

# Clstn1 Cas9-CKO Strategy

Designer: Reviewer:

**Design Date:** 

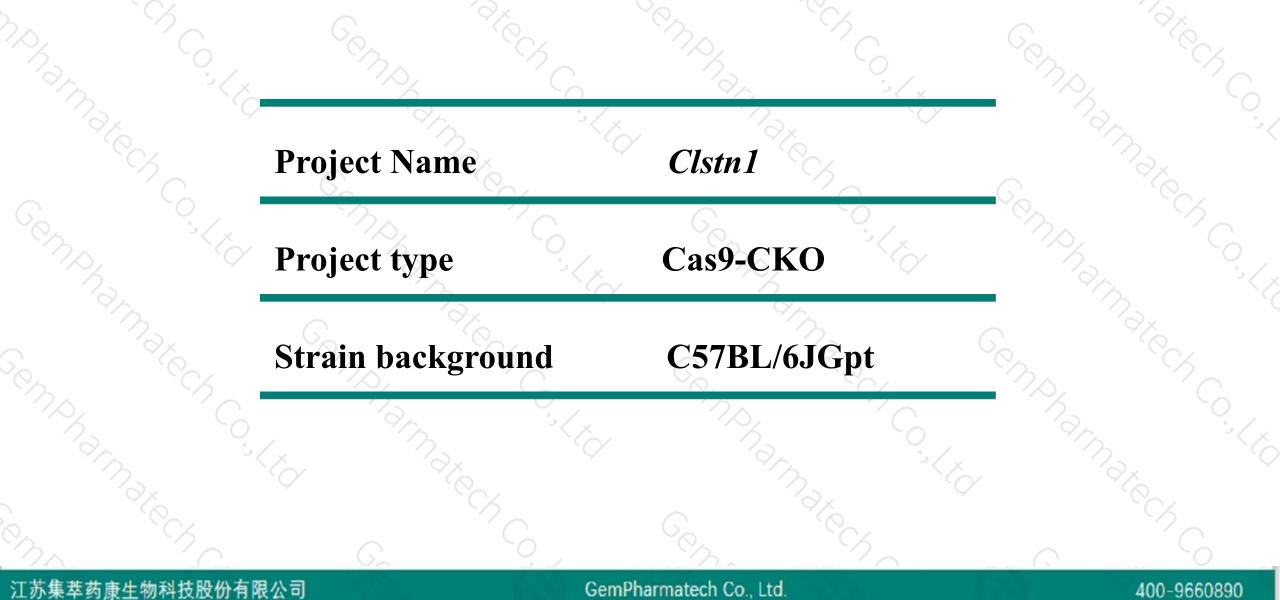
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2020-2-21

# **Project Overview**



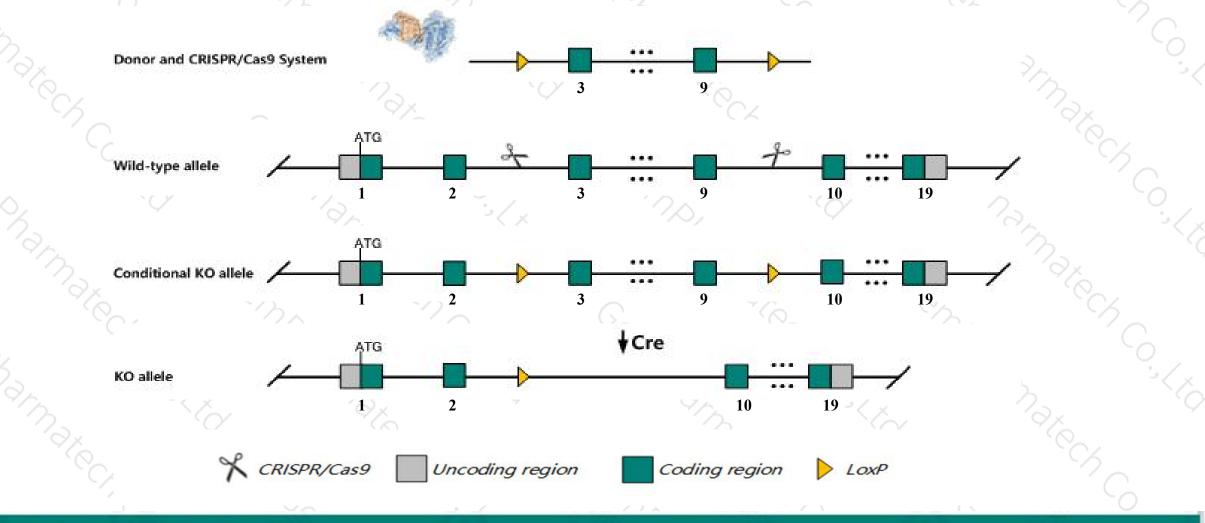


## **Conditional Knockout strategy**



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This model will use CRISPR/Cas9 technology to edit the *Clstn1* gene. The schematic diagram is as follows:



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The Clstn1 gene has 4 transcripts. According to the structure of Clstn1 gene, exon3-exon9 of Clstn1-201 (ENSMUST00000039144.6) transcript is recommended as the knockout region. The region contains 1142bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Clstn1* 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, Juvenile mice homozygous for a null allele show reduced basal excitatory synaptic transmission, abnormal excitatory postsynaptic currents, enhanced NMDA receptor-dependent long term potentiation, and delayed dendritic spine maturation in CA1 hippocampal pyramidal cells.
- The Clstn1 gene is located on the Chr4. 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)

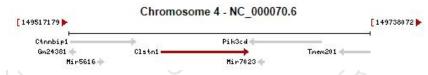


#### Clstn1 calsyntenin 1 [ Mus musculus (house mouse) ]

Gene ID: 65945, updated on 5-Jan-2020

Summary		2	
Official Symbol	Clstn1 provided by MGI		
Official Full Name	calsyntenin 1 provided by MGI		
Primary source	MGI:MGI:1929895		
See related	Ensembl:ENSMUSG0000039953		
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	Cst-1; Cstn1; 1810034E21Rik		
Expression	Broad expression in cortex adult (RPKM 93.0), frontal lobe adult (RPKM 80.3) and 23 other tissues See more		
Orthologs	human all		

<ul> <li>Genomic context</li> </ul>				
Location: 4; 4 E2 Exon count: 21				See Clstn1 in Genome Data Viewe
Annotation release	Status	Assembly	Chr	Location
<u>108</u> Build 37.2	current previous assembly	GRCm38.p6 ( <u>GCF_000001635.26</u> ) MGSCv37 ( <u>GCF_000001635.18</u> )	4	NC_000070.6 (149585111149648899) NC_000070.5 (148960747149022008)



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# **Transcript information (Ensembl)**



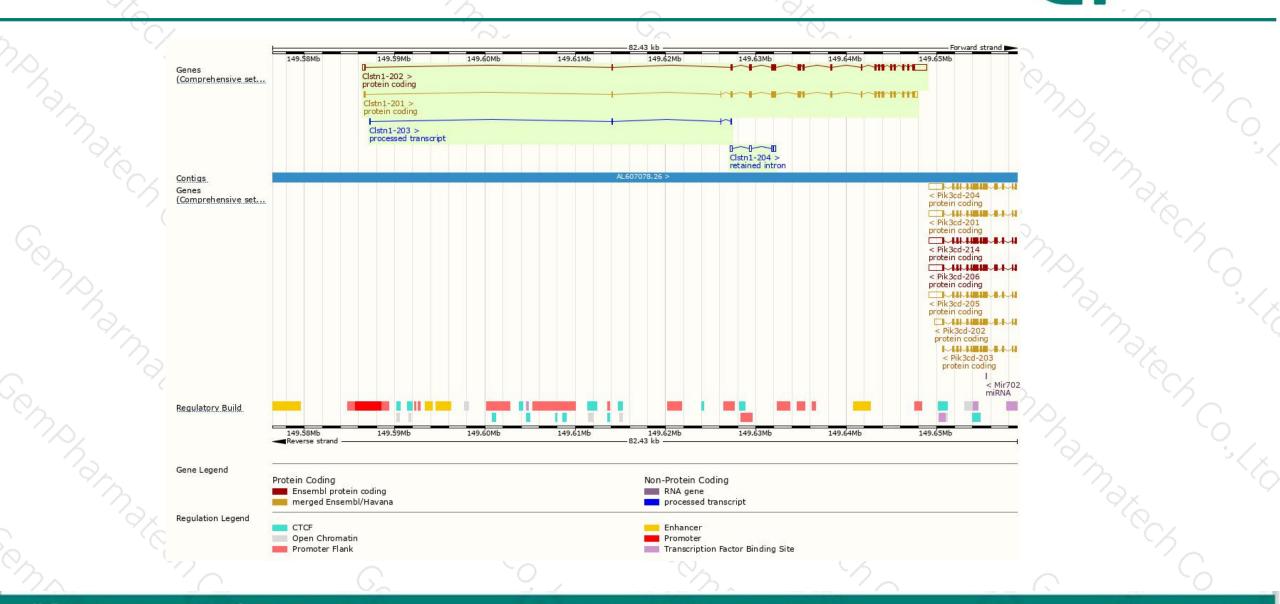
The gene has 4 transcripts, all transcripts are shown below:

Name 🍦	Transcript ID 🖕	bp 🍦	Protein 🖕	Biotype 🛔	CCDS	UniProt 🖕	Flags		
Clstn1-202	ENSMUST00000105691.7	<mark>44</mark> 59	<u>969aa</u>	Protein coding	CCDS71522	<u>Q9EPL2</u> 교	TSL:1	GENCODE basic	APPRIS ALT2
Clstn1-201	ENSMUST0000039144.6	3319	<u>979aa</u>	Protein coding	<u>CCDS18963</u> 교	<u>Q9EPL2</u> &	TSL:1	GENCODE basic	APPRIS P3
Clstn1-203	ENSMUST00000137232.1	358	No protein	Processed transcript	1.5	7	TSL:3		
Clstn1-204	ENSMUST00000151895.1	822	No protein	Retained intron		π.	TSL:2		

The strategy is based on the design of *Clstn1-201* transcript, The transcription is shown below



### **Genomic location distribution**



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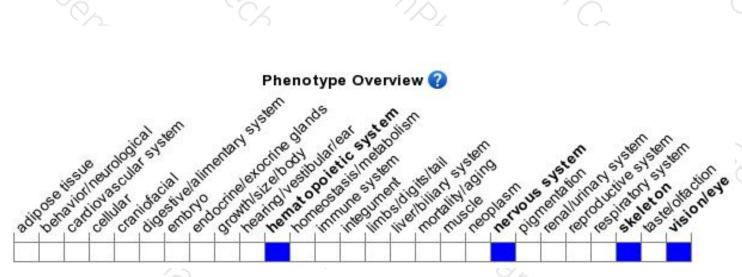
### **Protein domain**



$\langle \mathcal{O}_{\mathcal{L}} \rangle$				$\sim$						
73	ENSMUSP00000036 Transmembrane heli PDB-ENSP mappings MobiDB lite Low complexity (Seg) Coiled-coils (Ncoils) Cleavage site (Sign					-	-	-	=	200.
	Superfamily	Cadherin-like superfamily		Concanavalin A-like lee	ctin/glucanase domain supe	erfamily				
	SMART	Cadherin-like	-							
	Prints	Cadherin-like								
60	Pfam.		Cadherin-like	PF13385						
1	PROSITE profiles	PS50268								
	PANTHER	PTHR14139:SF4								`s /
		Calsyntenin								
	Gene3D	2.60.40.60		2,60,120,200						
	CDD	cd11304								
	All sequence SNPs/i	Sequence variants (dbSNP an	d all other sources)	U.L. I. I.U	1.1.1.1	C D.C. D	U. U. U. U	ET (= 300)	1 11 1	$\sim$
D,	Variant Legend	missense variant			splice regi	on variant			(	°0,
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## 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, Juvenile mice homozygous for a null allele show reduced basal excitatory synaptic transmission, abnormal excitatory postsynaptic currents, enhanced NMDA receptor-dependent long term potentiation, and delayed dendritic spine maturation in CA1 hippocampal pyramidal cells.

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



