

# *C5ar1* Cas9-CKO Strategy

Designer: Yanhua Shen

Design Date: 2019-08-06

# Project Overview

**Project Name**

*C5ar1*

**Project type**

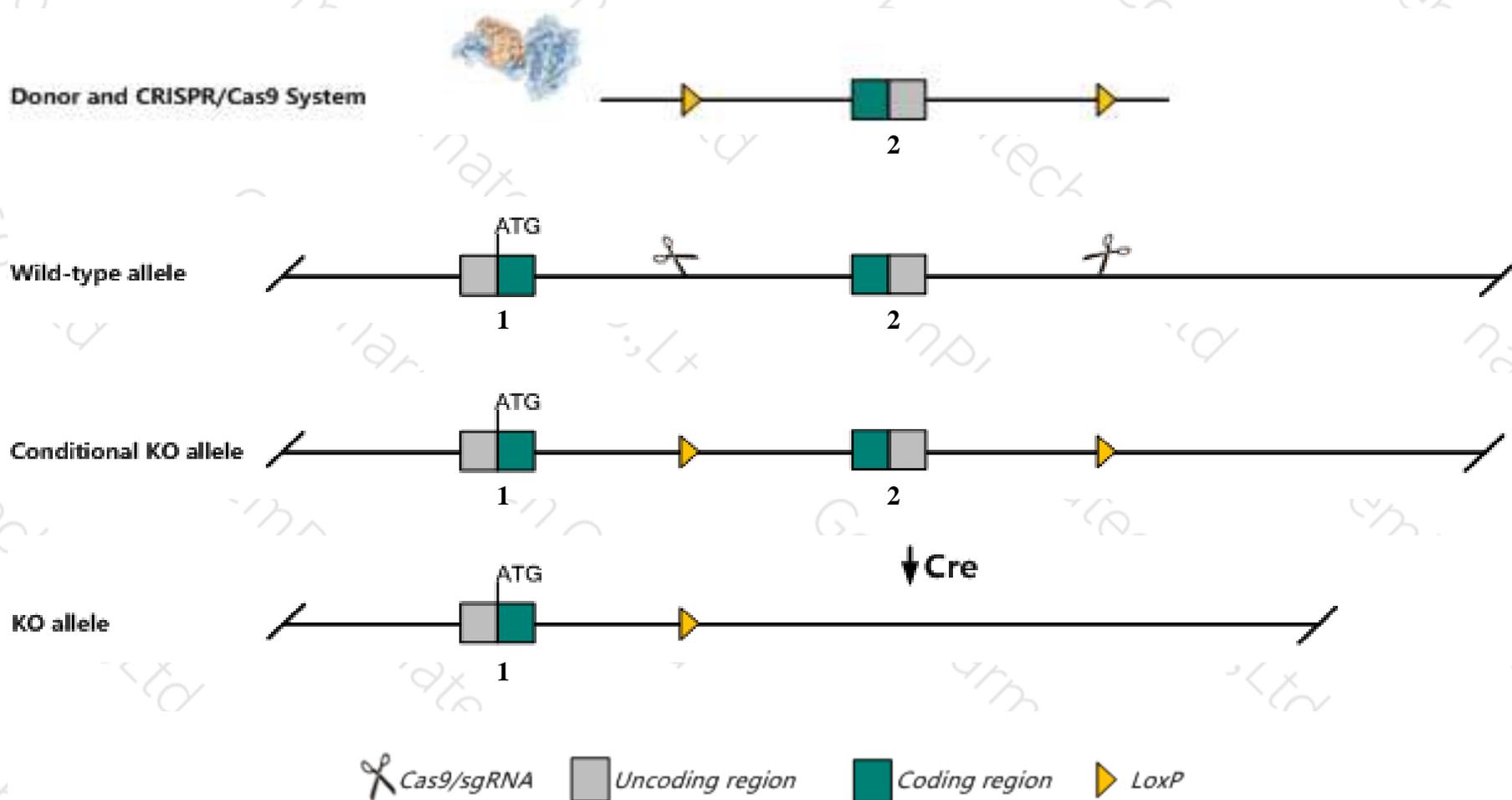
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *C5ar1* gene. The schematic diagram is as follows:



- The *C5ar1* gene has 3 transcripts. According to the structure of *C5ar1* gene, exon2 of *C5ar1-202* (ENSMUST00000168818.1) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *C5ar1* 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, Homozygous targeted mutants have impaired C5 responses that can show increased or decreased acute inflammation under different circumstances, and thus affect severity of disease or infection.
- The knockout region is about 2.6 kb away from the 5th end of the *C5ar2* gene, the insertion of the 3 end loxp may affect the regulation of the 5th end of the *C5ar2* gene; the insertion of the 3 end loxp may affect the 3 end regulation of the *C5ar1* gene.
- The *C5ar1* gene is located on the Chr7. 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)

## C5ar1 complement component 5a receptor 1 [Mus musculus (house mouse)]

Gene ID: 12273, updated on 19-Mar-2019

### Summary



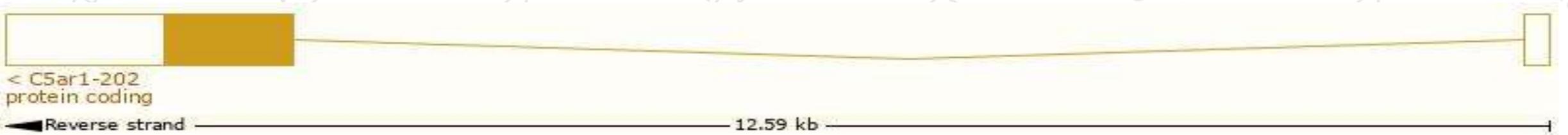
<b>Official Symbol</b>	C5ar1 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	complement component 5a receptor 1 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:88232</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000049130</a>
<b>Gene type</b>	protein coding
<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>	C5aR, C5r1, Cd88, D7Msu1
<b>Expression</b>	Broad expression in lung adult (RPKM 10.0), mammary gland adult (RPKM 8.9) and 20 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

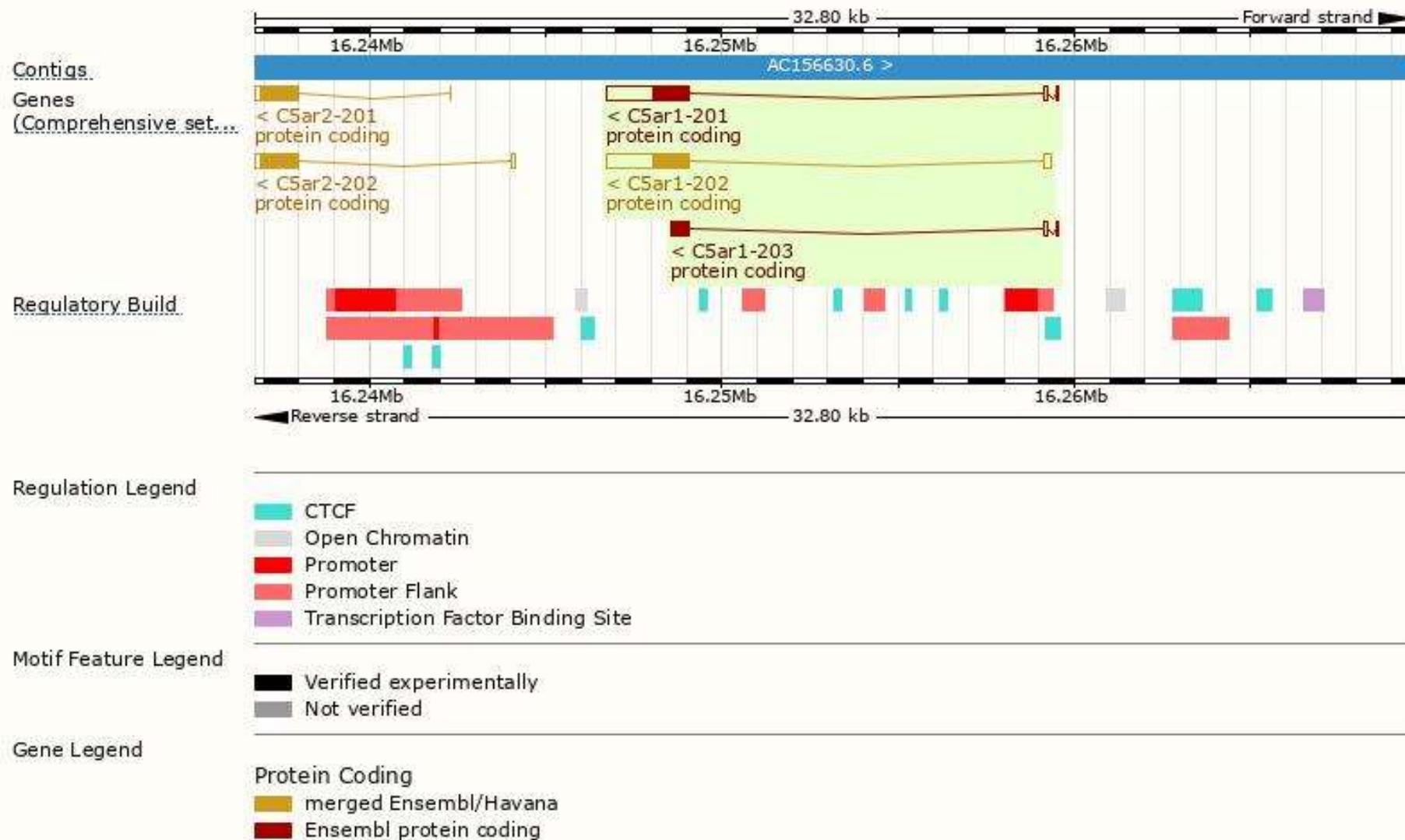
The gene has 3 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
C5ar1-202	<a href="#">ENSMUST00000168818.1</a>	2540	<a href="#">351aa</a>	Protein coding	<a href="#">CCDS20846</a>	<a href="#">P30993</a>	TSL:1 GENCODE basic APPRIS P1
C5ar1-201	<a href="#">ENSMUST00000050770.5</a>	2481	<a href="#">351aa</a>	Protein coding	<a href="#">CCDS20846</a>	<a href="#">P30993</a>	TSL:3 GENCODE basic APPRIS P1
C5ar1-203	<a href="#">ENSMUST00000209442.1</a>	674	<a href="#">181aa</a>	Protein coding	-	<a href="#">A0A1B0GT01</a>	CDS 3' incomplete TSL:3

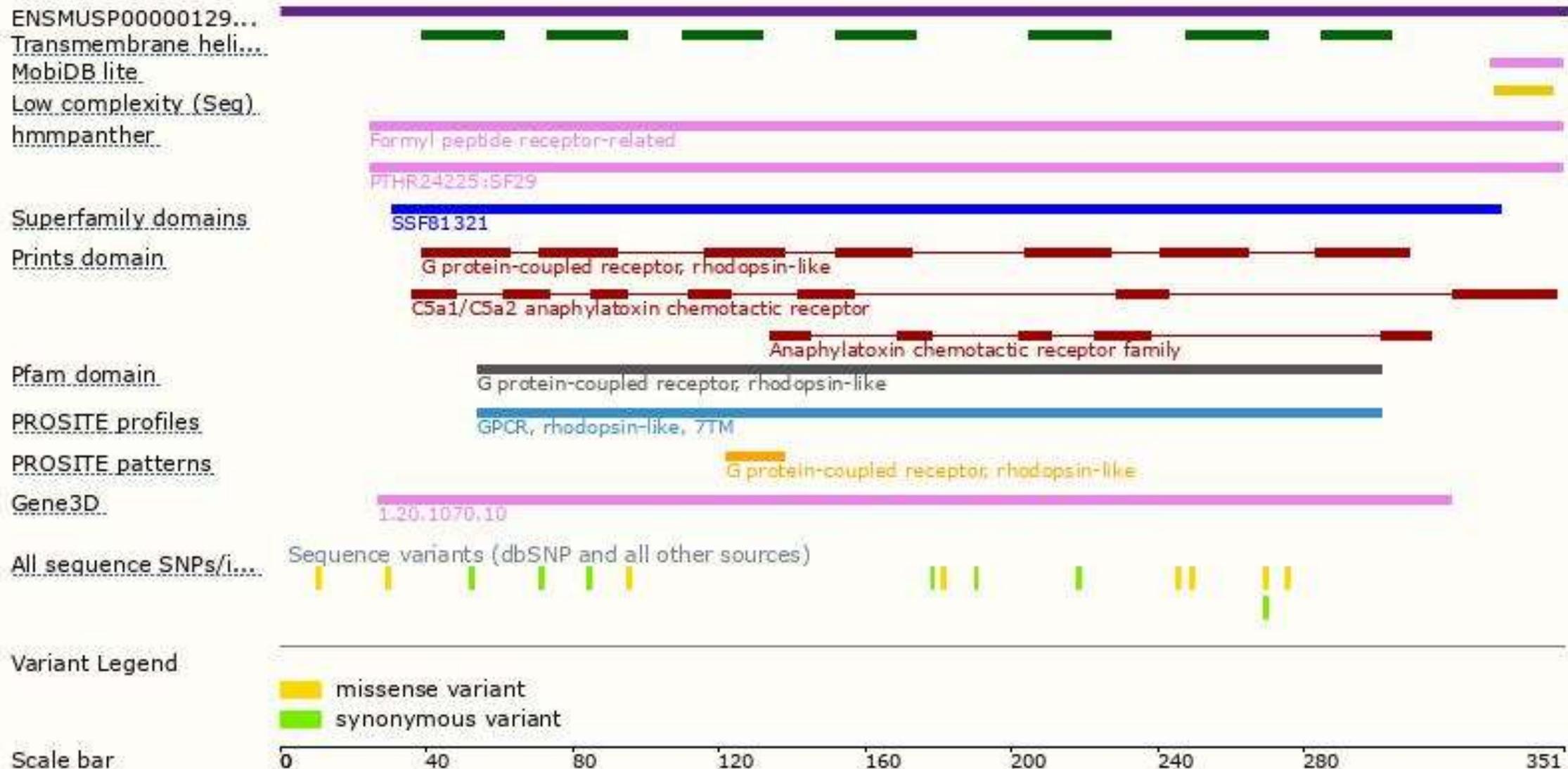
The strategy is based on the design of *C5ar1-202* transcript, The transcription is shown below



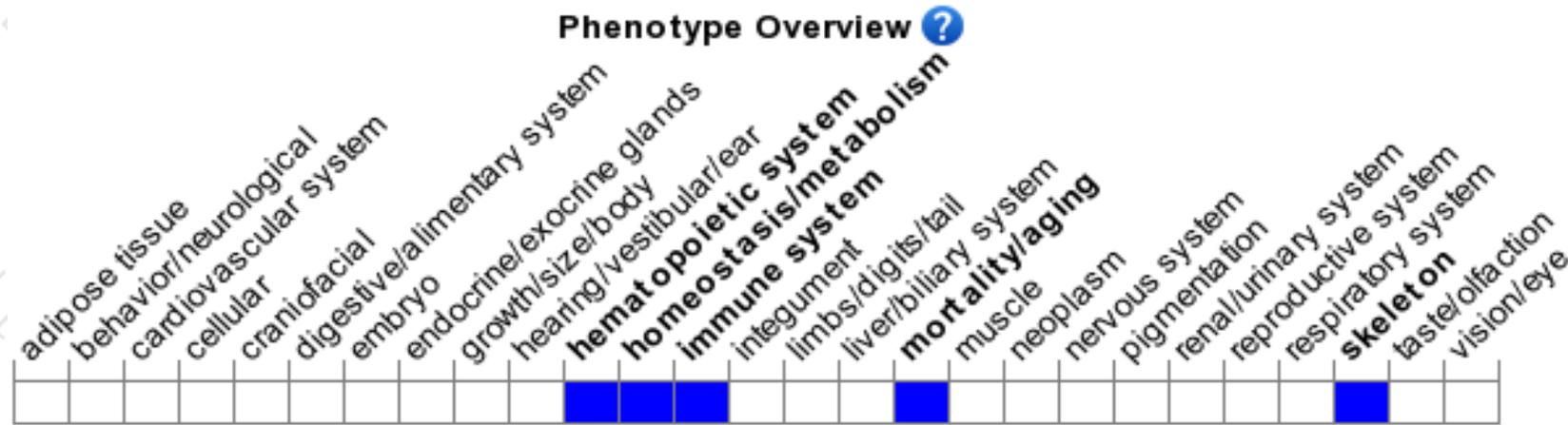
# Genomic location distribution



# 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, Homozygous targeted mutants have impaired C5 responses that can show increased or decreased acute inflammation under different circumstances, and thus affect severity of disease or infection.

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

Tel: 025-5864 1534

