

***Aaas* Cas9-CKO Strategy**

Designer: JiaYu

Reviewer: Xiaojing Li

Design Date: 2020-8-27

Project Overview

Project Name

Aaas

Project type

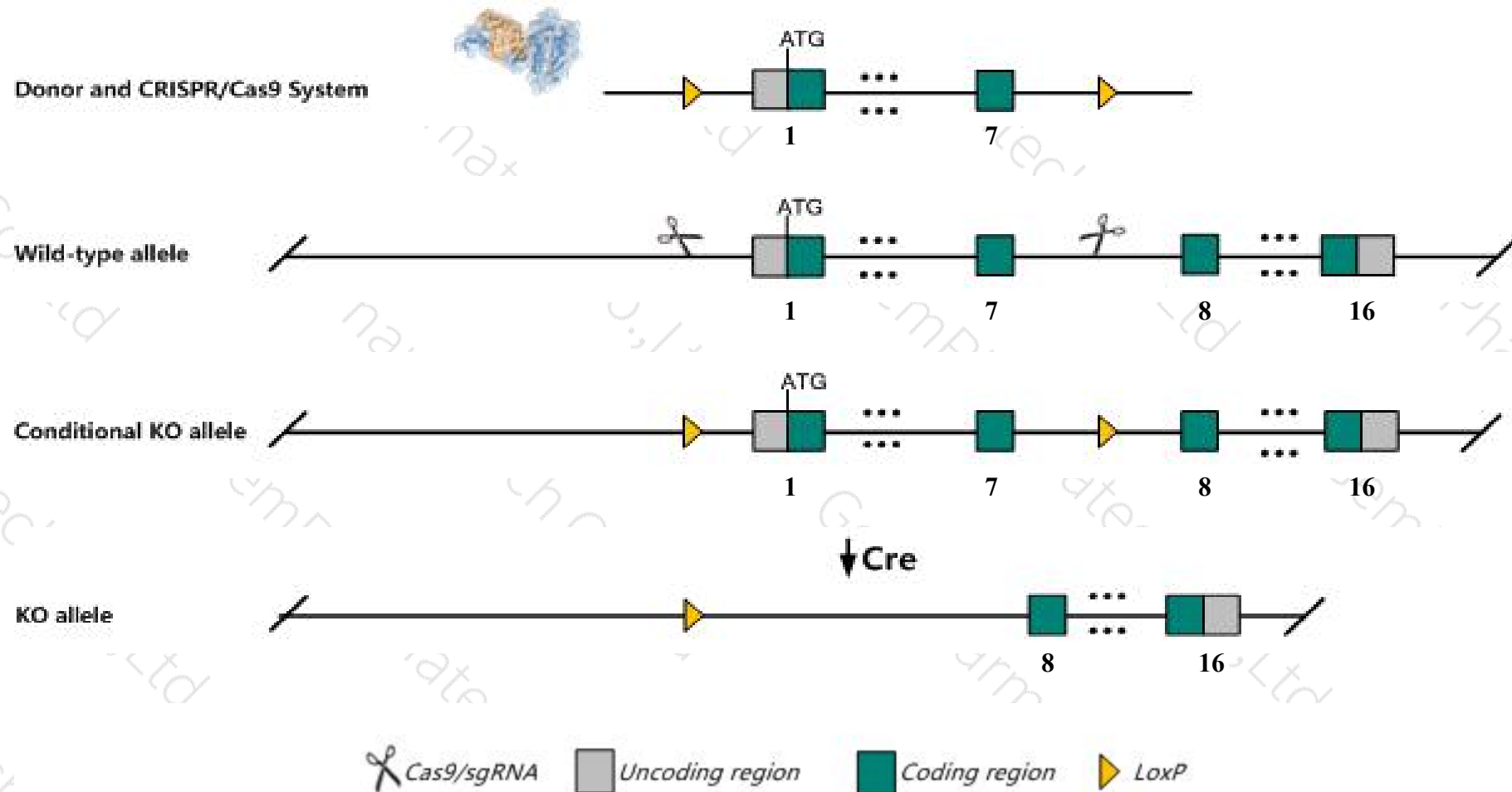
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Aaas* gene has 13 transcripts. According to the structure of *Aaas* gene, exon1-exon7 of *Aaas-201*(ENSMUST00000041208.8) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Aaas* 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.

- According to the existing MGI data, homozygous null mice display female infertility, mildly decreased exploratory behavior, and decreased body weight, but have normal adrenocortical function and do not develop severe neurological abnormalities.
- The flox region contain the Gm36246 gene, which may delete it after Cre.
- The *Aaas* gene is located on the Chr15. 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)

Aaas achalasia, adrenocortical insufficiency, alacrimia [Mus musculus (house mouse)]

Gene ID: 223921, updated on 13-Mar-2020

Summary



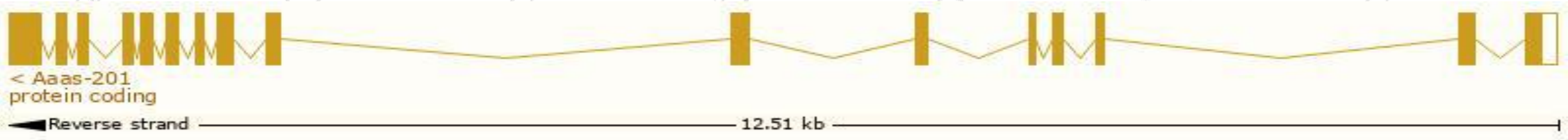
Official Symbol	Aaas provided by MGI
Official Full Name	achalasia, adrenocortical insufficiency, alacrimia provided by MGI
Primary source	MGI:MGI:2443767
See related	Ensembl:ENSMUSG00000036678
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AAA, ADRACALA, D030041N15Rik, GL003
Expression	Ubiquitous expression in thymus adult (RPKM 27.9), limb E14.5 (RPKM 21.3) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

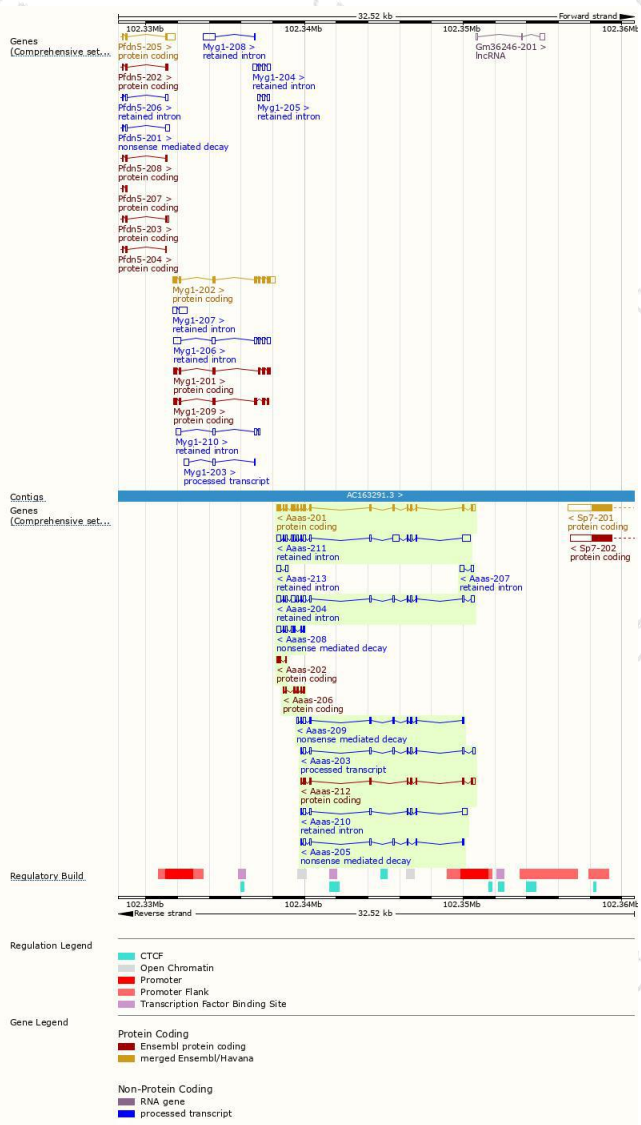
The gene has 13 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Aaas-201	ENSMUST00000041208.8	1811	546aa	Protein coding	CCDS27880	P58742	TSL:1 GENCODE basic APPRIS P1
Aaas-212	ENSMUST00000231061.1	1025	299aa	Protein coding	-	A0A2R8VI65	CDS 3' incomplete
Aaas-206	ENSMUST00000230239.1	440	147aa	Protein coding	-	A0A2R8VI45	CDS 5' and 3' incomplete
Aaas-202	ENSMUST00000228959.1	314	97aa	Protein coding	-	A0A2R8VHB1	CDS 5' incomplete
Aaas-209	ENSMUST00000230481.1	898	183aa	Nonsense mediated decay	-	A0A2R8VHX3	CDS 5' incomplete
Aaas-205	ENSMUST00000229900.1	781	64aa	Nonsense mediated decay	-	A0A2R8VHP6	CDS 5' incomplete
Aaas-208	ENSMUST00000230406.1	697	55aa	Nonsense mediated decay	-	A0A2R8VI59	CDS 5' incomplete
Aaas-203	ENSMUST00000229315.1	1021	No protein	Processed transcript	-	-	
Aaas-211	ENSMUST00000230812.1	2230	No protein	Retained intron	-	-	
Aaas-204	ENSMUST00000229589.1	1814	No protein	Retained intron	-	-	
Aaas-210	ENSMUST00000230710.1	959	No protein	Retained intron	-	-	
Aaas-207	ENSMUST00000230349.1	473	No protein	Retained intron	-	-	
Aaas-213	ENSMUST00000231099.1	398	No protein	Retained intron	-	-	

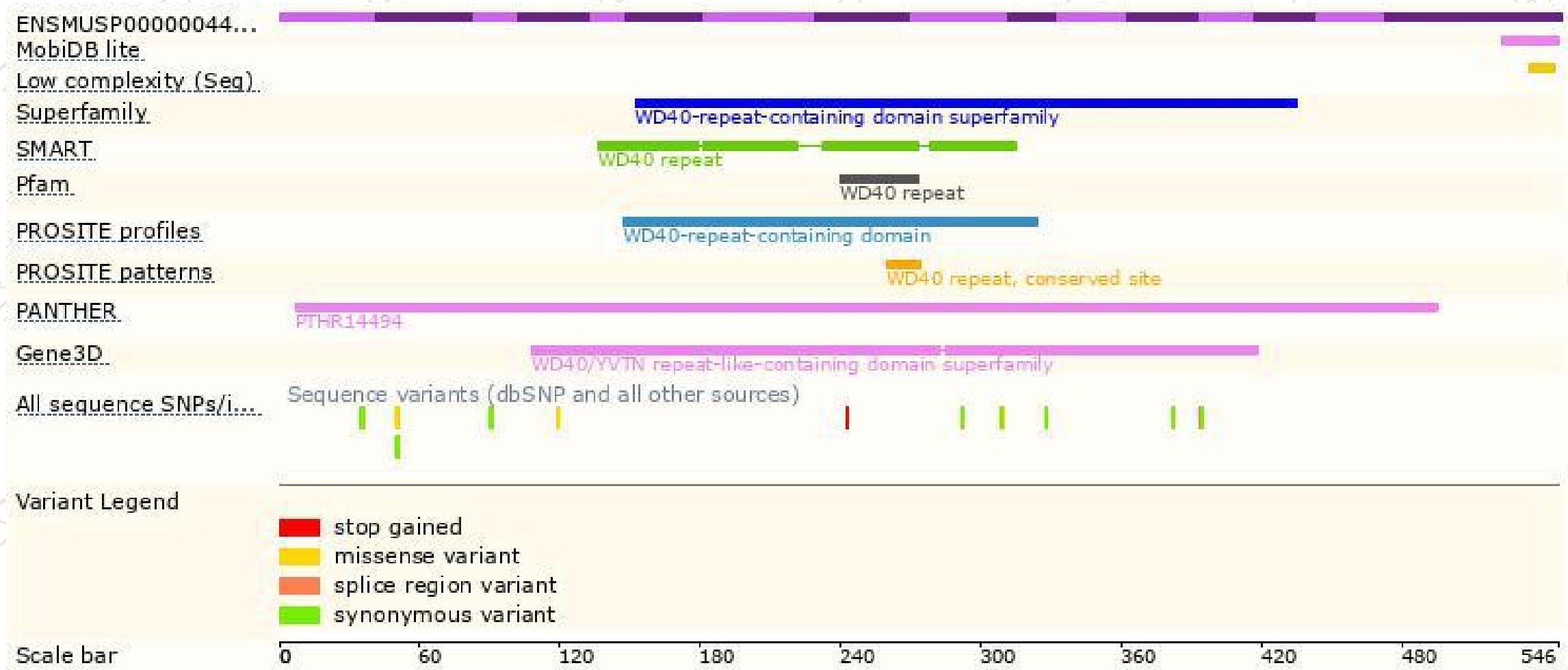
The strategy is based on the design of *Aaas-201* transcript,the transcription is shown below:



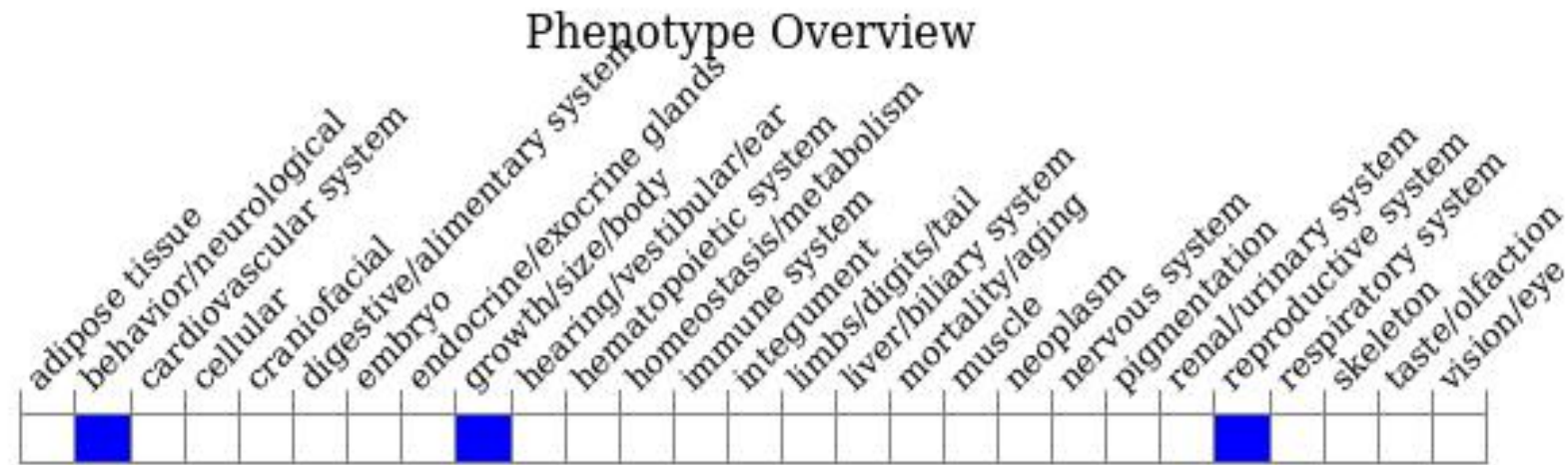
Genomic location distribution



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, homozygous null mice display female infertility, mildly decreased exploratory behavior, and decreased body weight, but have normal adrenocortical function and do not develop severe neurological abnormalities.

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

Tel: 025-5864 1534

