

# Ap4s1 Cas9-KO Strategy

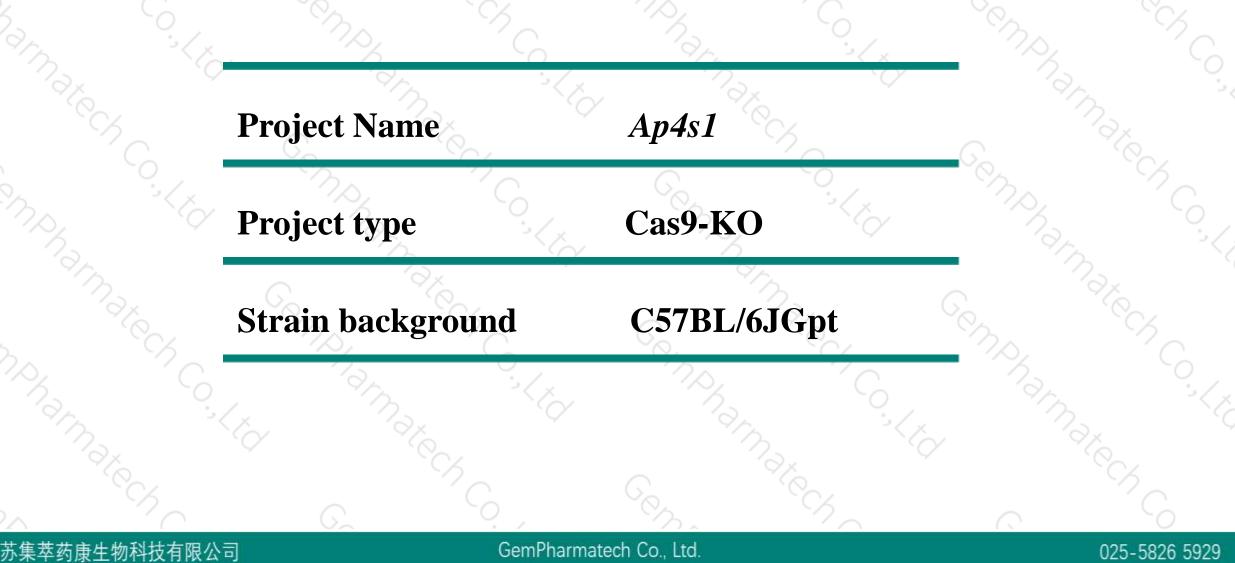
Designer: Reviewer:

**Design Date:** 

Daohua Xu Huimin Su 2019-11-14

# **Project Overview**

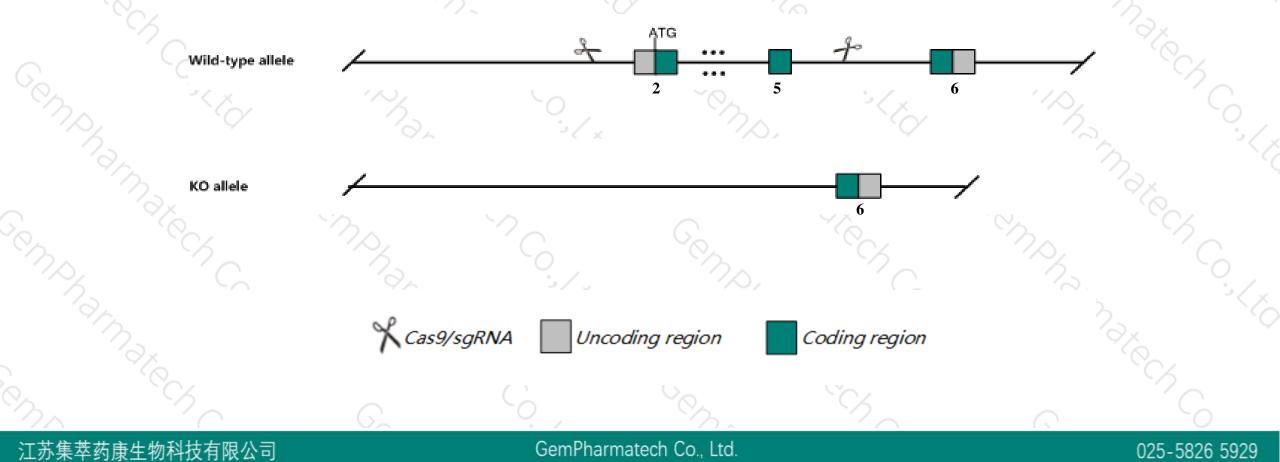




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This model will use CRISPR/Cas9 technology to edit the *Ap4s1* gene. The schematic diagram is as follows:





- The Ap4s1 gene has 2 transcripts. According to the structure of Ap4s1 gene, exon2-exon5 of Ap4s1-201 (ENSMUST0000021338.9) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify Ap4s1 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.





- The Ap4s1 gene is located on the Chr12. 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)**



025-5826 5929

### Ap4s1 adaptor-related protein complex AP-4, sigma 1 [Mus musculus (house mouse)]

Gene ID: 11782, updated on 31-Jan-2019

#### Summary

Official Symbol	Ap4s1 provided by MGI
Official Full Name	adaptor-related protein complex AP-4, sigma 1 provided by MGI
Primary source	MGI:MGI:1337065
See related	Ensembl:ENSMUSG00000020955
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	Al314282
Summary	This gene encodes the sigma subunit of the adaptor-related protein complex 4 which mediates intracellular membrane trafficking along the
	endocytic and secretory transport pathways. This complex contains four subunits, beta, epsilon, mu, and sigma, and belongs to a family of
	five adapter protein complexes, including three clathrin-associated complexes and two non clathrin-associated complexes, that localize to different intracellular compartments and mediate membrane vesicle trafficking using distinct pathways. In humans, loss-of-function mutations
	in this gene have been linked to specific adapter complex 4 deficiency disorders including hereditary spastic paraplegia. Alternate splicing
	results in multiple transcript variants. [provided by RefSeq, Jul 2016]
Expression	Ubiquitous expression in cerebellum adult (RPKM 9.3), frontal lobe adult (RPKM 8.9) and 28 other tissues <u>See more</u>
Orthologs	human all
5	

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# **Transcript information (Ensembl)**



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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ap4s1-201	ENSMUST00000021338.9	1035	<u>144aa</u>	Protein coding	CCDS36441	<u>Q9WVL1</u>	TSL:1 GENCODE basic APPRIS P1
Ap4s1-202	ENSMUST00000218820.1	757	<u>134aa</u>	Protein coding	-	A0A1W2P708	CDS 3' incomplete TSL:5

The strategy is based on the design of Ap4s1-201 transcript, The transcription is shown below

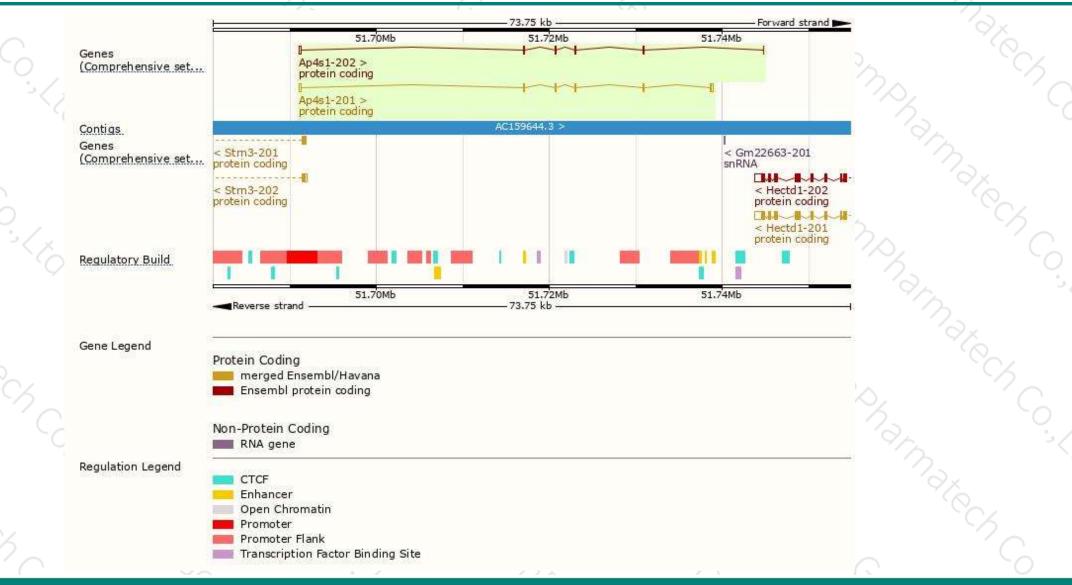
			- 47.9	0 kb		Forwa	ard strand
Ap4s1-201 > protein coding					- *)		U
$\sim$	( '	V.		10.		(5)	

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### **Genomic location distribution**





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# **Protein domain**



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ENSMUSP00000021		~ <u></u> .					2.	
Superfamily		omain superfam	ily.					
Pfam.	AP complex,	mu/sigma subu	nit					19
PIRSF	Adapter prot	ein complex, sig	ma subunit					
PANTHER	Adaptor prot	ein complex, sig	ma subunit					
C 2D	PTHR11753:	SF4						
Gene3D	3.30.450.60							
All sequence SNPs/i	Sequence v	ariants (dbSNF	and all other so	urces)	11			
All sequence SNPs/i Variant Legend	2	11		urces)	1			
	2	variants (dbSNF vmous variant 20		iurces) 60	80	'100	120	144
Variant Legend	2	/mous variant			80	100	120	144 <sup>°</sup>
Variant Legend	2	/mous variant			80	100	120	144

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



