

Aicda Cas9-CKO Strategy

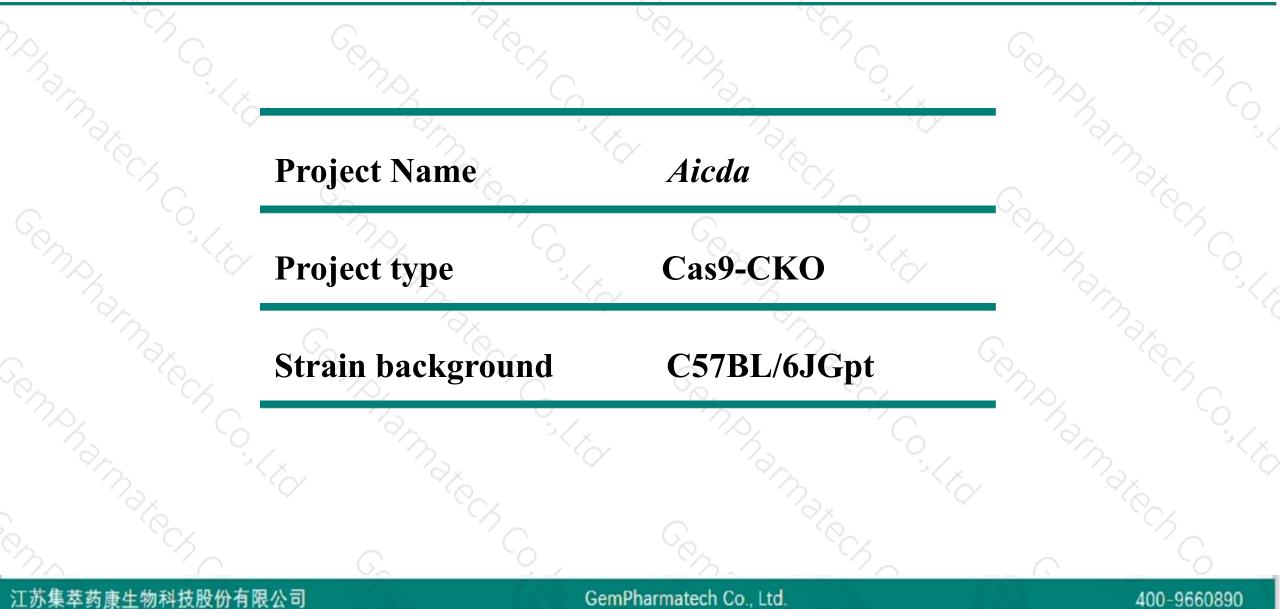
Designer: Reviewer:

Design Date:

Daohua Xu Huimin Su 2019-9-28

Project Overview

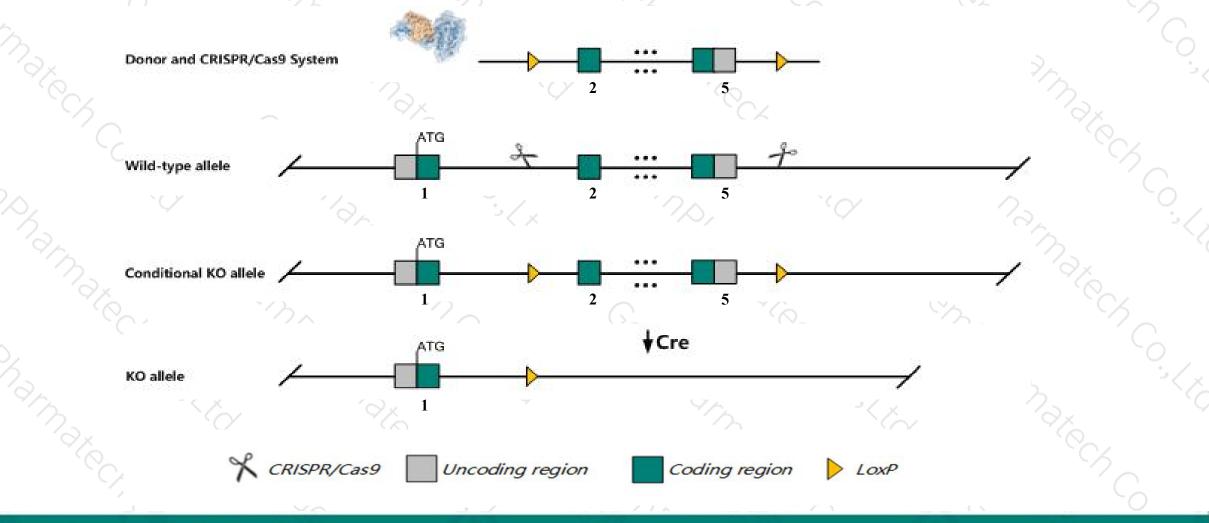




Conditional Knockout strategy



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



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The Aicda gene has 2 transcripts. According to the structure of Aicda gene, exon2-exon5 of Aicda-201 (ENSMUST00000043301.13) 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 *Aicda* 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, Homozygous mutation of this gene results in elevated IgM levels and impairment of B cell class switching.
- > The Aicda gene is located on the Chr6. 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)



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Aicda activation-induced cytidine deaminase [Mus musculus (house mouse)]

Gene ID: 11628, updated on 9-Apr-2019

Summary

Official Symbol	Aicda provided by MGI
Official Full Name	activation-induced cytidine deaminase provided by MGI
Primary source	MGI:MGI:1342279
See related	Ensembl:ENSMUSG0000040627
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	Aid, Arp2
Expression	Low expression observed in reference datasetSee more
Orthologs	human all

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Transcript information (Ensembl)



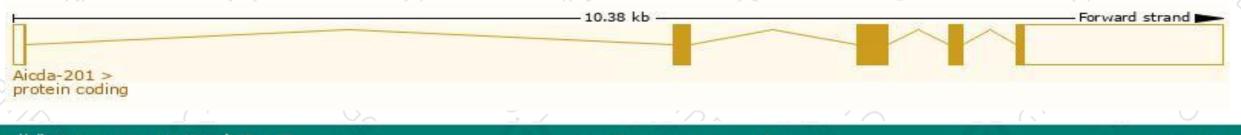
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The gene has 2 transcripts, all transcripts are shown below:

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Name Transcript ID		bp	Protein	Biotype	CCDS	UniProt	Flags		
Aicda-201	ENSMUST0000043301.13	2405	<u>198aa</u>	Protein coding	CCDS20497	Q9WVE0	TSL:1 GENCODE basic APPRIS P1		
Aicda-202	ENSMUST00000160685.1	1292	<u>60aa</u>	Protein coding		E0CXQ9	TSL:1 GENCODE basic		

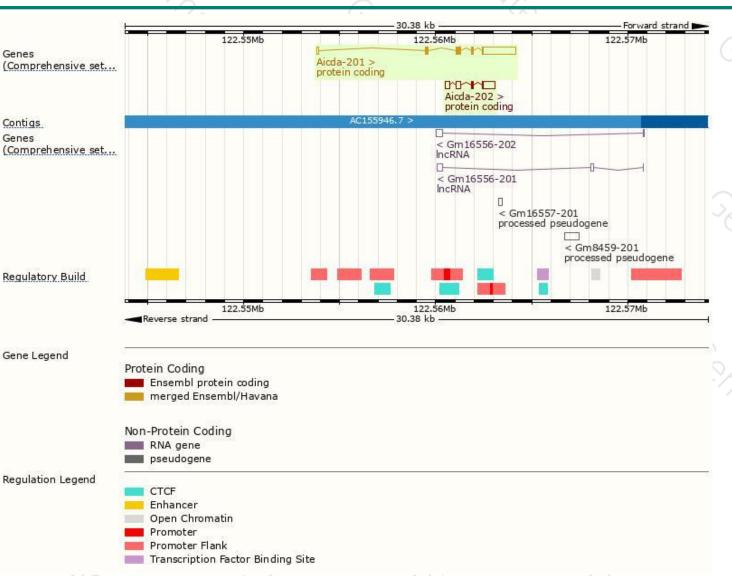
The strategy is based on the design of Aicda-201 transcript, The transcription is shown below



Genomic location distribution







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Protein domain

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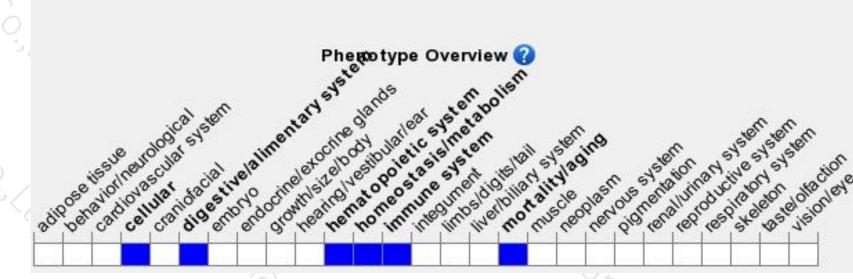
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ENSMUSP00000040			-	E.	- pu	3	* 1	10			
Superfamily			Cytidi	ne deaminas:	e-like						
Pfam.	PF18772										
PROSITE profiles		Cytidine ar	nd deaxycyt	idylate deam	inase domain						
PROSITE patterns				APOBEC/CMP	deaminase, a	inc-binding					
PANTHER	PTHR13857										-0
	PTHR13857:	SF10									
Gene3D	3.40.140.10										
CDD.	cd01283										
All sequence SNPs/i	Sequence v	variants (db:	SNP and a	ll other sour	ces)		1				2
Variant Legend	synon	ymous varia	ant								
Scale bar	0	20	40	60	80	100	120	140	160	1	.98
						10				- Co	

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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, Homozygous mutation of this gene results in elevated IgM levels and impairment of B cell class switching.



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



