Arfgef1 Cas9-CKO Strategy

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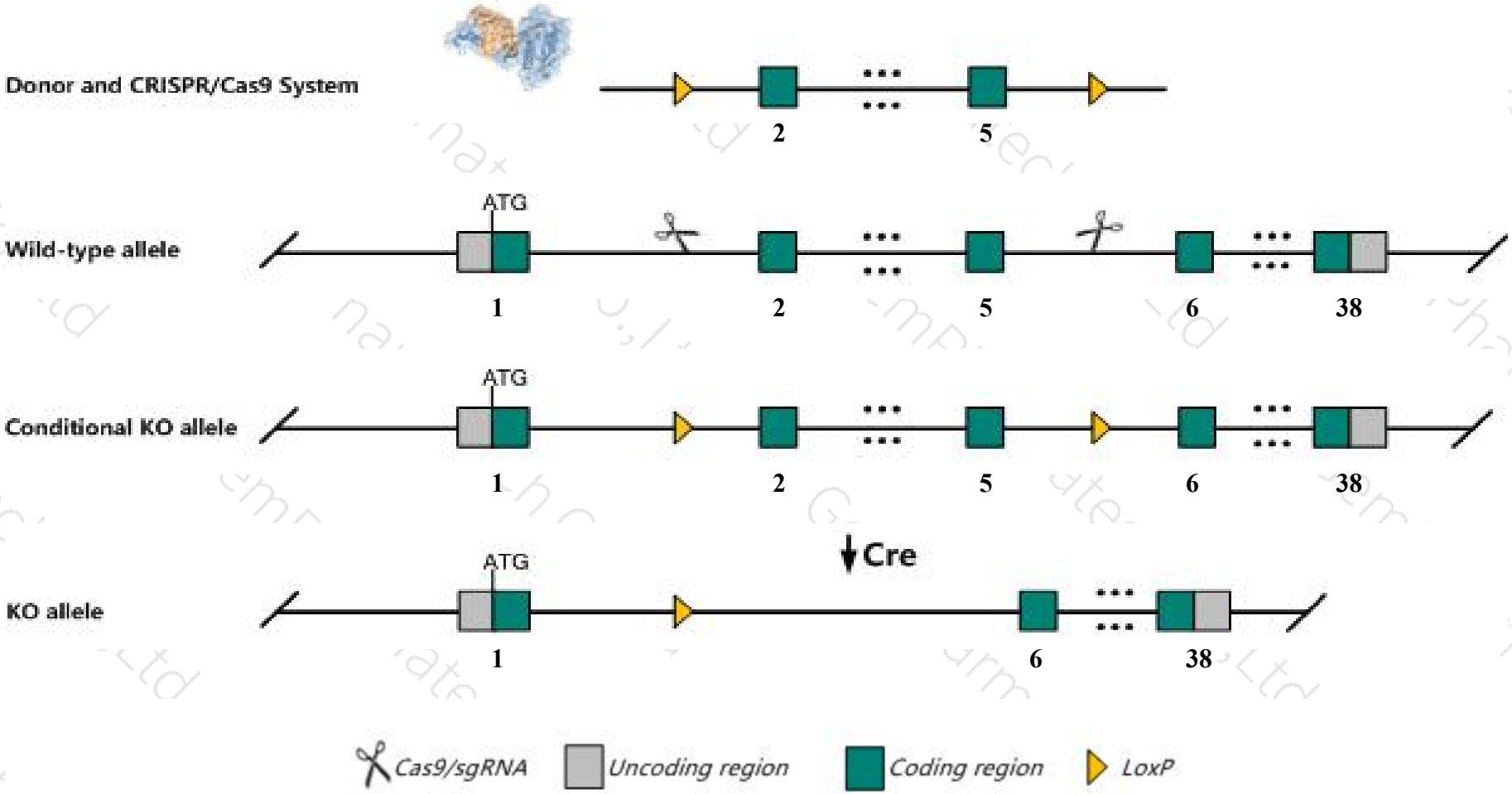
Project Overview

Project Name	***Arfgef1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Arfgef1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Arfgef1* gene has 8 transcripts. According to the structure of *Arfgef1* gene, exon2-exon5 of *Arfgef1-201*(ENSMUST00000088615.10) transcript is recommended as the knockout region.The region contains 515bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Arfgef1* 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 and cell types.

Notice

➢ According to the existing MGI data,mice homozygous for a null allele exhibit neonatal lethality, absent gastric milk and decreased brain size with increased neuron apoptosis, abnormal axon guidance and hypersensitivity to glutamate.

➢ The *Arfgef1* gene is located on the Chr1. 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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Arfgef1 ADP-ribosylation factor guanine nucleotide-exchange factor 1(brefeldin A-inhibited) [Mus musculus (house mouse)]

Gene ID: 211673, updated on 13-Mar-2020

Summary

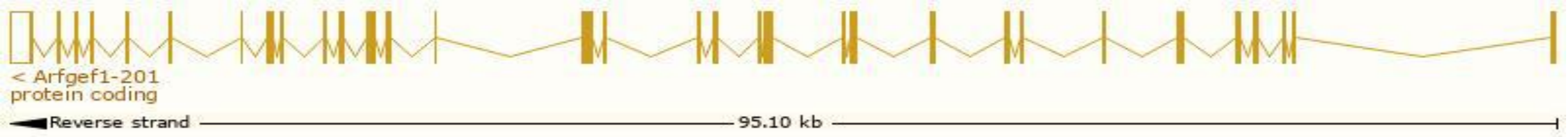
Official Symbol	Arfgef1 provided by MGI
Official Full Name	ADP-ribosylation factor guanine nucleotide-exchange factor 1(brefeldin A-inhibited) provided by MGI
Primary source	MGI:MGI:2442988
See related	Ensembl:ENSMUSG00000067851
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	ARFGEP1, BIG1, D130059B05Rik, D730028O18Rik, P200
Expression	Ubiquitous expression in bladder adult (RPKM 12.0), placenta adult (RPKM 10.3) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

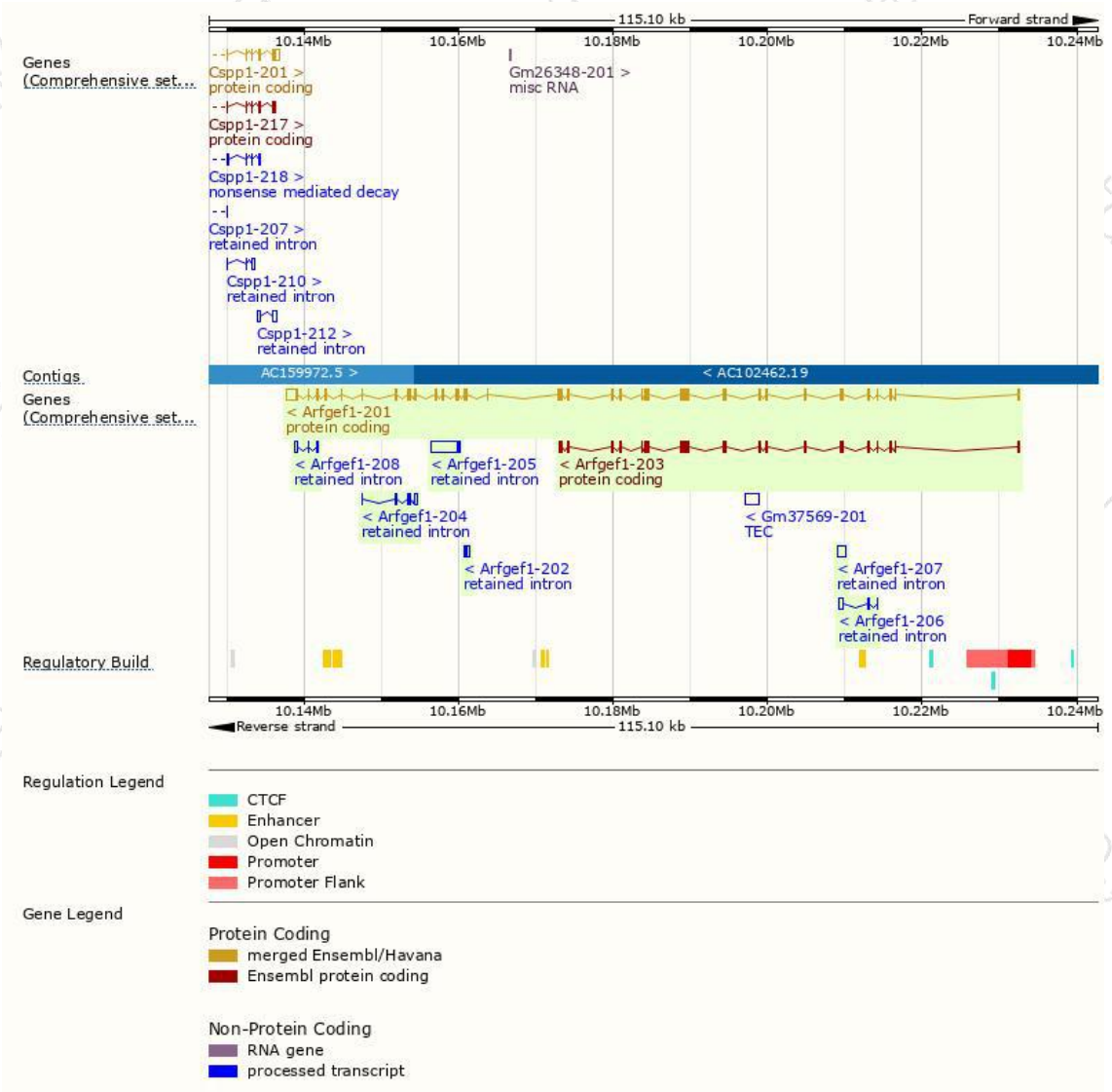
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arfgef1-201	ENSMUST00000088615.10	6978	1846aa	Protein coding	CCDS35512	G3X9K3	TSL:5 GENCODE basic APPRIS P1
Arfgef1-203	ENSMUST00000131556.1	3424	1054aa	Protein coding	-	D3YYK9	TSL:5 GENCODE basic
Arfgef1-205	ENSMUST00000156563.1	3526	No protein	Retained intron	-	-	TSL:1
Arfgef1-207	ENSMUST00000186813.1	1162	No protein	Retained intron	-	-	TSL:NA
Arfgef1-206	ENSMUST00000186720.1	810	No protein	Retained intron	-	-	TSL:5
Arfgef1-204	ENSMUST00000142497.1	729	No protein	Retained intron	-	-	TSL:2
Arfgef1-208	ENSMUST00000187417.1	568	No protein	Retained intron	-	-	TSL:2
Arfgef1-202	ENSMUST00000128834.1	458	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Arfgef1-201* transcript,the transcription is shown below:

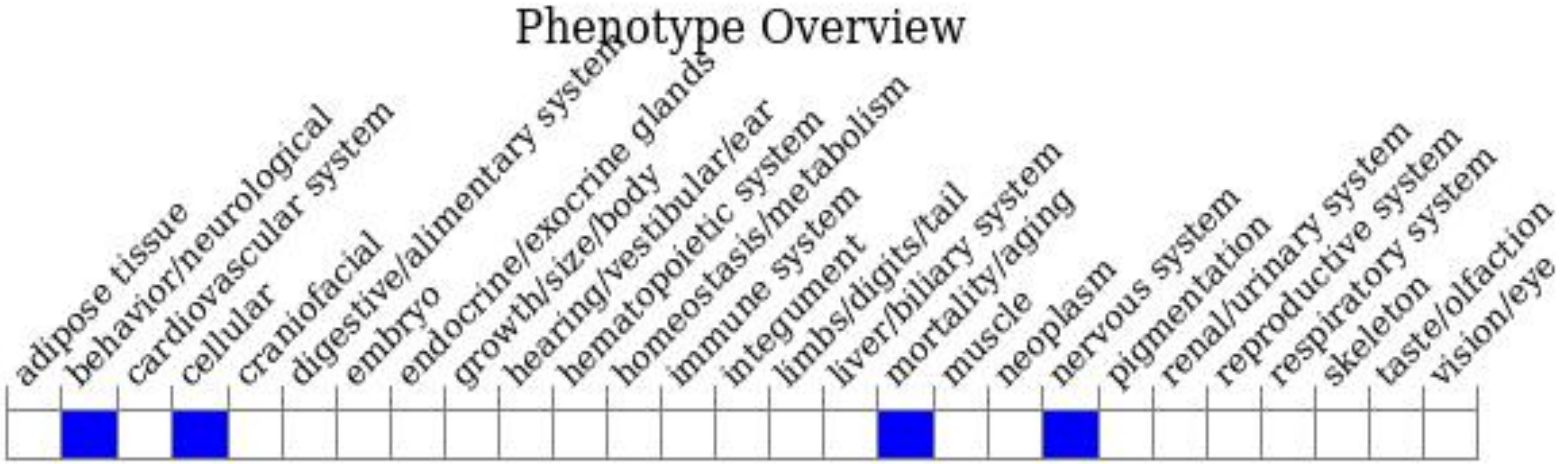


Genomic location distribution



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 null allele exhibit neonatal lethality, absent gastric milk and decreased brain size with increased neuron apoptosis, abnormal axon guidance and hypersensitivity to glutamate.

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

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