Deaf1 Cas9-CKO Strategy

Designer: Zihe Cui

Reviewer: Xiaojing Li

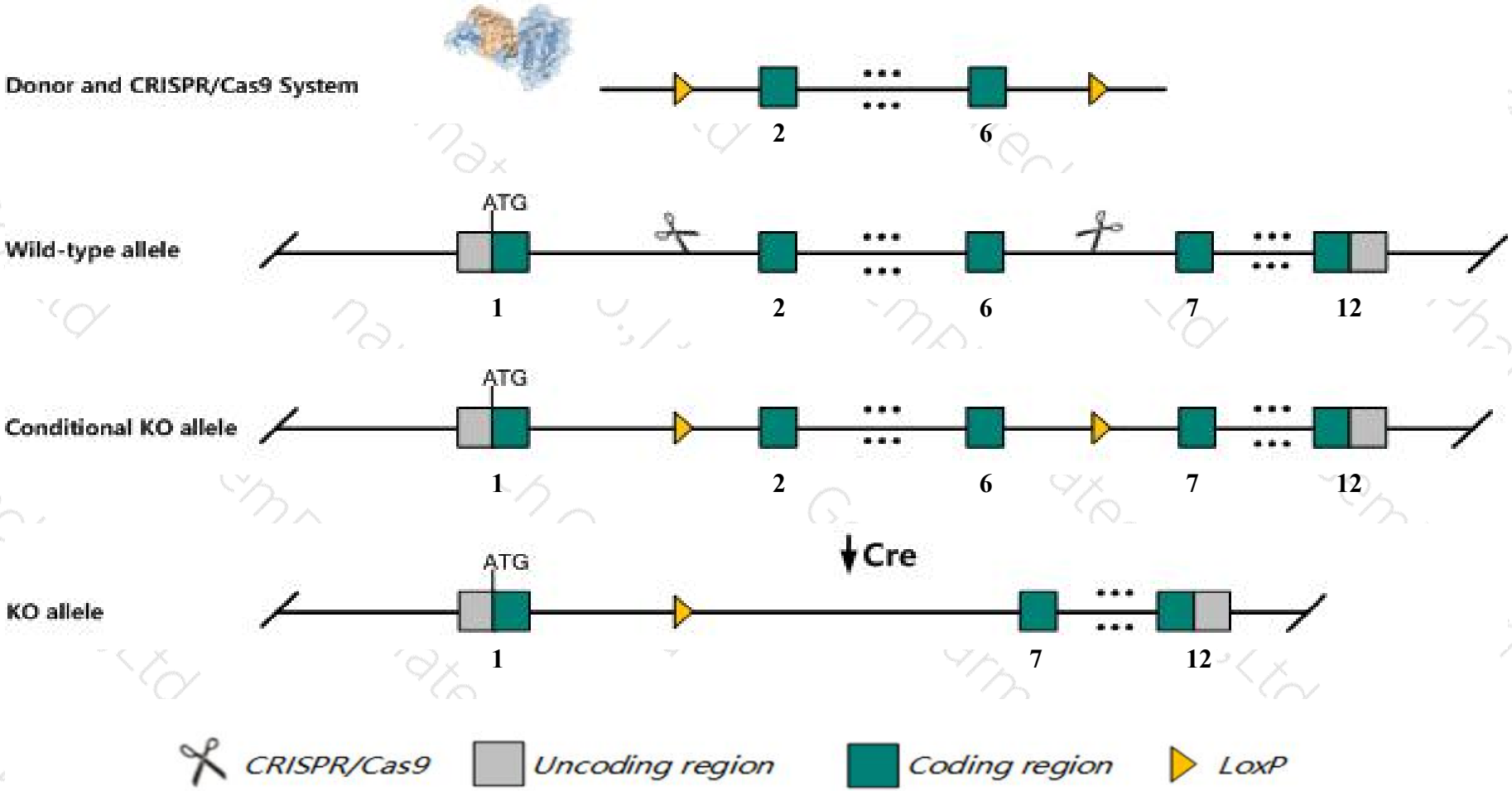
Design Date: 2020-9-27

Project Overview

Project Name	***Deaf1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Deaf1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Deaf1* gene has 9 transcripts. According to the structure of *Deaf1* gene, exon2-exon6 of *Deaf1-201*(ENSMUST00000080553.8) transcript is recommended as the knockout region.The region contains 581bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Deaf1* 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.

Notice

➢ According to the existing MGI data,mice homozygous for a knock-out allele exhibit frequent exencephaly associated with neonatal lethality, rib cage abnormalities, and a low frequency of homeotic transformations of cervical segments but no presphenoid bone or cranial nerve defects; non-exencephalic survivors are healthy and fertile.

➢ Transcript *Deaf1*-203 CDS 5' incomplete and effect of *Deaf1*-203 is unknown.

➢ The KO region is close to *Tmem80* gene.Knockout the region may affect the function of *Tmem80* gene.

➢ The *Deaf1* gene is located on the Chr7. 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）

集萃药康 GemPharmatech

Deaf1 DEAF1, transcription factor [*Mus musculus* (house mouse)]

Gene ID: 54006, updated on 26-Sep-2020

Summary

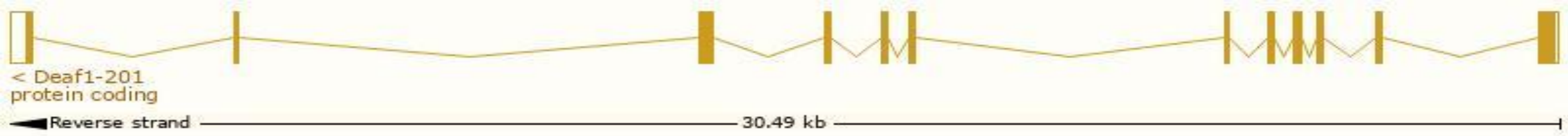
Official Symbol	Deaf1 provided by MGI
Official Full Name	DEAF1, transcription factor provided by MGI
Primary source	MGI:MGI:1858496
See related	Ensembl:ENSMUSG00000058886
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	NU; su; NUDR; AU042387; C230009B13Rik
Expression	Ubiquitous expression in CNS E14 (RPKM 6.3), ovary adult (RPKM 5.5) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

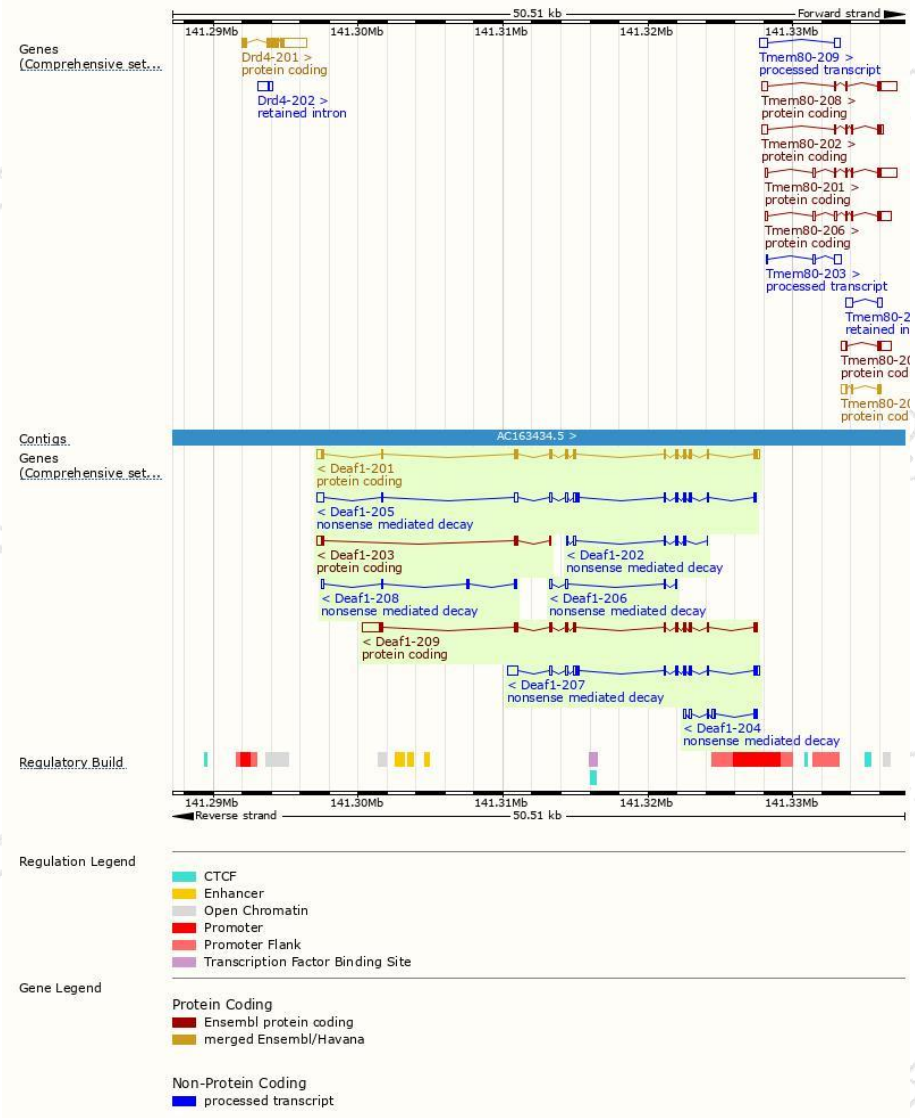
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Deaf1-201	ENSMUST00000080553.8	2135	566aa	Protein coding	CCDS40185	Q9Z1T5	TSL:1 GENCODE basic APPRIS P1
Deaf1-209	ENSMUST00000211537.1	2882	575aa	Protein coding	-	A0A1B0GRZ5	TSL:2 GENCODE basic
Deaf1-203	ENSMUST00000209600.1	725	132aa	Protein coding	-	A0A1B0GR05	CDS 5' incomplete TSL:2
Deaf1-207	ENSMUST00000210830.1	2265	308aa	Nonsense mediated decay	-	A0A1B0GRR4	TSL:1
Deaf1-205	ENSMUST00000210062.1	2121	283aa	Nonsense mediated decay	-	A0A1B0GT08	CDS 5' incomplete TSL:1
Deaf1-204	ENSMUST00000209608.1	837	120aa	Nonsense mediated decay	-	A0A1B0GS42	TSL:3
Deaf1-202	ENSMUST00000209397.1	593	116aa	Nonsense mediated decay	-	A0A1B0GRL7	CDS 5' incomplete TSL:3
Deaf1-208	ENSMUST00000211146.1	534	81aa	Nonsense mediated decay	-	A0A1B0GRN9	CDS 5' incomplete TSL:3
Deaf1-206	ENSMUST00000210816.1	406	53aa	Nonsense mediated decay	-	A0A1B0GSY7	CDS 5' incomplete TSL:5

The strategy is based on the design of *Deaf1-201* transcript,the transcription is shown below:

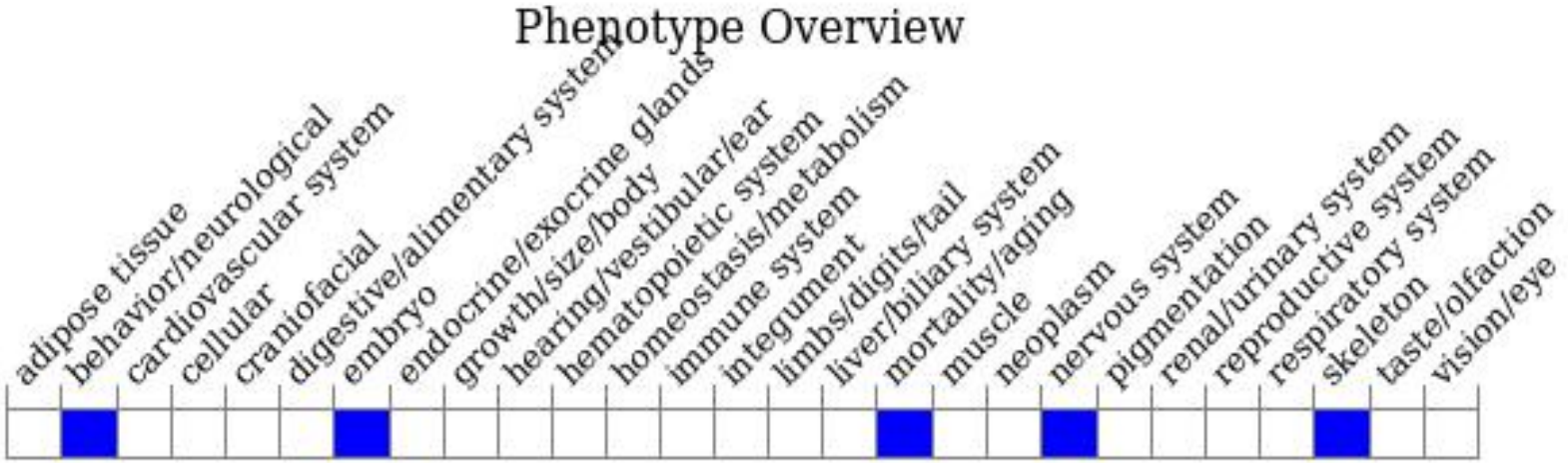


Genomic location distribution



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/).

According to the existing MGI data,mice homozygous for a knock-out allele exhibit frequent exencephaly associated with neonatal lethality, rib cage abnormalities, and a low frequency of homeotic transformations of cervical segments but no presphenoid bone or cranial nerve defects; non-exencephalic survivors are healthy and fertile.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd