Aqp4 Cas9-CKO Strategy

Designer: Huan Fan

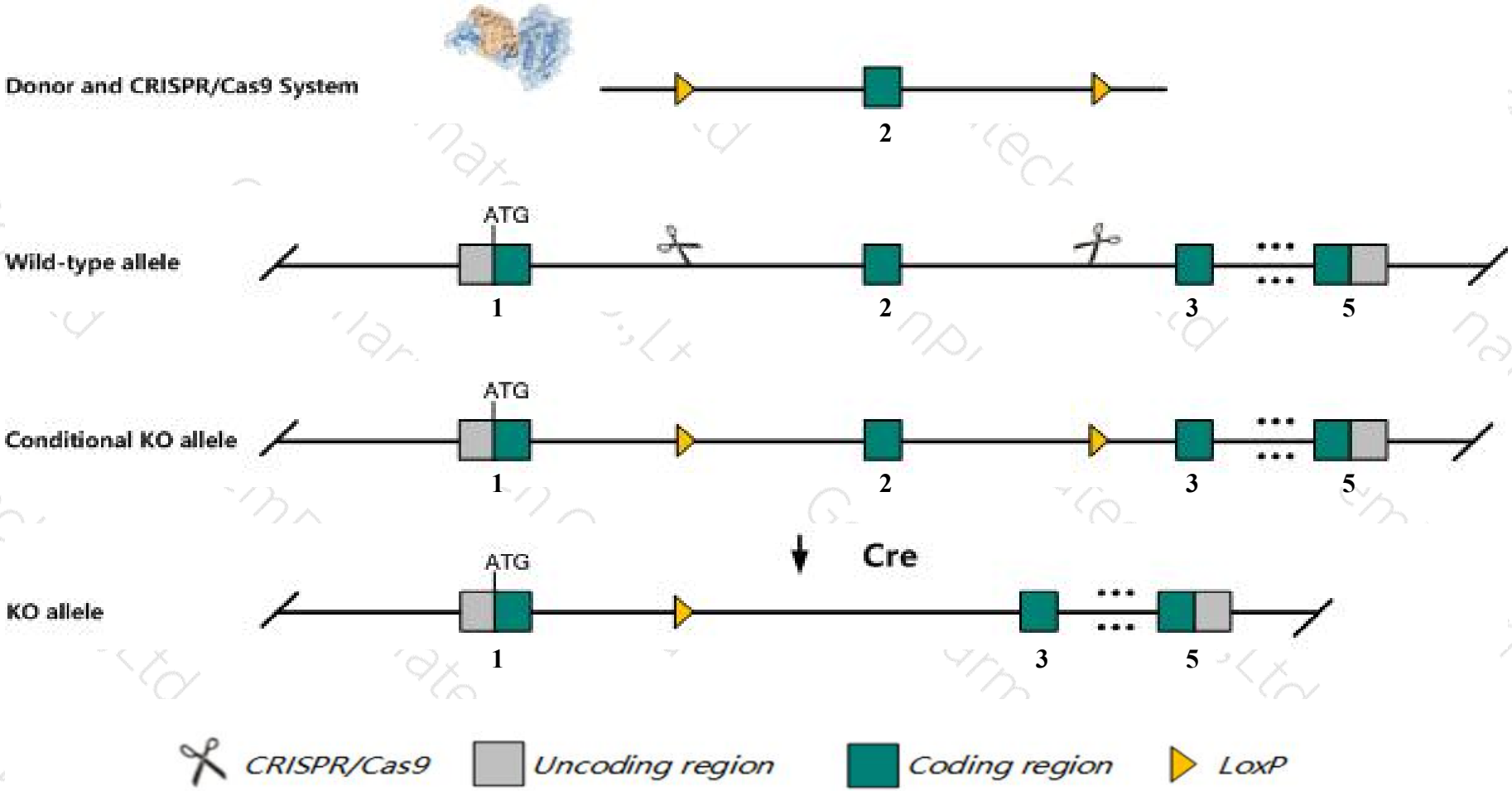
Design Date: 2019-8-23

Project Overview

Project Name	***Aqp4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Aqp4* gene. The schematic diagram is as follows:



Technical routes

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- The *Aqp4* gene has 11 transcripts. According to the structure of *Aqp4* gene, exon2 of *Aqp4-201* (ENSMUST00000079081.7) transcript is recommended as the knockout region.The region contains 415bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Aqp4* 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,Homozygotes for a targeted mutation exhibit decreased urine osmolality associated with reduced water permeability in inner medullary collecting ducts, increased survival rates and reduced brain edema after acute water intoxication and ischemic stroke, aswell as significant hearing impairment.

- The *Aqp4* gene is located on the Chr18. 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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Aqp4 aquaporin 4 [Mus musculus (house mouse)]

Gene ID: 11829, updated on 9-Apr-2019

Summary

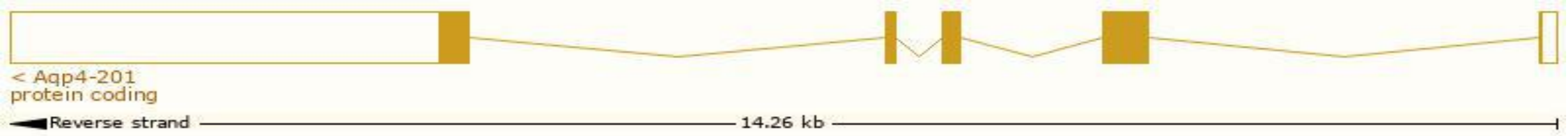
Official Symbol	Aqp4 provided by MGI
Official Full Name	aquaporin 4 provided by MGI
Primary source	MGI:MGI:107387
See related	Ensembl:ENSMUSG00000024411
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	WCH4
Summary	This gene encodes a member of the aquaporin family of intrinsic membrane proteins that function as water-selective channels in the plasma membranes of many cells. This protein is the predominant aquaporin found in brain and has an important role in brain water homeostasis. Alternatively spliced transcript variants encoding different isoforms have been described for this gene. A recent study provided evidence for translational readthrough in this gene and expression of an additional C-terminally extended isoform via the use of an alternative in-frame translation termination codon. [provided by RefSeq, Dec 2015]
Expression	Biased expression in cerebellum adult (RPKM 26.4), frontal lobe adult (RPKM 12.8) and 4 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

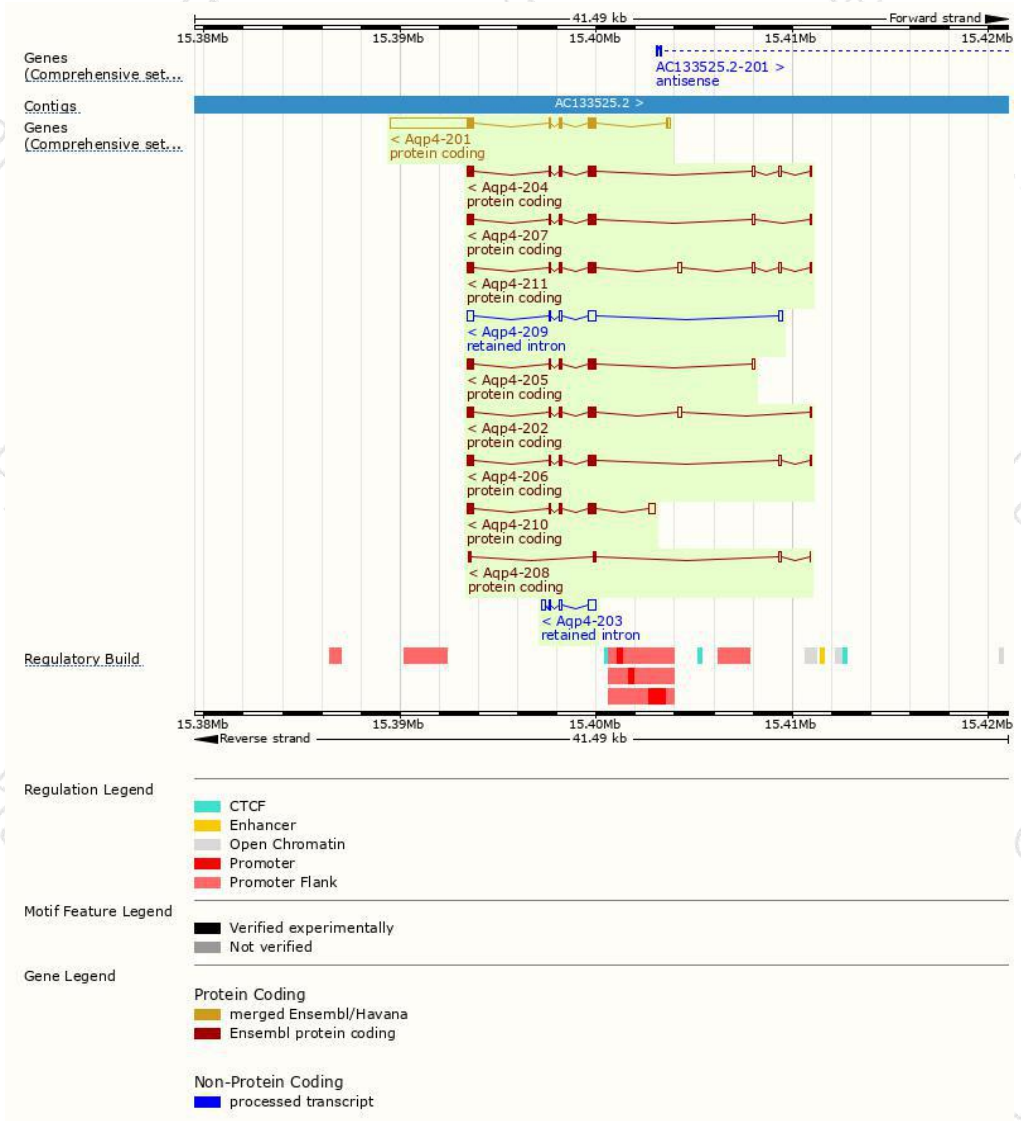
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Aqp4-201	ENSMUST00000079081.7	5054	323aa	Protein coding	CCDS29073	A0A0R4J0Z3	TSL:1 GENCODE basic APPRIS P2
Aqp4-211	ENSMUST00000235044.1	1511	301aa	Protein coding	-	Q50H70	GENCODE basic APPRIS ALT1
Aqp4-204	ENSMUST00000234391.1	1327	301aa	Protein coding	-	Q50H70	GENCODE basic APPRIS ALT1
Aqp4-210	ENSMUST00000234643.1	1288	301aa	Protein coding	-	Q50H70	GENCODE basic APPRIS ALT1
Aqp4-202	ENSMUST00000234053.1	1275	301aa	Protein coding	-	Q50H70	GENCODE basic APPRIS ALT1
Aqp4-206	ENSMUST00000234473.1	1221	301aa	Protein coding	-	Q50H70	GENCODE basic APPRIS ALT1
Aqp4-207	ENSMUST00000234518.1	1197	301aa	Protein coding	-	Q50H70	GENCODE basic APPRIS ALT1
Aqp4-205	ENSMUST00000234466.1	1106	301aa	Protein coding	-	Q50H70	GENCODE basic APPRIS ALT1
Aqp4-208	ENSMUST00000234533.1	453	83aa	Protein coding	-	-	CDS 3' incomplete
Aqp4-209	ENSMUST00000234624.1	1218	No protein	Retained intron	-	-	
Aqp4-203	ENSMUST00000234140.1	902	No protein	Retained intron	-	-	

The strategy is based on the design of *Aqp4-201* transcript,The transcription is shown below

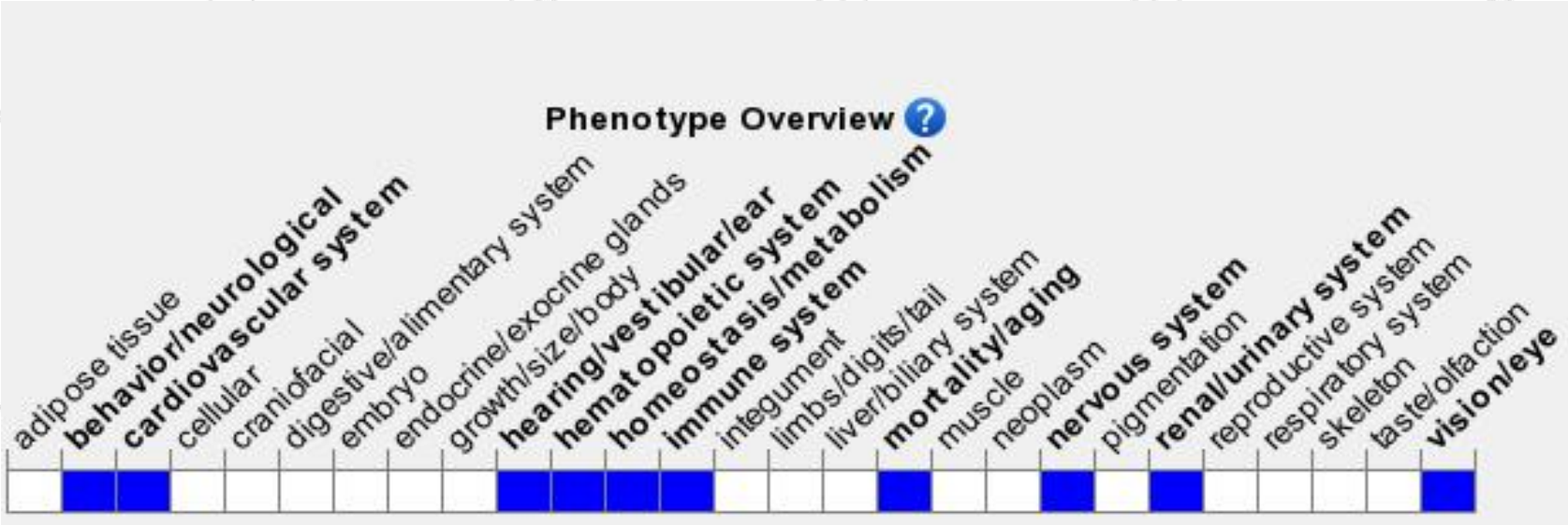


Genomic location distribution



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,Homozygotes for a targeted mutation exhibit decreased urine osmolality associated with reduced water permeability in inner medullary collecting ducts, increased survival rates and reduced brain edema after acute water intoxication and ischemic stroke, aswell as significant hearing impairment.

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

Tel: 400-9660890

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GemPharmatech Co.,Ltd