Cfap65 Cas9-CKO Strategy

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Project Overview

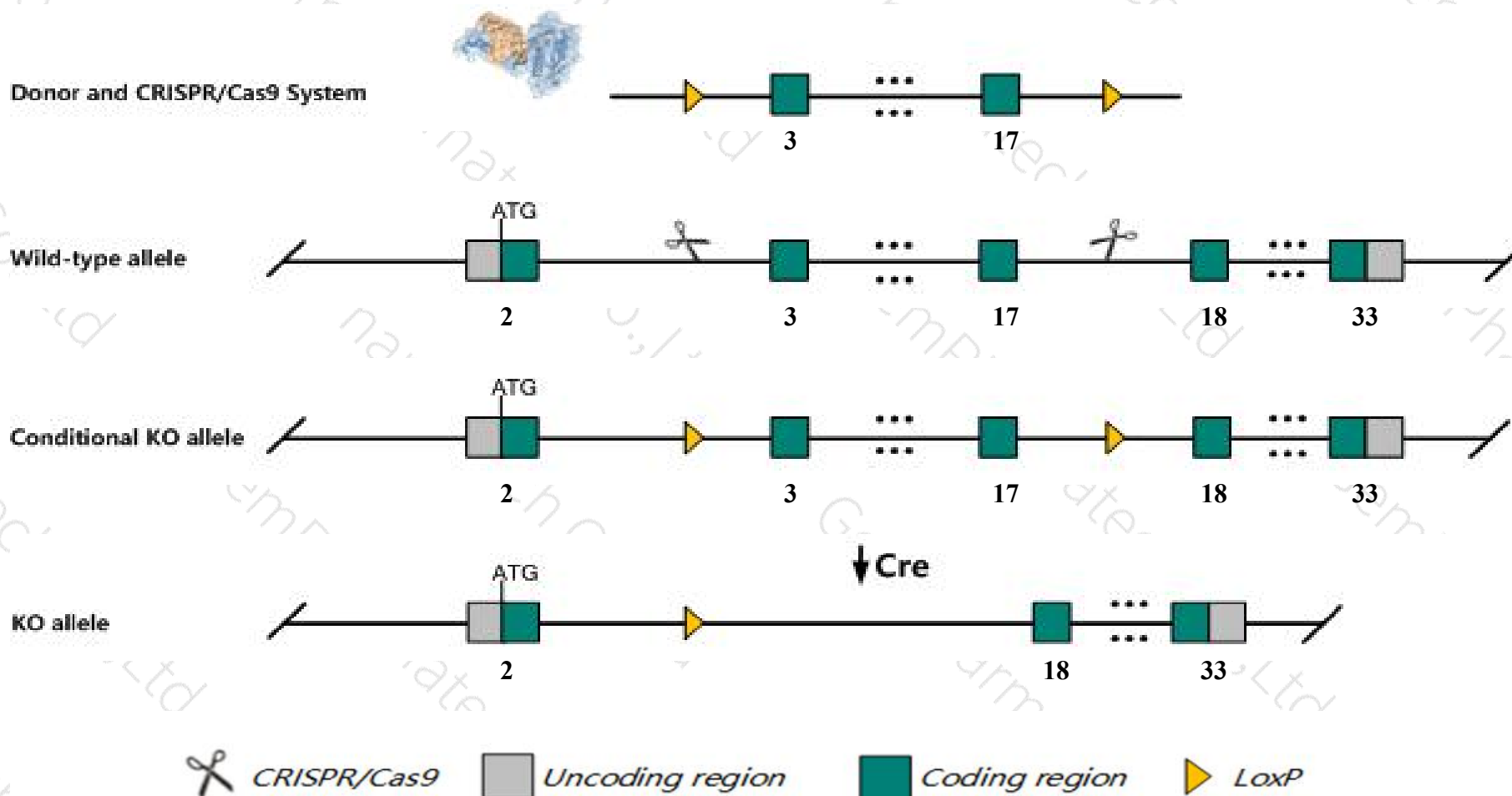|	
Project Name	*Cfap65*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cfap65* gene. The schematic diagram is as follows:

Technical routes

➢ The *Cfap65* gene has 3 transcripts. According to the structure of *Cfap65* gene, exon3-exon17 of *Cfap65-201* (ENSMUST00000094844.3) transcript is recommended as the knockout region.The region contains Ens 2914bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Cfap65* 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.

➢ The *Cfap65* gene is located on the Chr1. 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）

Cfap65 cilia and flagella associated protein 65 [Mus musculus (house mouse)]
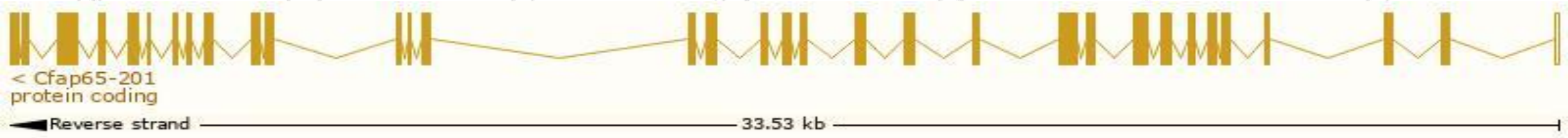
Gene ID: 241116, updated on 13-Mar-2020

▲Summary

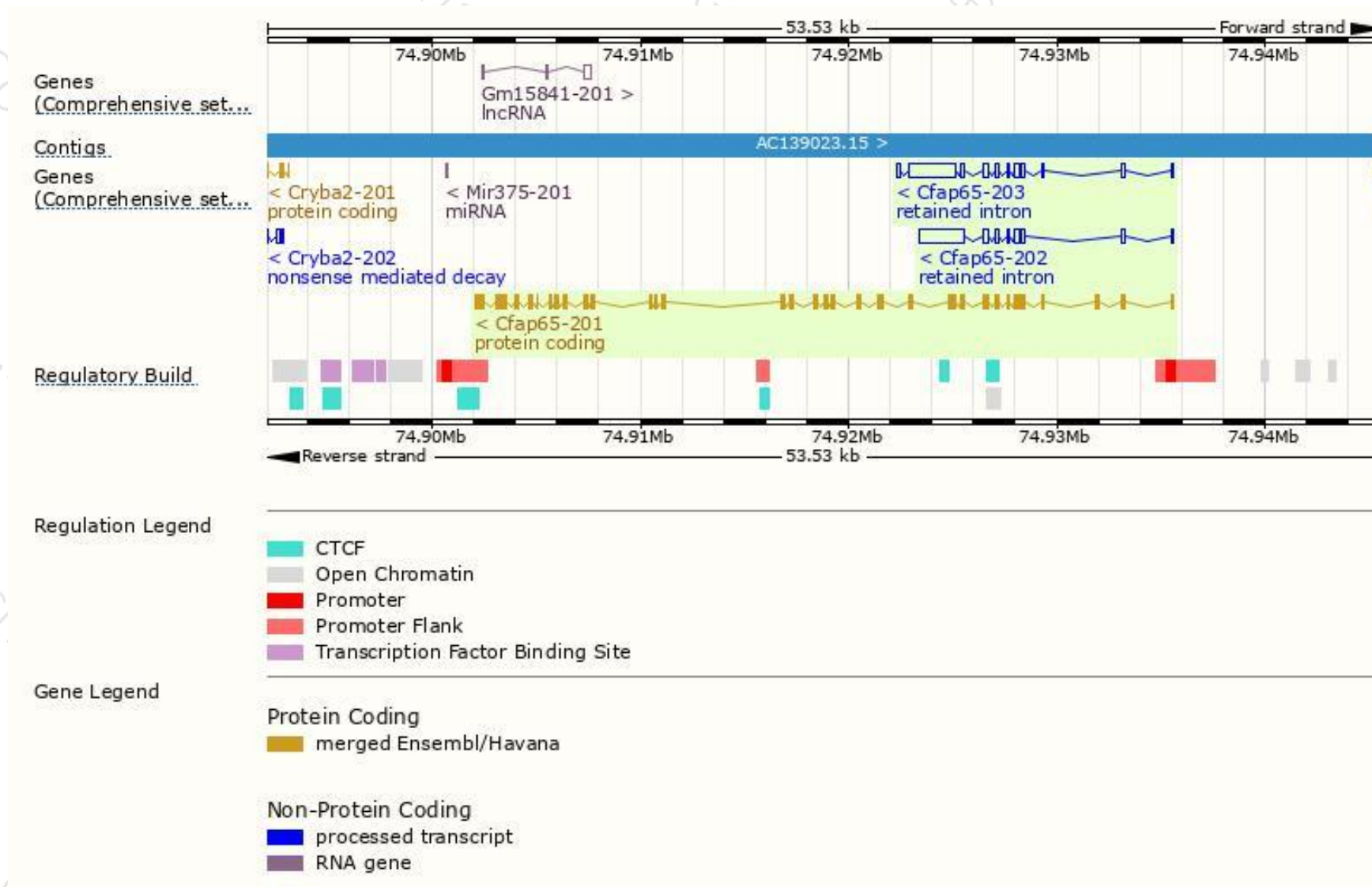|	
Official Symbol	Cfap65 provided by MGI
Official Full Name	cilia and flagella associated protein 65 provided byMGI
Primary source	MGI:MGI:2444274
See related	Ensembl:ENSMUSG00000047021
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A230051F10, B230363K08Rik, Ccdc108
Expression	Biased expression in testis adult (RPKM 56.8), lung adult (RPKM 7.4) and 1 other tissueSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cfap65-201	ENSMUST00000094844.3	5727	1847aa	Protein coding	CCDS15060	Q3V0B4	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Cfap65-203	ENSMUST00000139950.7	3947	No protein	Retained intron	-	-	TSL:1
Cfap65-202	ENSMUST00000130489.1	3425	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Cfap65-201* transcript,the transcription is shown below：

< Cfap65-201
protein coding

◄—Reverse strand——————————————————— 33.53 kb ——————————————————

Genomic location distribution

Protein domain

ENSMUSP00000092...	
Transmembrane heli...	
MobiDB lite	
Low complexity (Seq).	
Coiled-coils (Ncoils)	
PANTHER	PTHR46127
Gene3D	Immunoglobulin-like fold
All sequence SNPs/i...	Sequence variants (dbSNP and all other sources)

Variant Legend

- inframe insertion
- missense variant
- splice region variant
- synonymous variant

Scale bar: 0　200　400　600　800　1000　1200　1400　1600　1847
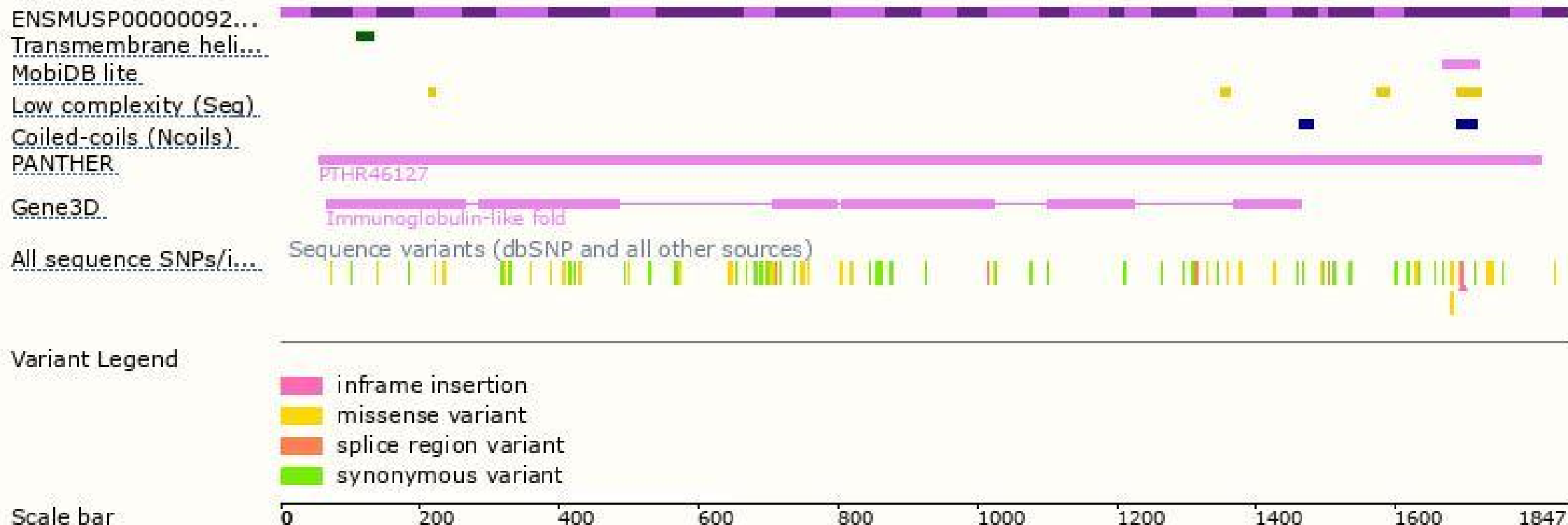
If you have any questions, you are welcome to inquire.
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