U2af2 Cas9-CKO Strategy

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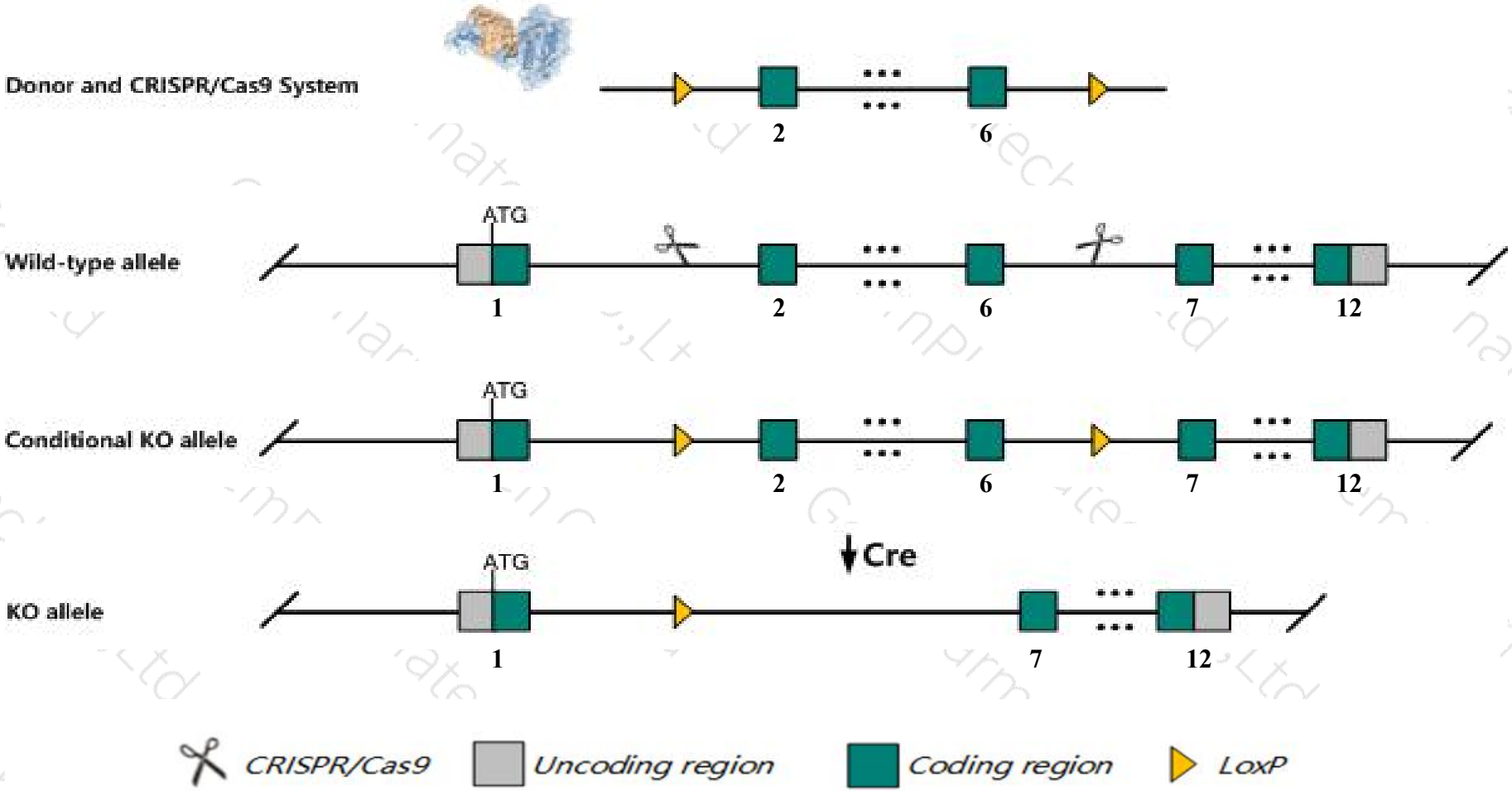
Project Overview

Project Name	***U2af2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *U2af2* gene. The schematic diagram is as follows:



Technical routes

- The *U2af2* gene has 9 transcripts. According to the structure of *U2af2* gene, exon2-exon6 of *U2af2-209* (ENSMUST00000209099.1) transcript is recommended as the knockout region.The region contains 554bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *U2af2* 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

➢ The *U2af2* gene is located on the Chr7. 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.

➢ The insertion of 5'Loxp may affect the 5-terminal regulaiton of *U2af2-202* transcript.

➢ 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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U2af2 U2 small nuclear ribonucleoprotein auxiliary factor (U2AF) 2 [*Mus musculus* (house mouse)]

Gene ID: 22185, updated on 9-Feb-2020

Summary

Official Symbol	U2af2 provided by MGI
Official Full Name	U2 small nuclear ribonucleoprotein auxiliary factor (U2AF) 2 provided by MGI
Primary source	MGI:MGI:98886
See related	Ensembl:ENSMUSG00000030435
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	65kDa
Expression	Ubiquitous expression in CNS E11.5 (RPKM 88.3), CNS E14 (RPKM 83.6) and 28 other tissues See more
Orthologs	human all

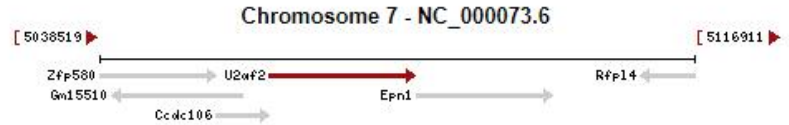
Genomic context

Location: 7; 7 A1

See U2af2 in Genome Data Viewer

Exon count: 14

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	7	NC_000073.6 (5060885..5079945)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	7	NC_000073.5 (5013784..5031541)

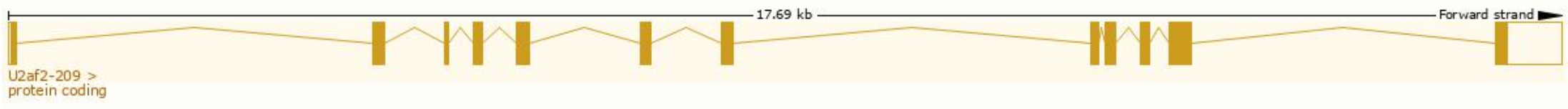


Transcript information（Ensembl）

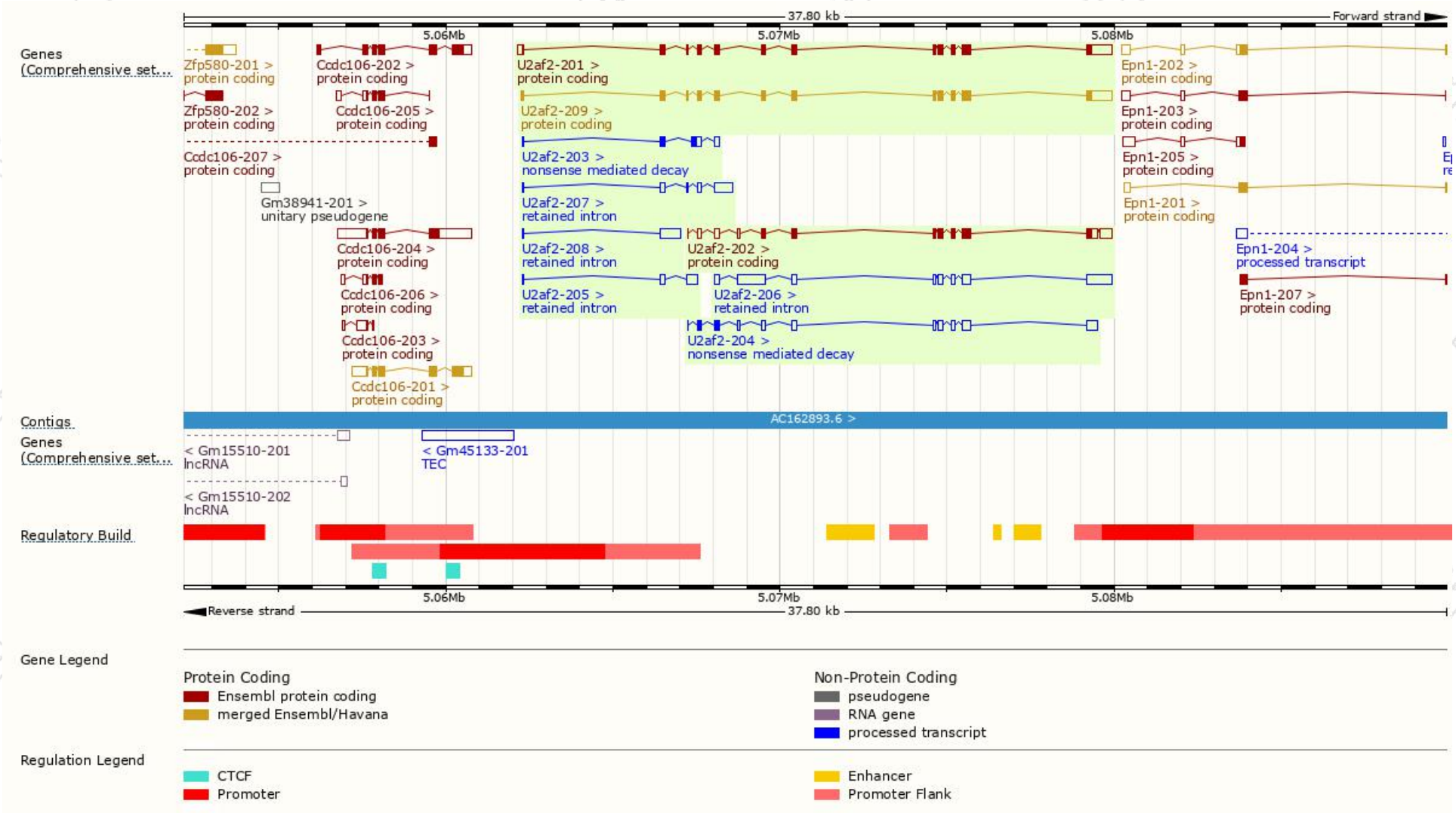
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
U2af2-201	ENSMUST00000005041.14	2183	471aa	Protein coding	CCDS85208	Q80XR5	TSL:1 GENCODE basic APPRIS ALT1
U2af2-209	ENSMUST00000209099.1	2088	475aa	Protein coding	CCDS57476	P26369	TSL:1 GENCODE basic APPRIS P3
U2af2-202	ENSMUST00000165399.1	1810	307aa	Protein coding	-	Q3KQM4	TSL:5 GENCODE basic
U2af2-204	ENSMUST00000207498.1	1455	95aa	Nonsense mediated decay	-	A0A140LJK3	CDS 5' incomplete TSL:5
U2af2-203	ENSMUST00000207097.1	626	111aa	Nonsense mediated decay	-	A0A140LJ08	TSL:3
U2af2-206	ENSMUST00000207926.1	2412	No protein	Retained intron	-	-	TSL:2
U2af2-207	ENSMUST00000207960.1	904	No protein	Retained intron	-	-	TSL:2
U2af2-208	ENSMUST00000208419.1	684	No protein	Retained intron	-	-	TSL:2
U2af2-205	ENSMUST00000207523.1	486	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *U2af2-209* transcript,The transcription is shown below



Genomic location distribution



Protein domain

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

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