

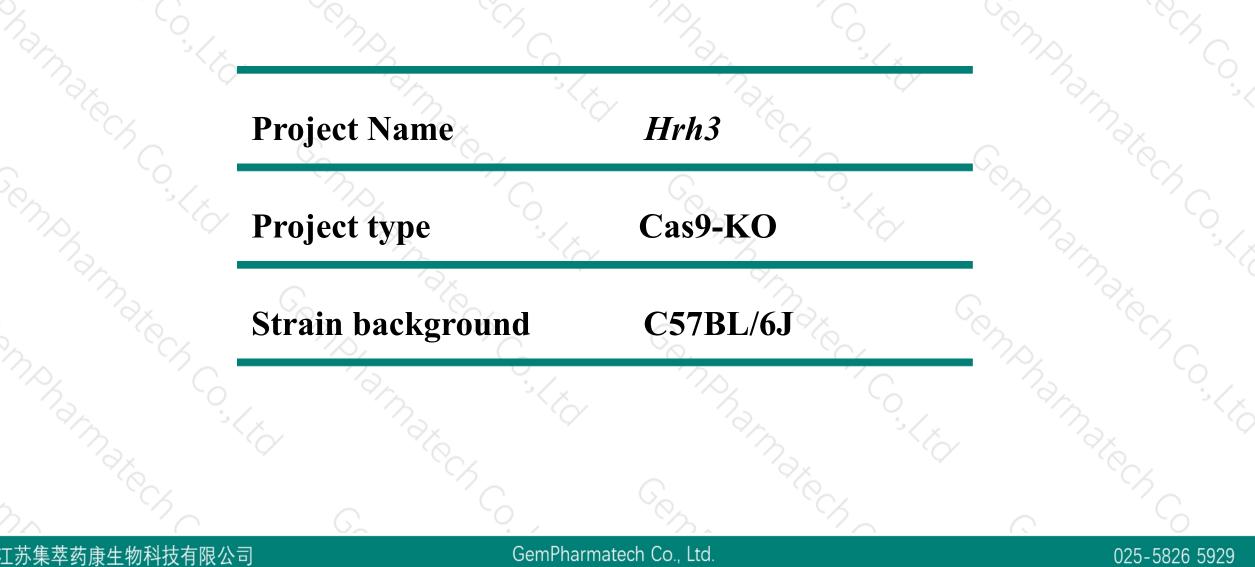
# ND Harmate Cho-1ty Hrh3 Cas9-KO Strategy Enphamate F Conplanated Co-ty

Emphamaten C. Lt. JiaYu

empharmatech Co.

## **Project Overview**





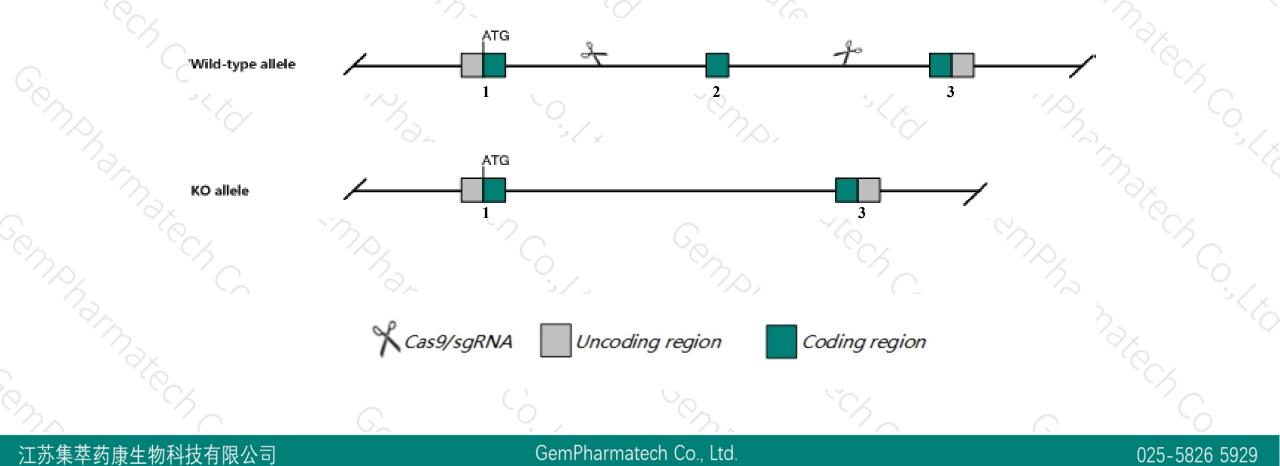
江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.

# **Knockout strategy**

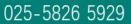


This model will use CRISPR/Cas9 technology to edit the *Hrh3* gene. The schematic diagram is as follows:





- The *Hrh3* gene has 8 transcripts. According to the structure of *Hrh3* gene, exon2 of *Hrh3-201* (ENSMUST00000056480.9) transcript is recommended as the knockout region. The region contains 167bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Hrh3* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.



025-5826 5929

- According to the existing MGI data, Homozygotes for a targeted null mutation exhibit reduced locomotor activity and body temperature, and attenuated behavioral responses to the drugs thioperamide, methamphetamine, and scopolamine.
- The Hrh3 gene is located on the Chr2. 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.

Notice

# **Gene information (NCBI)**



< ?

#### Hrh3 histamine receptor H3 [Mus musculus (house mouse)]

Gene ID: 99296, updated on 12-Mar-2019

#### Summary

Official Symbol	Hrh3 provided by MGI
Official Full Name	histamine receptor H3 provided by MGI
Primary source	MGI:MGI:2139279
See related	Ensembl:ENSMUSG00000039059
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
	Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW049250, Eae8, H3R, HH3R
Expression	Biased expression in frontal lobe adult (RPKM 17.2), cortex adult (RPKM 16.0) and 8 other tissuesSee more
Orthologs	human all

#### 江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.



## **Transcript information (Ensembl)**



025-5826 5929

#### The gene has 8 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hrh3-201	ENSMUST0000056480.9	2706	<u>445aa</u>	Protein coding	CCDS17169	P58406 Q540P3	TSL:1 GENCODE basic APPRIS P2
Hrh3-202	ENSMUST00000163215.7	1401	<u>466aa</u>	Protein coding	-	<u>E9Q540</u>	TSL:5 GENCODE basic
Hrh3-205	ENSMUST00000165762.7	1368	<u>455aa</u>	Protein coding	-	<u>E9Q292</u>	TSL:5 GENCODE basic APPRIS ALT2
Hrh3-203	ENSMUST00000164442.1	1242	<u>413aa</u>	Protein coding	-	E9Q5S3	TSL:5 GENCODE basic APPRIS ALT2
Hrh3-204	ENSMUST00000165248.7	1224	<u>407aa</u>	Protein coding	-	<u>E9Q7T5</u>	TSL:5 GENCODE basic APPRIS ALT2
Hrh3-208	ENSMUST00000171736.7	906	<u>301aa</u>	Protein coding	-	<u>E9Q522</u>	TSL:5 GENCODE basic
Hrh3-207	ENSMUST00000166724.1	1574	<u>94aa</u>	Nonsense mediated decay	-	E9PZM9	TSL:1
Hrh3-206	ENSMUST00000166392.1	1040	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Hrh3-201* transcript, The transcription is shown below

#### < Hrh3-201 protein coding

Reverse strand

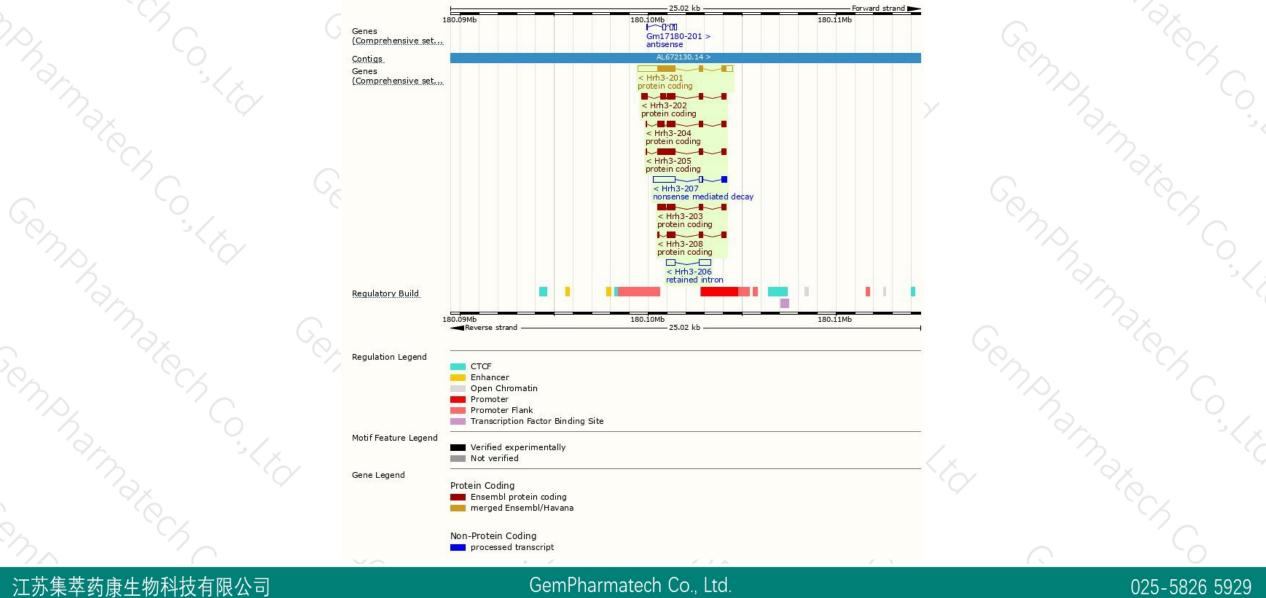
江苏集萃药康生物科技有限公司

#### GemPharmatech Co., Ltd.

5.02 kb

### **Genomic location distribution**





江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.

## **Protein domain**



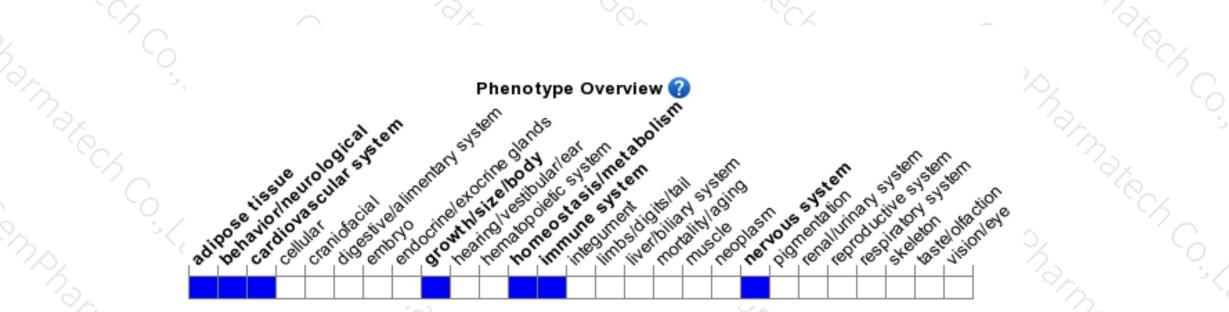
江苏集萃药康生物科技有

All sequence SNPs/i	Seau	ience vari	ants (dbS	NP and	all othe	SOUTHEST							
			.1070.10	ND	all atha						-	 	
Gene3D					G proteii	n-coupled re	eceptor, rhod	lopsin-like		1.20.120	0.10		
PROSITE profiles PROSITE patterns		G	SPCR, rhodo		_							-	
Pfam domain		10000	<mark>tein-couple</mark> 3 protein-co		100	p <mark>sin-like</mark> hodopsin-lik	(e						-0-36
Prints domain	Hista	nine H3 re	ceptor							-			
Superfamily domains SMART domains	PTI	IR44152 SSF813 G p	and the second se	pled rece	eptor, rhe	od opsin-like				-	_		
MobiDB lite Low complexity (Seg) Conserved Domains hmmpanther		IR44152:S	F4					-	-	-			

### Mouse phenotype description(MGI)



025-5826 5929



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, Homozygotes for a targeted null mutation exhibit reduced locomotor activity and body temperature, and attenuated behavioral responses to the drugs thioperamide, methamphetamine, and scopolamine.



If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



