

# Alg14 Cas9-KO Strategy

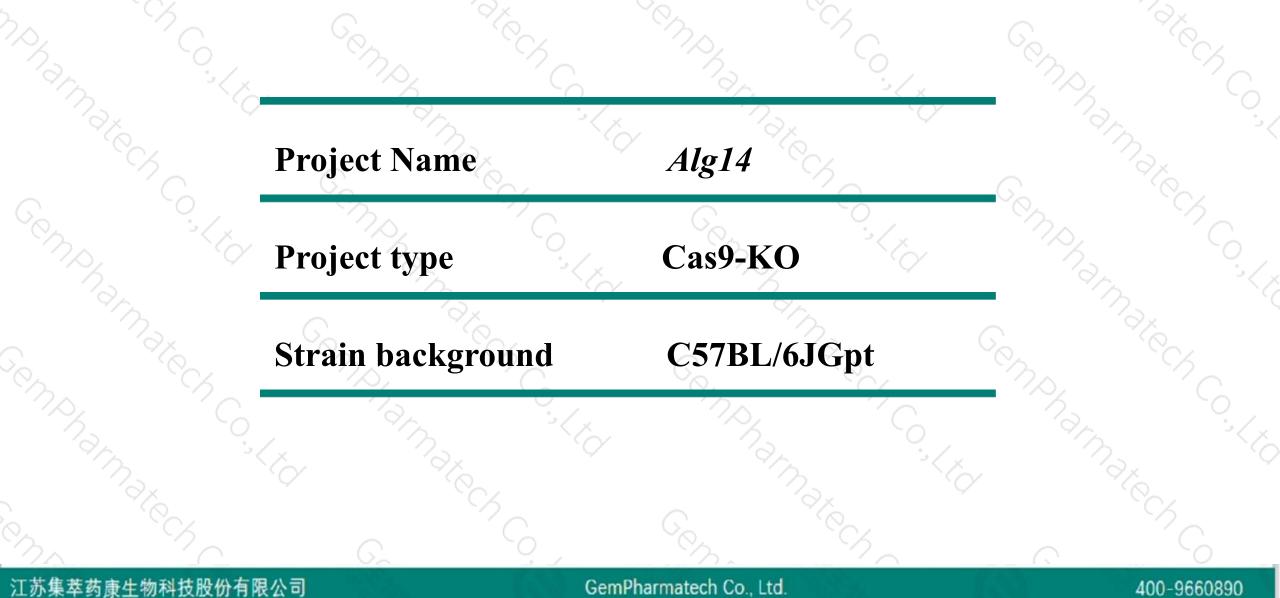
**Designer:** Jing Jin

**Reviewer:** Xiaojing Li

Design Date: 2018-10-25

### **Project Overview**

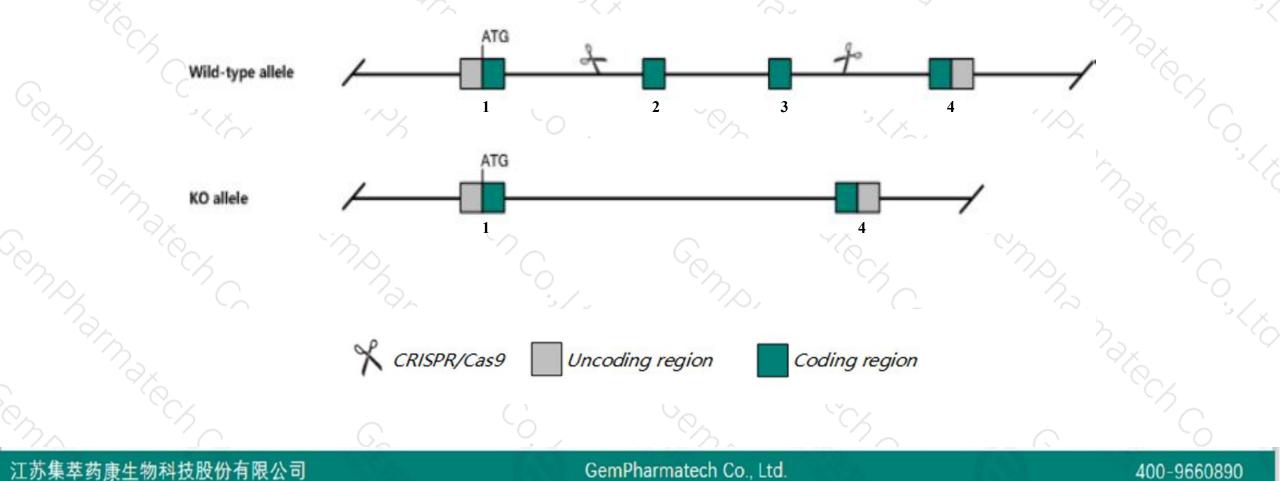




### **Knockout** strategy



This model will use CRISPR/Cas9 technology to edit the *Alg14* gene. The schematic diagram is as follows:





> The *Alg14* gene has 4 transcripts. According to the structure of *Alg14* gene, exon2-exon3 of *Alg14*-201(ENSMUST00000039442.11) transcript is recommended as the knockout region. The region contains 287bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Alg14* gene. The brief process is as follows: CRISPR/Cas9 system 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.



- > According to the existing MGI data,mice homozygous for a knock-out allele show complete embryonic lethality between implantation and somite formation and failure of blastocysts to hatch from the zona pellucida with apparent necrosis during outgrowth culture.
- > The Alg14 gene is located on the Chr3. 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)**



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### Alg14 asparagine-linked glycosylation 14 [Mus musculus (house mouse)]

Gene ID: 66789, updated on 13-Mar-2020

#### Summary

Official Symbol	Alg14 provided by MGI
<b>Official Full Name</b>	asparagine-linked glycosylation 14 provided by MGI
<b>Primary source</b>	MGI:MGI:1914039
See related	Ensembl:ENSMUSG0000039887
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	5430428G01Rik, AI854024
Expression	Ubiquitous expression in lung adult (RPKM 8.4), subcutaneous fat pad adult (RPKM 8.1) and 28 other tissuesSee more
Orthologs	human all

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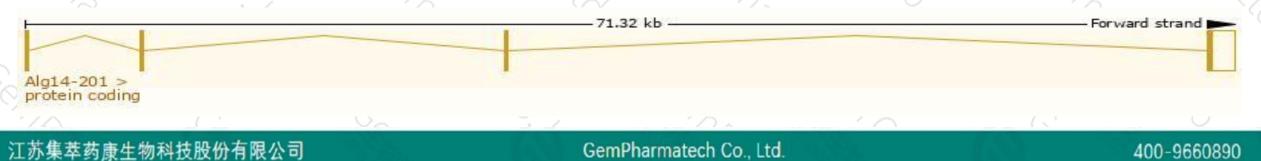
# **Transcript information (Ensembl)**



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

Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags		
ENSMUST0000039442.11	2037	<u>217aa</u>	Protein coding	CCDS17801	<u>Q9D081</u>	TSL:1 GENCODE basic APPRIS P1		
ENSMUST00000198341.1	831	<u>133aa</u>	Protein coding	£	A0A0G2JEH5	CDS 5' incomplete TSL:2		
ENSMUST00000199554.1	441	<u>92aa</u>	Protein coding	<u>2</u>	A0A0G2JEQ0	TSL:3 GENCODE basic		
ENSMUST00000198235.1	474	<u>60aa</u>	Nonsense mediated decay		A0A0G2JF20	TSL:3		
	ENSMUST0000039442.11 ENSMUST00000198341.1 ENSMUST00000199554.1	ENSMUST0000039442.11         2037           ENSMUST00000198341.1         831           ENSMUST00000199554.1         441	ENSMUST0000039442.11         2037         217aa           ENSMUST00000198341.1         831         133aa           ENSMUST00000199554.1         441         92aa	ENSMUST0000039442.112037217aaProtein codingENSMUST00000198341.1831133aaProtein codingENSMUST00000199554.144192aaProtein coding	ENSMUST0000039442.112037217aaProtein codingCCDS17801ENSMUST0000198341.1831133aaProtein coding-ENSMUST0000199554.144192aaProtein coding-	ENSMUST0000039442.112037217aaProtein codingCCDS17801Q9D081ENSMUST0000198341.1831133aaProtein coding-A0A0G2JEH5ENSMUST0000199554.144192aaProtein coding-A0A0G2JEQ0		

The strategy is based on the design of *Alg14-201* transcript, the transcription is shown below:



### **Genomic location distribution**

Genes

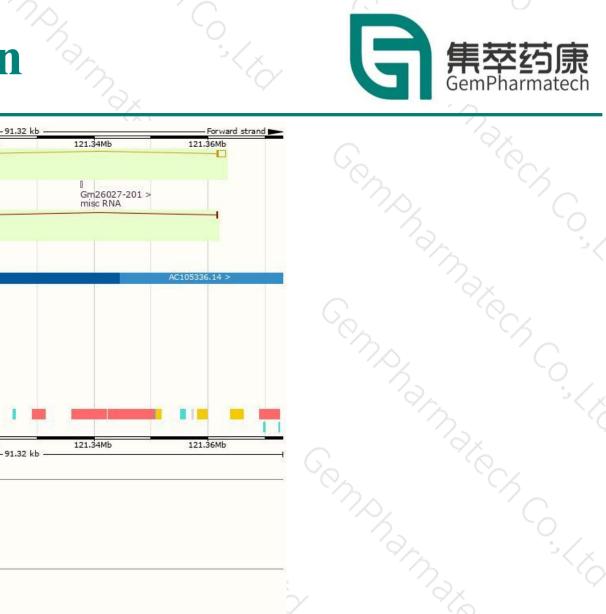
Contigs Genes

(Comprehensive set...

(Comprehensive set...

**Regulatory Build** 

Regulation Legend



Gene Legend

Protein Coding merged Ensembl/Havana Ensembl protein coding

Transcription Factor Binding Site

121.30Mb

nonsense mediated decay

Alg14-201 > protein coding

Alg14-202 >

Alg14-204 > protein coding Alg14-203 > protein coding

< Tmem56-204 protein coding

< Tmem56-205 protein coding

< Tmem56-206 processed transcript

< Tmem56-202 processed transcript

Reverse strand

CTCF Enhancer Open Chromatin Promoter Promoter Flank

H

121.32Mb

< AC161210.5

121.32Mb

121.30Mb

Non-Protein Coding

RNA gene
processed transcript

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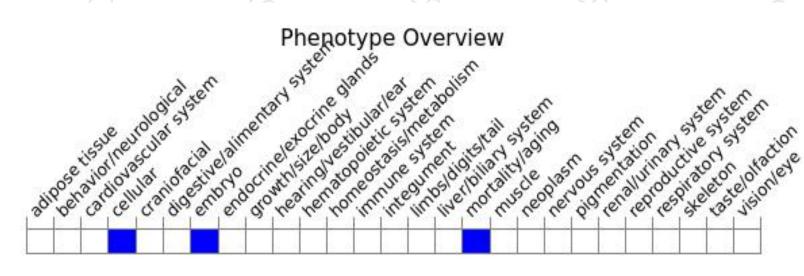
### **Protein domain**



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0	<u>Pfam</u>		Oligosaccharide biosynthesis protein Alg14-like										
00	PANTHER	Oli	gosaccharid	e biosynthes	sis protein A	lg14-like							
	Gene3D			3.40.50.200	20	004 TAUADANARA A							<u>`</u> 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,mice homozygous for a knock-out allele show complete embryonic lethality between implantation and somite formation and failure of blastocysts to hatch from the zona pellucida with apparent necrosis during outgrowth culture.

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



