Fastkd2 Cas9-CKO Strategy

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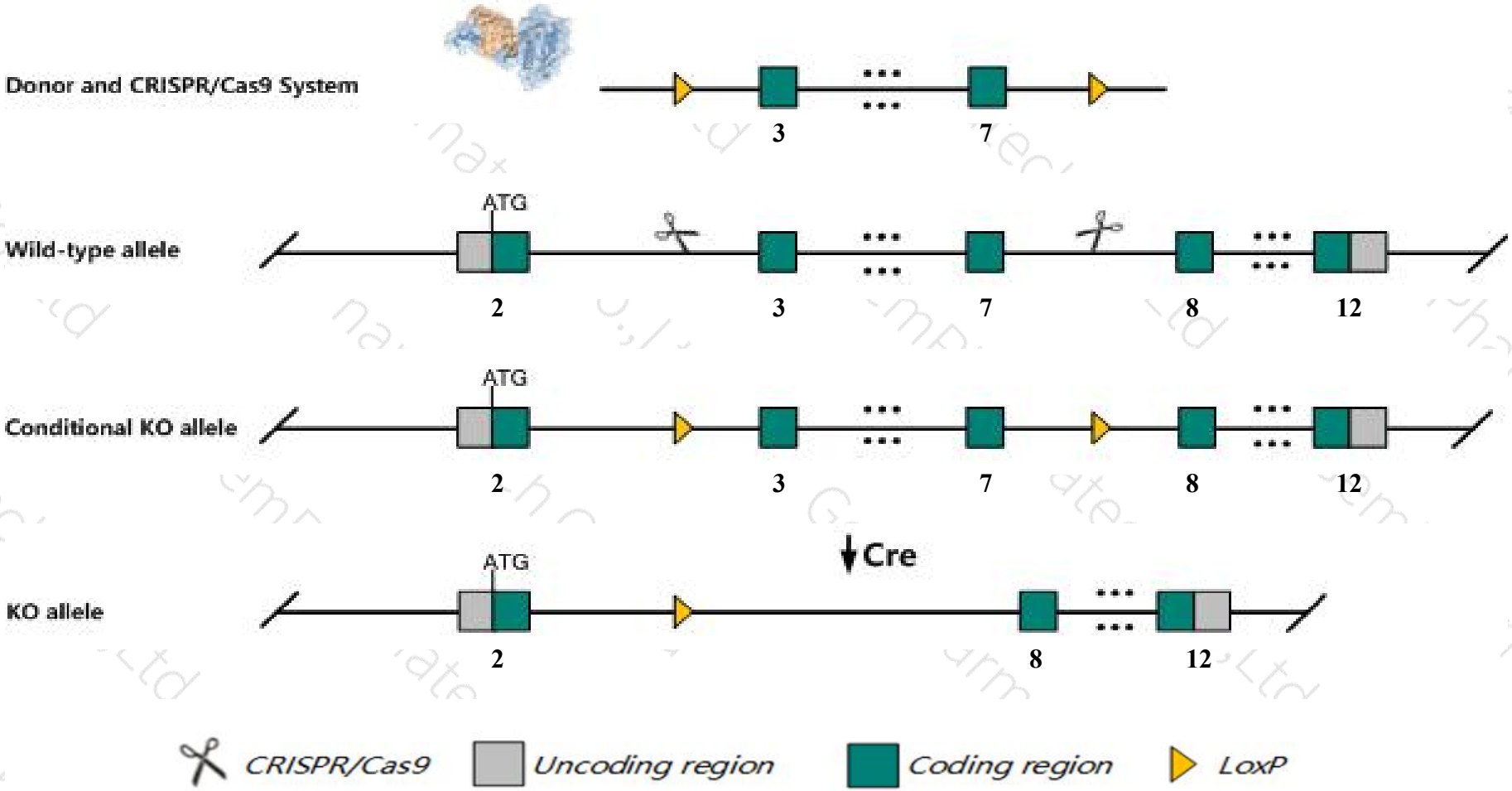
Project Overview

Project Name	***Fastkd2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Fastkd2* gene. The schematic diagram is as follows:



Technical routes

- The *Fastkd2* gene has 6 transcripts. According to the structure of *Fastkd2* gene, exon3-exon7 of *Fastkd2-201* (ENSMUST00000027103.6) transcript is recommended as the knockout region.The region contains 650bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Fastkd2* 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 *Fastkd2* 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.

- The knockout region is about 3.9kb from the 5-terminal of Mdh1b gene, which may affect its 5-terminal regulatory function.

- The insertion position of loxP was about 3.9 KB away from the 5-terminal of Mdh1b gene, and the knockout might affect its 5-terminal regulatory function.

- 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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Fastkd2 FAST kinase domains 2 [Mus musculus (house mouse)]

Gene ID: 75619, updated on 31-Jan-2019

Summary

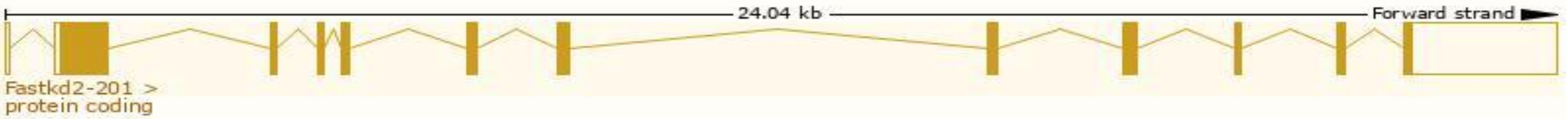
Official Symbol	Fastkd2 provided by MGI
Official Full Name	FAST kinase domains 2 provided byMGI
Primary source	MGI:MGI:1922869
See related	Ensembl:ENSMUSG00000025962
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2810421I24Rik
Expression	Ubiquitous expression in placenta adult (RPKM 6.3), CNS E11.5 (RPKM 5.2) and 24 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

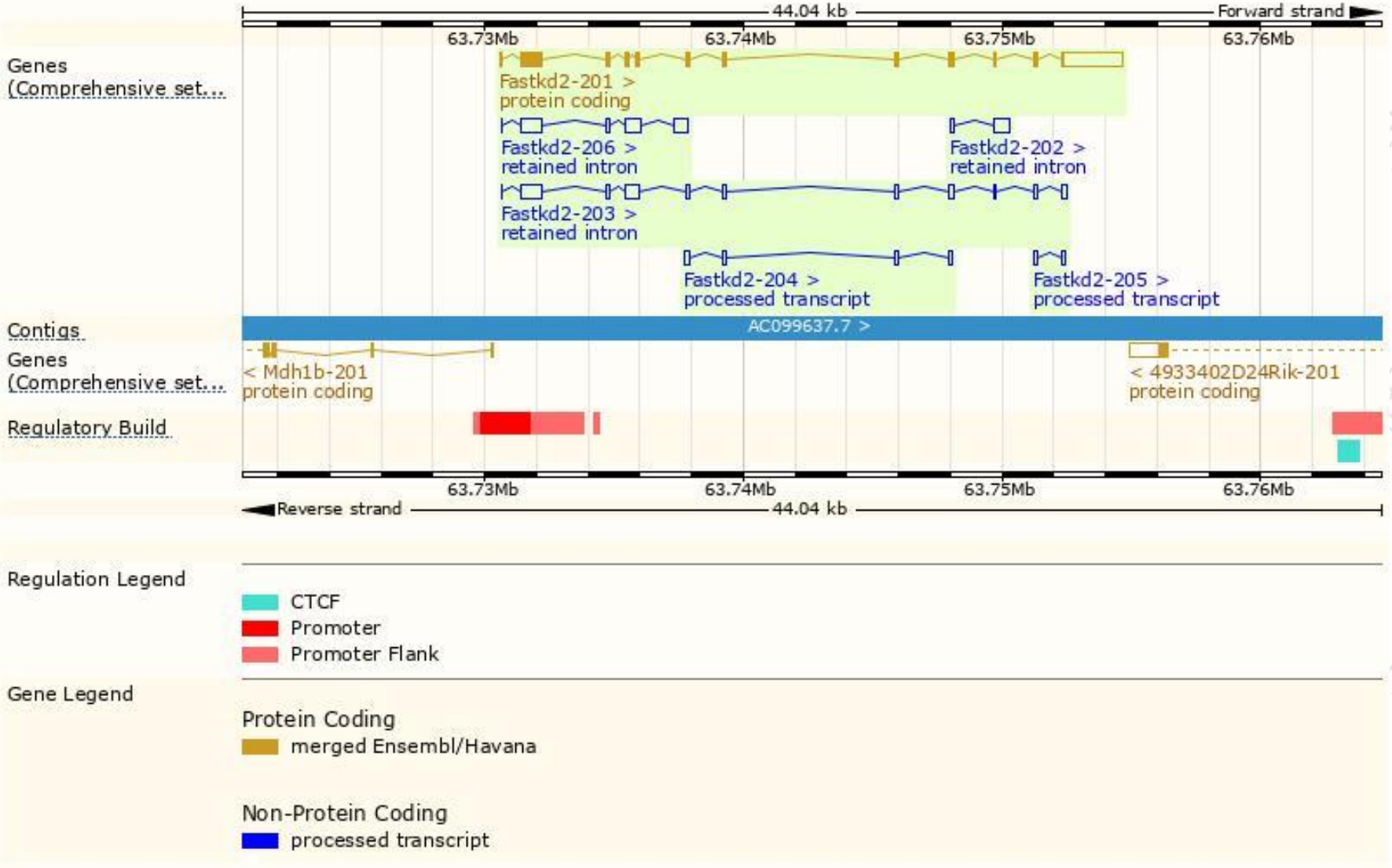
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fastkd2-201	ENSMUST00000027103.6	4486	689aa	Protein coding	CCDS15001	Q922E6	TSL:1 GENCODE basic APPRIS P1
Fastkd2-203	ENSMUST00000128621.7	2613	No protein	Retained intron	-	-	TSL:1
Fastkd2-206	ENSMUST00000148758.7	2117	No protein	Retained intron	-	-	TSL:5
Fastkd2-202	ENSMUST00000123945.1	741	No protein	Retained intron	-	-	TSL:2
Fastkd2-204	ENSMUST00000131478.1	642	No protein	lncRNA	-	-	TSL:2
Fastkd2-205	ENSMUST00000134454.1	327	No protein	lncRNA	-	-	TSL:2

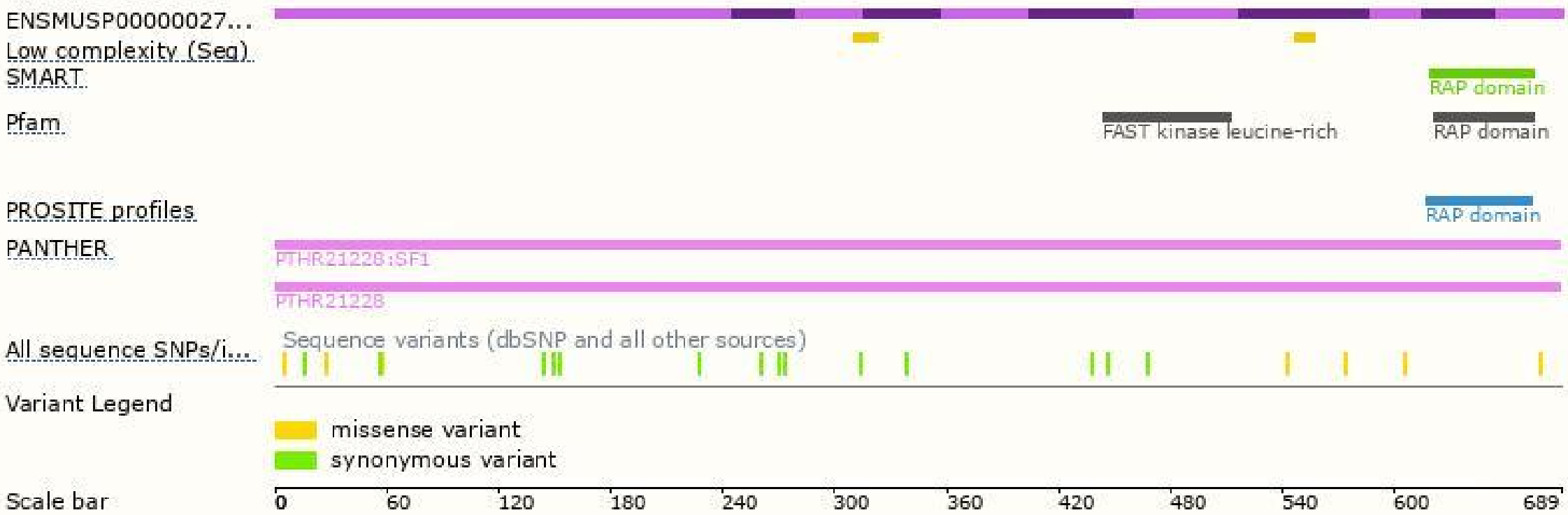
The strategy is based on the design of *Fastkd2-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



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

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