

Afdn Cas9-CKO Strategy

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Reviewer:

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Design Date:

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Project Overview

Project Name

Afdn

Project type

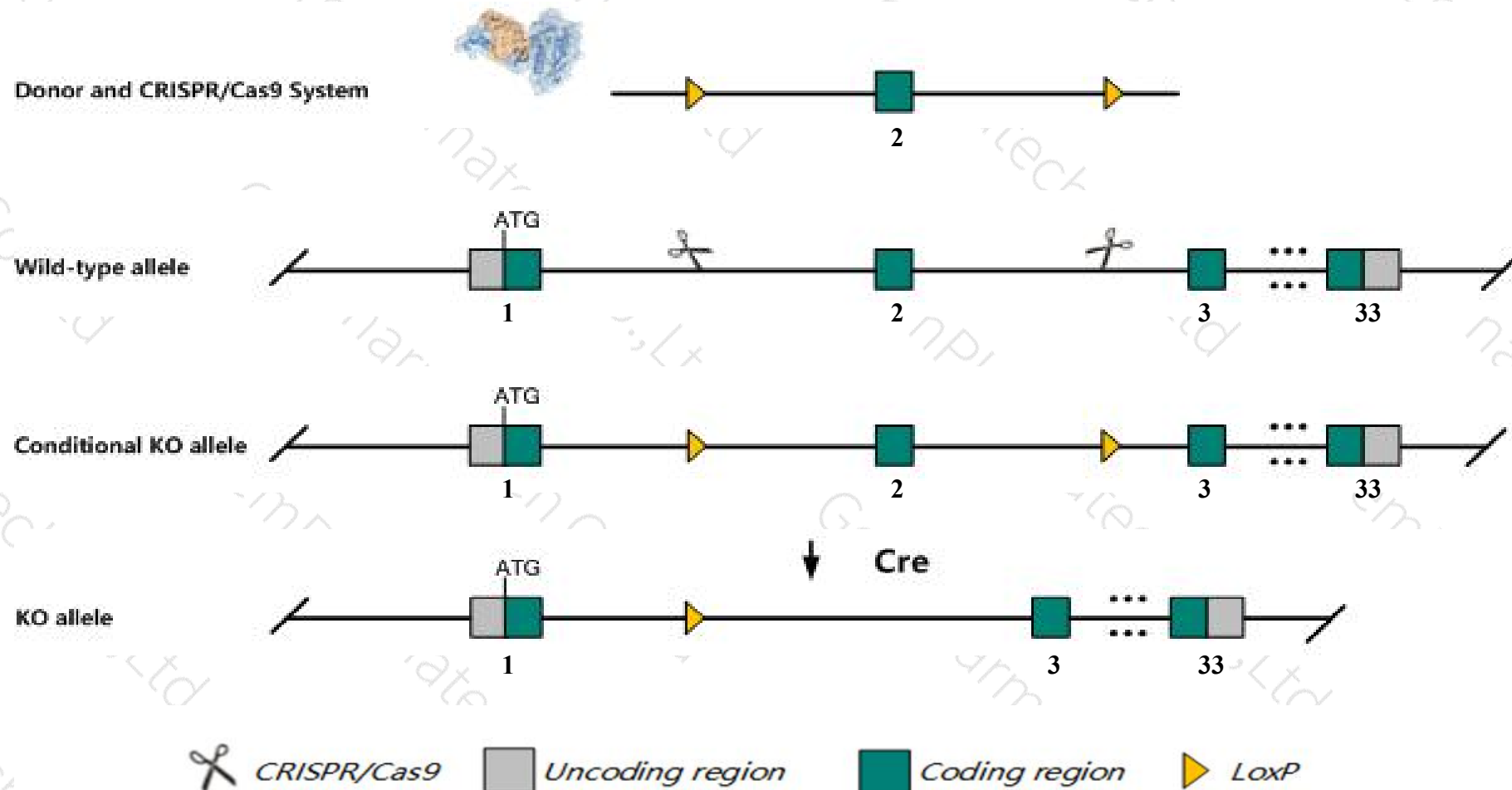
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Afdn* gene has 16 transcripts. According to the structure of *Afdn* gene, exon2 of *Afdn-212* (ENSMUST00000139666.7) transcript is recommended as the knockout region. The region contains 196bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Afdn* 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 null mice display embryonic lethality, abnormal ectoderm development including disrupted cell junctions, and absence of the somites, notochord, allantois, and neural folds.
- The *Afdn* gene is located on the Chr17. 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)

Afdn afadin, adherens junction formation factor [Mus musculus (house mouse)]

Gene ID: 17356, updated on 2-Apr-2019

Summary



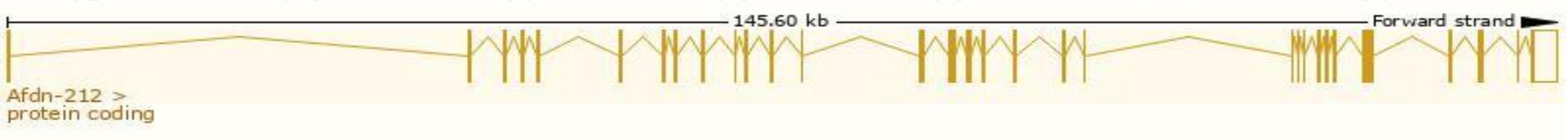
Official Symbol	Afdn provided by MGI
Official Full Name	afadin, adherens junction formation factor provided by MGI
Primary source	MGI:MGI:1314653
See related	Ensembl:ENSMUSG00000068036
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5033403D15Rik, AF6, Af-6, Afadin, Gm314, I-afadin, Mlt4, S-afadin
Expression	Ubiquitous expression in CNS E14 (RPKM 14.2), whole brain E14.5 (RPKM 13.1) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

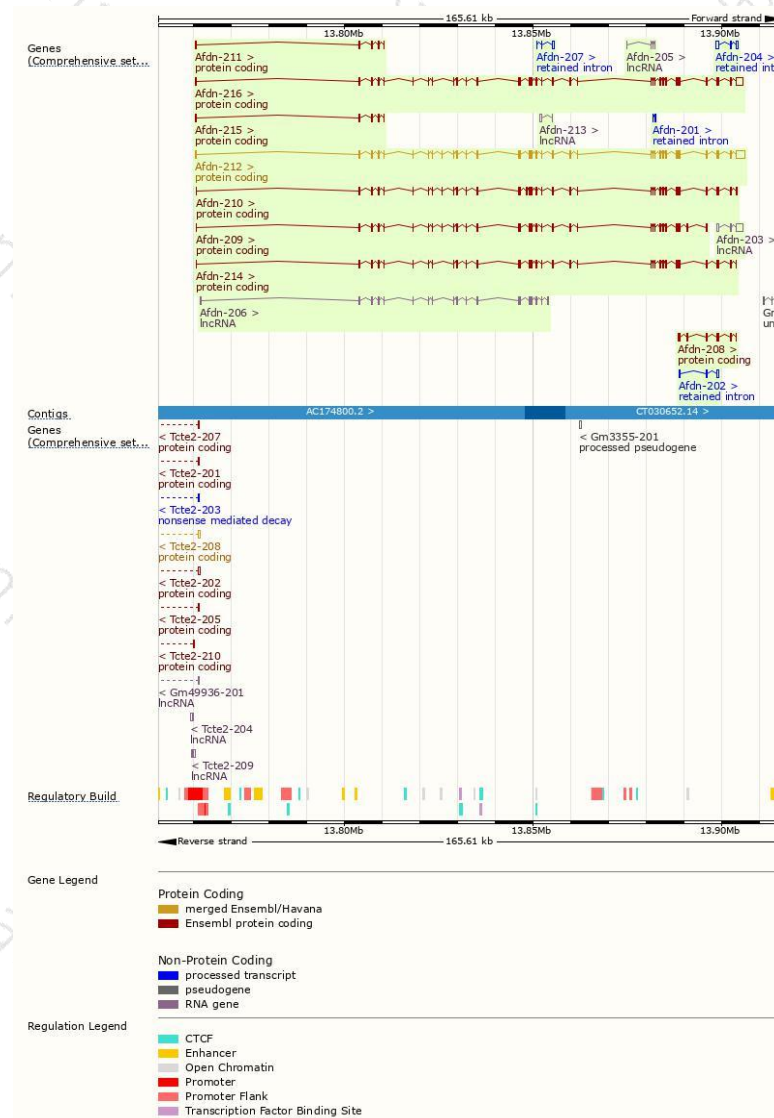
The gene has 16 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Afdn-212	ENSMUST00000139666.7	7732	1820aa	Protein coding	CCDS49955	Q9QZQ1	TSL:2 GENCODE basic APPRIS P2
Afdn-216	ENSMUST00000170827.8	7338	1805aa	Protein coding	-	E9Q852	TSL:5 GENCODE basic APPRIS ALT2
Afdn-210	ENSMUST00000137784.7	5684	1827aa	Protein coding	-	E9Q9C3	TSL:5 GENCODE basic APPRIS ALT2
Afdn-214	ENSMUST00000150848.7	5418	1805aa	Protein coding	-	Q9QZQ1	TSL:5 GENCODE basic APPRIS ALT2
Afdn-209	ENSMUST00000137708.7	5153	1663aa	Protein coding	-	E9PYX7	TSL:5 GENCODE basic APPRIS ALT2
Afdn-208	ENSMUST00000137531.2	1210	380aa	Protein coding	-	F7C3I9	CDS 5' incomplete TSL:3
Afdn-211	ENSMUST00000139347.7	822	229aa	Protein coding	-	D3YUD2	CDS 3' incomplete TSL:5
Afdn-215	ENSMUST00000156591.7	798	221aa	Protein coding	-	D3Z7L2	CDS 3' incomplete TSL:3
Afdn-204	ENSMUST00000133422.7	1167	No protein	Retained intron	-	-	TSL:2
Afdn-202	ENSMUST00000124188.1	610	No protein	Retained intron	-	-	TSL:2
Afdn-207	ENSMUST00000137503.1	593	No protein	Retained intron	-	-	TSL:3
Afdn-201	ENSMUST00000123030.1	427	No protein	Retained intron	-	-	TSL:3
Afdn-206	ENSMUST00000137495.7	3246	No protein	lncRNA	-	-	TSL:1
Afdn-203	ENSMUST00000124922.1	2662	No protein	lncRNA	-	-	TSL:1
Afdn-213	ENSMUST00000141640.1	779	No protein	lncRNA	-	-	TSL:2
Afdn-205	ENSMUST00000136087.1	241	No protein	lncRNA	-	-	TSL:5

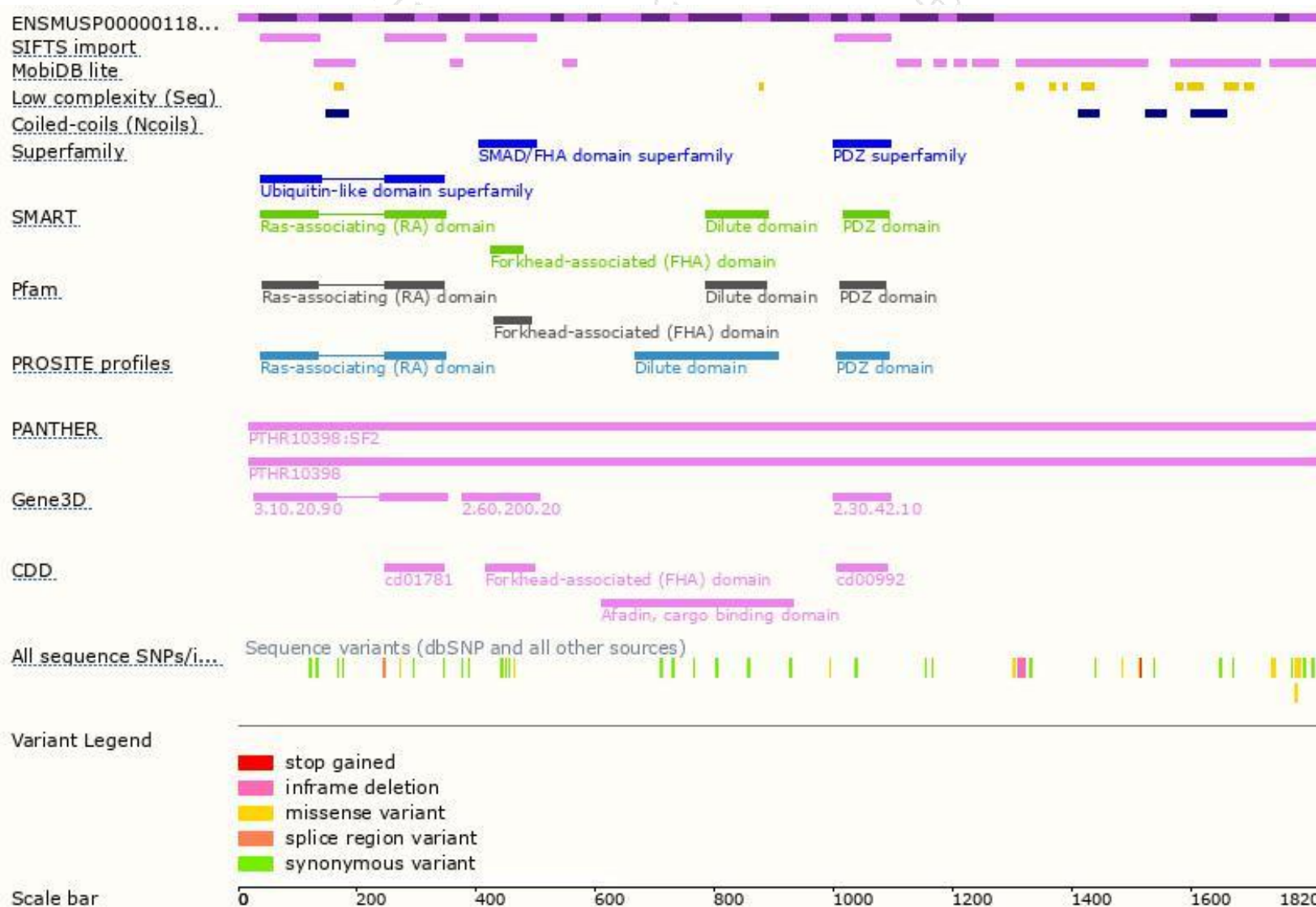
The strategy is based on the design of *Afdn-212* transcript,The transcription is shown below



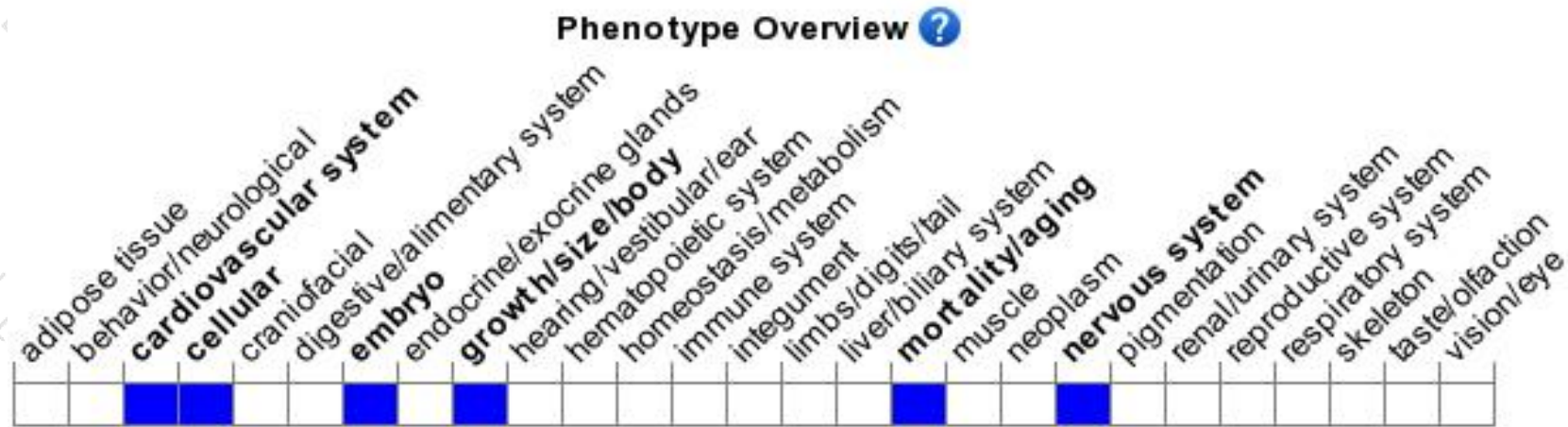
Genomic location distribution



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, Homozygous null mice display embryonic lethality, abnormal ectoderm development including disrupted cell junctions, and absence of the somites, notochord, allantois, and neural folds.

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

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