

Alg1 Cas9-CKO Strategy

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Reviewer: Xiaojing Li

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Project Overview



Project Name Alg1

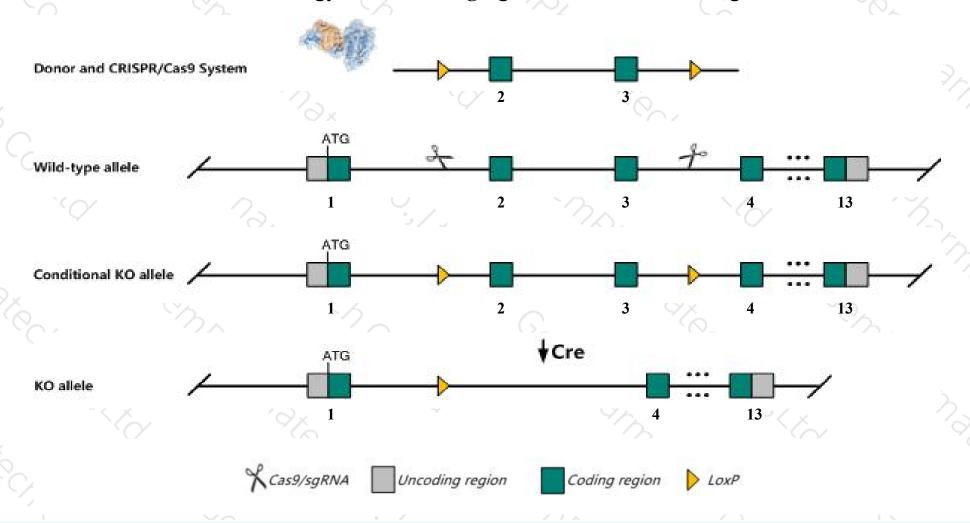
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Alg1 gene. The schematic diagram is as follows:



Technical routes



- ➤ The Alg1 gene has 5 transcripts. According to the structure of Alg1 gene, exon2-exon3 of Alg1202(ENSMUST00000100196.8) transcript is recommended as the knockout region. The region contains 182bp coding sequence.

 Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Alg1* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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.

Notice



- > The Alg1 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)



Alg1 asparagine-linked glycosylation 1 (beta-1,4-mannosyltransferase) [Mus musculus (house mouse)]

Gene ID: 208211, updated on 13-Mar-2020

Summary



Official Symbol Alg1 provided by MGI

Official Full Name asparagine-linked glycosylation 1 (beta-1,4-mannosyltransferase) provided by MGI

Primary source MGI:MGI:2384774

See related Ensembl:ENSMUSG00000039427

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 HMAT1, HMT1

Expression Ubiquitous expression in adrenal adult (RPKM 34.2), ovary adult (RPKM 29.7) and 28 other tissuesSee more

Orthologs <u>human</u> all

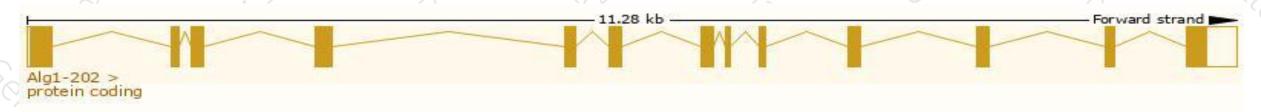
Transcript information (Ensembl)



The gene has 5 transcripts, all transcripts are shown below:

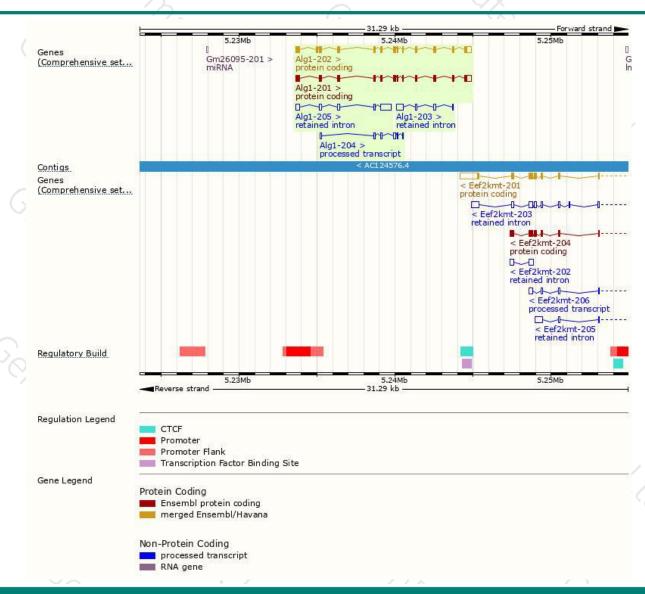
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Alg1-202	ENSMUST00000100196.8	1755	482aa	Protein coding	CCDS49753	Q921Q3	TSL:1 GENCODE basic APPRIS P2
Alg1-201	ENSMUST00000049207.8	1669	<u>456aa</u>	Protein coding	940	Q921Q3	TSL:2 GENCODE basic APPRIS ALT2
Alg1-204	ENSMUST00000147530.1	519	No protein	Processed transcript	851	2	TSL:3
Alg1-205	ENSMUST00000153730.7	1246	No protein	Retained intron			TSL:3
Alg1-203	ENSMUST00000142902.1	708	No protein	Retained intron	125	-	TSL:3

The strategy is based on the design of *Alg1-202* transcript, the transcription is shown below:



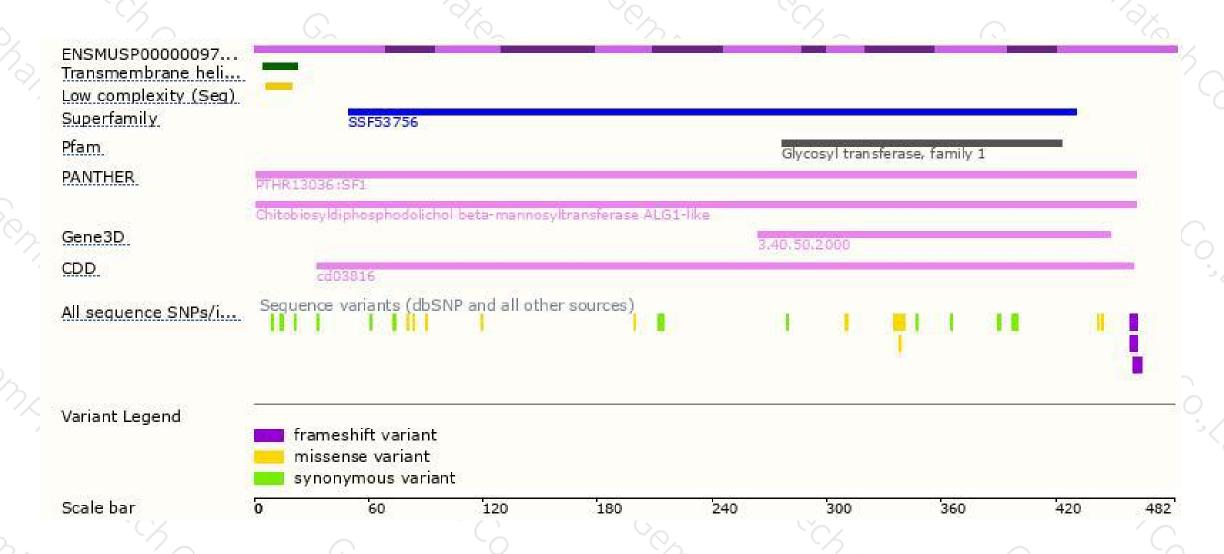
Genomic location distribution





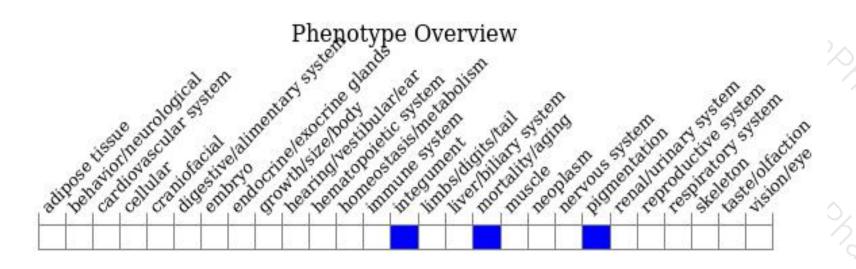
Protein domain





Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).



If you have any questions, you are welcome to inquire.

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