

A1cf Cas9-CKO Strategy

Designer:

Huimin Su

Reviewer:

Ruirui Zhang

Design Date:

2019/10/21

Project Overview

Project Name

A1cf

Project type

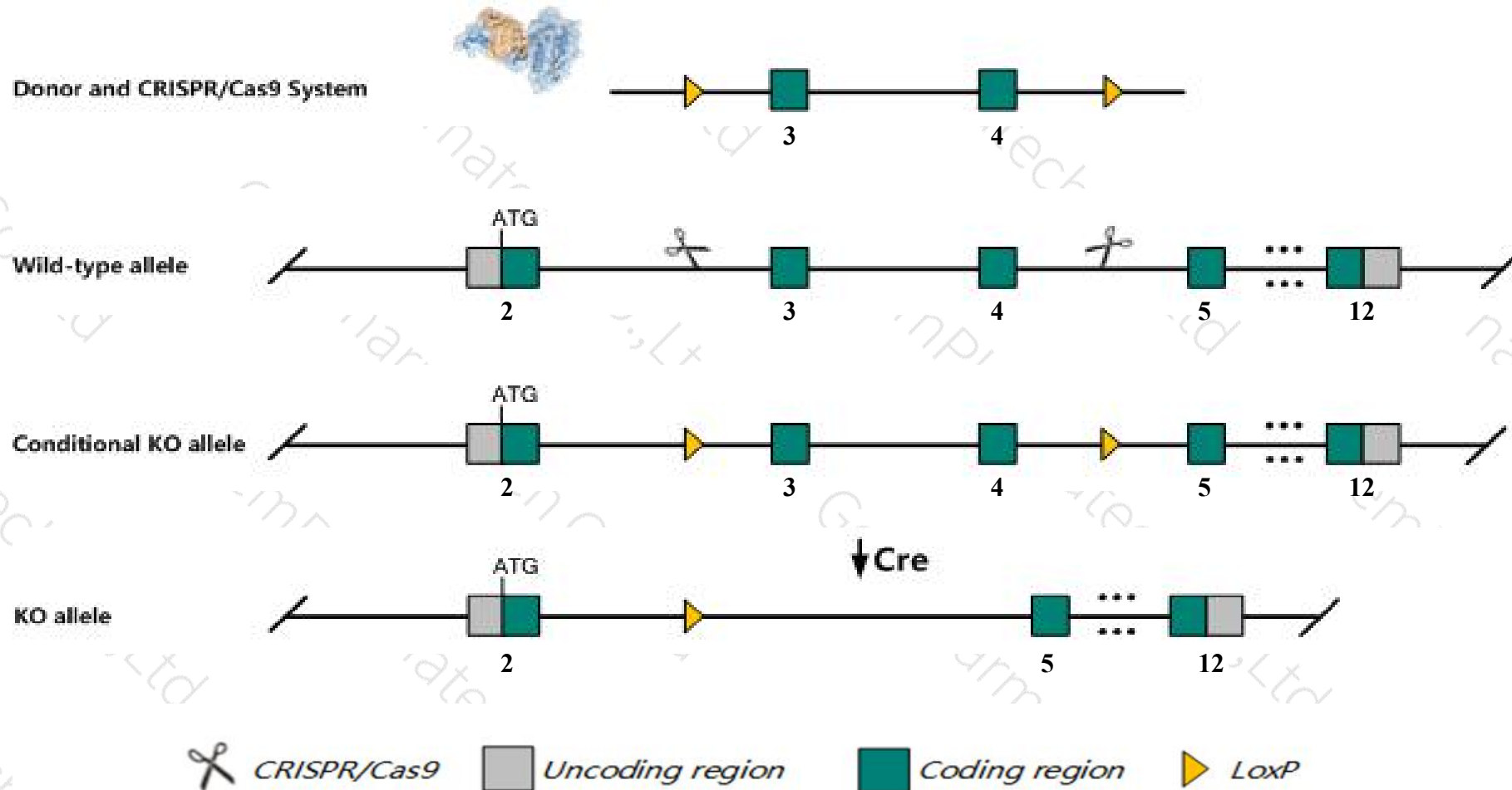
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Alcf* gene has 5 transcripts. According to the structure of *Alcf* gene, exon3-exon4 of *Alcf*-201 (ENSMUST00000075838.7) transcript is recommended as the knockout region. The region contains 266bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Alcf* 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, Embryos homozygous for a targeted deletion of this gene are detectable only until the blastocyst stage (E3.5) and isolated mutant blastocysts fail to proliferate in vitro.
- The distance between exon4 of *Alcf* and *Gm19241* is about 2.4kb, and the knockout of *Alcf* may affect the 5-terminal regulation of *Gm19241*.
- The *Alcf* gene is located on the Chr19. 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)

A1cf APOBEC1 complementation factor [*Mus musculus* (house mouse)]

Gene ID: 69865, updated on 22-Oct-2019

Summary

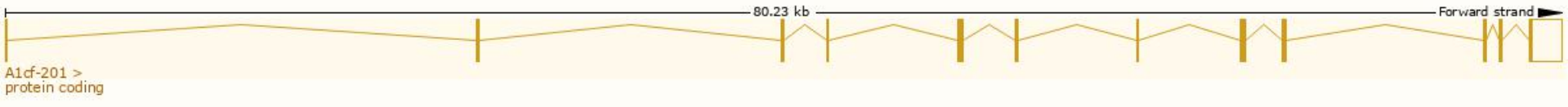
| | |
|---------------------------|---|
| Official Symbol | A1cf provided by MGI |
| Official Full Name | APOBEC1 complementation factor provided by MGI |
| Primary source | MGI:MGI:1917115 |
| See related | Ensembl:ENSMUSG00000052595 |
| Gene type | protein coding |
| RefSeq status | VALIDATED |
| Organism | Mus musculus |
| Lineage | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| Also known as | ASP; Acf; MCM; mer; ACF64; ACF65; MerCreMer; 1810073H04Rik; 9130016M20Rik; alpha-MHC-MerCreMer; alphaMHC-Cre-Mer-Cre; Tg(Myh6-cre/Esr1*)1Jmk |
| Expression | Biased expression in liver adult (RPKM 13.0), liver E18 (RPKM 9.1) and 8 other tissues See more |
| Orthologs | human all |

Transcript information (Ensembl)

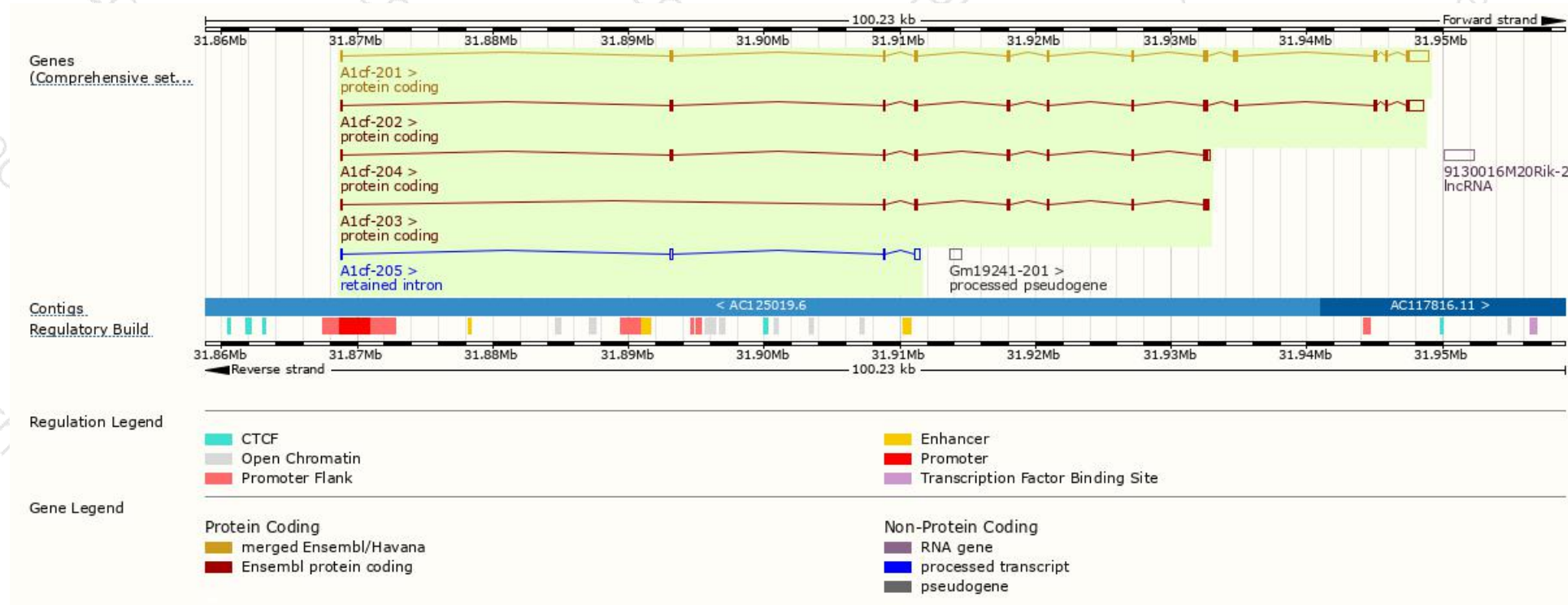
The gene has 5 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|----------|--------------------------------------|------|-----------------------|-----------------|---------------------------|----------------------------|-------------------------------|
| A1cf-201 | ENSMUST00000075838.7 | 3414 | 595aa | Protein coding | CCDS37959 | Q5YD48 | TSL:1 GENCODE basic APPRIS P2 |
| A1cf-202 | ENSMUST00000224304.1 | 2968 | 587aa | Protein coding | - | Q5YD48 | GENCODE basic APPRIS ALT1 |
| A1cf-204 | ENSMUST00000224564.1 | 1432 | 384aa | Protein coding | - | Q5YD48 | GENCODE basic |
| A1cf-203 | ENSMUST00000224400.1 | 1204 | 300aa | Protein coding | - | A0A286YDF7 | GENCODE basic |
| A1cf-205 | ENSMUST00000225165.1 | 696 | No protein | Retained intron | - | - | - |

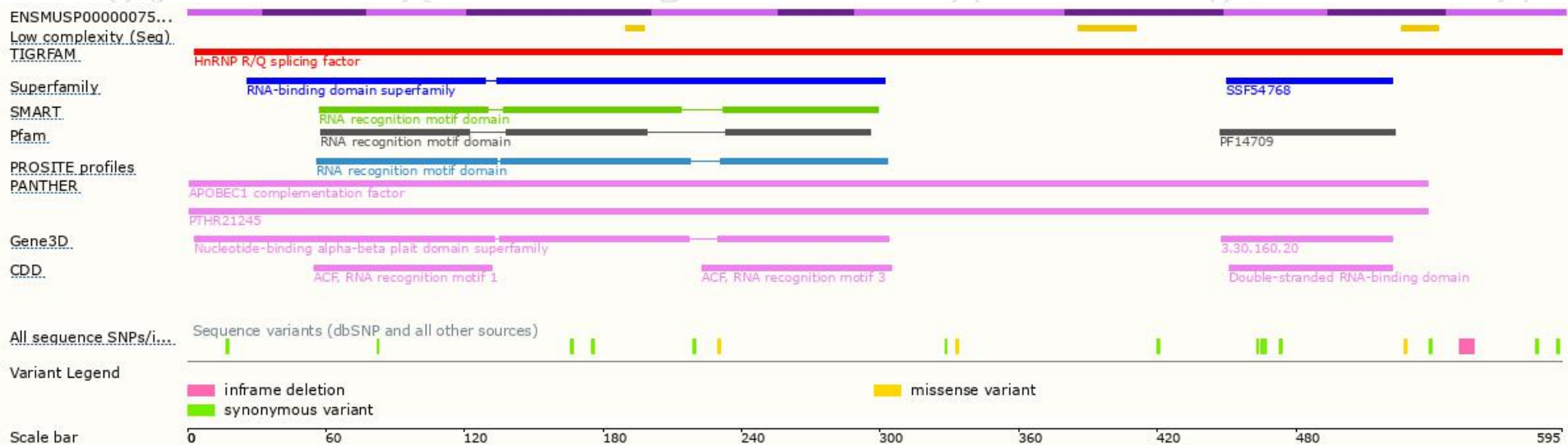
The strategy is based on the design of *A1cf-201* transcript,The transcription is shown below



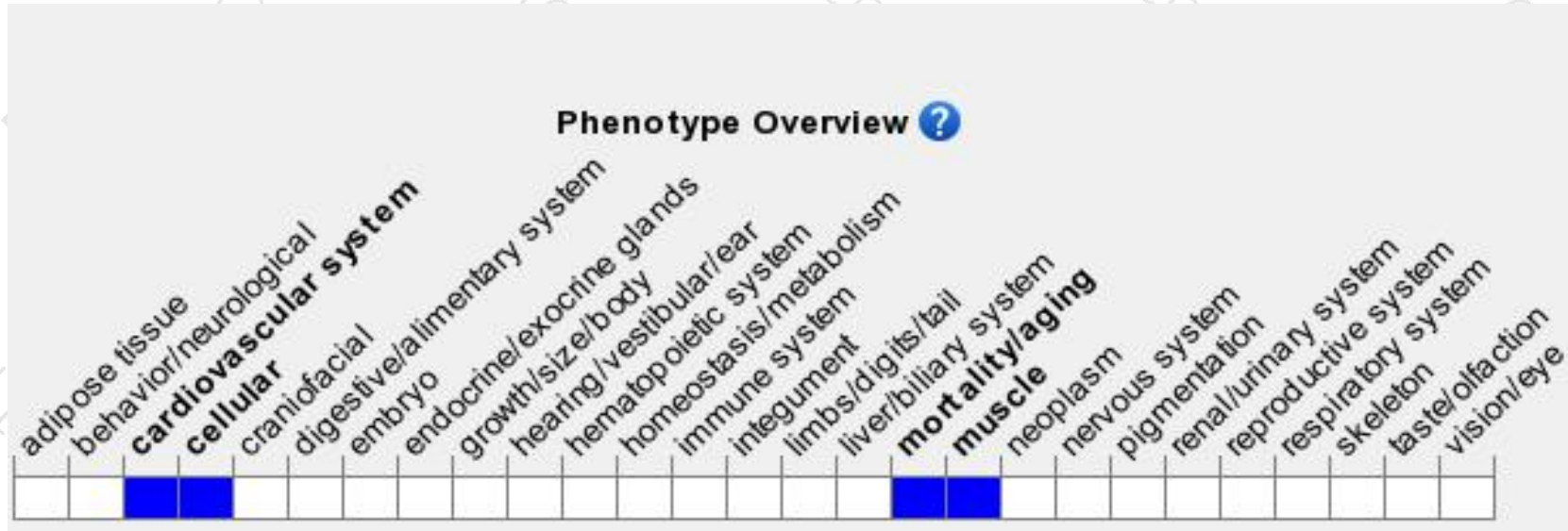
Genomic location distribution



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, Embryos homozygous for a targeted deletion of this gene are detectable only until the blastocyst stage (E3.5) and isolated mutant blastocysts fail to proliferate in vitro.

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

Tel: 400-9660890

