

Tsg101 Cas9-CKO Strategy

Designer:

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Design Date:

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Project Overview

Project Name

Tsg101

Project type

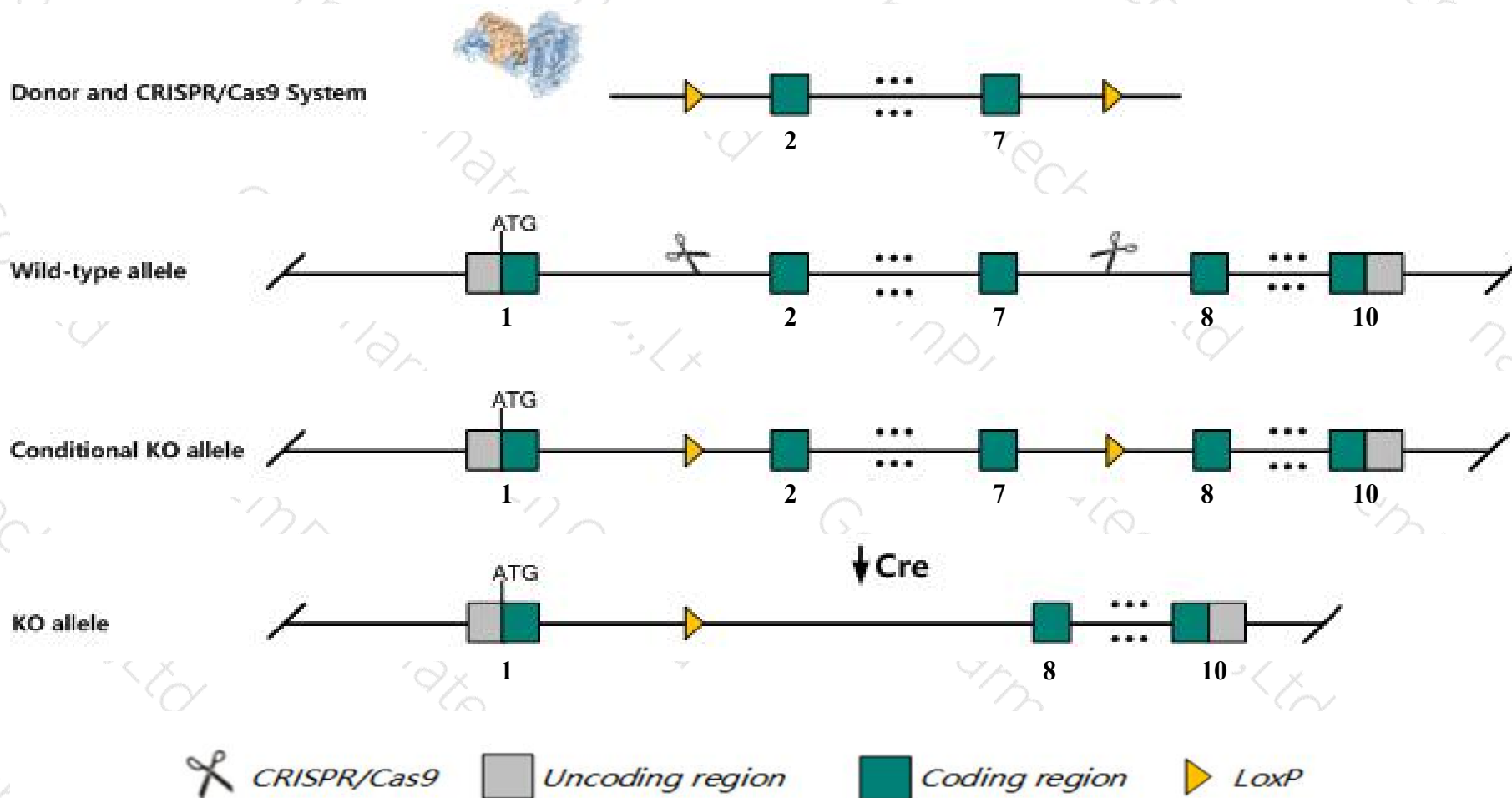
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tsg101* gene. The schematic diagram is as follows:



- The *Tsg101* gene has 9 transcripts. According to the structure of *Tsg101* gene, exon2-exon7 of *Tsg101-201* (ENSMUST00000014546.14) transcript is recommended as the knockout region. The region contains 601bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Tsg101* 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

- According to the existing MGI data, Homozygotes for targeted null mutations exhibit reduced growth, fail to form mesoderm, accumulate p53 protein and die by embryonic day 6.5. Homozygotes for a mammary gland-specific knockout show impaired mammaryogenesis and are unable to nurse their pups.
- The distance between *Gm45628* and knockout region is about 2kb, and the 5-terminal regulation of *Gm45628* may be affected.
- The *Tsg101* gene is located on the Chr7. 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)

Tsg101 tumor susceptibility gene 101 [Mus musculus (house mouse)]

Gene ID: 22088, updated on 31-Jan-2019

Summary

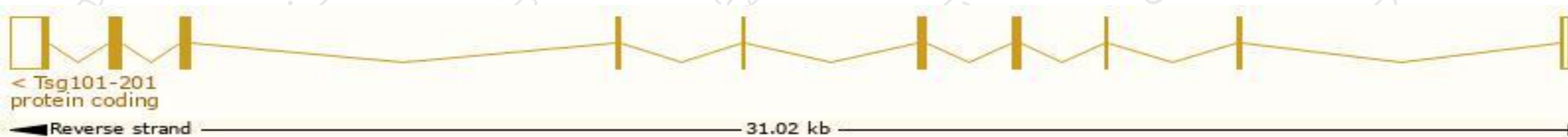
Official Symbol	Tsg101 provided by MGI
Official Full Name	tumor susceptibility gene 101 provided by MGI
Primary source	MGI:MGI:106581
See related	Ensembl:ENSMUSG00000014402
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI255943, CC2
Expression	Ubiquitous expression in CNS E11.5 (RPKM 29.9), CNS E14 (RPKM 28.1) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

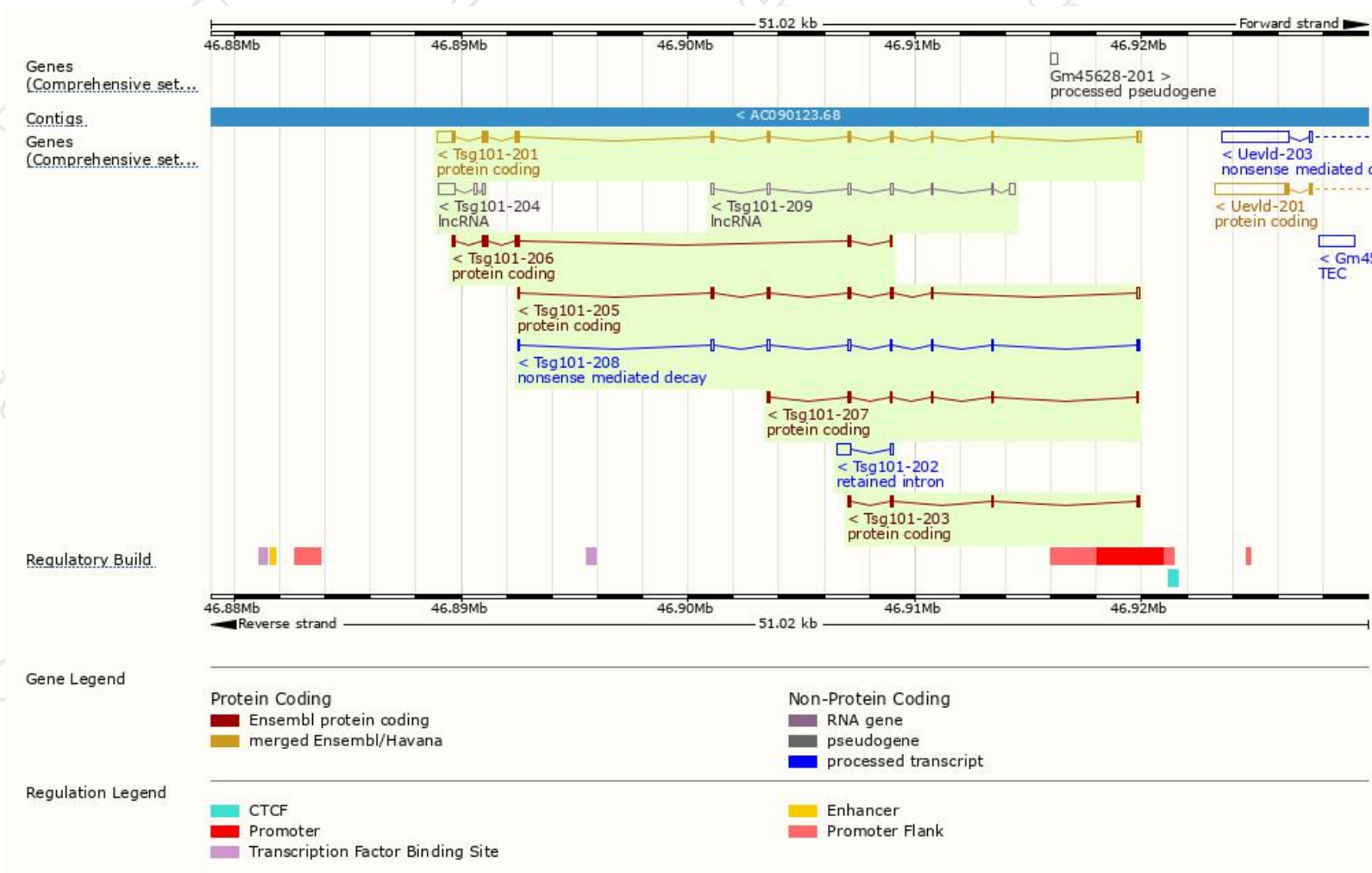
The gene has 9 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tsg101-201	ENSMUST00000014546.14	1949	391aa	Protein coding	CCDS21291	Q3UCW0 Q61187	TSL:1 GENCODE basic APPRIS P1
Tsg101-206	ENSMUST00000209538.1	791	253aa	Protein coding	-	A0A1B0GS09	CDS 5' incomplete TSL:3
Tsg101-205	ENSMUST00000156335.8	661	145aa	Protein coding	-	D3Z0S9	CDS 3' incomplete TSL:5
Tsg101-203	ENSMUST00000143413.2	493	139aa	Protein coding	-	D3Z2V5	CDS 3' incomplete TSL:5
Tsg101-207	ENSMUST00000210664.1	453	148aa	Protein coding	-	A0A1B0GS10	CDS 3' incomplete TSL:3
Tsg101-208	ENSMUST00000211076.1	712	66aa	Nonsense mediated decay	-	A0A1B0GRX2	TSL:3
Tsg101-204	ENSMUST00000146130.1	897	No protein	Processed transcript	-	-	TSL:5
Tsg101-209	ENSMUST00000211595.1	807	No protein	Processed transcript	-	-	TSL:3
Tsg101-202	ENSMUST00000140693.1	730	No protein	Retained intron	-	-	TSL:1

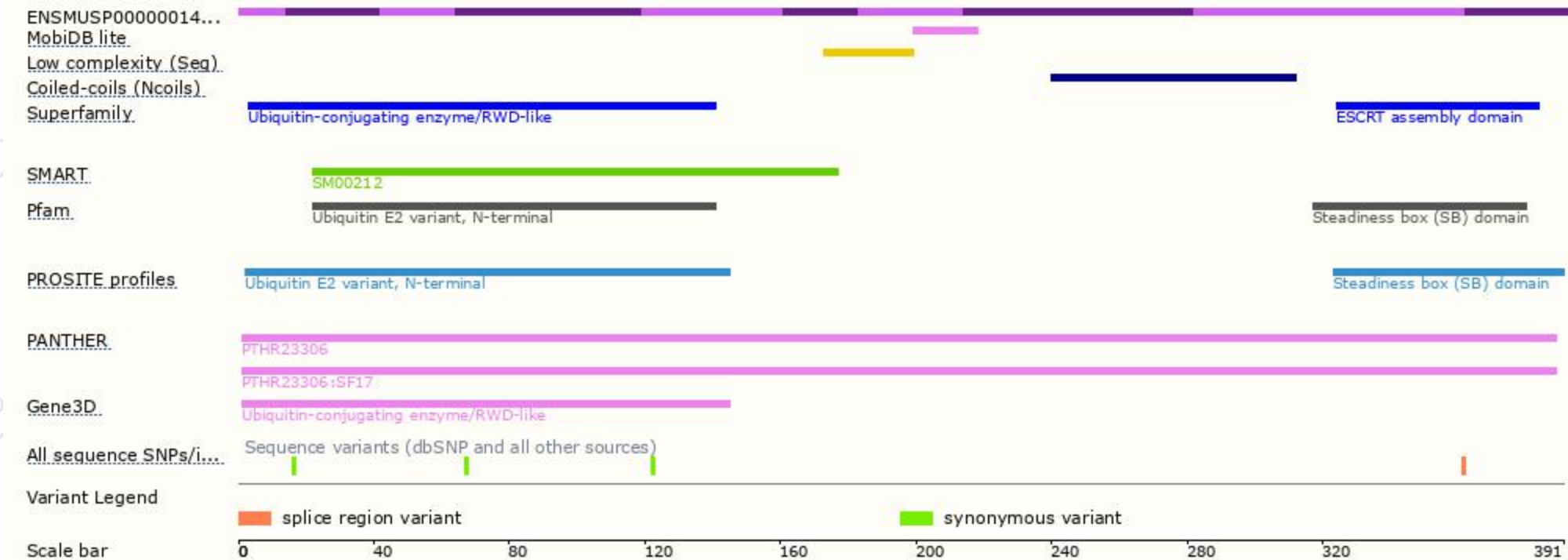
The strategy is based on the design of *Tsg101-201* transcript, The transcription is shown below



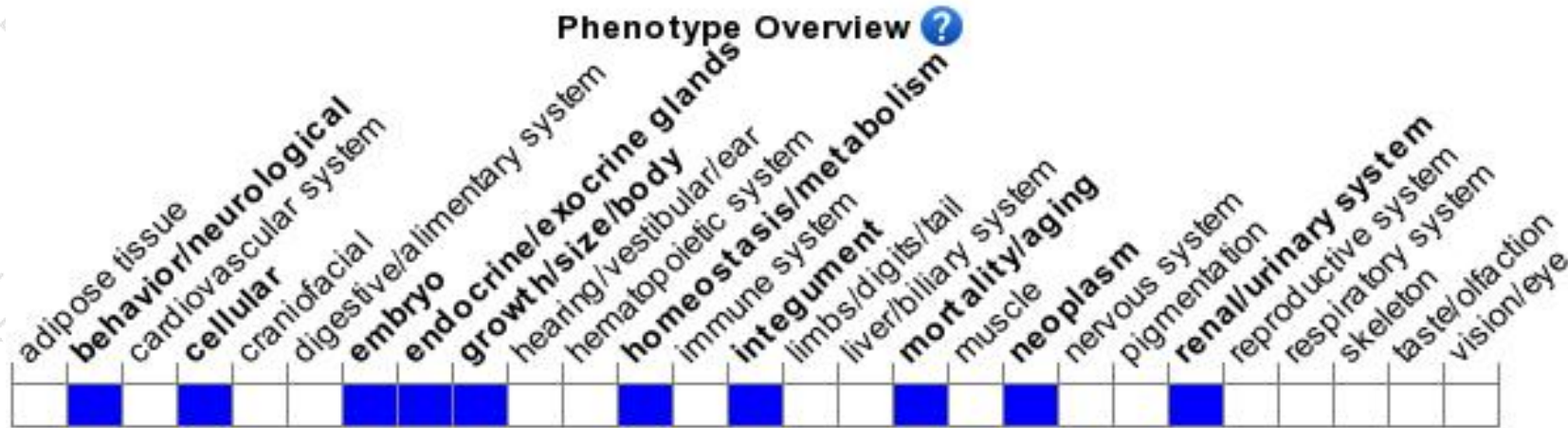
Genomic location distribution



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/>).

According to the existing MGI data, Homozygotes for targeted null mutations exhibit reduced growth, fail to form mesoderm, accumulate p53 protein and die by embryonic day 6.5. Homozygotes for a mammary gland-specific knockout show no mammary gland development, no mammary gland involution, and are unable to nurse their pups.

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

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