Wdr44 Cas9-CKO Strategy

Designer: Zihe Cui

Reviewer: Huimin Su

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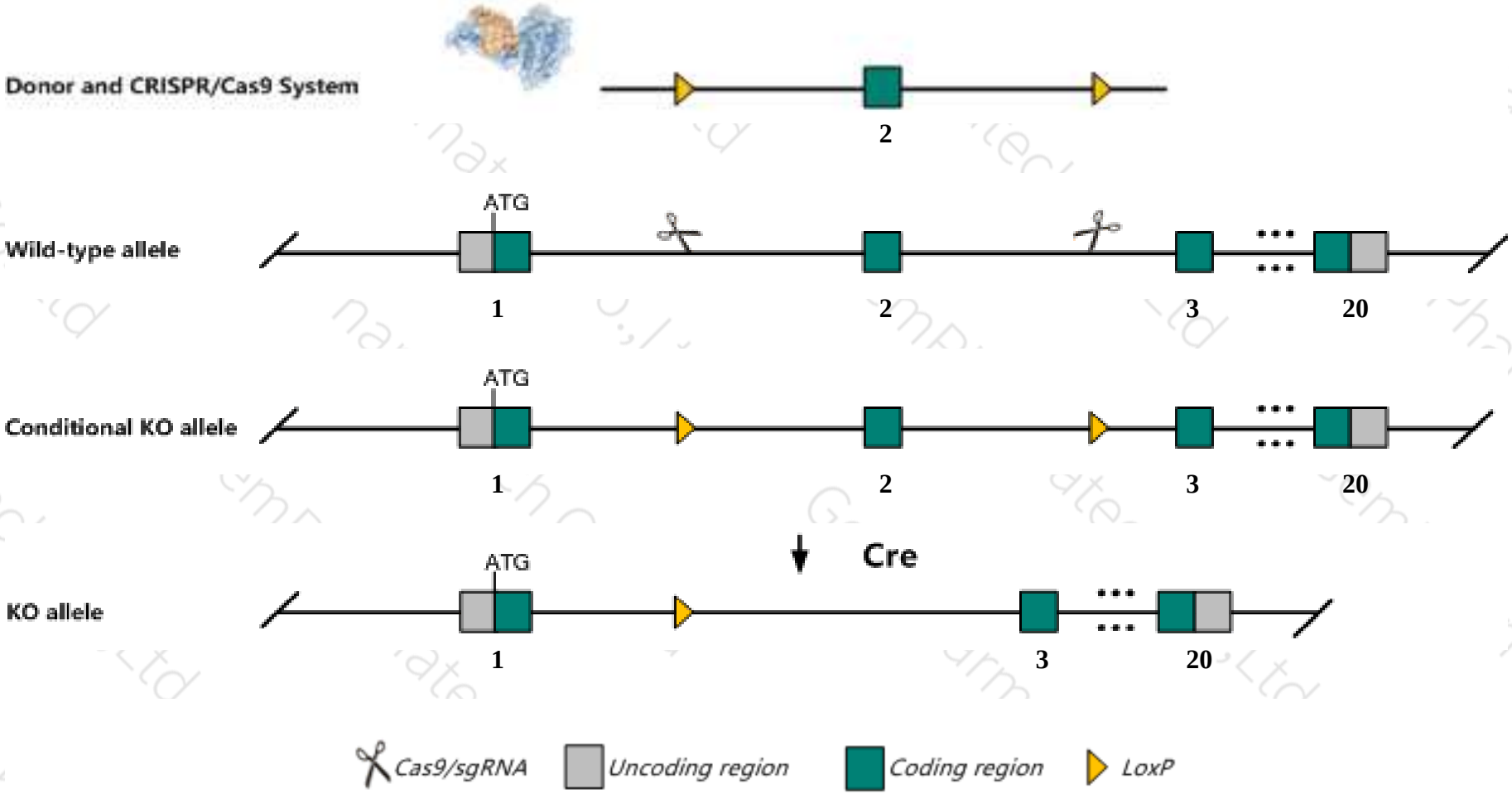
Project Overview

Project Name	***Wdr44***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Wdr44* gene. The schematic diagram is as follows:



Technical routes

➢ The *Wdr44* gene has 5 transcripts. According to the structure of *Wdr44* gene, exon2 of *Wdr44-201*(ENSMUST00000035766.12) transcript is recommended as the knockout region.The region contains 34bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Wdr44* 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,male chimeras hemizygous for a gene trapped allele appear normal at E9.5.

➢ The *Wdr44* gene is located on the ChrX. 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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Wdr44 WD repeat domain 44 [Mus musculus (house mouse)]

Gene ID: 72404, updated on 13-Mar-2020

Summary

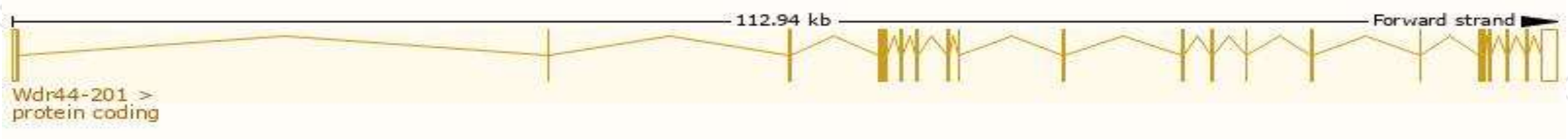
Official Symbol	Wdr44 provided by MGI
Official Full Name	WD repeat domain 44 provided byMGI
Primary source	MGI:MGI:1919654
See related	Ensembl:ENSMUSG00000036769
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2610034K17Rik, RAB11BP, RPH11
Summary	This gene encodes a protein containing multiple WD repeats. The encoded protein may play a role in vesicle trafficking. Alternate splicing results in multiple transcript variants. [provided by RefSeq, Mar 2015]
Expression	Ubiquitous expression in bladder adult (RPKM 2.9), CNS E18 (RPKM 2.3) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

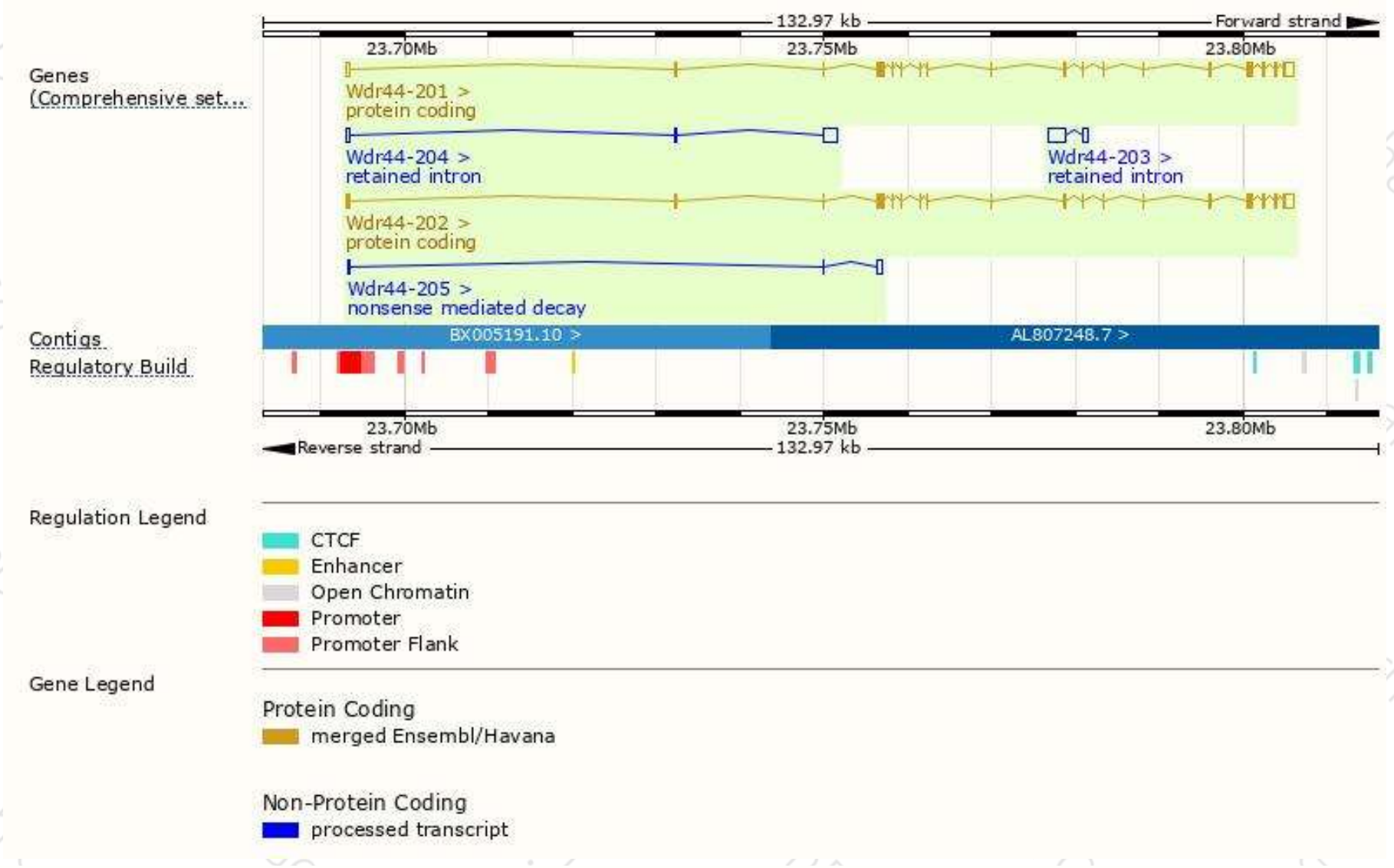
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Wdr44-201	ENSMUST00000035766.12	4167	915aa	Protein coding	CCDS40892	Q6NVE8	TSL:1 GENCODE basic APPRIS P1
Wdr44-202	ENSMUST00000101670.2	4002	915aa	Protein coding	CCDS40892	Q6NVE8	TSL:1 GENCODE basic APPRIS P1
Wdr44-205	ENSMUST00000149483.1	1046	43aa	Nonsense mediated decay	-	D6RHR5	TSL:1
Wdr44-203	ENSMUST00000135445.1	2723	No protein	Retained intron	-	-	TSL:1
Wdr44-204	ENSMUST00000142600.1	2291	No protein	Retained intron	-	-	TSL:1

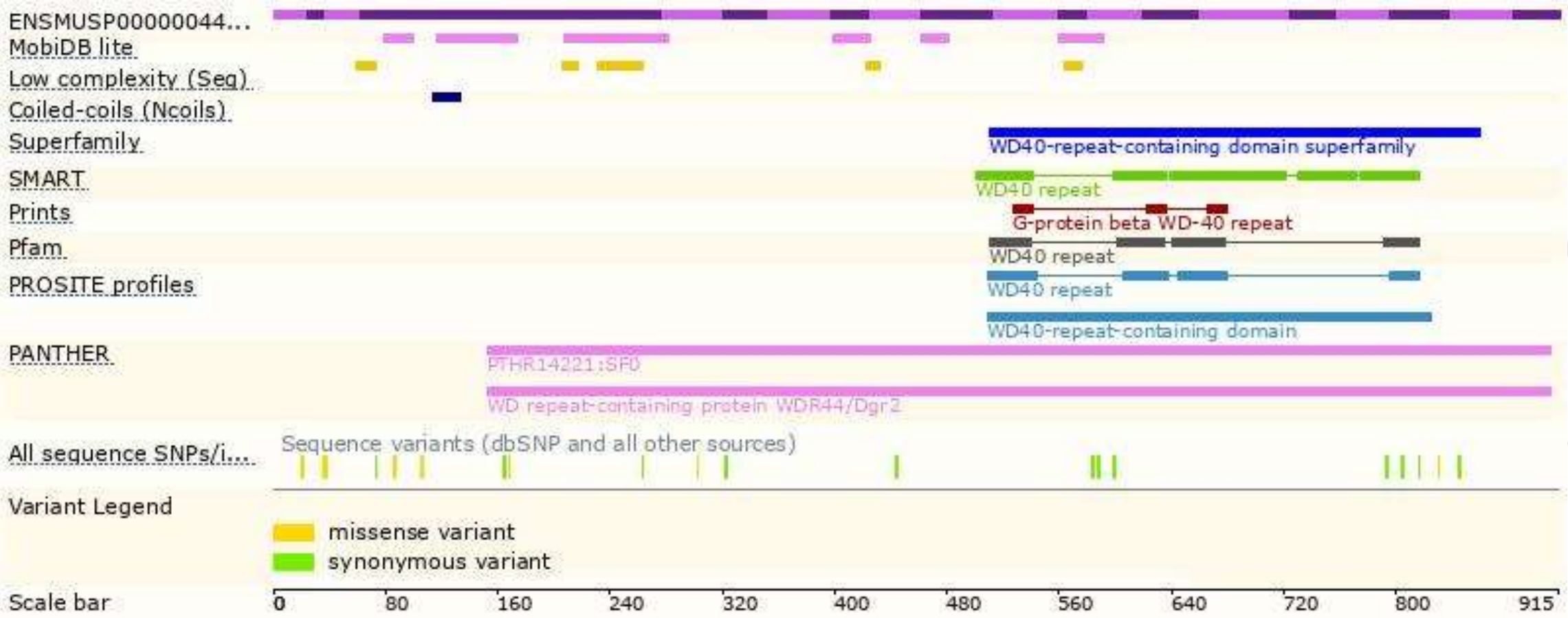
The strategy is based on the design of *Wdr44-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

Tel: 025-5864 1534

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