

Apaf1 Cas9-CKO Strategy

Designer: Lixin Lv

Project Overview

Project Name

Apaf1

Project type

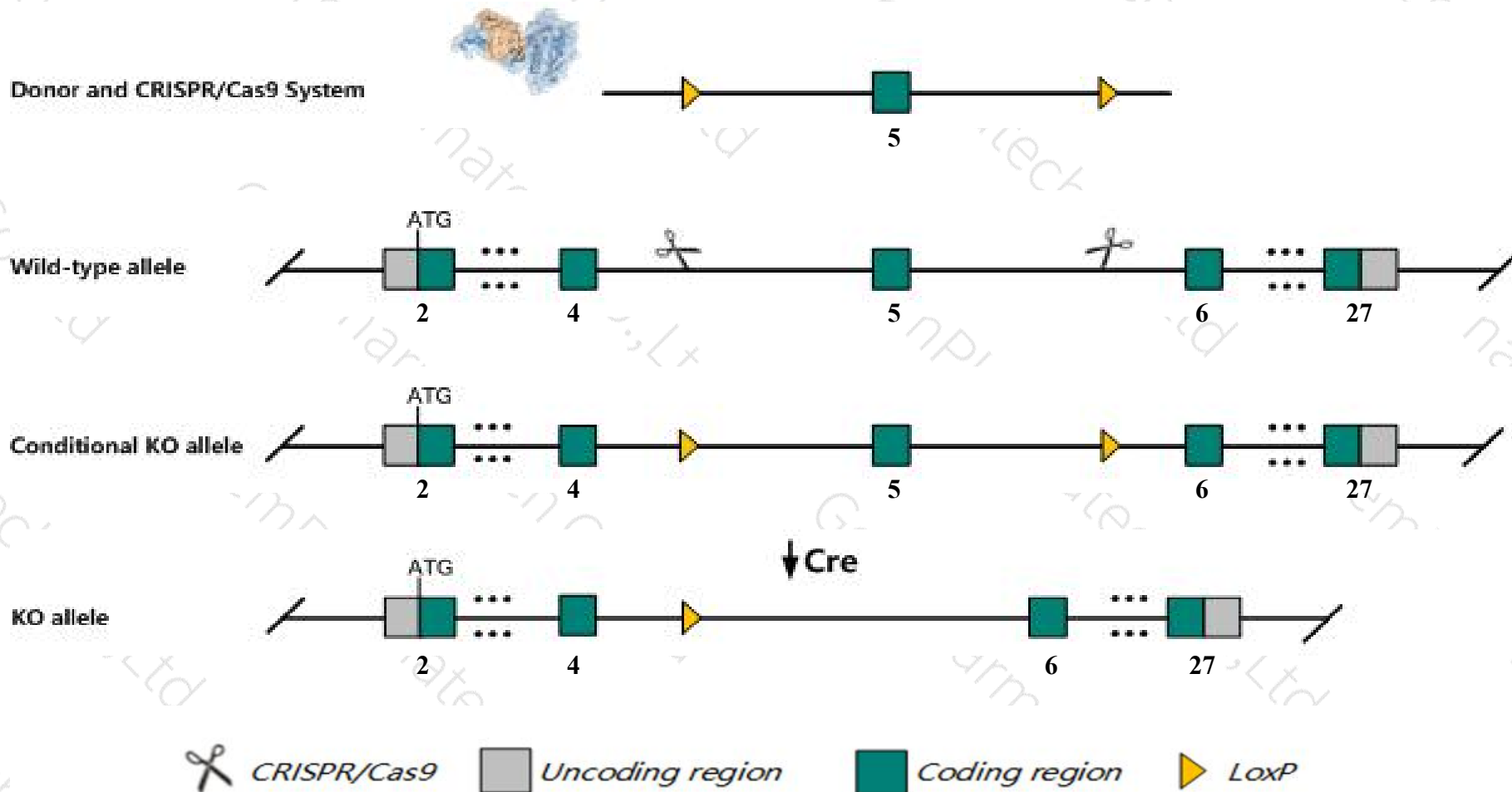
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Apaf1* gene. The schematic diagram is as follows:



Technical routes

- The *Apaf1* gene has 8 transcripts. According to the structure of *Apaf1* gene, exon5 of *Apaf1*-208 (ENSMUST00000162618.7) transcript is recommended as the knockout region. The region contains 184bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Apaf1* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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, Homozygotes for targeted null mutations have defects in apoptosis resulting in brain overgrowth, craniofacial defects, interdigit webbing and altered lens and retina. Most mutants die by embryonic day 16.5 or perinatally, and male survivors are sterile.
- The *Apaf1* gene is located on the Chr10. 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)

Apaf1 apoptotic peptidase activating factor 1 [Mus musculus (house mouse)]

Gene ID: 11783, updated on 26-Mar-2019

Summary



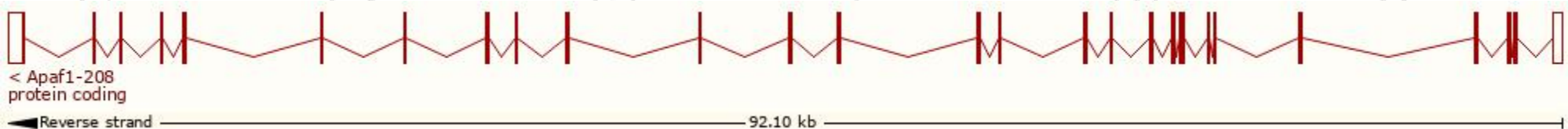
Official Symbol	Apaf1 provided by MGI
Official Full Name	apoptotic peptidase activating factor 1 provided by MGI
Primary source	MGI:MGI:1306796
See related	Ensembl:ENSMUSG00000019979
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	6230400I06Rik, Apaf-1, Apaf1l, fog, mKIAA0413
Expression	Ubiquitous expression in limb E14.5 (RPKM 8.3), large intestine adult (RPKM 7.7) and 27 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

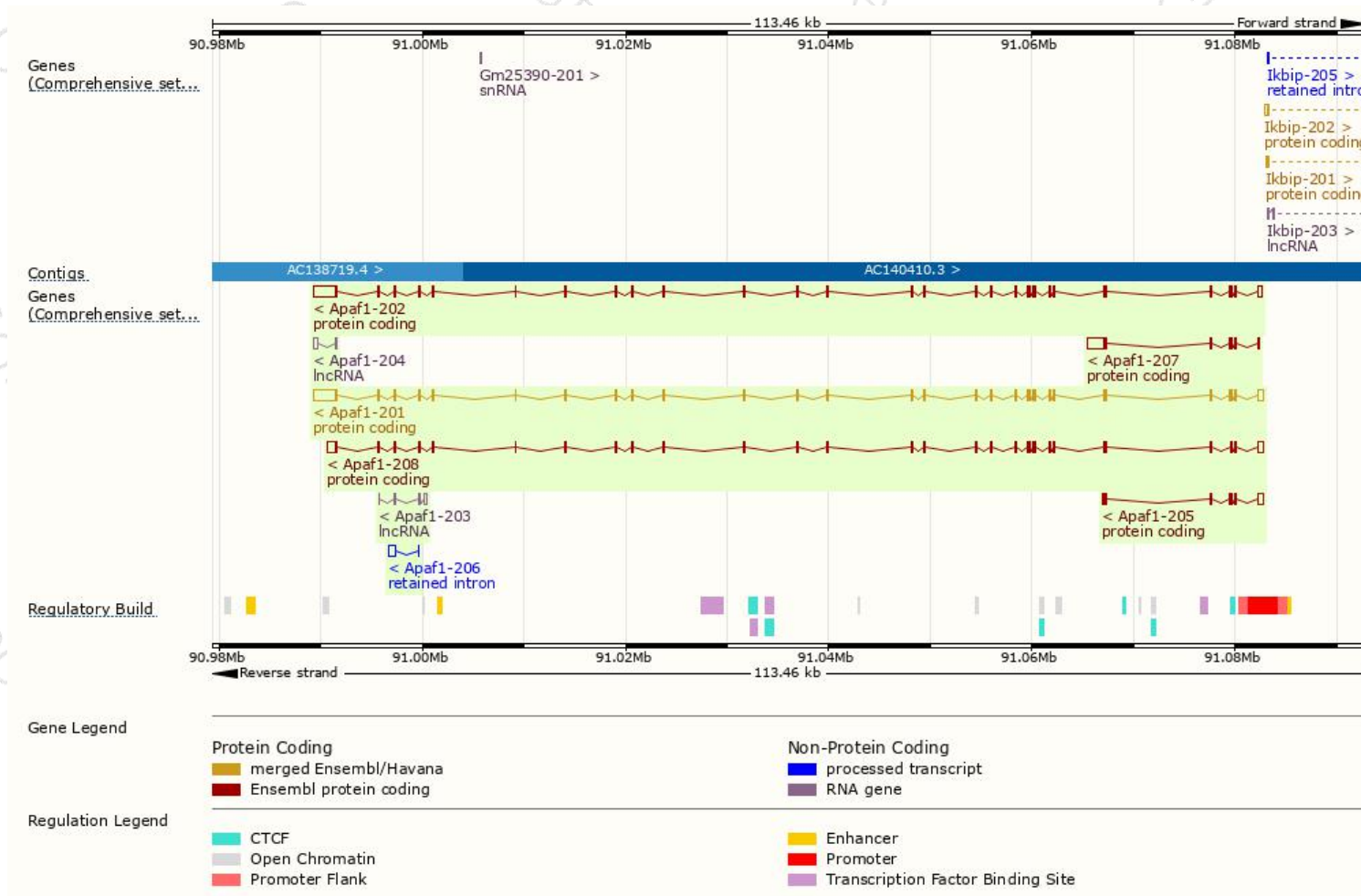
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Apaf1-201	ENSMUST00000020157.12	6577	1249aa	Protein coding	CCDS36030	O88879	TSL:1 GENCODE basic APPRIS P1
Apaf1-202	ENSMUST000000159110.7	6433	1249aa	Protein coding	CCDS36030	O88879	TSL:1 GENCODE basic APPRIS P1
Apaf1-208	ENSMUST000000162618.7	5151	1238aa	Protein coding	CCDS70095	G3XA09	TSL:1 GENCODE basic
Apaf1-207	ENSMUST000000161987.7	2710	258aa	Protein coding	-	Q80VR5	TSL:1 GENCODE basic
Apaf1-205	ENSMUST000000160788.1	1596	258aa	Protein coding	-	Q80VR5	TSL:1 GENCODE basic
Apaf1-206	ENSMUST000000161095.1	666	No protein	Retained intron	-	-	TSL:3
Apaf1-203	ENSMUST000000159457.1	597	No protein	lncRNA	-	-	TSL:3
Apaf1-204	ENSMUST000000160725.1	511	No protein	lncRNA	-	-	TSL:3

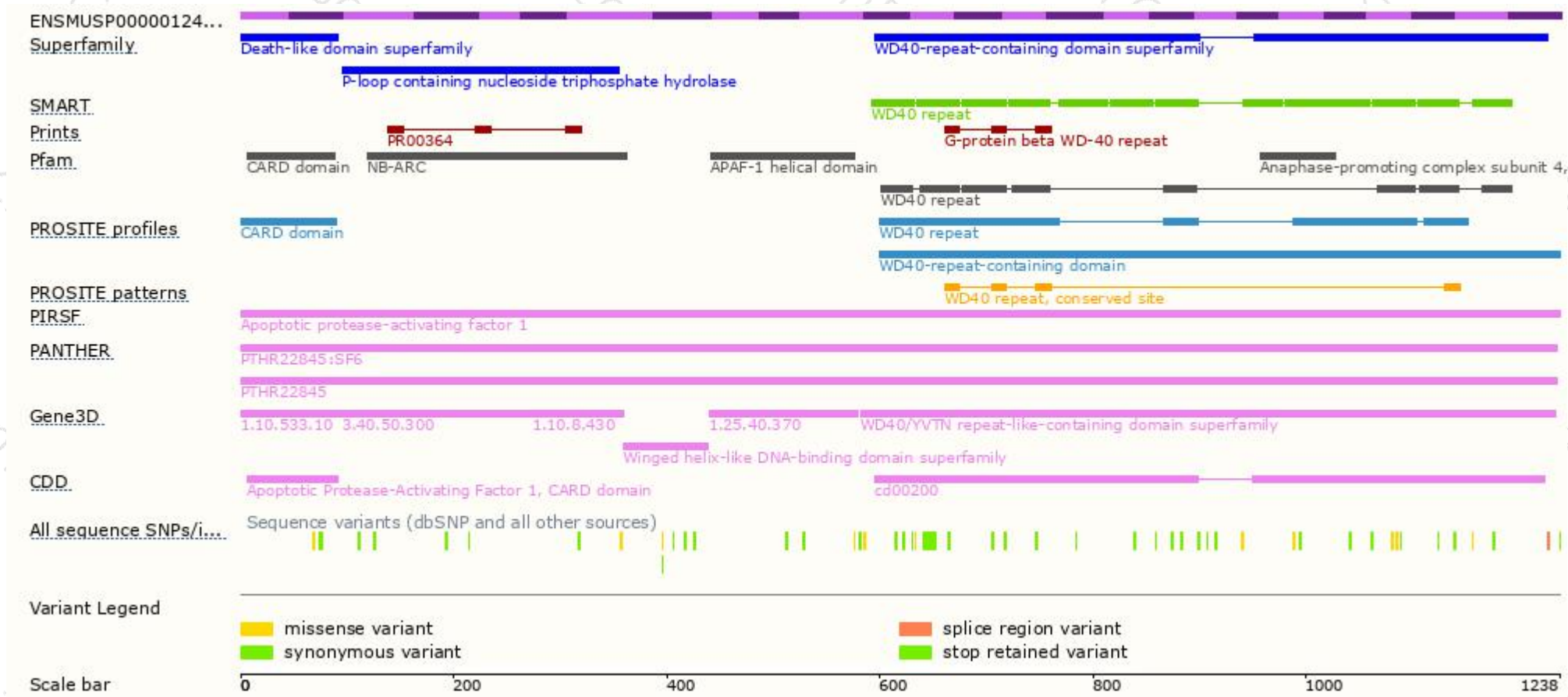
The strategy is based on the design of *Apaf1-208* transcript,The transcription is shown below



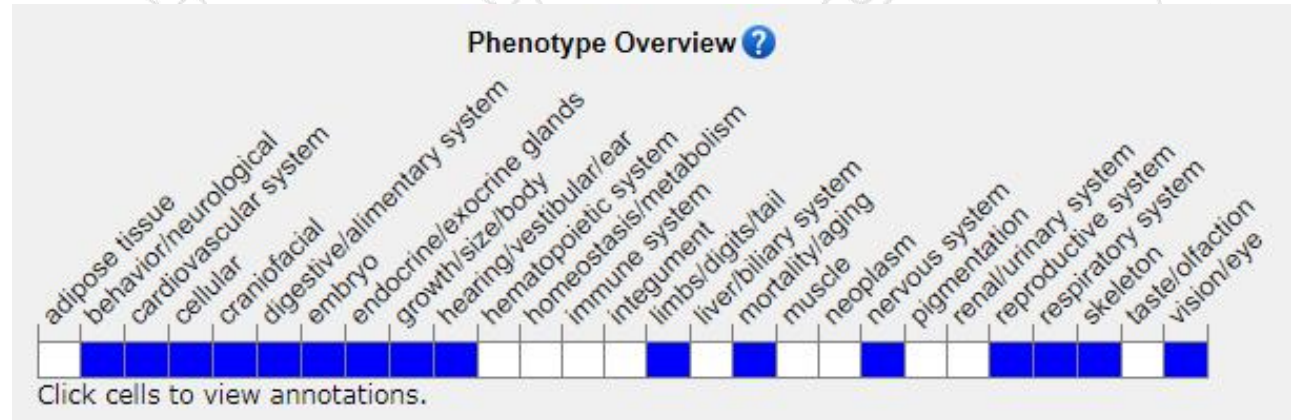
Genomic location distribution



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/>).

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

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