Ift20 Cas9-KO Strategy

Designer:Daohua Xu

Reviewer: Xueting Zhang

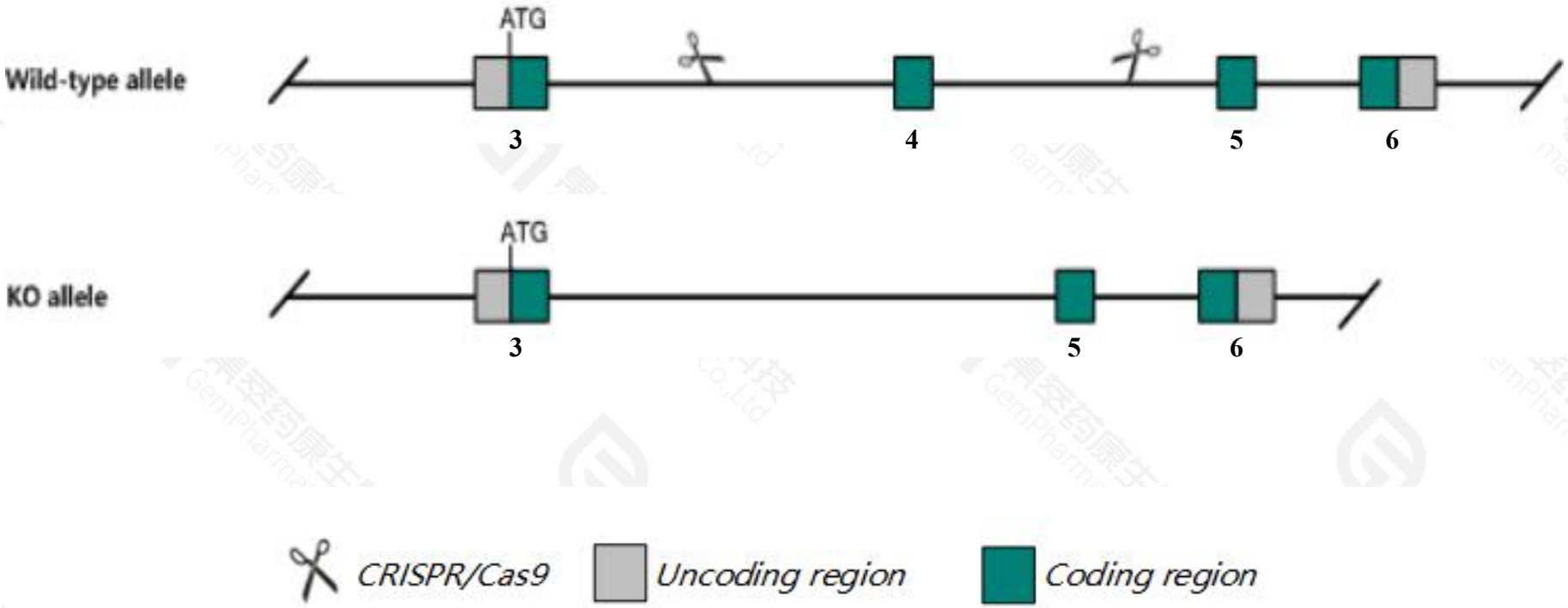
Design Date: 2021-10-13

Project Overview

Project Name	***Ift20***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ift20* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ift20* gene has 5 transcripts. According to the structure of *Ift20* gene, exon4 of *Ift20-203*(ENSMUST00000128788.8) transcript is recommended as the knockout region.The region contains 86bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ift20* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

➢ According to the existing MGI data,mice homozygous for a null mutation die before birth. Mice with conditional loss in renal collecting ducts lack primary cilia and develop renal cysts.

➢ This strategy may affect the 5-terminal regulation of *Tnfaip1* gene.

➢ The *Ift20* gene is located on the Chr11. 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 gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Ift20 intraflagellar transport 20 [Mus musculus (house mouse)]

Gene ID: 55978, updated on 12-Feb-2021

Summary

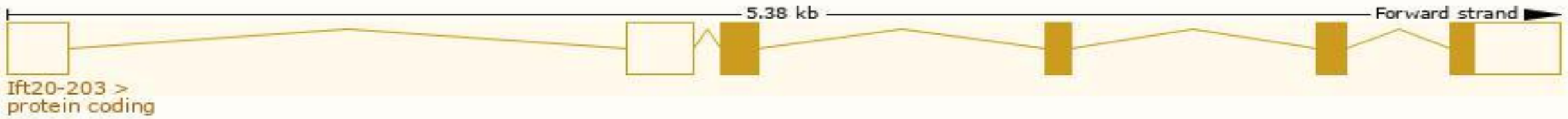
Official Symbol	Ift20 provided by MGI
Official Full Name	intraflagellar transport 20 provided byMGI
Primary source	MGI:MGI:1915585
See related	Ensembl:ENSMUSG00000001105
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	0610009H04Rik, AU015496, mIFT20
Expression	Ubiquitous expression in duodenum adult (RPKM 42.4), small intestine adult (RPKM 37.8) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

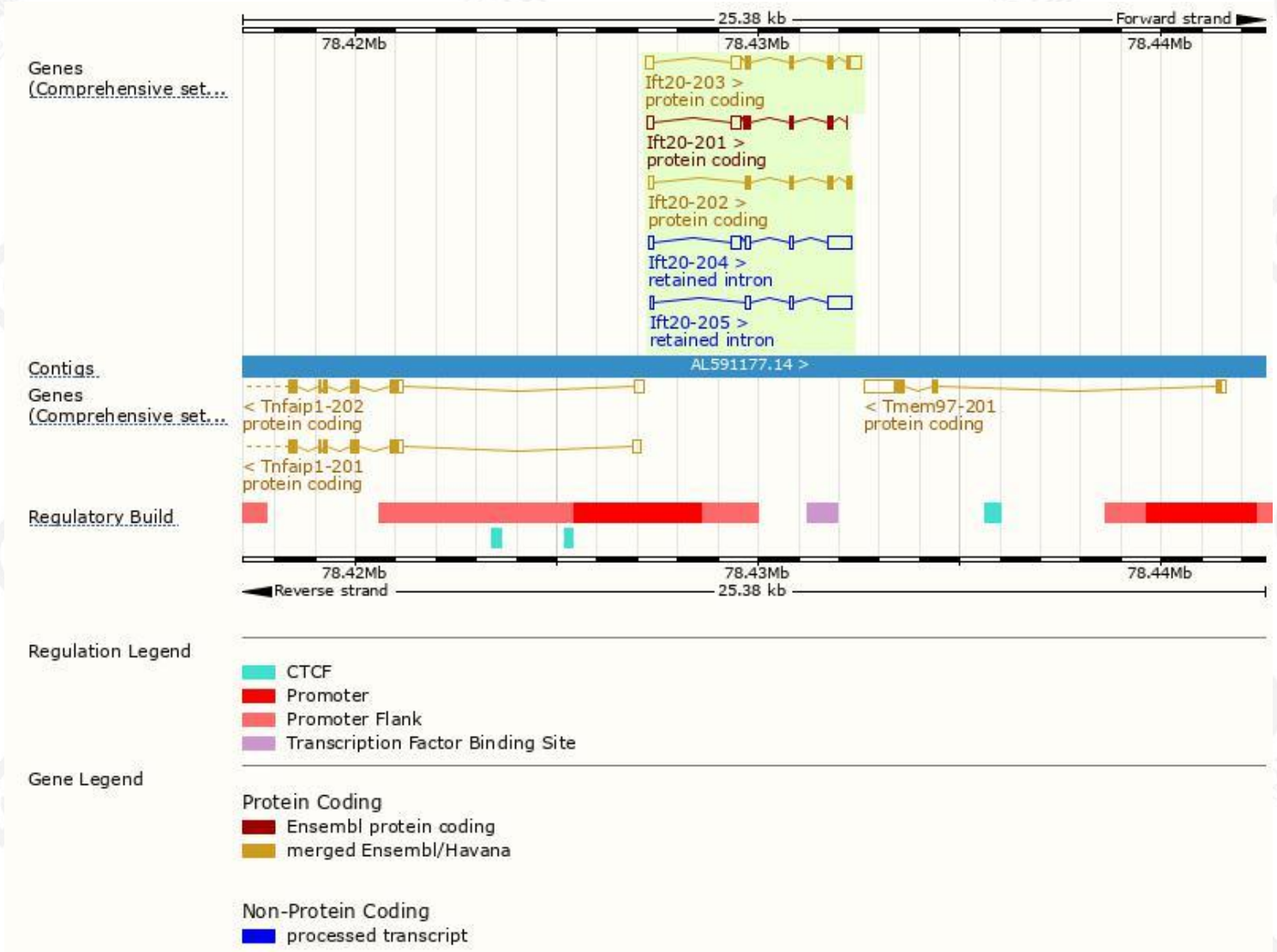
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ift20-203	ENSMUST00000128788.8	1141	132aa	Protein coding	CCDS25111		TSL:1 , GENCODE basic , APPRIS P1 ,
Ift20-202	ENSMUST00000108275.2	582	132aa	Protein coding	CCDS25111		TSL:1 , GENCODE basic , APPRIS P1 ,
Ift20-201	ENSMUST00000050366.15	735	111aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Ift20-204	ENSMUST00000137120.8	1167	No protein	Retained intron	-		TSL:1 ,
Ift20-205	ENSMUST00000152016.2	909	No protein	Retained intron	-		TSL:2 ,

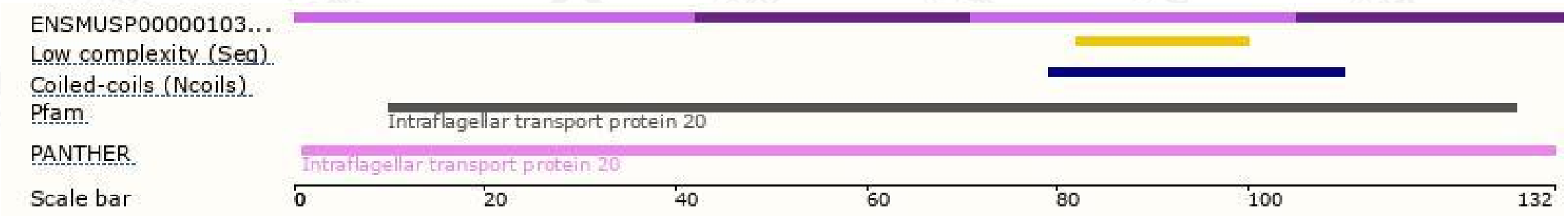
The strategy is based on the design of *Ift20-203* transcript,the transcription is shown below:



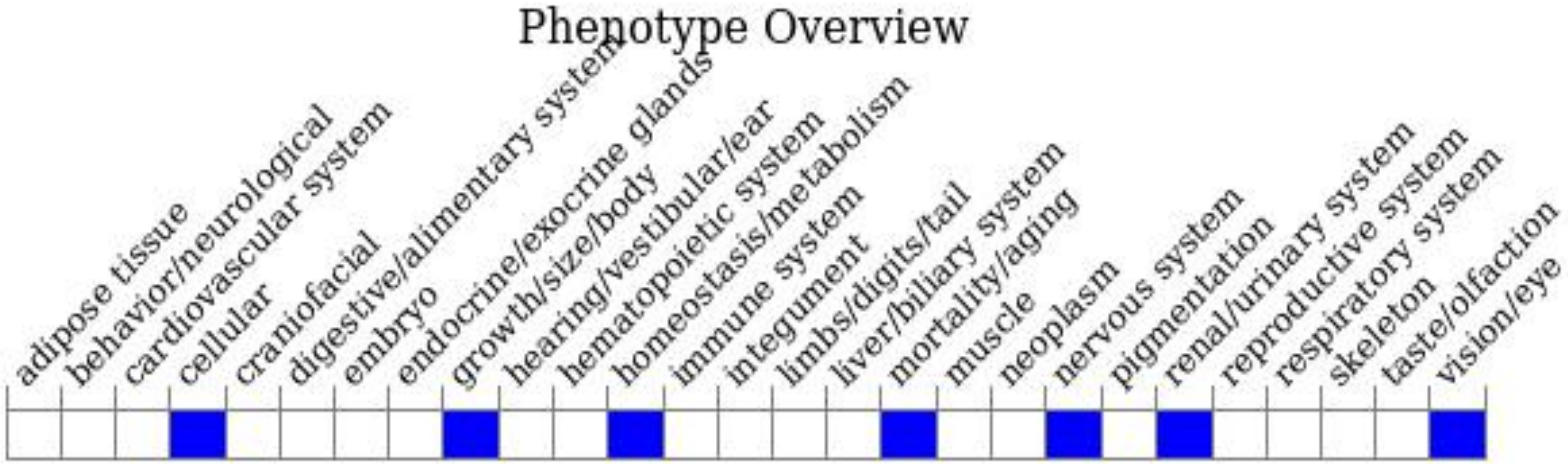
Genomic location distribution



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.
Tel: 400-9660890

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