A3galt2 Cas9-CKO Strategy

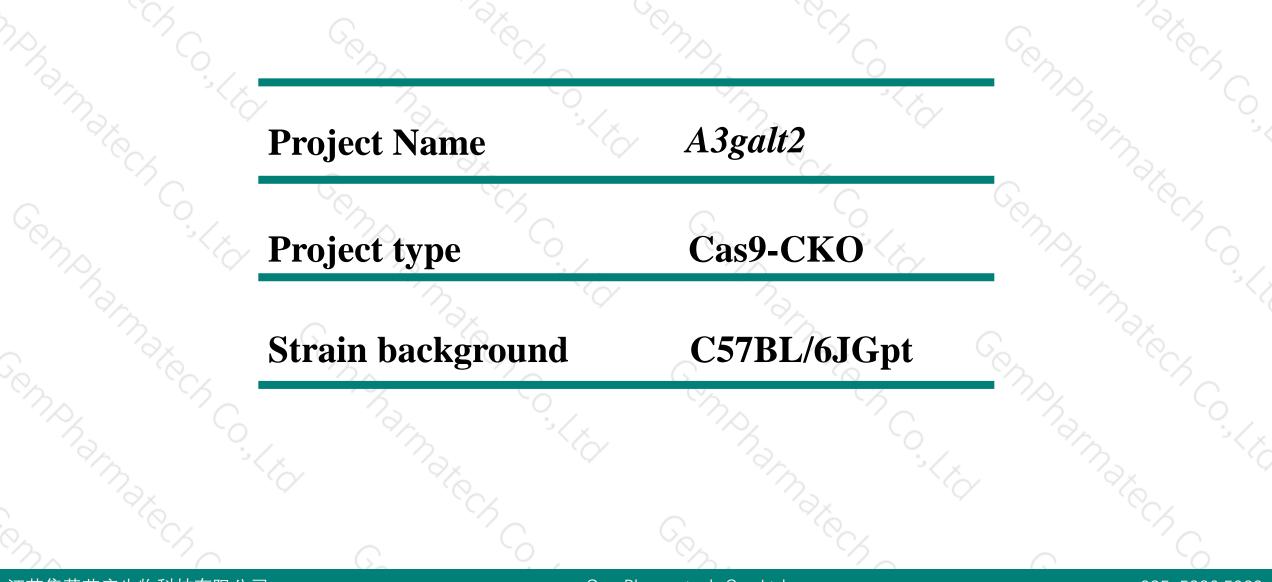
Designer: Reviewer :

Design Date:

Daohua Xu Huimin Su 2020-3-23

Project Overview

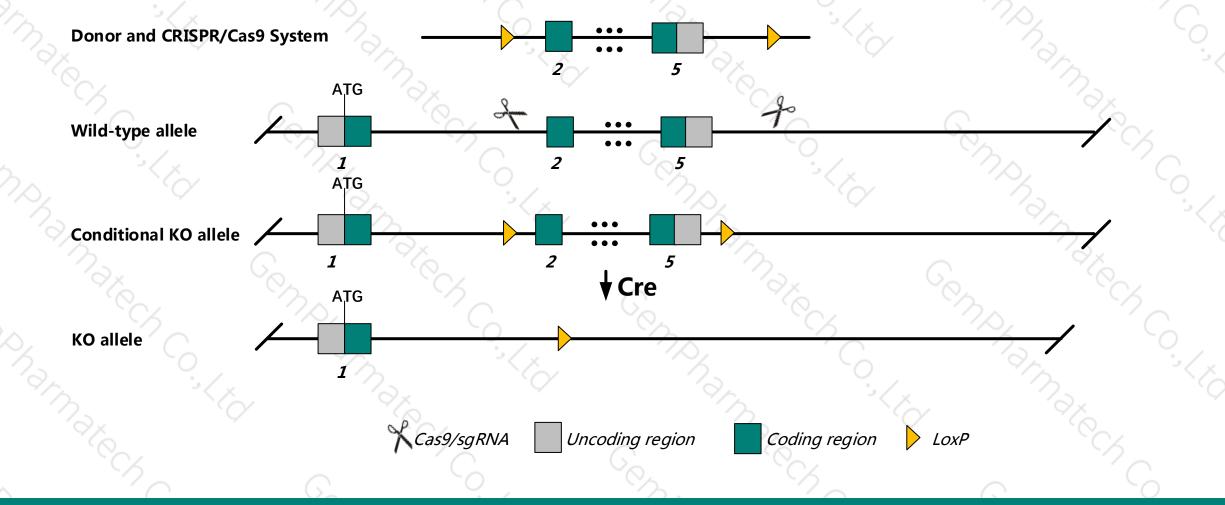




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *A3galt2* gene. The schematic diagram is as follows:



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Technical routes



- The *A3galt2* gene has 2 transcripts. According to the structure of *A3galt2* gene, exon2-exon5 of *A3galt2*-201 (
 ENSMUST00000030585.7) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify A3galt2 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 or cell types.



- According to the existing MGI data, Mice homozygous for a knock-out allele are viable, fertile and behaviorally normal with no detectable alterations in the development and function of invariant natural killer T cells.
- The A3galt2 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)



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A3galt2 alpha 1,3-galactosyltransferase 2 (isoglobotriaosylceramide synthase) [*Mus musculus* (house mouse)] Gene ID: 215493, updated on 13-Mar-2020

Summary

Official SymbolA3galt2 provided by MGIOfficial Full Namealpha 1,3-galactosyltransferase 2 (isoglobotriaosylceramide synthase) provided by MGIPrimary sourceMGI:MGI:2685279See relatedEnsembl:ENSMUSG0000028794Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Murinae; Mus; MusAlso known asi6b3; Gm433; i6b3SExpressionBroad expression in whole brain E14.5 (RPKM 1.3), CNS E14 (RPKM 1.1) and 17 other tissues See more
human all

Transcript information (Ensembl)



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

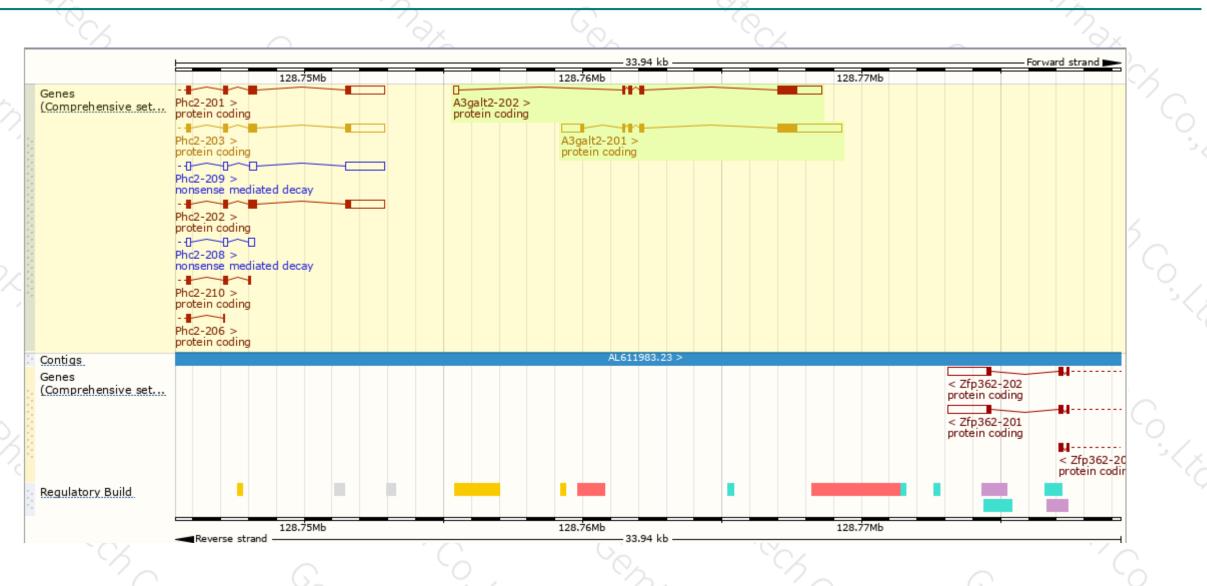
\sim .	1				1.1.2			Z 1			
1	Name 🖕	Transcript ID 💧	bp 🖕	Protein 🖕	Biotype	CCDS 🖕	UniProt 🖕		Flags	÷	5
	A3galt2-201	ENSMUST0000030585.7	3390	<u>370aa</u>	Protein coding	<u>CCDS18675</u> &	<u>Q3V1N9</u> ₽	TSL:1	GENCODE basic	APPRIS P2	
	A3galt2-202	ENSMUST00000106077.7	2114	<u>339aa</u>	Protein coding	-	<u>Q3V1N9</u> ₽	TSL:5	GENCODE basic	APPRIS ALT2	

The strategy is based on the design of A3galt2-201 transcript, The transcription is shown below

A3galt2-201 protein codir	l > ng		10.4	04 kb		Forward strand	
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Genomic location distribution



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Protein domain



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Superfamily			Nucleo	otide-diphospho-sugar tra	insferases						· I.
Pfam			Gly	cosyl transferase, family	6						
PANTHER		PTHR10462:SF33									
		Glycosyl transferas	se, family 6								-6
Gene3D			Nucleot	tide-diphospho-sugar tran	nsferases						
CDD			c	d02515							
All sequence SNPs/	Sequence var	ants (dbSNP and a	II other source	s)							_
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Variant Legend Scale bar	missense	variant	80	120	splic			280	320		370
Variant Legend	missense	variant ous variant	•						320	Na Ko	370

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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 are viable, fertile and behaviorally normal with no detectable alterations in the development and function of invariant natural killer T cells.

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



