

Diaph2 Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Diaph2

Project type

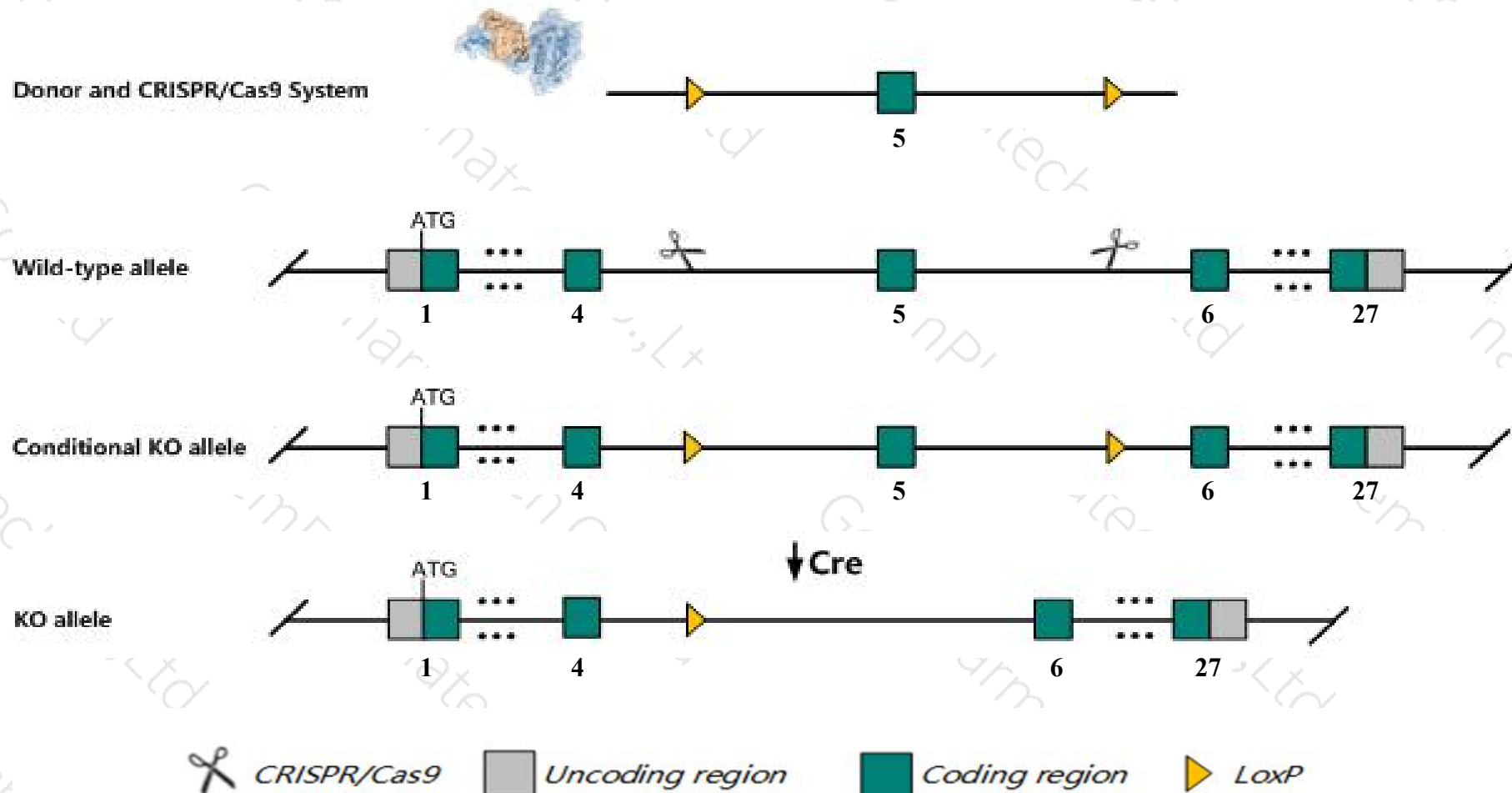
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Diaph2* gene. The schematic diagram is as follows:



- The *Diaph2* gene has 7 transcripts. According to the structure of *Diaph2* gene, exon5 of *Diaph2-201* (ENSMUST00000037854.14) transcript is recommended as the knockout region. The region contains 140bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Diaph2* 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, Male chimeras hemizygous for a gene trapped allele appear normal at E10.5. Chimeras hemizygous for another gene trapped allele exhibit hemorrhage, cardiac defects, and brain malformations from E11 onwards.
- The *Diaph2* gene is located on the ChrX. 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)

Diaph2 diaphanous related formin 2 [*Mus musculus* (house mouse)]

Gene ID: 54004, updated on 12-Aug-2019

Summary

Official Symbol Diaph2 provided by [MGI](#)
Official Full Name diaphanous related formin 2 provided by [MGI](#)
Primary source [MGI:MGI:1858500](#)
See related [Ensembl:ENSMUSG00000034480](#)
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 Dia3; Drf2; Diap2; E430022I22Rik
Expression Ubiquitous expression in bladder adult (RPKM 3.8), lung adult (RPKM 1.9) and 27 other tissues [See more](#)
Orthologs [human](#) [all](#)

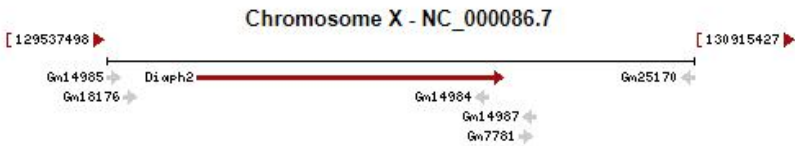
Genomic context

Location: X; X E3

See Diaph2 in [Genome Data Viewer](#)

Exon count: 29

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	X	NC_000086.7 (129749668..130465834)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	X	NC_000086.6 (126284278..126995733)

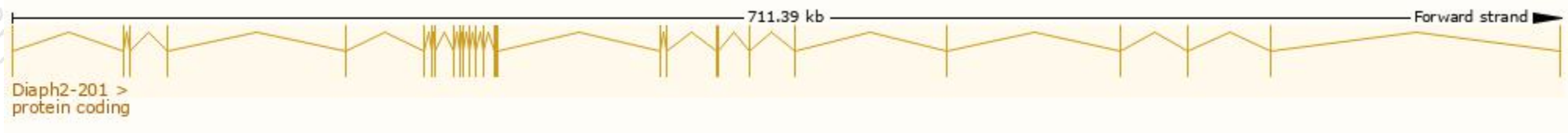


Transcript information (Ensembl)

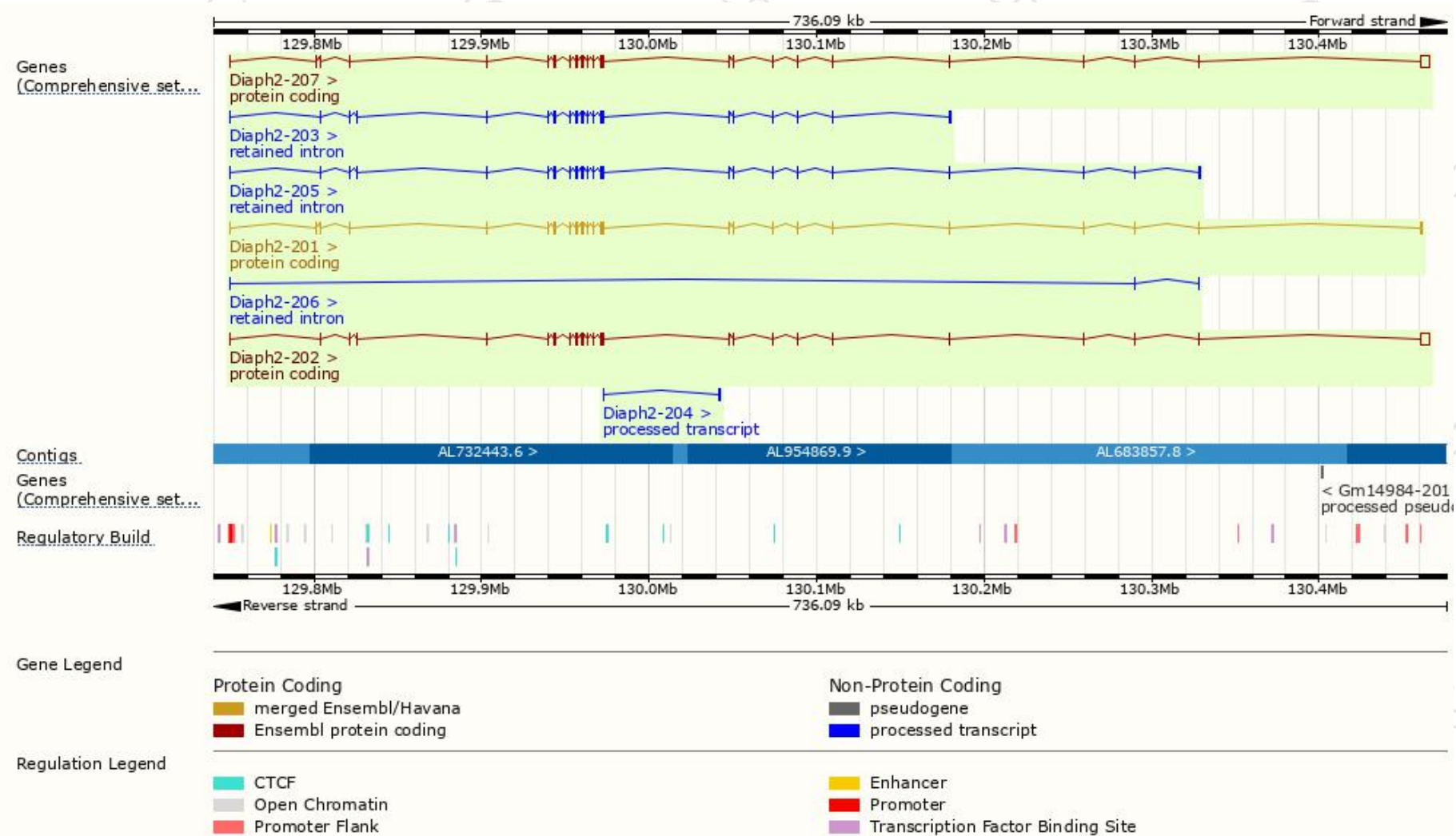
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Diaph2-201	ENSMUST00000037854.14	3742	1102aa	Protein coding	CCDS53180	Q6W4W7	TSL:1 GENCODE basic APPRIS P2
Diaph2-207	ENSMUST000000167619.8	8439	1102aa	Protein coding	-	E9Q4U7	TSL:5 GENCODE basic APPRIS ALT2
Diaph2-202	ENSMUST000000113320.2	8196	1098aa	Protein coding	-	O70566	TSL:5 GENCODE basic APPRIS ALT2
Diaph2-204	ENSMUST000000133457.1	960	No protein	Processed transcript	-	-	TSL:1
Diaph2-203	ENSMUST000000127577.7	4480	No protein	Retained intron	-	-	TSL:2
Diaph2-205	ENSMUST000000143668.7	4208	No protein	Retained intron	-	-	TSL:2
Diaph2-206	ENSMUST000000155333.1	994	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Diaph2-201* transcript,The transcription is shown below



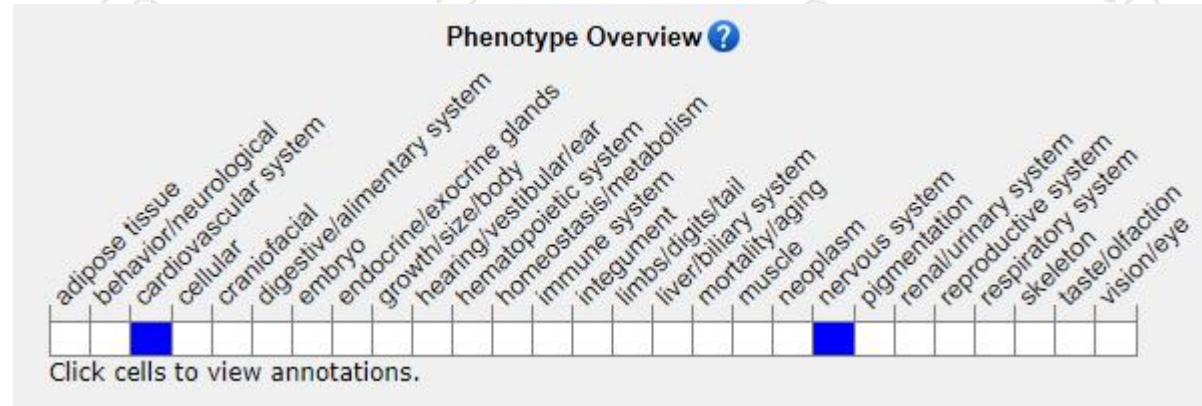
Genomic location distribution



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, Male chimeras hemizygous for a gene trapped allele appear normal at E10.5.

Chimeras hemizygous for another gene trapped allele exhibit hemorrhage, cardiac defects, and brain malformations from E11 onwards.

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

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