>
NEW	Try the new <a href="#">Gene table</a>
	Try the new <a href="#">Transcript table</a>

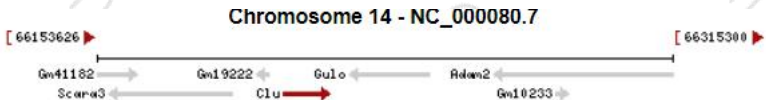
### Genomic context

Location: 14 D1; 14 34.36 cM

See Clu in [Genome Data Viewer](#)

Exon count: 11

Annotation release	Status	Assembly	Chr	Location
<a href="#">109</a>	current	GRCm39 ( <a href="#">GCF_000001635.27</a> )	14	NC_000080.7 (66205932..66218997)
108.20200622	previous assembly	GRCm38.p6 ( <a href="#">GCF_000001635.26</a> )	14	NC_000080.6 (65968483..65981548)
Build 37.2	previous assembly	MGSCv37 ( <a href="#">GCF_000001635.18</a> )	14	NC_000080.5 (66587320..66600382)

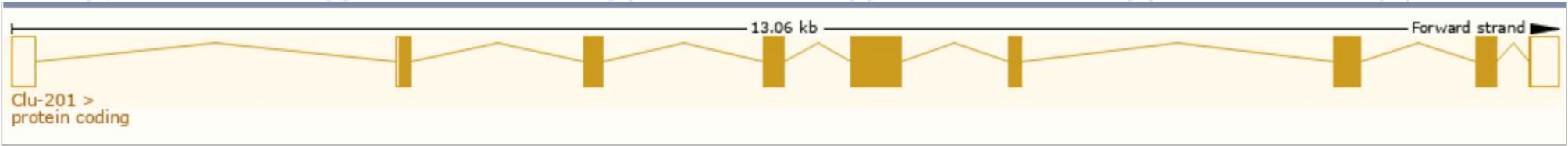


# Transcript information (Ensembl)

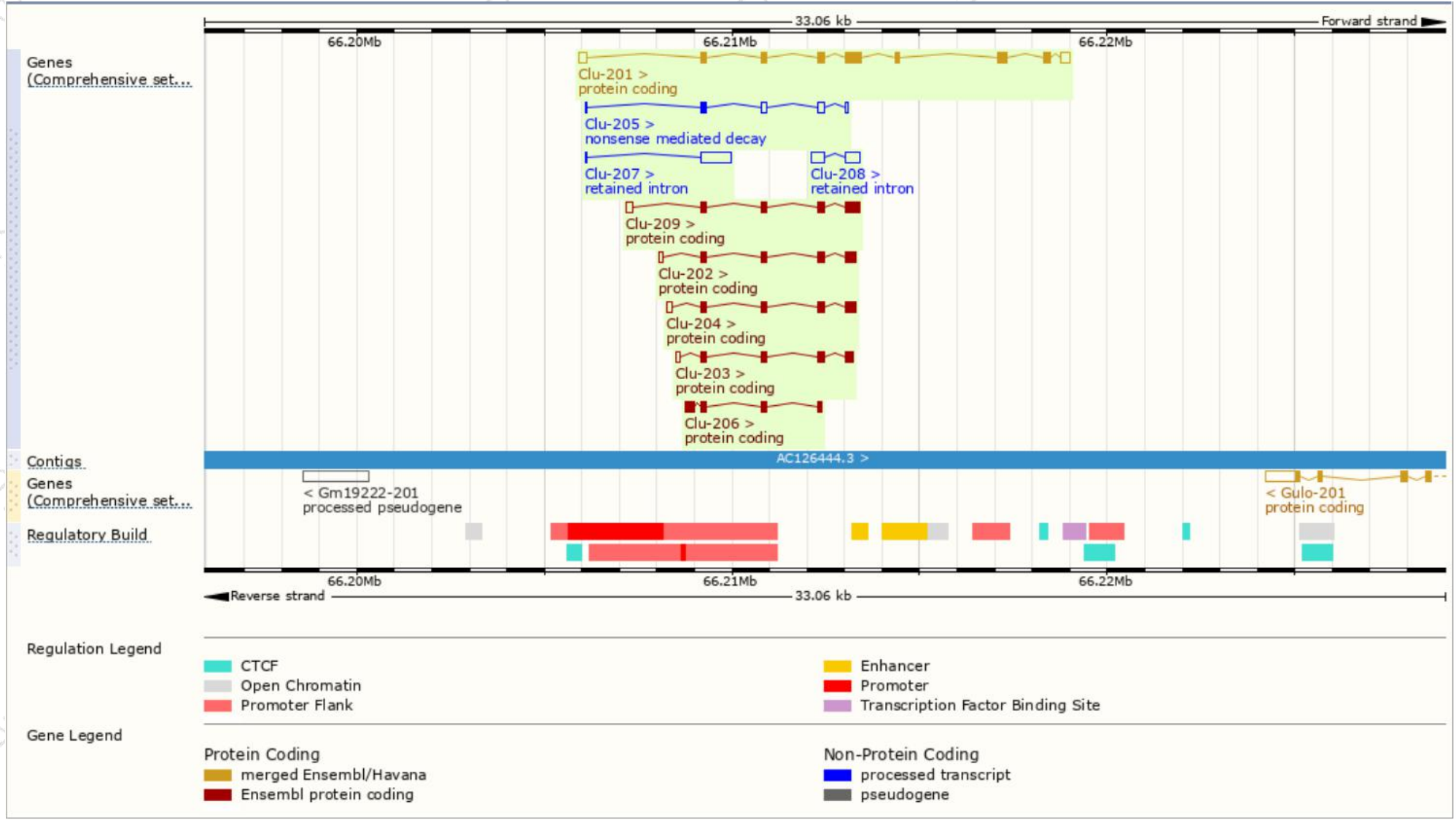
The gene has 9 transcripts, and all the transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Clu-201	<a href="#">ENSMUST00000022616.14</a>	1810	<a href="#">448aa</a>	Protein coding	<a href="#">CCDS36957</a>	<a href="#">Q06890</a> <a href="#">Q549A5</a>	TSL:1 GENCODE basic APPRIS P1
Clu-209	<a href="#">ENSMUST00000153460.8</a>	1004	<a href="#">265aa</a>	Protein coding	-	<a href="#">E9Q8Y5</a>	CDS 3' incomplete TSL:2
Clu-204	<a href="#">ENSMUST00000138191.8</a>	844	<a href="#">225aa</a>	Protein coding	-	<a href="#">E9PUU2</a>	CDS 3' incomplete TSL:5
Clu-202	<a href="#">ENSMUST00000127387.8</a>	811	<a href="#">233aa</a>	Protein coding	-	<a href="#">E9PXG5</a>	CDS 3' incomplete TSL:3
Clu-203	<a href="#">ENSMUST00000128539.8</a>	756	<a href="#">203aa</a>	Protein coding	-	<a href="#">E9Q9B8</a>	CDS 3' incomplete TSL:2
Clu-206	<a href="#">ENSMUST00000144619.2</a>	632	<a href="#">209aa</a>	Protein coding	-	<a href="#">E9Q2G2</a>	CDS 3' incomplete TSL:3
Clu-205	<a href="#">ENSMUST00000138665.2</a>	558	<a href="#">39aa</a>	Nonsense mediated decay	-	<a href="#">D6RFP9</a>	TSL:5
Clu-207	<a href="#">ENSMUST00000146990.2</a>	841	No protein	Retained intron	-	-	TSL:2
Clu-208	<a href="#">ENSMUST00000152903.2</a>	739	No protein	Retained intron	-	-	TSL:2

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

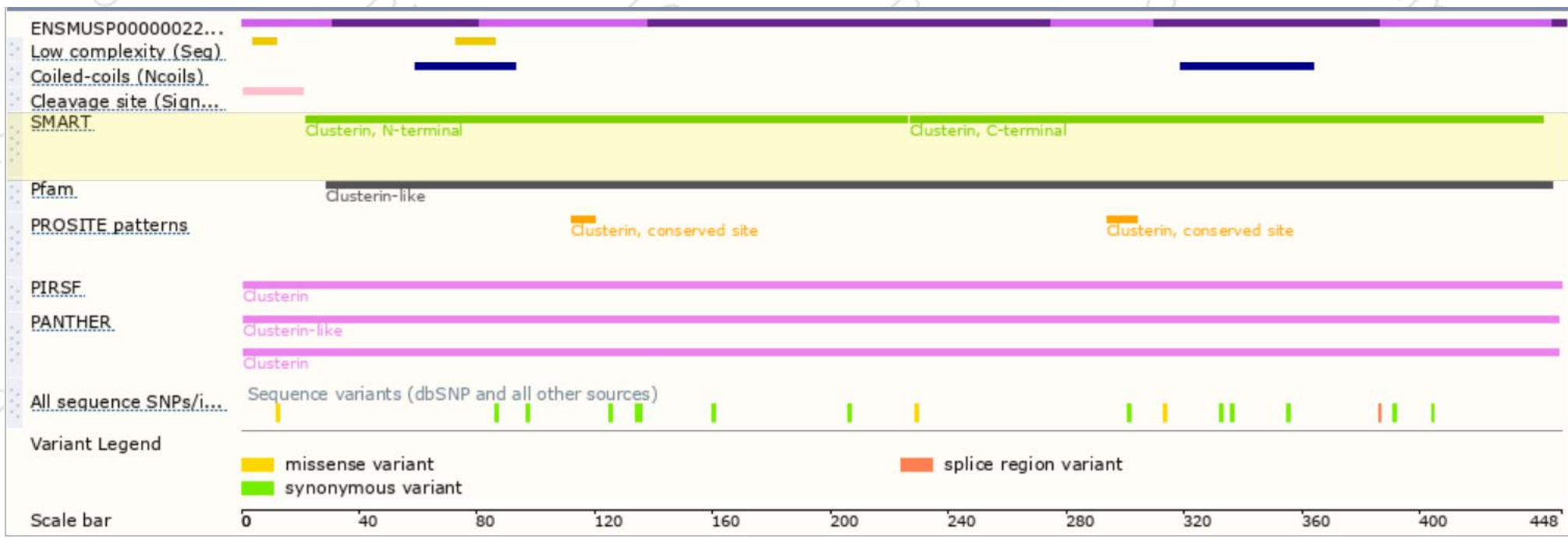


# Genomic location distribution

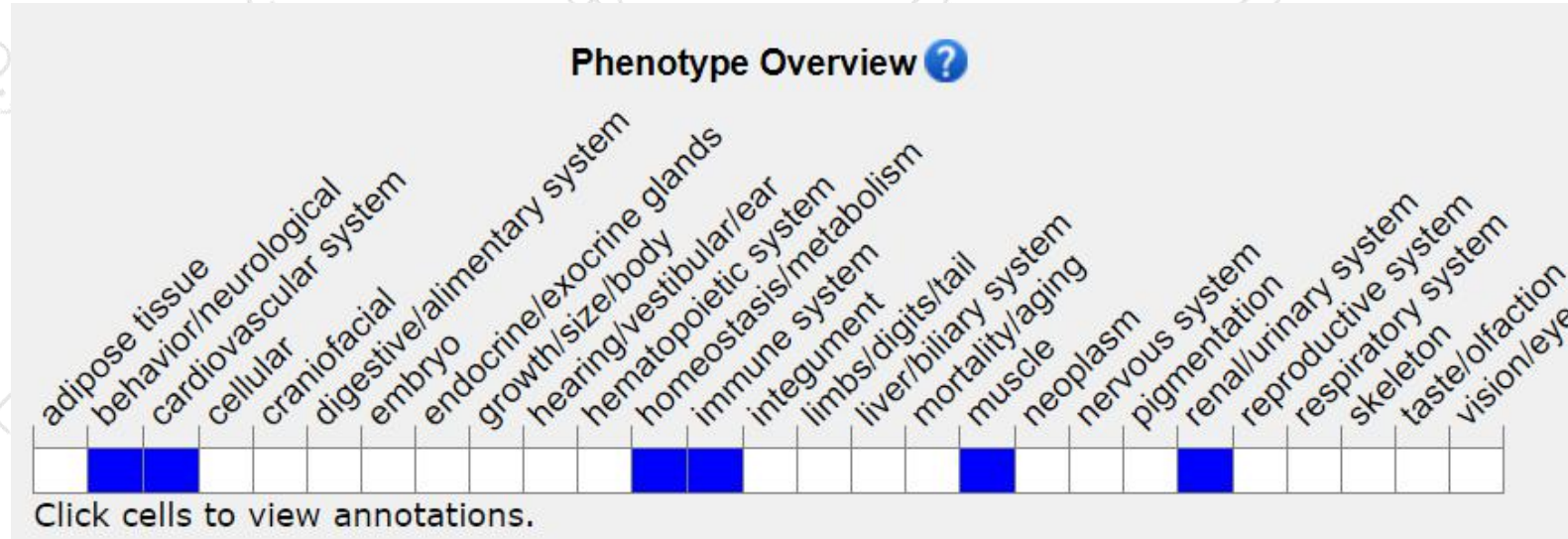




# Protein domain



# Mouse phenotype description(MGI)



*Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/marker/MGI:88423>) .*

Homozygous inactivation of this gene leads to progressive renal glomerulopathy and increased severity of myosin-induced autoimmune myocarditis.

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

