Zfand2a Cas9-CKO Strategy

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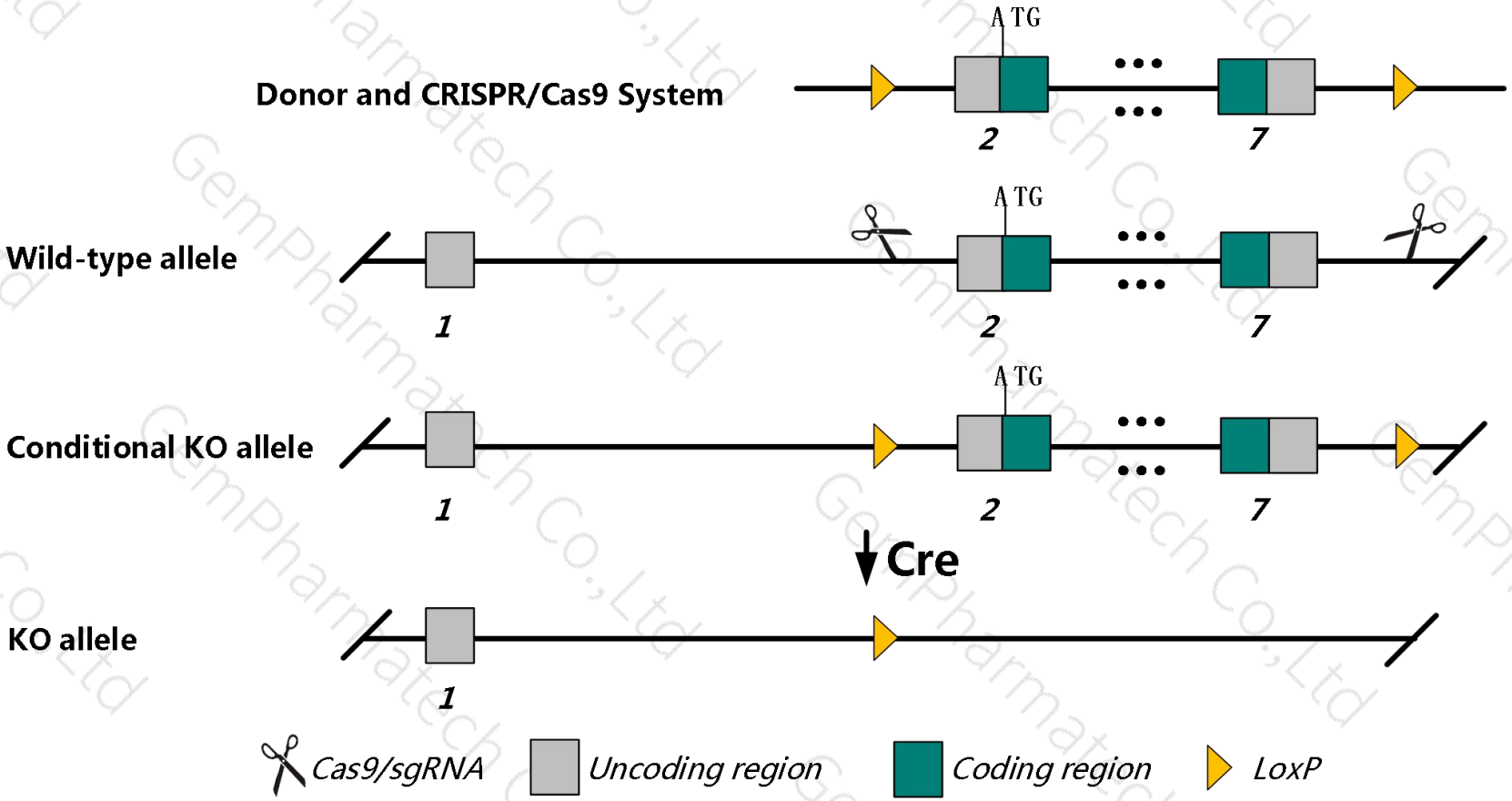
Project Overview

Project Name	***Zfand2a***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Zfand2a* gene. The schematic diagram is as follows:



Technical routes

➢ The *Zfand2a* gene has 5 transcripts. According to the structure of *Zfand2a* gene, exon2-exon7 of *Zfand2a-201*(ENSMUST00000079996.12) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Zfand2a* 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.

Notice

➢ According to the existing MGI data,homozygous mutant mouse embryonic fibroblasts accumulate more polyubiquitylated proteins than wild-type cells and display higher levels of stress when exposed to arsenite, while isolated proteasomes are relatively impaired in substrate degradation in vitro.

➢ The *Zfand2a* gene is located on the Chr5. 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）

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Zfand2a zinc finger, AN1-type domain 2A [Mus musculus (house mouse)]

Gene ID: 100494, updated on 13-Mar-2020

Summary

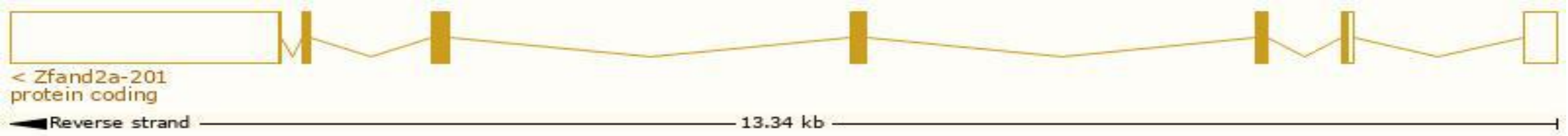
Official Symbol	Zfand2a provided by MGI
Official Full Name	zinc finger, AN1-type domain 2A provided by MGI
Primary source	MGI:MGI:2140729
See related	Ensembl:ENSMUSG00000053581
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AA407930, AU016206, Airap
Expression	Ubiquitous expression in cortex adult (RPKM 12.9), frontal lobe adult (RPKM 11.4) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

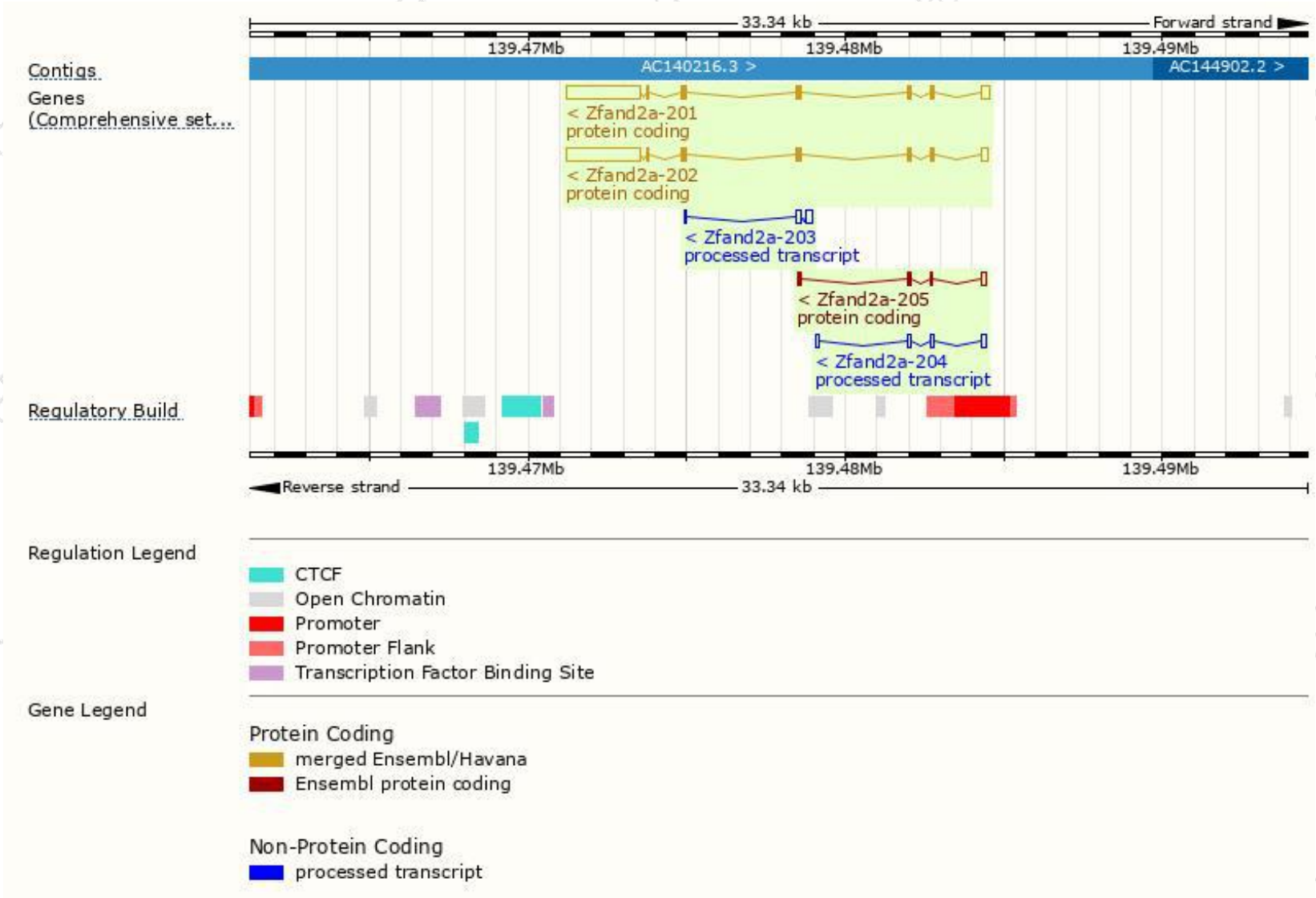
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Zfand2a-201	ENSMUST00000079996.12	3169	171aa	Protein coding	CCDS19812	Q9JII7	TSL:1 GENCODE basic APPRIS P1
Zfand2a-202	ENSMUST00000110851.7	3121	171aa	Protein coding	CCDS19812	Q9JII7	TSL:1 GENCODE basic APPRIS P1
Zfand2a-205	ENSMUST00000150992.1	463	89aa	Protein coding	-	D3YUL0	CDS 3' incomplete TSL:5
Zfand2a-204	ENSMUST00000143889.1	433	No protein	Processed transcript	-	-	TSL:2
Zfand2a-203	ENSMUST00000132454.1	376	No protein	Processed transcript	-	-	TSL:3

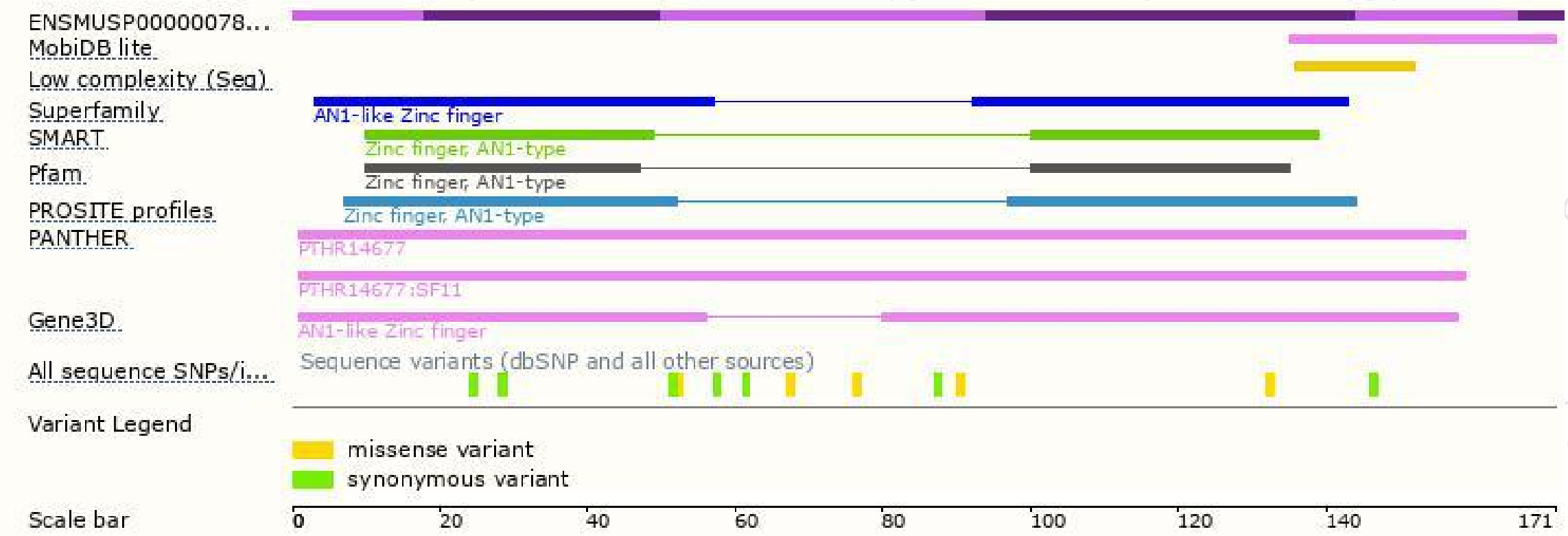
The strategy is based on the design of *Zfand2a-201* transcript,the transcription is shown below:



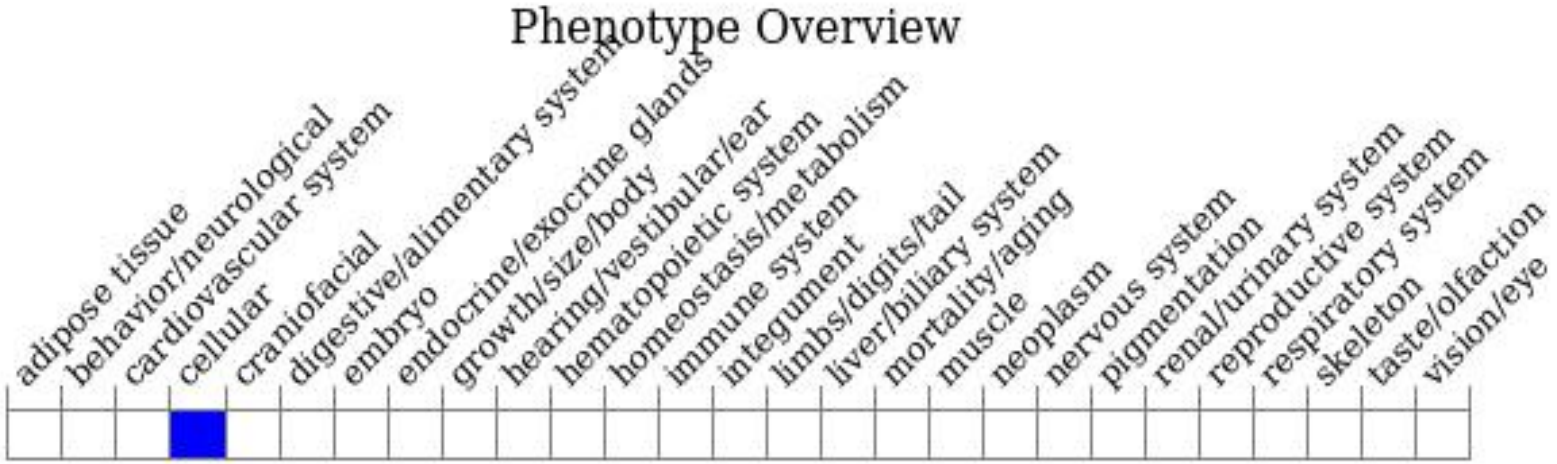
Genomic location distribution



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/).

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

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