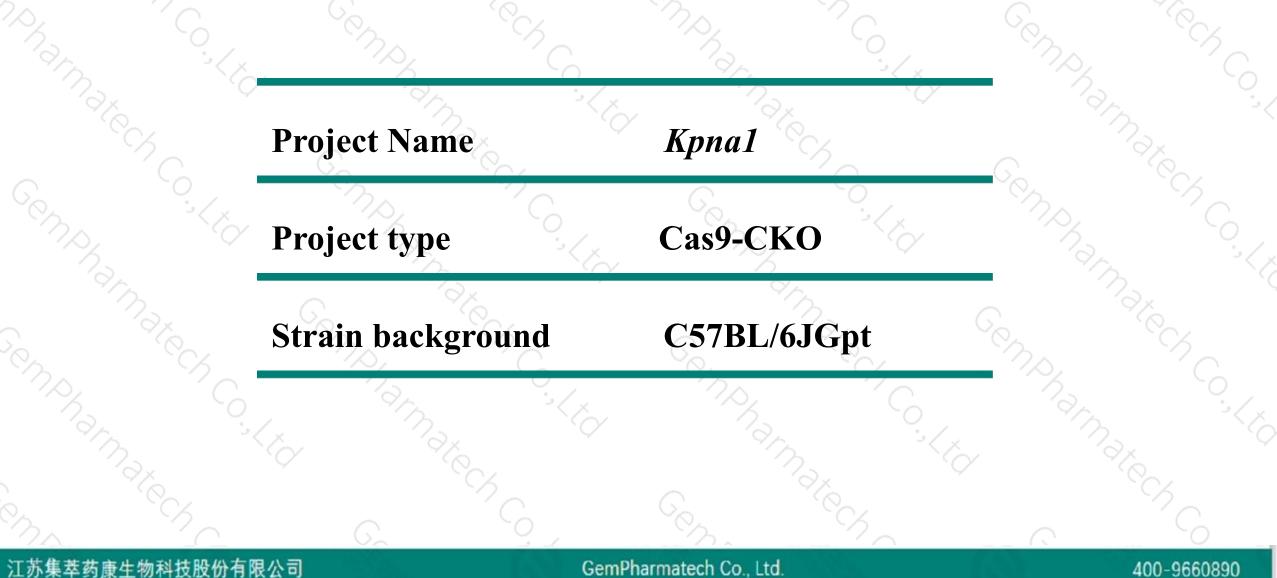


Kpnal Cas9-CKO Strategy

Designer:Xueting Zhang Reviewer:Yanhua Shen Date:2020-1-14

Project Overview



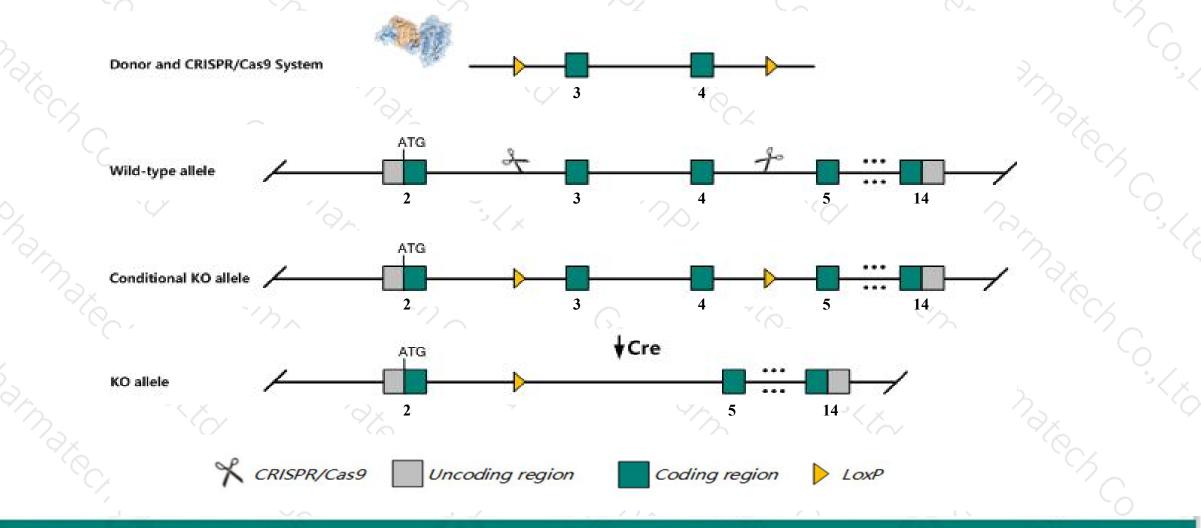


Conditional Knockout strategy



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



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The Kpnal gene has 10 transcripts. According to the structure of Kpnal gene, exon3-exon4 of Kpnal-201 (ENSMUST0000004054.12) transcript is recommended as the knockout region. The region contains 208bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Kpna1* 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 a gene trap allele do not display any nervous system or behavioral abnormalities.
- ≻Transcript *Kpna1*-202&204&206 may not be affected.
- The Kpnal gene is located on the Chr16. 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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0	pna1 karyopherii ene ID: 16646, updated on	the second se	1 [<i>Mus musculus</i> (house mous	se)]					
	Summary				* ?	0			
	Official Full Name Primary source See related Gene type RefSeq status Organism Lineage Also known as	MGI:MGI:103560 Ensembl:ENSMUSG00000022905 protein coding VALIDATED Mus musculus Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia Myomorpha; Muroidea; Murinae; Mus; Mus NPI1; Rch2; IPOA5; mSRP1; AW494490 Ubiquitous expression in CNS E18 (RPKM 11.6), cortex adult (RPKM 11.3) and 28 other tissues <u>See more</u>							
°~, e	Genomic context				≈ ?				
Location: 16; 16 B3 See Kpna1 in Gen Exon count: 15									
	Annotation release	Status	Assembly	Chr	Location				
7	<u>108</u> Build 37.2	current previous assembly	GRCm38.p6 (<u>GCF_000001635.26</u>) MGSCv37 (<u>GCF_000001635.18</u>)	16 16	NC_000082.6 (3598328836037136) NC_000082.5 (3598344936036248)				

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Transcript information (Ensembl)



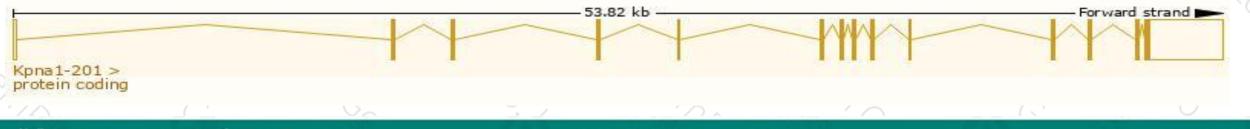
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The gene has 10 transcripts, all transcripts are shown below:

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Kpna1-201	ENSMUST0000004054.12	5039	<u>538aa</u>	Protein coding	CCDS28144	<u>Q60960</u>	TSL:1 GENCODE basic APPRIS P1
Kpna1-205	ENSMUST00000173555.7	442	<u>119aa</u>	Protein coding	-	G3UWJ8	CDS 3' incomplete TSL:5
Kpna1-202	ENSMUST00000172534.7	2960	<u>45aa</u>	Nonsense mediated decay	-	G3UYD2	TSL:5
Kpna1-207	ENSMUST00000173696.7	1740	<u>182aa</u>	Nonsense mediated decay	-	G3UZK5	TSL:5
Kpna1-209	ENSMUST00000174500.7	780	<u>145aa</u>	Nonsense mediated decay		G3UXT6	TSL:5
Kpna1-210	ENSMUST00000174737.1	532	<u>98aa</u>	Nonsense mediated decay	-	G3UXW7	CDS 5' incomplete TSL:5
Kpna1-208	ENSMUST00000173715.1	2664	No protein	Retained intron	-	2	TSL:1
Kpna1-204	ENSMUST00000173469.1	815	No protein	Retained intron	-	2	TSL:2
Kpna1-203	ENSMUST00000172991.1	667	No protein	Retained intron			TSL:1
Kpna1-206	ENSMUST00000173641.1	690	No protein	IncRNA		-	TSL:5

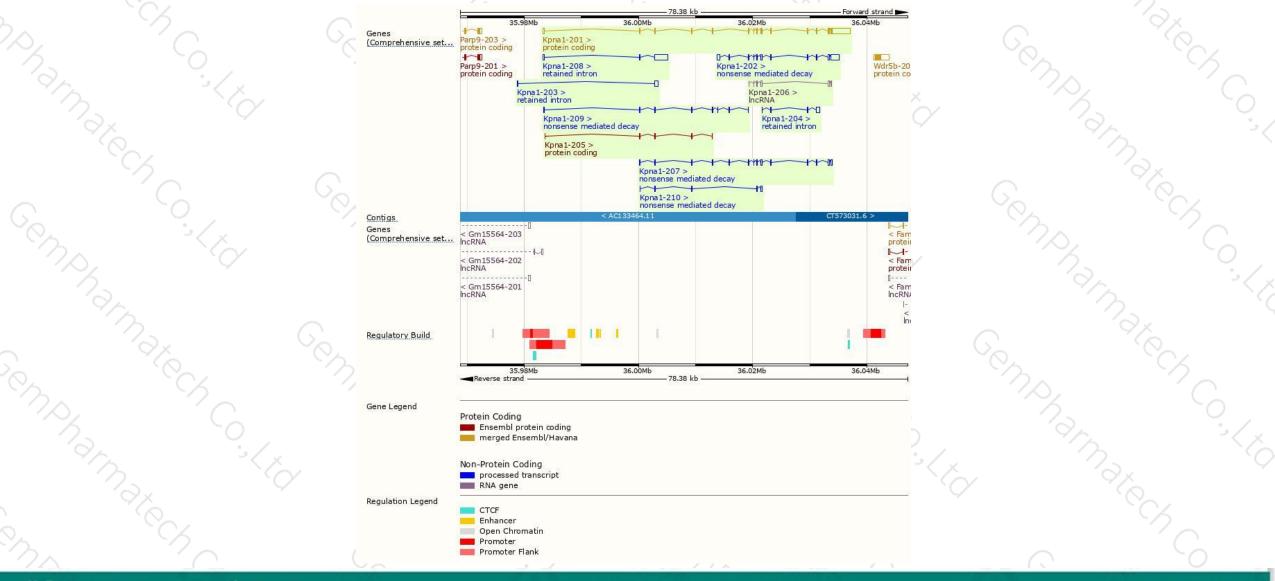
The strategy is based on the design of Kpna1-201 transcript, The transcription is shown below



Genomic location distribution



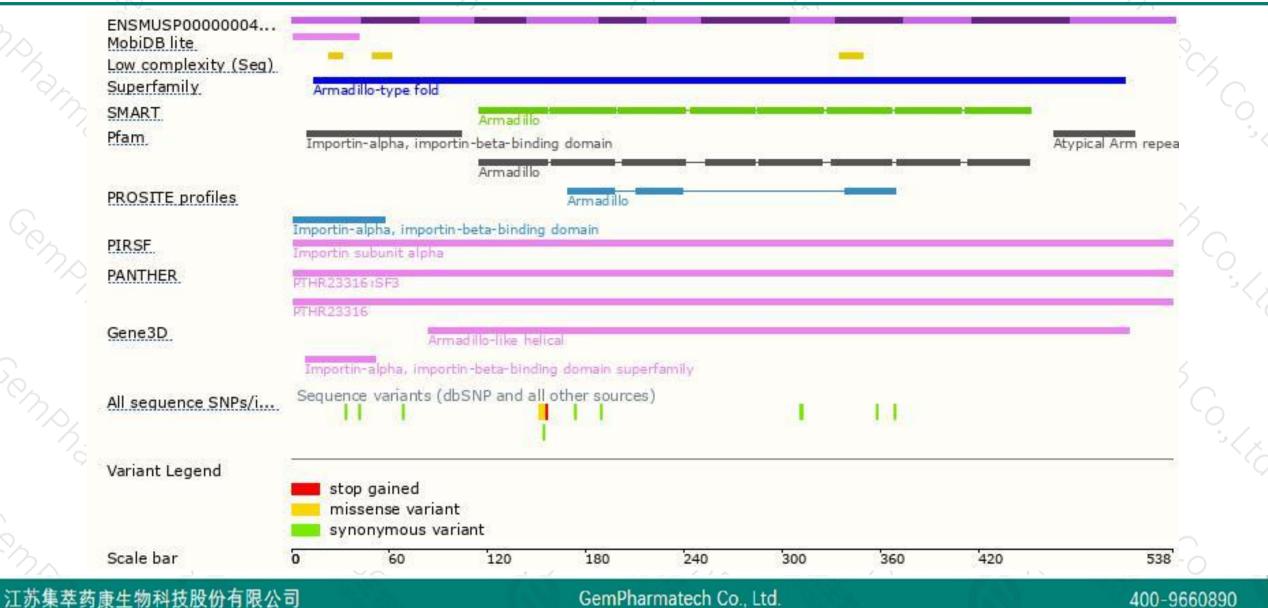
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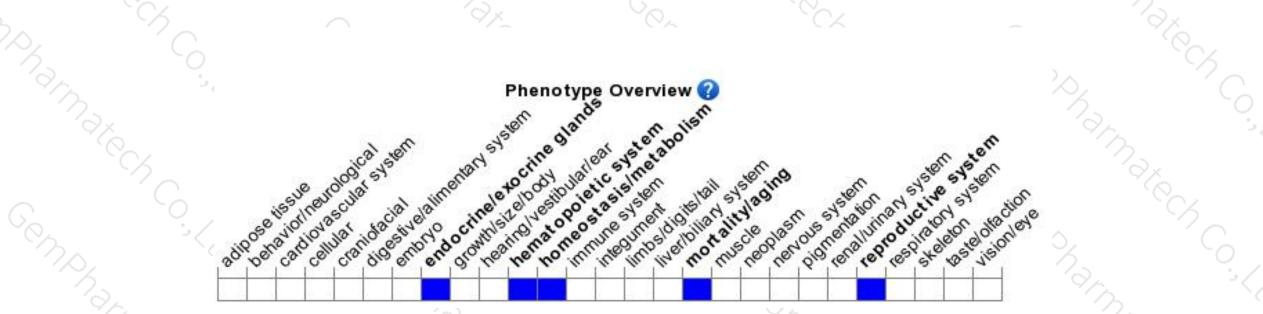
Protein domain





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 gene trap allele do not display any nervous system or behavioral abnormalities.



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



