

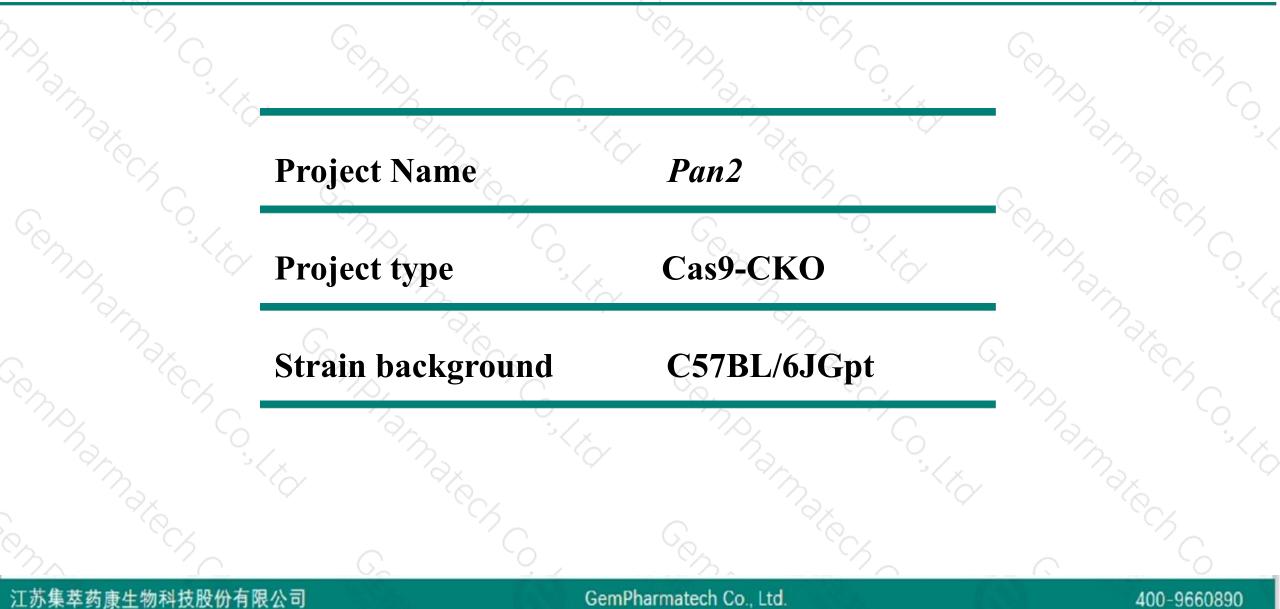
# anphamatech Co. nphamaker College Pan2 Cas9-CKO Strategy Romphamater Coste

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## **Project Overview**

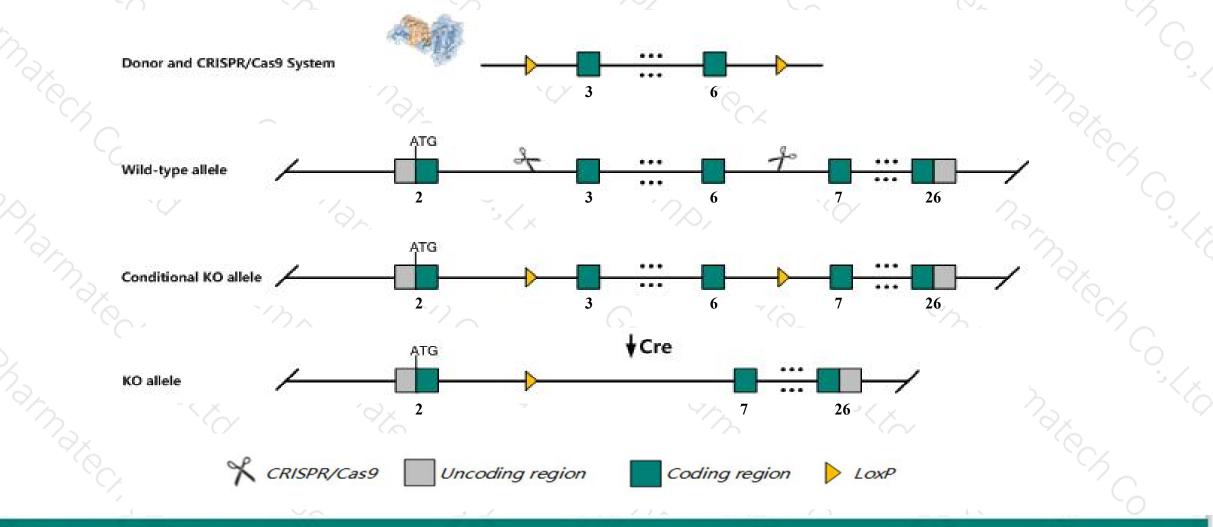




### **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Pan2* gene. The schematic diagram is as follows:



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400-9660890



The Pan2 gene has 7 transcripts. According to the structure of Pan2 gene, exon3-exon6 of Pan2-201 (ENSMUST00000005825.7) transcript is recommended as the knockout region. The region contains 637bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Pan2* 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

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.



- > According to the existing MGI data, Mice homozygous for an ENU-induced allele exhibit embryonic lethality.
- The Pan2 gene is located on the Chr10. 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)**



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### Pan2 PAN2 poly(A) specific ribonuclease subunit [Mus musculus (house mouse)]

Gene ID: 103135, updated on 31-Jan-2019

#### Summary

Pan2 provided by MGI
PAN2 poly(A) specific ribonuclease subunit provided by MGI
MGI:MGI:1918984
Ensembl:ENSMUSG0000005682
protein coding
VALIDATED
Mus musculus
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Muridae; Murinae; Mus; Mus
1200014O24Rik, Al047843, AW742773, Usp52, mKIAA0710
Ubiquitous expression in thymus adult (RPKM 19.4), limb E14.5 (RPKM 15.5) and 28 other tissues See more
human all

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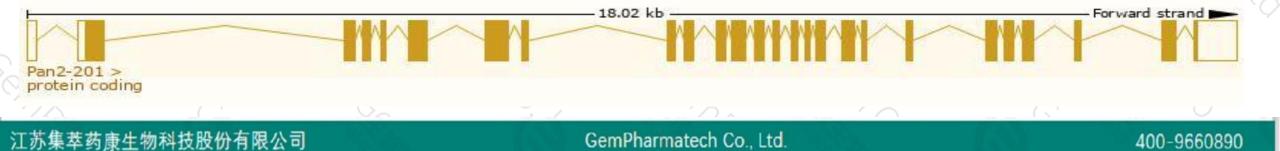
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### The gene has 7 transcripts, all transcripts are shown below:

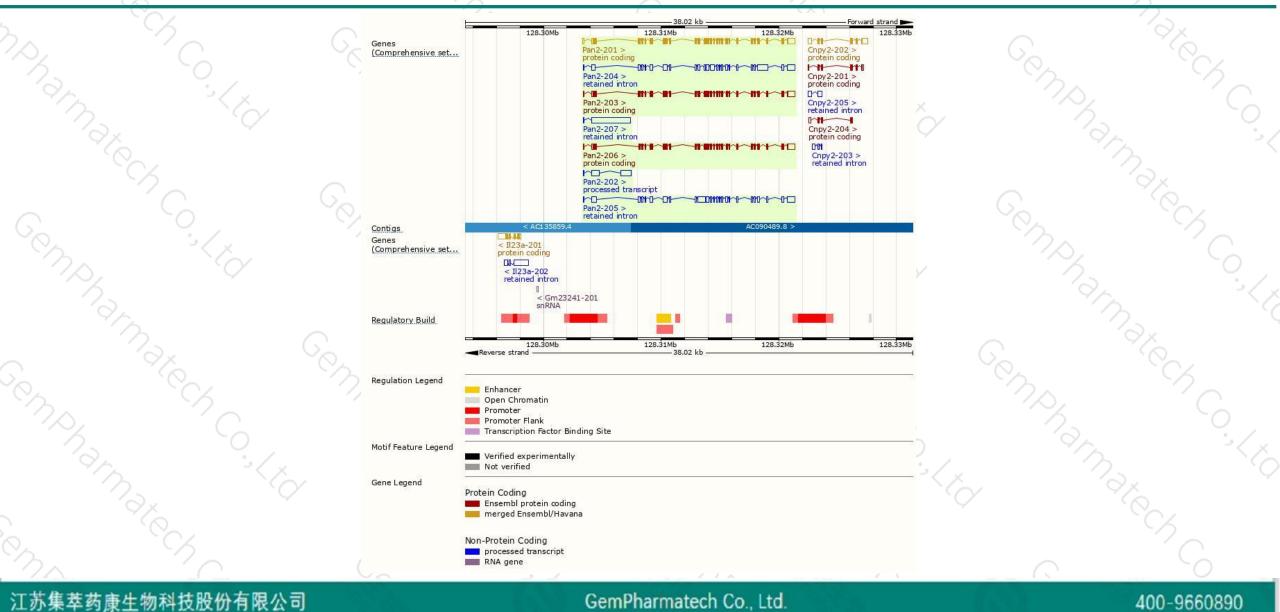
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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pan2-201	ENSMUST0000005825.7	4445	<u>1200aa</u>	Protein coding	CCDS24271	Q8BGF7	TSL:1 GENCODE basic APPRIS P2
Pan2-203	ENSMUST00000218315.1	4379	<u>1191aa</u>	Protein coding	-	Q8BGF7	TSL:1 GENCODE basic APPRIS ALT2
Pan2-206	ENSMUST00000219721.1	4316	<u>1173aa</u>	Protein coding	4	Q8BGF7	TSL:1 GENCODE basic APPRIS ALT2
Pan2-202	ENSMUST00000218137.1	1601	No protein	Processed transcript	2	22	TSL:1
Pan2-204	ENSMUST00000218496.1	5051	No protein	Retained intron		-	TSL:2
Pan2-205	ENSMUST00000219255.1	4759	No protein	Retained intron	-	*	TSL:2
Pan2-207	ENSMUST00000219801.1	3410	No protein	Retained intron	2	-	TSL:1

The strategy is based on the design of Pan2-201 transcript, The transcription is shown below



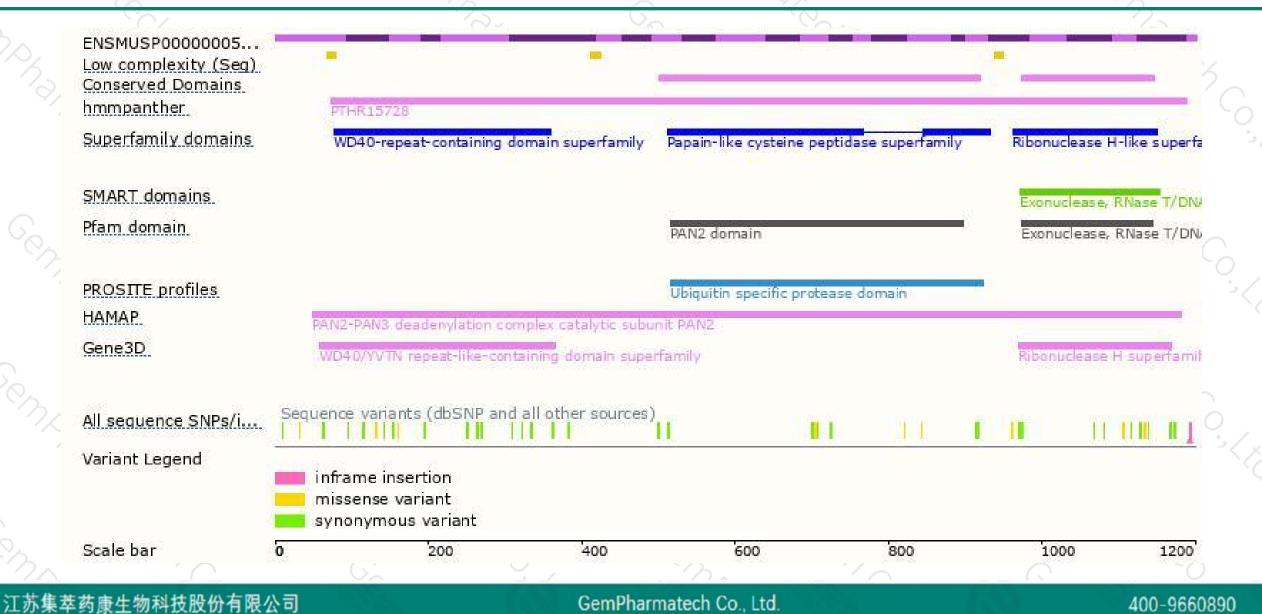
### **Genomic location distribution**





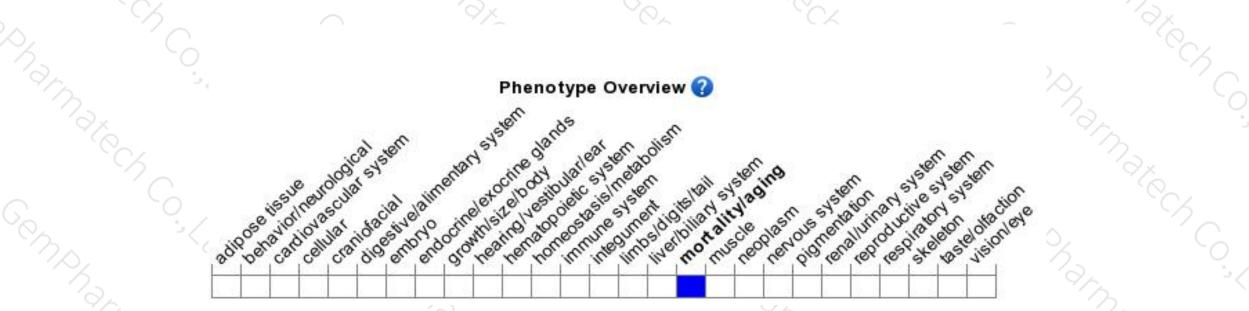
### **Protein domain**





### 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 an ENU-induced allele exhibit embryonic lethality.



If you have any questions, you are welcome to inquire. Tel: 400-9660890



