Wdfy4 Cas9-CKO *Strategy*

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Project Overview

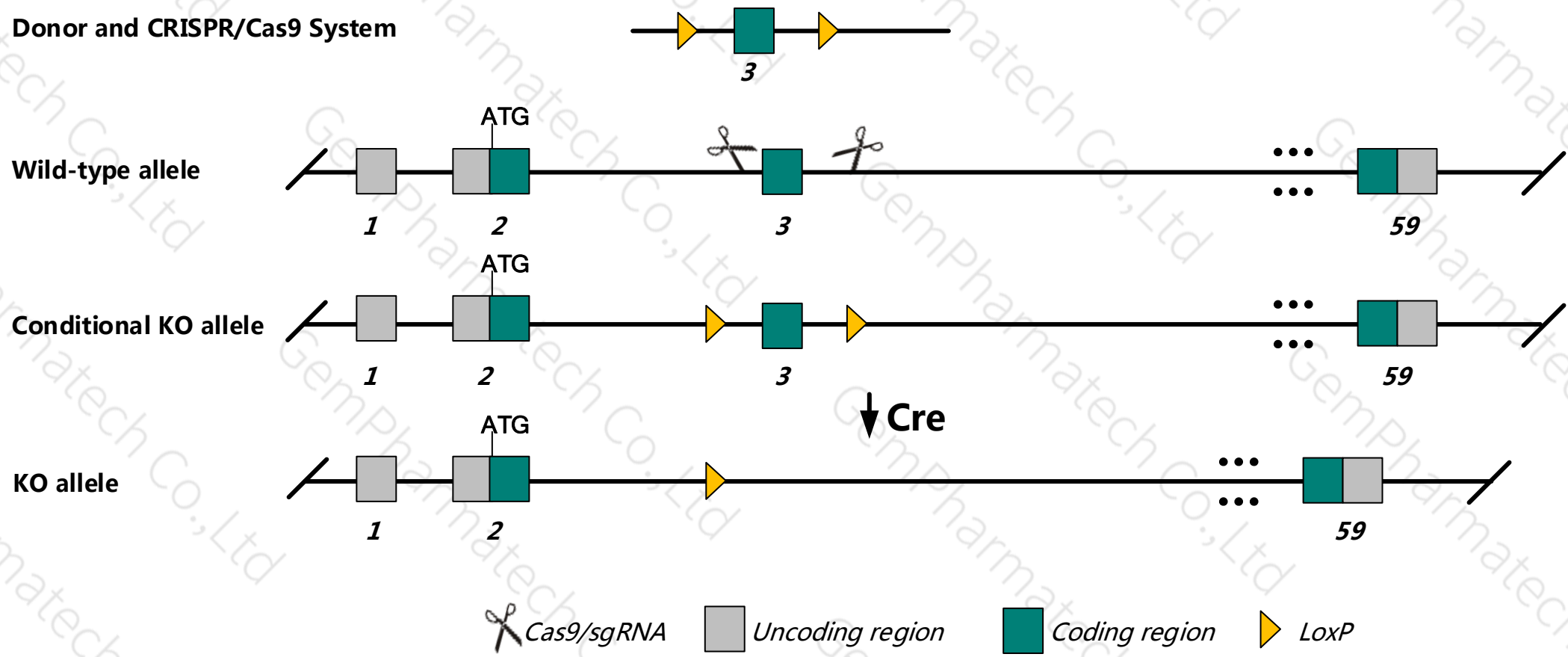|	
Project Name	*Wdfy4*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Wdfy4* gene. The schematic diagram is as follows:

Technical routes

➢ The *Wdfy4* gene has 4 transcripts.According to the structure of *Wdfy4* gene, exon3 of *Wdfy4*-201 (ENSMUST00000061753.14) transcript is recommended as the knockout region.The region contains 115bp coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Wdfy4* 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 or cell types.

Notice

- Transcript *Wdfy4-203,Wdfy4-204* may not be affected.

- The *Wdfy4* gene is located on the Chr14. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

Wdfy4   WD repeat and FYVE domain containing 4 [*Mus musculus* (house mouse)]

Gene ID: 545030, updated on 5-Aug-2018

▲ **Summary**

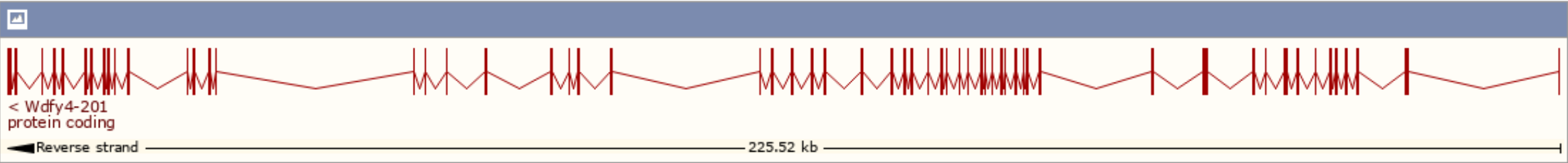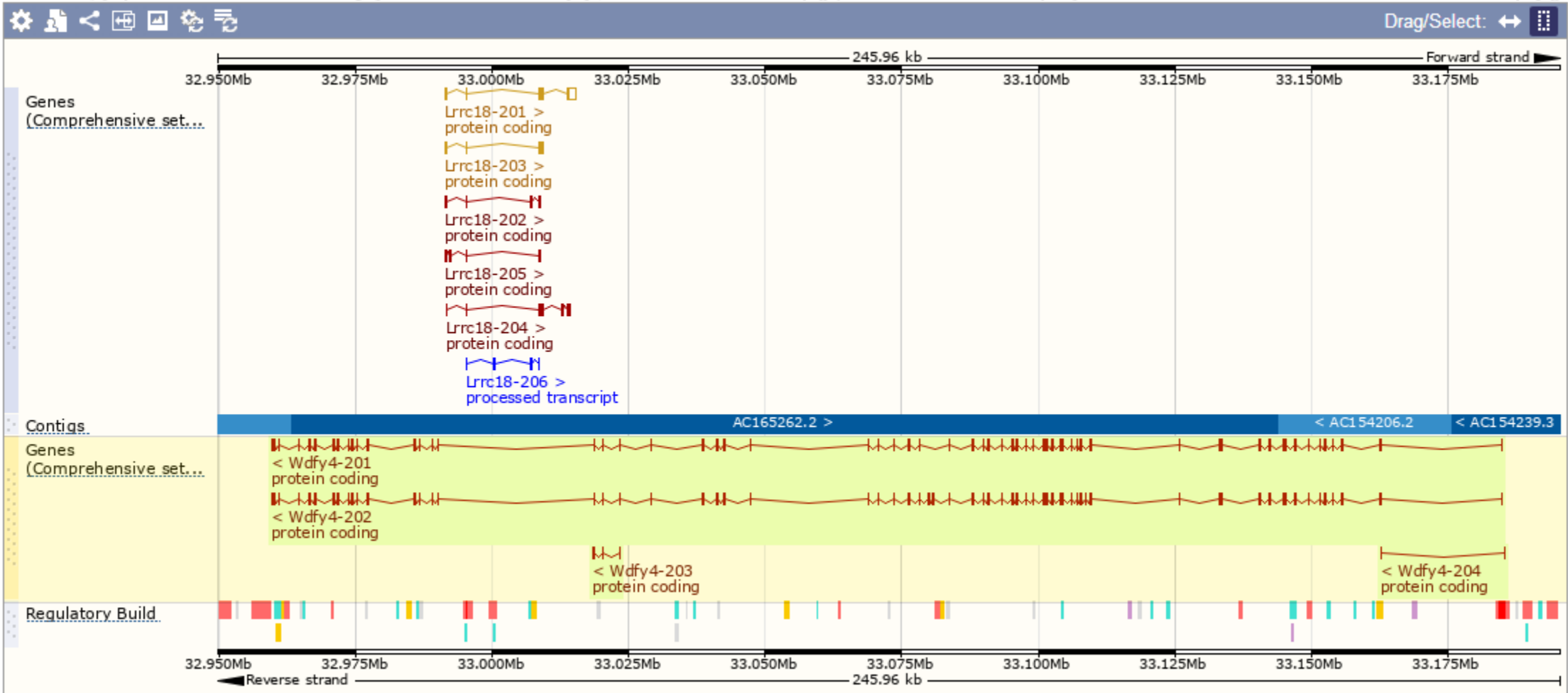|	
Official Symbol	Wdfy4 provided by MGI
Official Full Name	WD repeat and FYVE domain containing 4 provided by MGI
Primary source	MGI:MGI:3584510
See related	Ensembl:ENSMUSG00000051506 Vega:OTTMUSG00000027929
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Gm18810
Expression	Biased expression in spleen adult (RPKM 29.1), mammary gland adult (RPKM 6.4) and 4 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

The gene has 4 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
Wdfy4-201	ENSMUST00000061753.14	9681	3024aa	Protein coding	CCDS49441	E9PV60	NM_001146022 NP_001139494	TSL:5 GENCODE basic APPRIS P2
Wdfy4-202	ENSMUST00000130509.9	10158	3183aa	Protein coding	-	E9Q2M9	-	TSL:5 GENCODE basic APPRIS ALT2
Wdfy4-203	ENSMUST00000132153.1	437	60aa	Protein coding	-	F6X8K3	-	CDS 5' incomplete TSL:3
Wdfy4-204	ENSMUST00000159606.1	347	65aa	Protein coding	-	E0CXD5	-	CDS 3' incomplete TSL:3

The strategy is based on the design of *Wdfy4*-201 transcript, The transcription is shown below

< Wdfy4-201
protein coding

◄ Reverse strand ——————————————— 225.52 kb ———————————————

Genomic location distribution

Protein domain

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