

# Ahil Cas9-CKO Strategy

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**Design Date:** 2019-8-8

# **Project Overview**



**Project Name** 

Ahi1

**Project type** 

Cas9-CKO

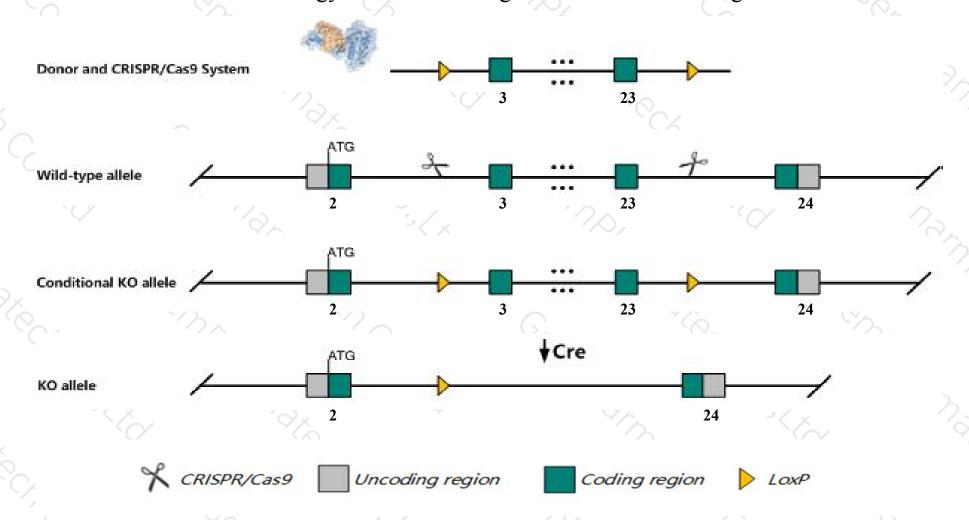
Strain background

C57BL/6JGpt

# Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Ahi1* gene. The schematic diagram is as follows:



### Technical routes



- ➤ The *Ahi1* gene has 7 transcripts. According to the structure of *Ahi1* gene, exon3-exon23 of *Ahi1-201*(ENSMUST00000105525.11) transcript is recommended as the knockout region. The region contains 2830bp coding sequence.

  Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ahi1* 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, Mouse embryonic fibroblasts homozygous for one knock-out allele exhibit reduced and abnormal cilia. Mice homozygous for another knock-out allele exhibit premature death and abnormal kidney morphology and physiology.
- > The *Ahi1* 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)



#### Ahi1 Abelson helper integration site 1 [Mus musculus (house mouse)]

Gene ID: 52906, updated on 10-Feb-2019

#### Summary

☆ ?

Official Symbol Ahi1 provided by MGI

Official Full Name Abelson helper integration site 1 provided by MGI

Primary source MGI:MGI:87971

See related Ensembl: ENSMUSG00000019986

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 1700015F03Rik, Ahi-1, D10Bwg0629e

Expression Biased expression in CNS E18 (RPKM 44.9), frontal lobe adult (RPKM 34.7) and 6 other tissuesSee more

Orthologs <u>human</u> all

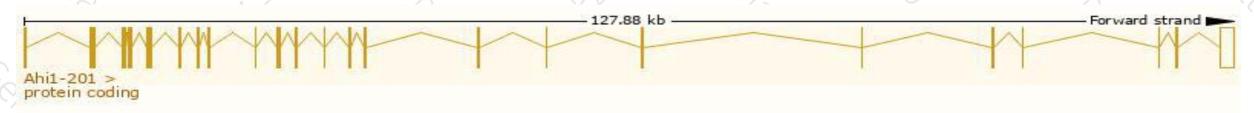
# Transcript information (Ensembl)



The gene has 7 transcripts, all transcripts are shown below:

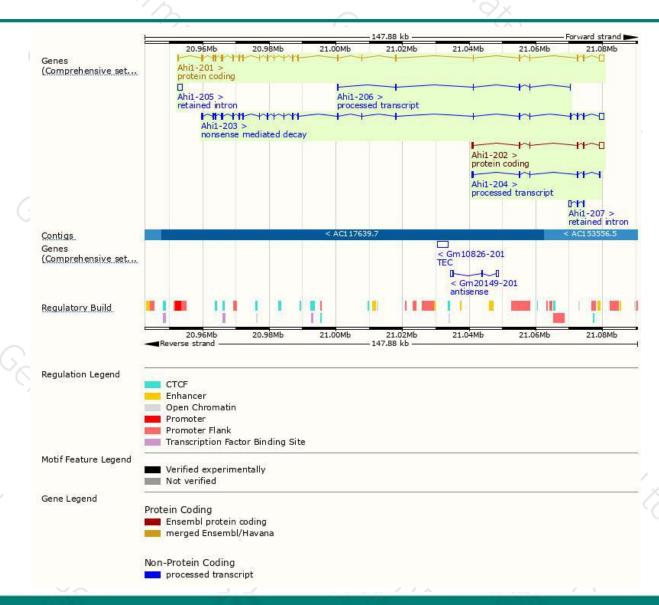
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ahi1-201	ENSMUST00000105525.11	4851	<u>1047aa</u>	Protein coding	CCDS35860	E9QP54	TSL:1 GENCODE basic APPRIS P1
Ahi1-202	ENSMUST00000163505.2	2067	<u>142aa</u>	Protein coding	CCDS48512	E9Q552	TSL:1 GENCODE basic
Ahi1-203	ENSMUST00000213104.1	4829	<u>1005aa</u>	Nonsense mediated decay		A0A1L1SQE2	TSL:2
Ahi1-204	ENSMUST00000213852.1	1038	No protein	Processed transcript	100	20	TSL:1
Ahi1-206	ENSMUST00000214893.1	788	No protein	Processed transcript	-	14	TSL:5
Ahi1-205	ENSMUST00000214745.1	1375	No protein	Retained intron	. *	<del>.</del> *:	TSL:NA
Ahi1-207	ENSMUST00000215543.1	790	No protein	Retained intron	-	20	TSL:5

The strategy is based on the design of *Ahi1-201* transcript, The transcription is shown below



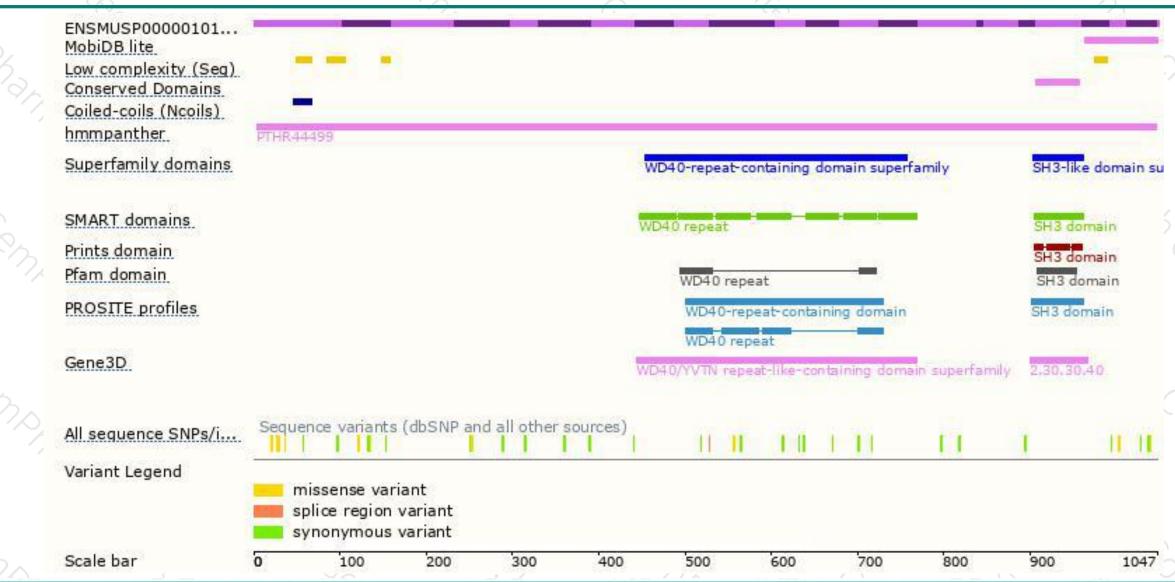
### Genomic location distribution





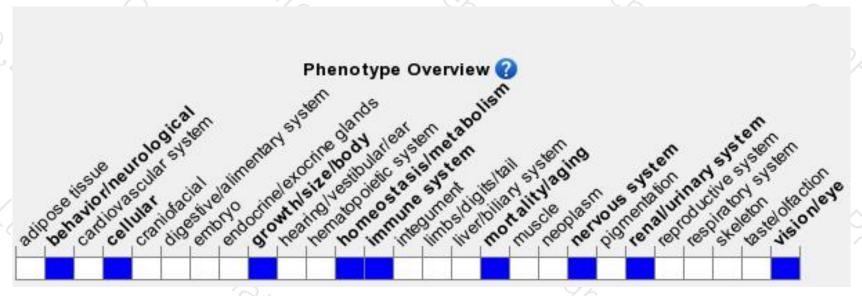
### Protein domain





# 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, Mouse embryonic fibroblasts homozygous for one knock-out allele exhibit reduced and abnormal cilia. Mice homozygous for another knock-out allele exhibit premature death and abnormal kidney morphology physiology.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





