Wnt4 Cas9-CKO *Strategy*

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Project Overview

Project Name	***Wnt4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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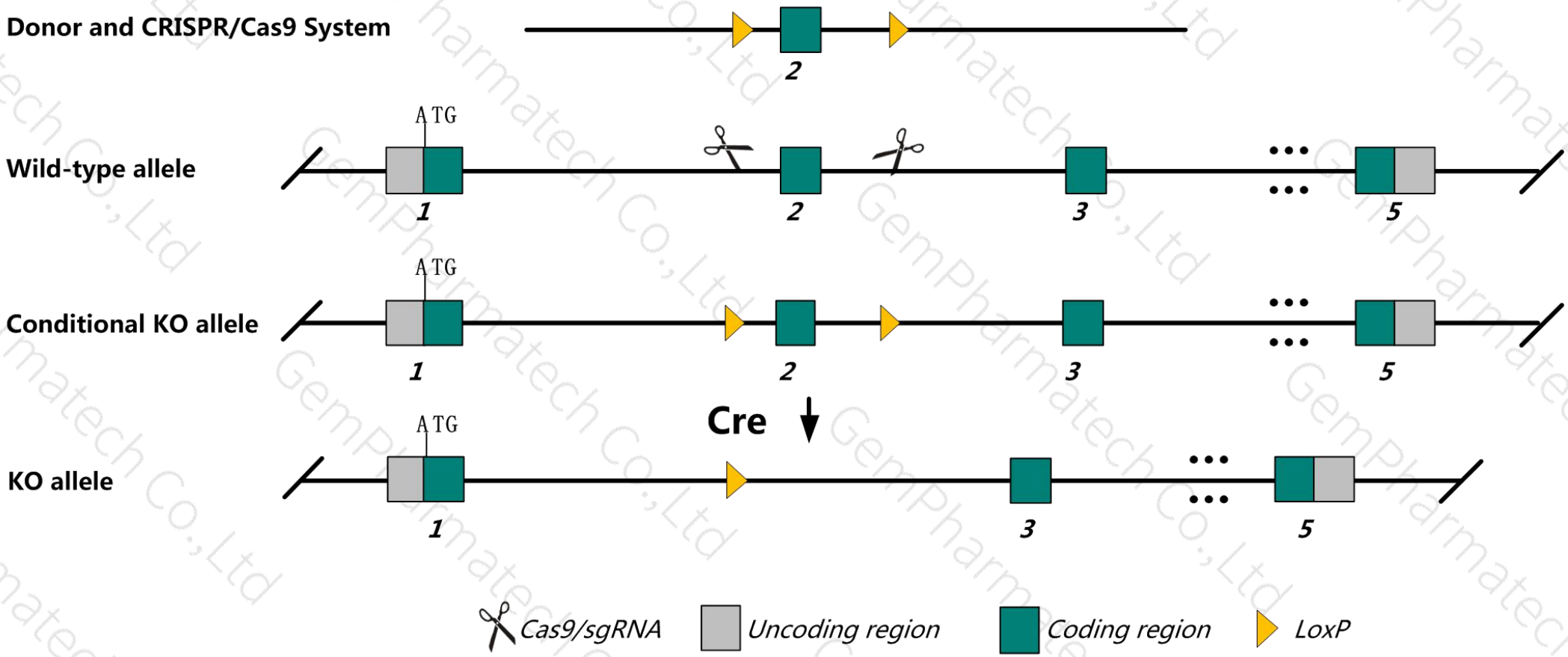
This model will use CRISPR/Cas9 technology to edit the *Wnt4* gene. The schematic diagram is as follows:



Technical routes

- The *Wnt4* gene has 1 transcript.According to the structure of *Wnt4* gene, exon2 of *Wnt4*-201 transcript is recommended as the knockout region.The region contains 236bp coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Wnt4* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 or cell types.

Notice

- According to the existing MGI data，Homozygous mutants exhibit impaired development of the kidney, pituitary gland, and female reproductive system. Mutants die within 24 hours of birth.
- The *Wnt4* gene is located on the Chr4. 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）

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Wnt4 wingless-type MMTV integration site family, member 4 [*Mus musculus* (house mouse)]

Gene ID: 22417, updated on 25-Jun-2019

Summary

Official Symbol	Wnt4 provided by MGI
Official Full Name	wingless-type MMTV integration site family, member 4 provided by MGI
Primary source	MGI:MGI:98957
See related	Ensembl:ENSMUSG00000036856
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	Wnt-4
Expression	Biased expression in adrenal adult (RPKM 48.0), ovary adult (RPKM 25.7) and 13 other tissues See more
Orthologs	human all

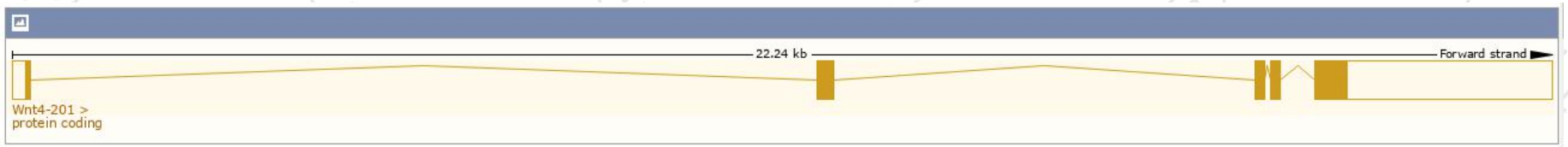
Transcript information（Ensembl）

The gene has 1 transcript, and all transcripts are shown below:

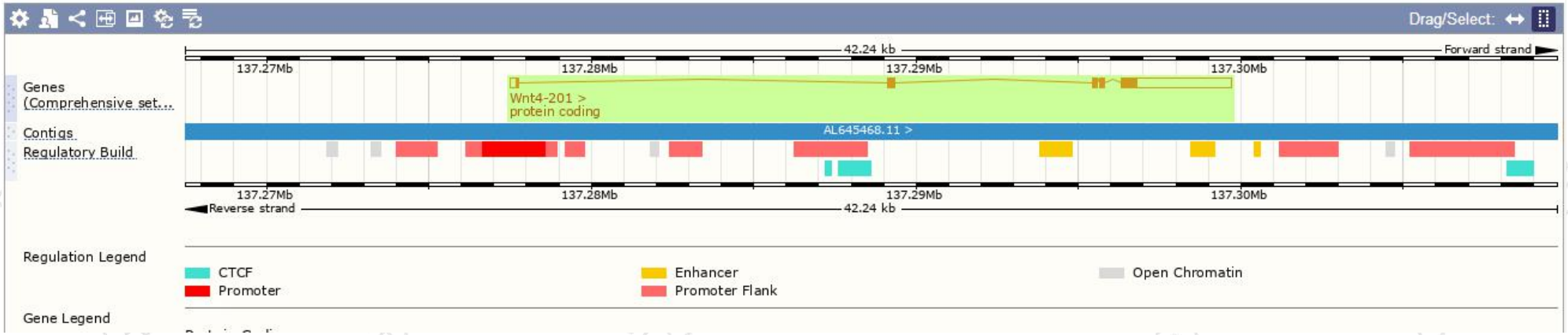
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Wnt4-201	ENSMUST00000045747.4	4194	351aa	Protein coding	CCDS18815	P22724 Q3ZB23	TSL:1 GENCODE basic APPRIS P1

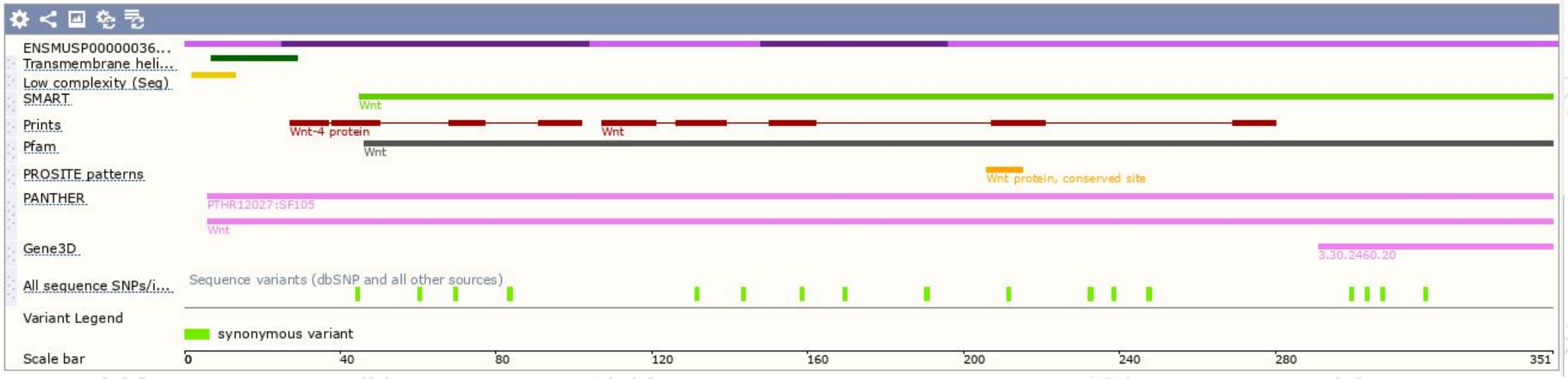
The strategy is based on the design of *Wnt4*-201 transcript,The transcription is shown below



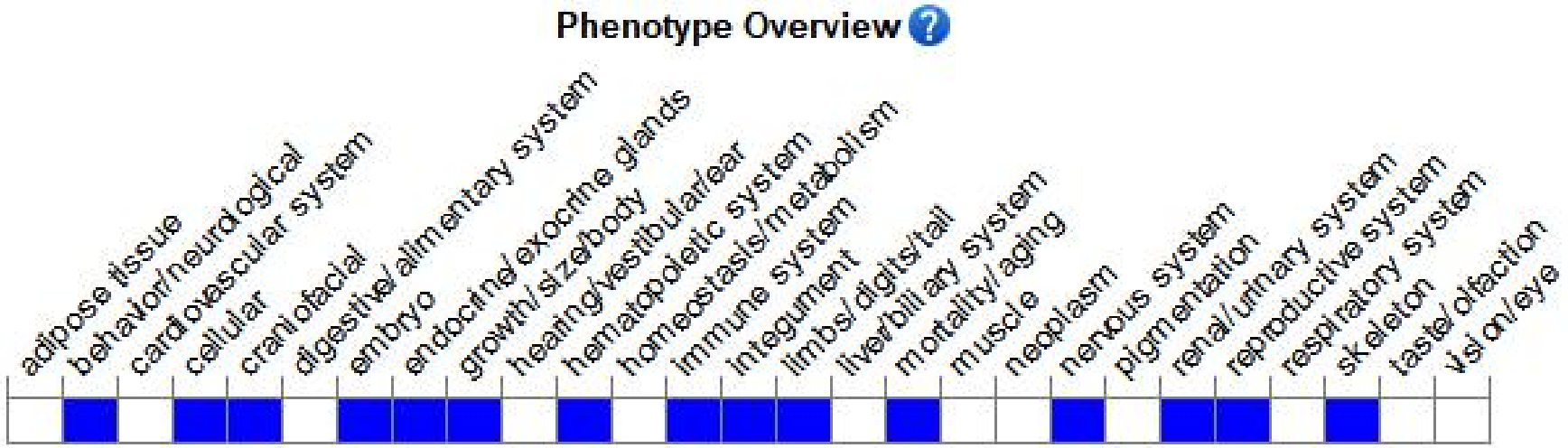
Genomic location distribution



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,Mutations in this locus affect cell-cycle regulation and apoptos is. Null homozygotes show high, early-onset tumor incidence; some have persistent hyaloid vasculature and cataracts. Truncated or temperature-sensitive alleles cause early aging phenotypes.

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