Ang Cas9-KO Strategy

Designer: Jinling Wang

Reviewer: Yumeng Wang

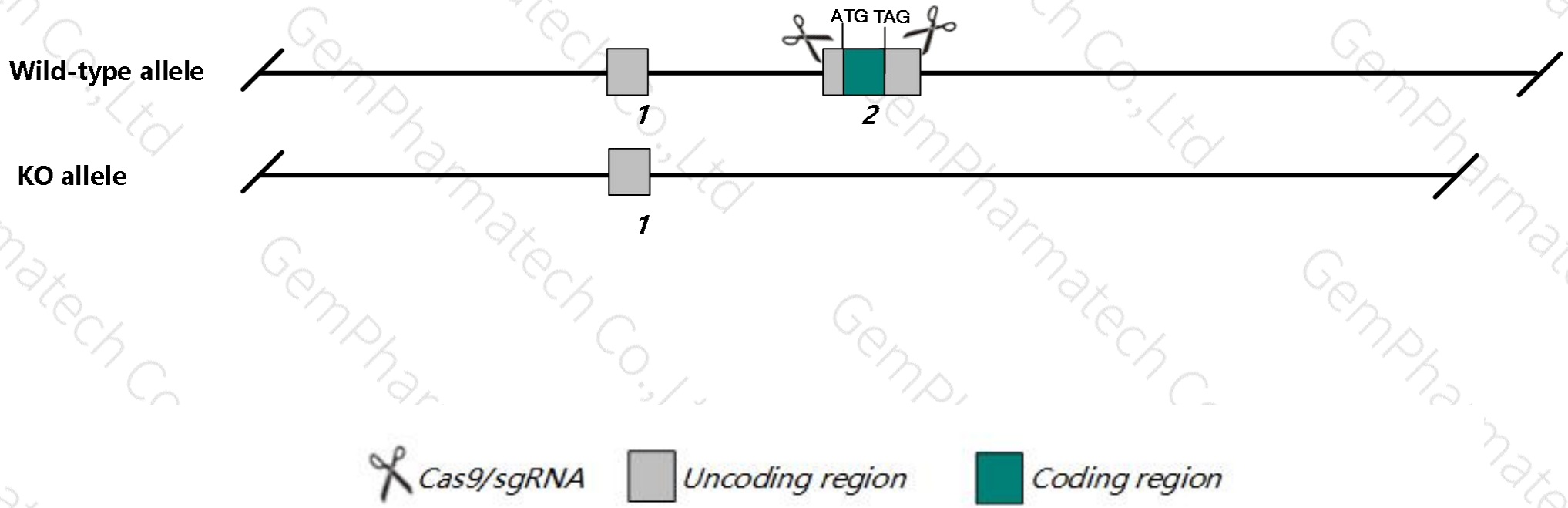
Design Date: 2022-3-17

Project Overview

Project Name	***Ang***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

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



Technical routes

➢ The *Ang* gene has 2 transcripts. According to the structure of *Ang* gene, exon2 of *Ang-202*(ENSMUST00000171688.8) transcript is recommended as the knockout region.The region contains all 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 *Ang* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice

➢ According to the existing MGI data,knock-out mice show increased proliferative capacity of hematopoietic stem/progenitor cells and decreased proliferative capacity of myeloid-restricted progenitor cells and develop leukopenia.

➢ The KO region contains functional region of the Rnase4 gene. Knockout the region may affect the function of Rnase4 gene.

➢ The *Ang* gene is located on the Chr14. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

集萃药康 GemPharmatech

Ang angiogenin, ribonuclease, RNase A family, 5 [Mus musculus (house mouse)]

Gene ID: 11727, updated on 13-Mar-2020

Summary

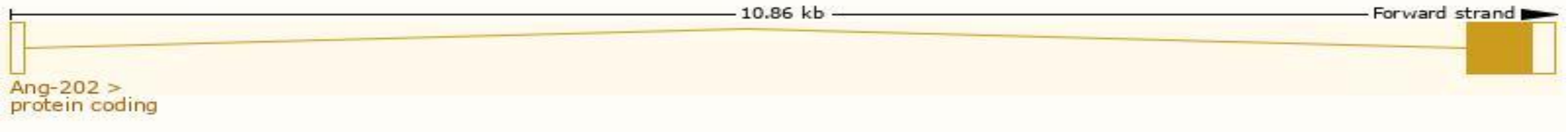
Official Symbol	Ang provided by MGI
Official Full Name	angiogenin, ribonuclease, RNase A family, 5 provided by MGI
Primary source	MGI:MGI:88022
See related	Ensembl:ENSMUSG00000072115
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI385586, Ang1, Rnase5, Rnase5a
Summary	This gene encodes a member of the pancreatic ribonuclease A superfamily and is a potent inducer of neovascularization. The encoded protein is a secreted multifunctional tRNA-specific ribonuclease that promotes angiogenesis in response to angiogenetic stimuli such as hypoxia, mediates stress-induced translational repression by cleaving cellular tRNAs, stimulates cell proliferation by mediating rRNA transcription in prostate cancer cells, and is involved in neurite pathfinding. This gene resides in a cluster of highly related genes. It shares dual promoters and 5' exons with the ribonuclease, RNase A family 4 gene. Two alternatively spliced variants, with different 5' exons but the same coding exon, have been identified. Multiple pseudogenes have been found for this gene. [provided by RefSeq, Jun 2009]
Expression	Biased expression in liver adult (RPKM 249.9), lung adult (RPKM 38.7) and 4 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

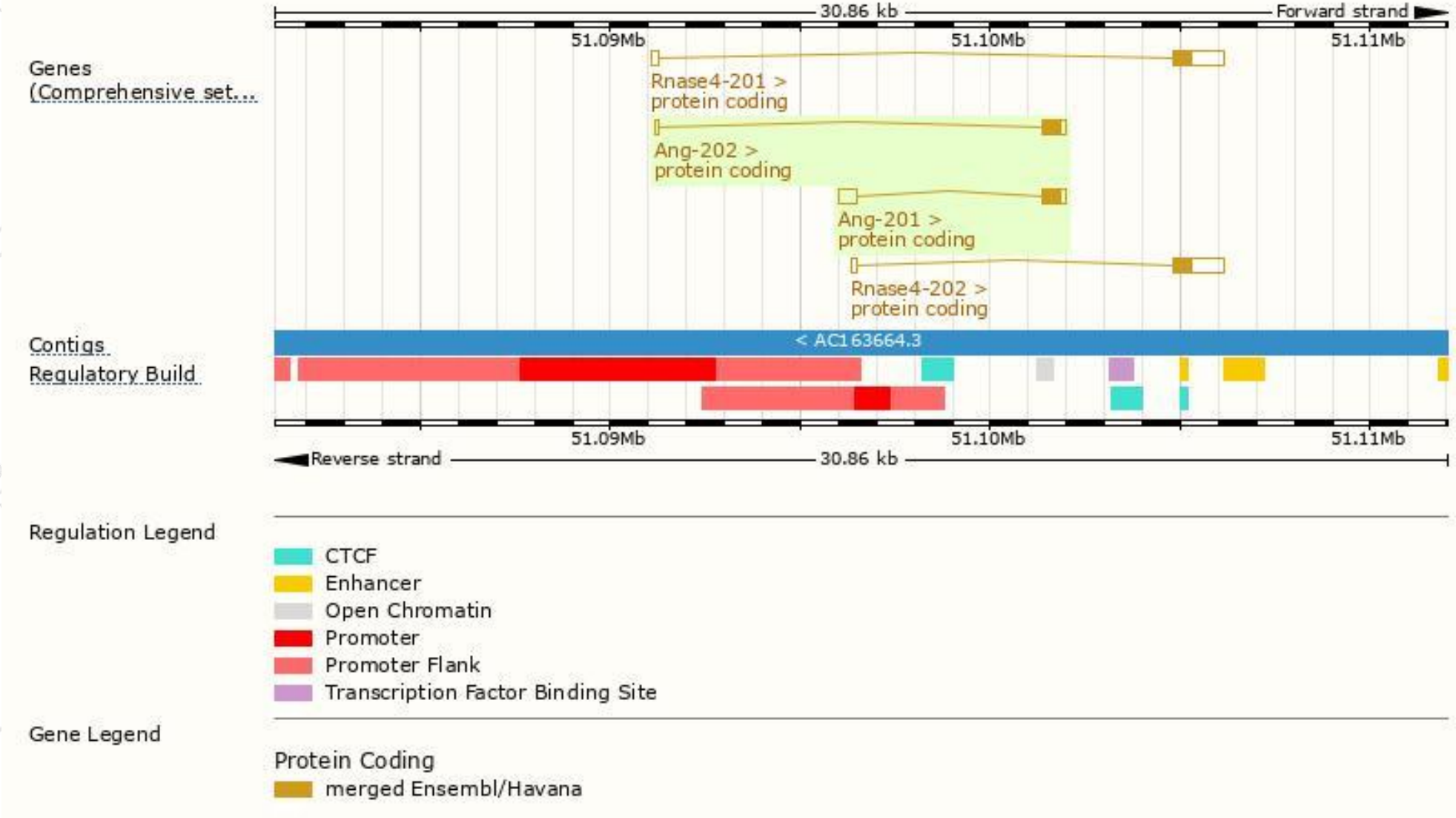
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ang-201	ENSMUST00000069011.8	1094	145aa	Protein coding	CCDS27034	P21570 Q3TBG7	TSL:1 GENCODE basic APPRIS P1
Ang-202	ENSMUST00000171688.8	728	145aa	Protein coding	CCDS27034	P21570 Q3TBG7	TSL:1 GENCODE basic APPRIS P1

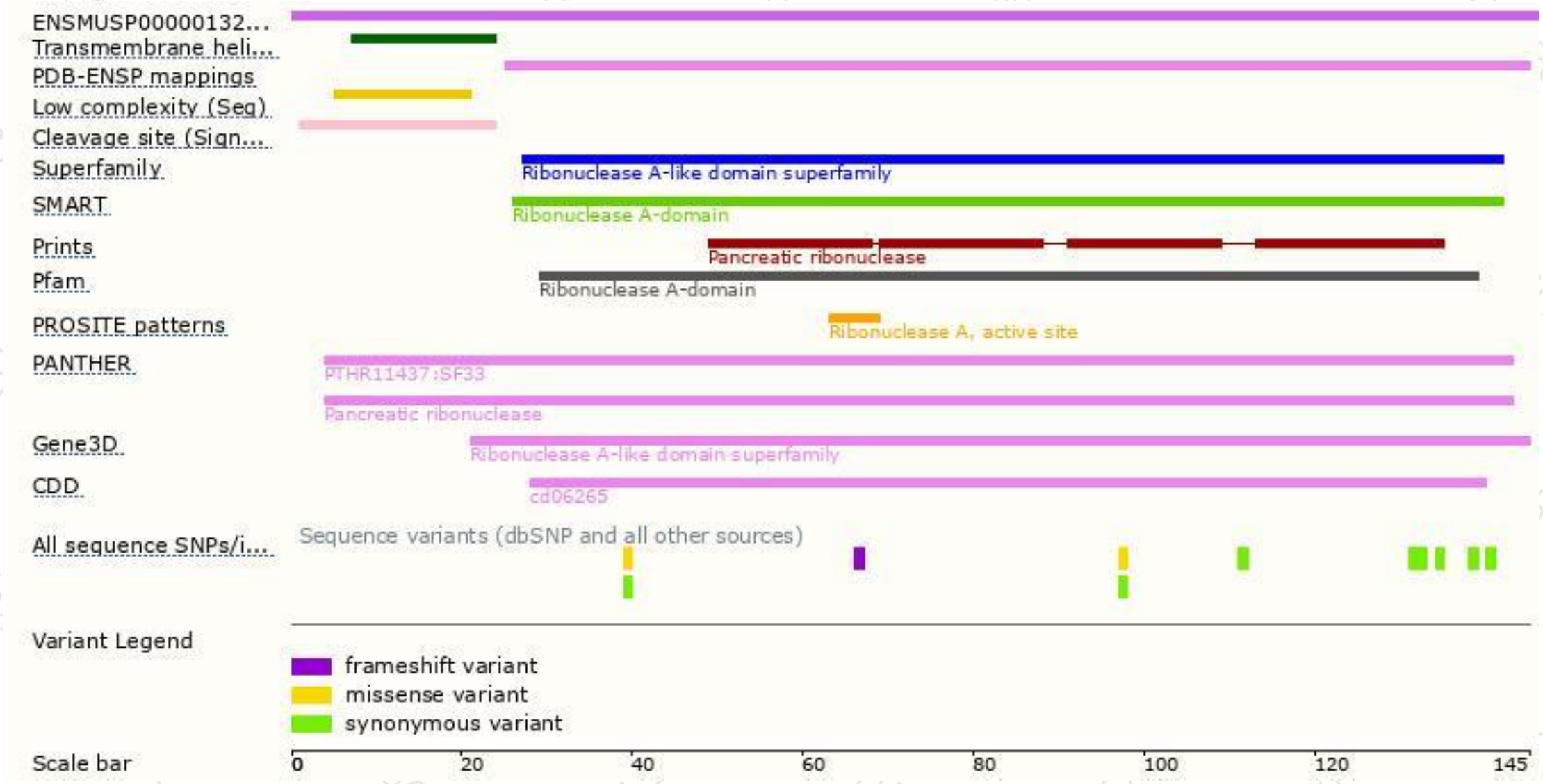
The strategy is based on the design of *Ang-202* transcript,the transcription is shown below:



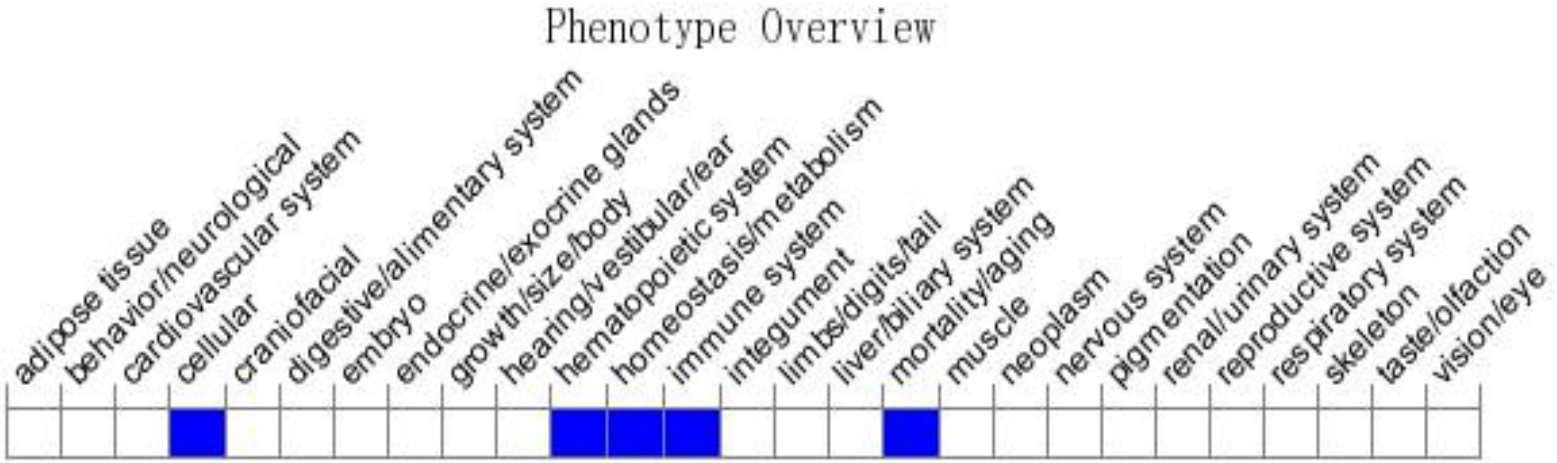
Genomic location distribution



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,knock-out mice show increased proliferative capacity of hematopoietic stem/progenitor cells and decreased proliferative capacity of myeloid-restricted progenitor cells and develop leukopenia.

集萃药康
GemPharmatech

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