

Ash1l Cas9-CKO Strategy

Designer:

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Design Date:

2019-8-6

Project Overview

Project Name

Ash1l

Project type

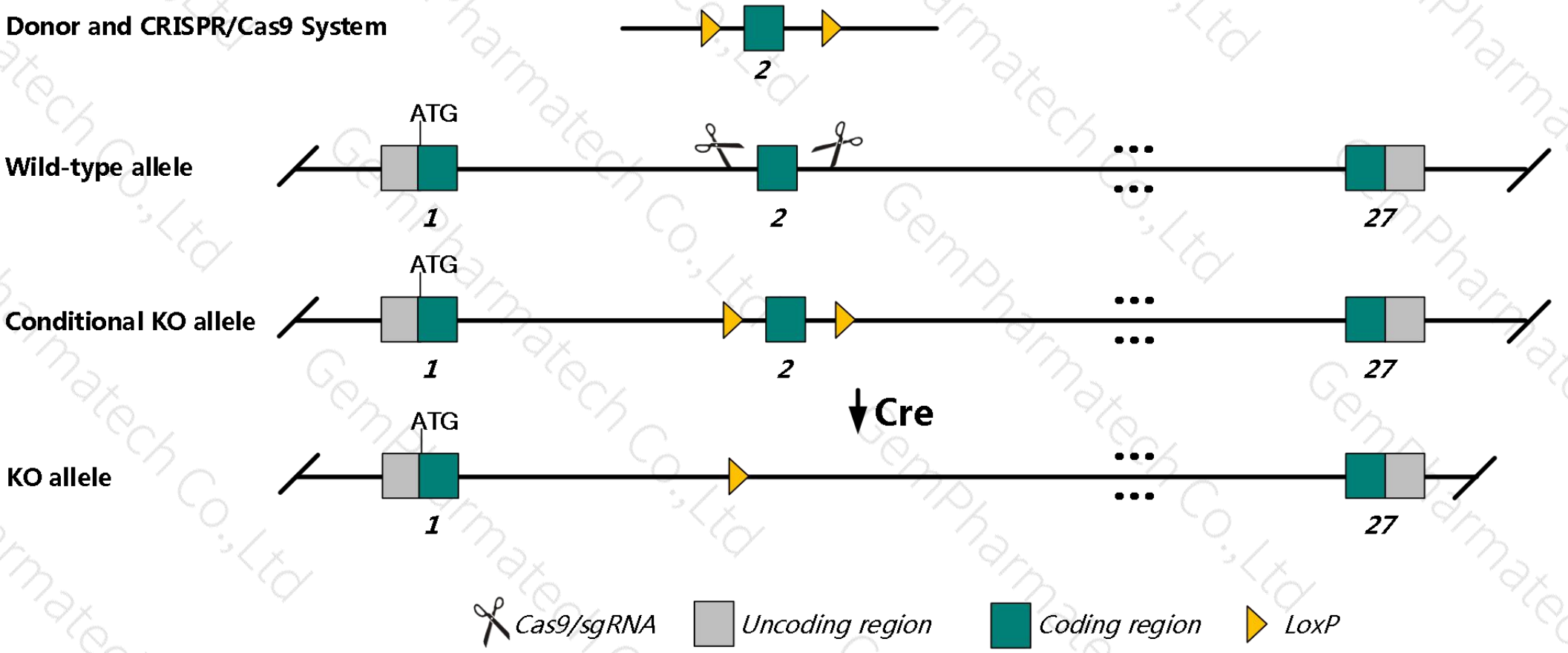
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ash1l* gene. The schematic diagram is as follows:



- The *Ash1l* gene has 4 transcripts. According to the structure of *Ash1l* gene, exon2 of *Ash1l*-201 (ENSMUST00000090933.4) transcript is recommended as the knockout region. The region contains 4558bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ash1l* 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 or cell types.

- According to the existing MGI data , Homozygotes for a transposon-induced allele are more susceptible to endotoxin shock, sepsis, and autoimmune disease. Homozygotes for a hypomorphic allele show reduced growth and postnatal lethality; surviving adults lack Meibomian glands and show vertebral, reproductive organ, and fertility defects.
- The *Ash1l* gene is located on the Chr3. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Ash1l ASH1 like histone lysine methyltransferase [*Mus musculus* (house mouse)]






Gene ID: 192195, updated on 2-Oct-2018

Summary

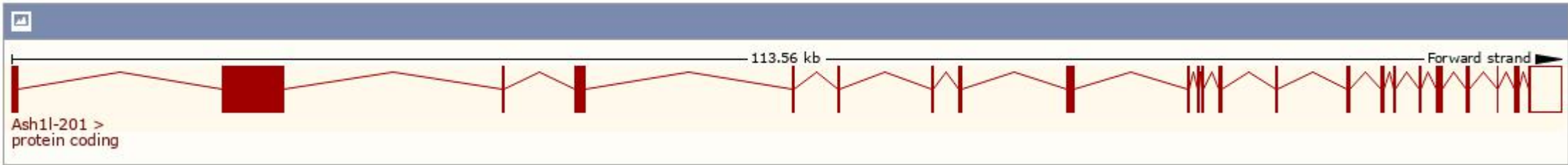
Official Symbol	Ash1l provided by MGI
Official Full Name	ASH1 like histone lysine methyltransferase provided by MGI
Primary source	MGI:MGI:2183158
See related	Ensembl:ENSMUSG000000028053 Vega:OTTMUSG000000047367
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<i>Mus musculus</i>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Ash1; Kmt2h; 8030453L17Rik; E430018P19Rik
Expression	Ubiquitous expression in bladder adult (RPKM 8.0), cerebellum adult (RPKM 8.0) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

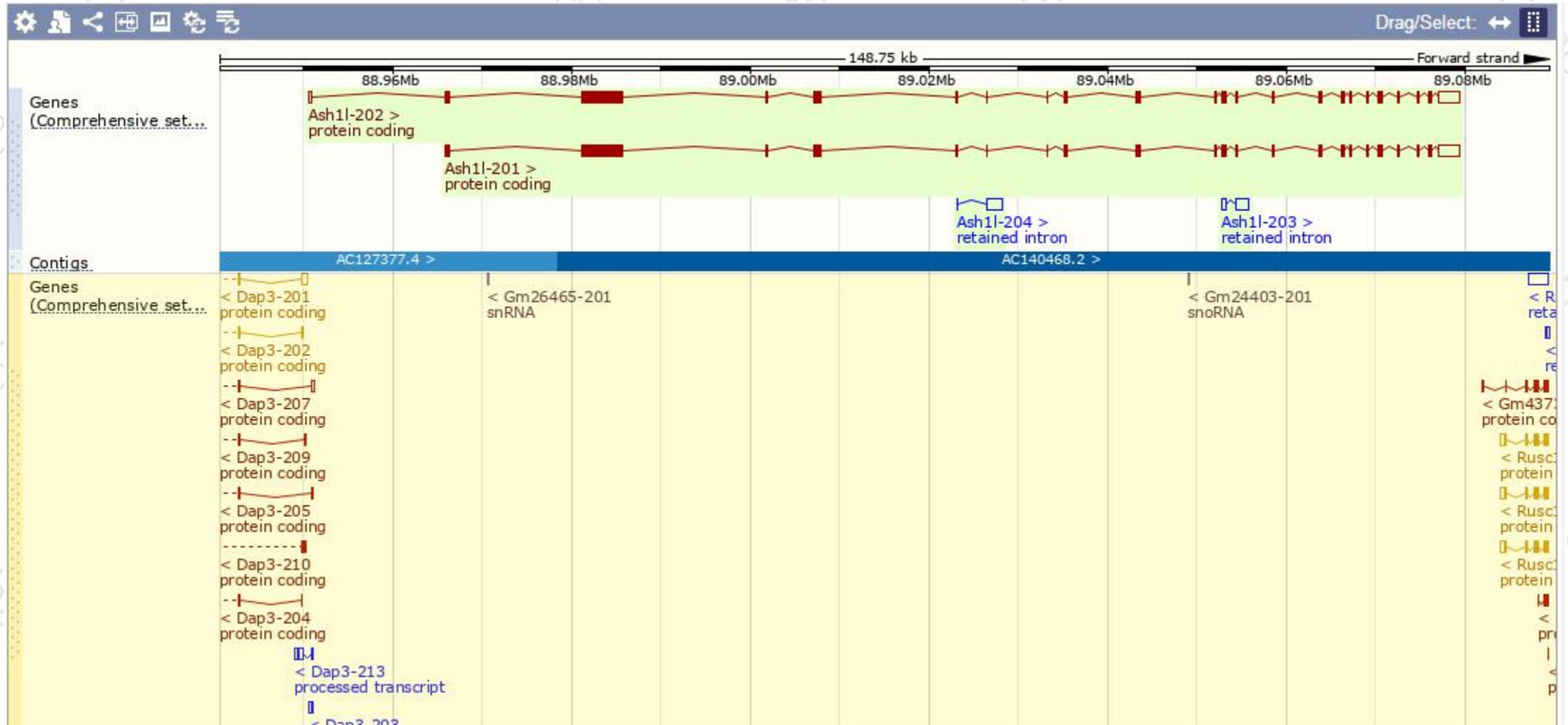
The gene has 4 transcripts, and all transcripts are shown below:

Show/hide columns (1 hidden)								Filter			
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags			
Ash1l-202	ENSMUST00000186583.6	11644	2958aa	 Protein coding	CCDS17487	Q99MY8	-	TSL:5	GENCODE basic	APPRIS P1	
Ash1l-201	ENSMUST00000090933.4	11268	2958aa	 Protein coding	CCDS17487	Q99MY8	NM_138679 NP_619620	TSL:5	GENCODE basic	APPRIS P1	
Ash1l-204	ENSMUST00000198846.1	1885	No protein	 Retained intron	-	-	-	TSL:2			
Ash1l-203	ENSMUST00000189824.1	1788	No protein	 Retained intron	-	-	-	TSL:1			

The strategy is based on the design of *Ash1l*-201 transcript, The transcription is shown below



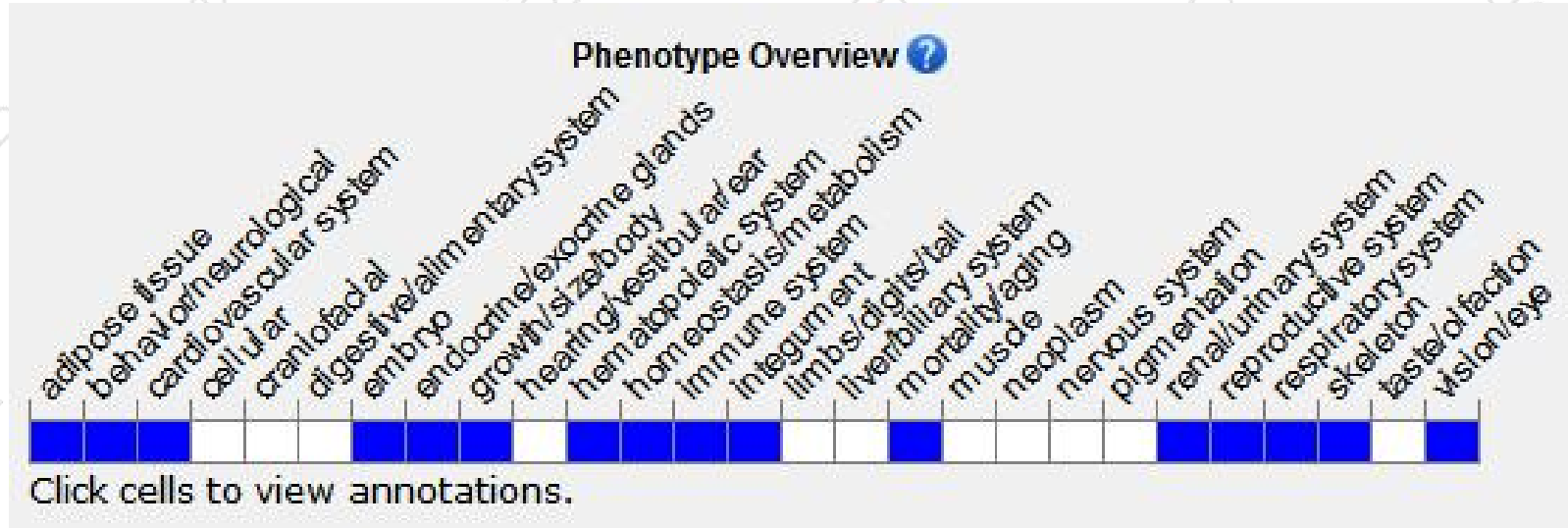
Genomic location distribution



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, Homozygotes for a transposon-induced allele are more susceptible to endotoxin shock, sepsis, and autoimmune disease. Homozygotes for a hypomorphic allele show reduced growth and postnatal lethality; surviving adults lack Meibomian glands and show vertebral, reproductive organ, and fertility defects.

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