Gdf9 Cas9-CKO *Strategy*

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Project Overview

Project Name	*Gdf9*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Gdf9* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele

Cas9/sgRNA   Uncoding region   Coding region   LoxP

Technical routes

➤ The *Gdf9* gene has 3 transcripts.According to the structure of *Gdf9* gene, exon2 of *Gdf9*-201 (ENSMUST00000018382.6) transcript is recommended as the knockout region.The region contains most of the coding sequence.Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Gdf9* 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

➢ According to the existing MGI data，Females homozygous for a targeted null mutation are sterile with a block in folliculogenesis. Mutant oocytes exhibit perinuclear organelle aggregation, unusual Golgi complexes, absence of cortical granules, and impaired granulosa cell connections.

➢ The *Gdf9* gene is located on the Chr11. 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）

Gdf9 **growth differentiation factor 9 [*Mus musculus* (house mouse)]**

Gene ID: 14566, updated on 12-Mar-2019

▲ Summary

Official Symbol	Gdf9 provided by MGI
Official Full Name	growth differentiation factor 9 provided by MGI
Primary source	MGI:MGI:95692
See related	Ensembl:ENSMUSG00000018238
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	Gdf-9
Summary	This gene encodes a secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Ligands of this family bind various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression. The encoded preproprotein is proteolytically processed to generate each subunit of the disulfide-linked homodimer. This protein regulates ovarian function. Female mice that are homozygous null for this gene are sterile with impaired folliculogenesis. [provided by RefSeq, Jul 2016]
Expression	Biased expression in ovary adult (RPKM 37.3) and testis adult (RPKM 3.0) See more
Orthologs	human all

Transcript information（Ensembl）

The gene has 3 transcripts, and all transcripts are shown below:

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gdf9-201	ENSMUST00000018382.6	1793	441aa	Protein coding	CCDS24679	Q07105 Q3UWR9	TSL:1 GENCODE basic APPRIS P1
Gdf9-203	ENSMUST00000156037.1	429	No protein	lncRNA	-	-	TSL:5
Gdf9-202	ENSMUST00000145369.1	382	No protein	lncRNA	-	-	TSL:3

The strategy is based on the design of *Gdf9*-201 transcript,The transcription is shown below
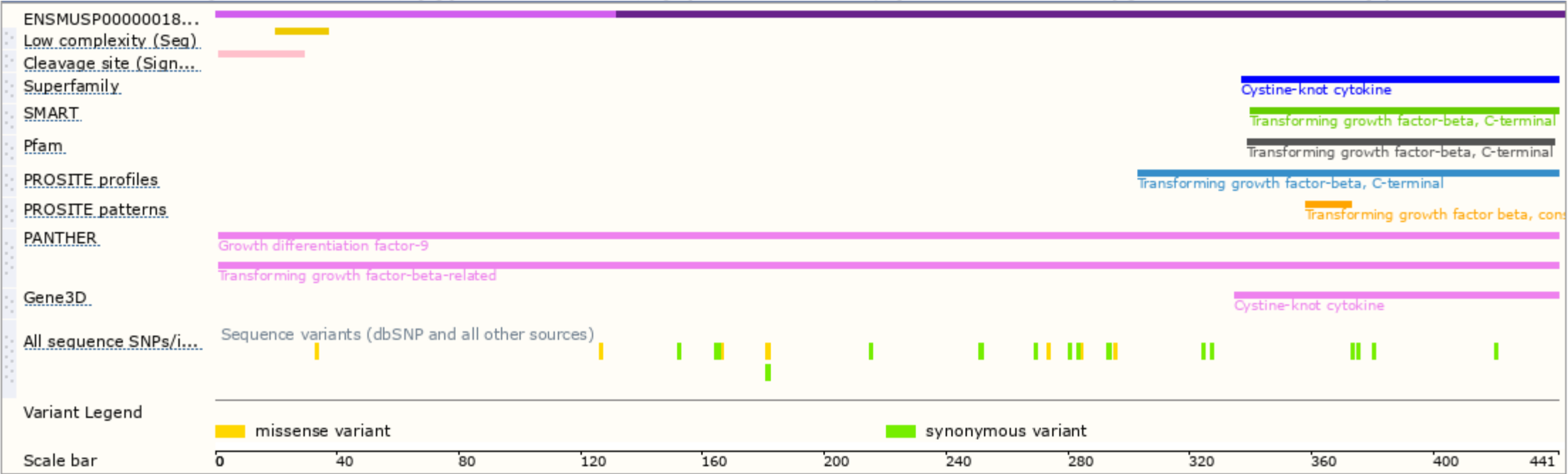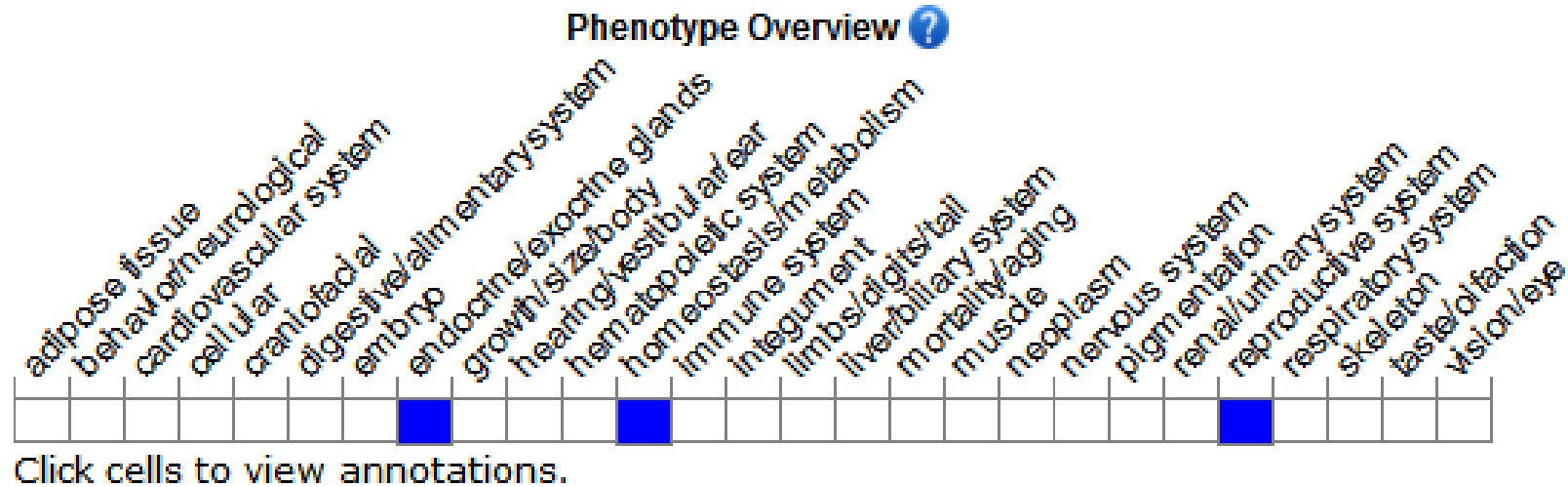
Genomic location distribution
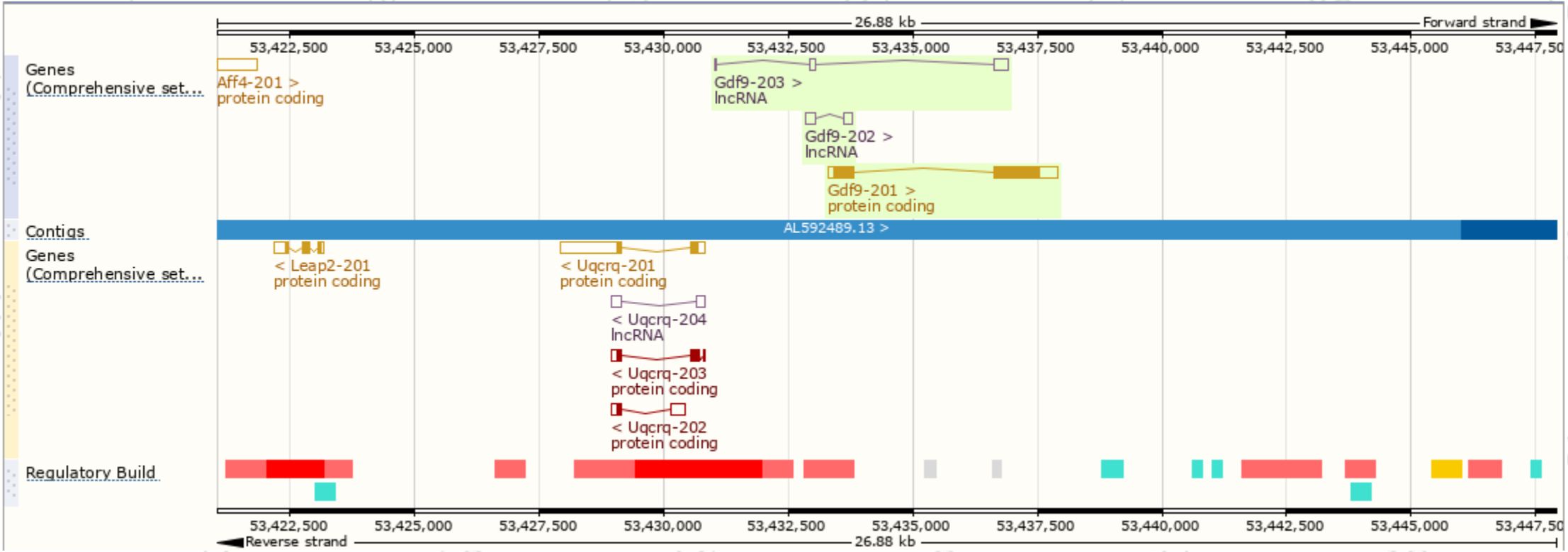
Protein domain

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,Females homozygous for a targeted null mutation are sterile with a block in folliculogenesis. Mutant oocytes exhibit perinuclear organelle aggregation, unusual Golgi complexes, absence of cortical granules, and impaired granulosa cell connections.

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