Stat3 Cas9-KO Strategy

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Design Date: 2019-8-15

Project Overview

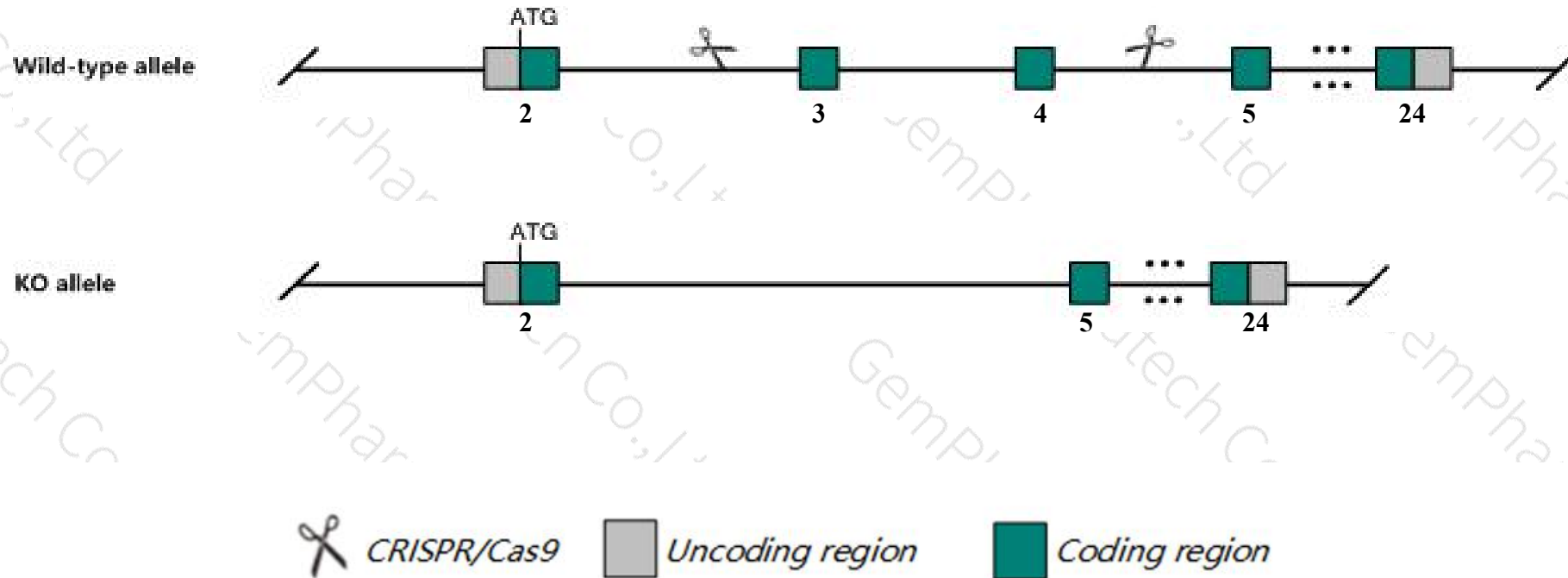|	
Project Name	*Stat3*
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Stat3* gene. The schematic diagram is as follows:

Technical routes

➢ The *Stat3* gene has 14 transcripts. According to the structure of *Stat3* gene, exon3-exon4 of *Stat3-203* (ENSMUST00000127638.7) transcript is recommended as the knockout region.The region contains 244bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Stat3* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

Notice

➢ According to the existing MGI data,Homozygotes for targeted null mutations die at embryonic day 6.5-7.5. Conditional, tissue specific mutants are variably viable and show diverse defects including obesity, diabetes, thermal dysregulation and infertility.

➢ The *Stat3* 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 gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Stat3 signal transducer and activator of transcription 3 [Mus musculus (house mouse)]

Gene ID: 20848, updated on 9-Apr-2019

▲Summary

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Official Symbol	Stat3 provided by MGI
Official Full Name	signal transducer and activator of transcription 3 provided by MGI
Primary source	MGI:MGI:103038
See related	Ensembl:ENSMUSG00000004040
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	1110034C02Rik, AW109958, Aprf
Summary	The protein encoded by this gene is a member of the STAT protein family. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein is activated through phosphorylation in response to various cytokines and growth factors including IFNs, EGF, IL5, IL6, HGF, LIF and BMP2. This protein mediates the expression of a variety of genes in response to cell stimuli, and thus plays a key role in many cellular processes such as cell growth and apoptosis. The small GTPase Rac1 has been shown to bind and regulate the activity of this protein. PIAS3 protein is a specific inhibitor of this protein. Alternative splicing results in multiple transcript variants encoding distinct isoforms. [provided by RefSeq, Sep 2015]
Expression	Ubiquitous expression in lung adult (RPKM 48.1), mammary gland adult (RPKM 46.4) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 14 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Stat3-202	ENSMUST00000103114.7	6042	722aa	Protein coding	CCDS25441	P42227 Q6GU23	TSL:1 GENCODE basic APPRIS ALT1
Stat3-203	ENSMUST00000127638.7	4516	770aa	Protein coding	CCDS25440	P42227	TSL:1 GENCODE basic APPRIS P4
Stat3-201	ENSMUST00000092671.11	2927	769aa	Protein coding	CCDS48934	P42227	TSL:1 GENCODE basic APPRIS ALT1
Stat3-207	ENSMUST00000138438.1	2506	744aa	Protein coding	-	B7ZC18	TSL:5 GENCODE basic
Stat3-214	ENSMUST00000156645.1	651	No protein	Processed transcript	-	-	TSL:5
Stat3-208	ENSMUST00000146971.1	1995	No protein	Retained intron	-	-	TSL:1
Stat3-205	ENSMUST00000137093.7	1079	No protein	Retained intron	-	-	TSL:2
Stat3-210	ENSMUST00000152601.1	826	No protein	Retained intron	-	-	TSL:1
Stat3-212	ENSMUST00000154170.1	672	No protein	Retained intron	-	-	TSL:3
Stat3-206	ENSMUST00000137367.1	646	No protein	Retained intron	-	-	TSL:2
Stat3-211	ENSMUST00000152623.1	609	No protein	Retained intron	-	-	TSL:3
Stat3-209	ENSMUST00000151544.1	408	No protein	Retained intron	-	-	TSL:3
Stat3-213	ENSMUST00000155972.1	396	No protein	Retained intron	-	-	TSL:3
Stat3-204	ENSMUST00000130686.1	349	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Stat3-203* transcript,The transcription is shown below

< Stat3-203
protein coding

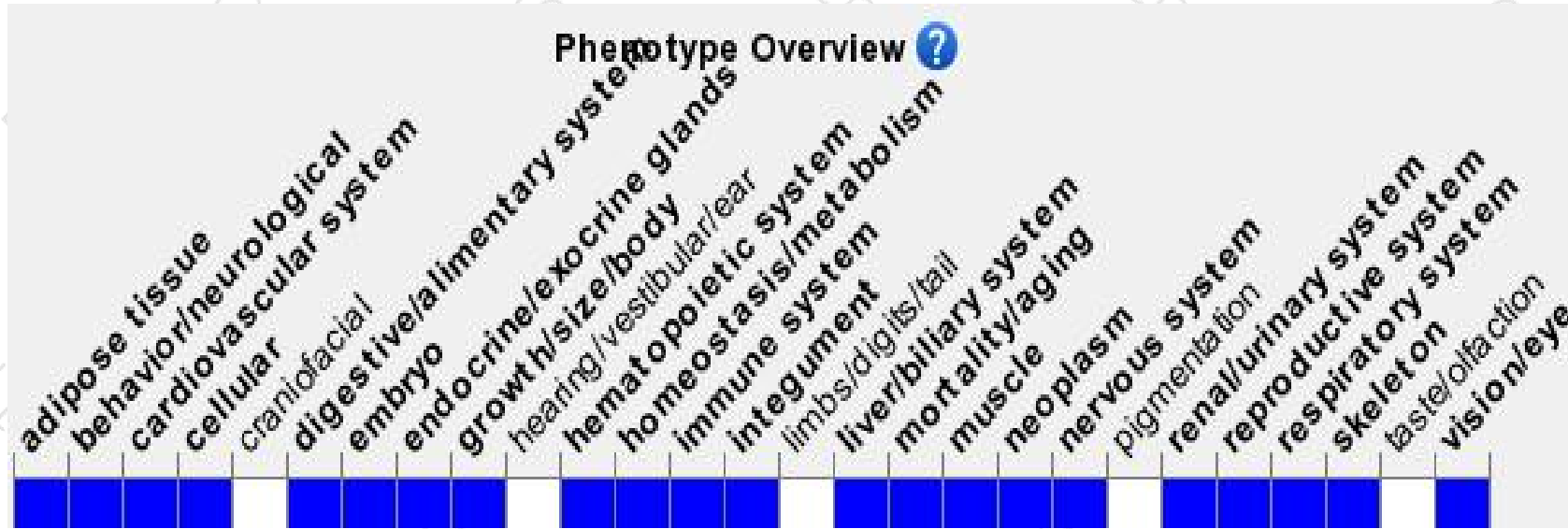◄ Reverse strand ————————————————————— 52.73 kb ———————————————————
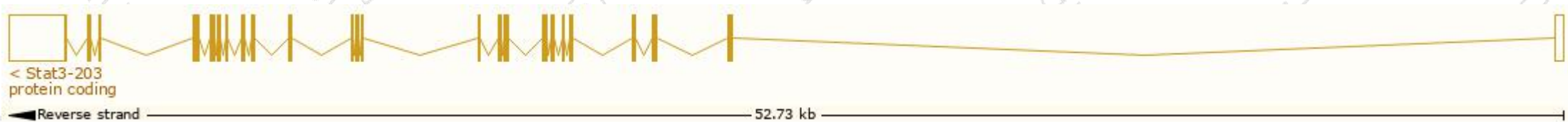
Genomic location distribution
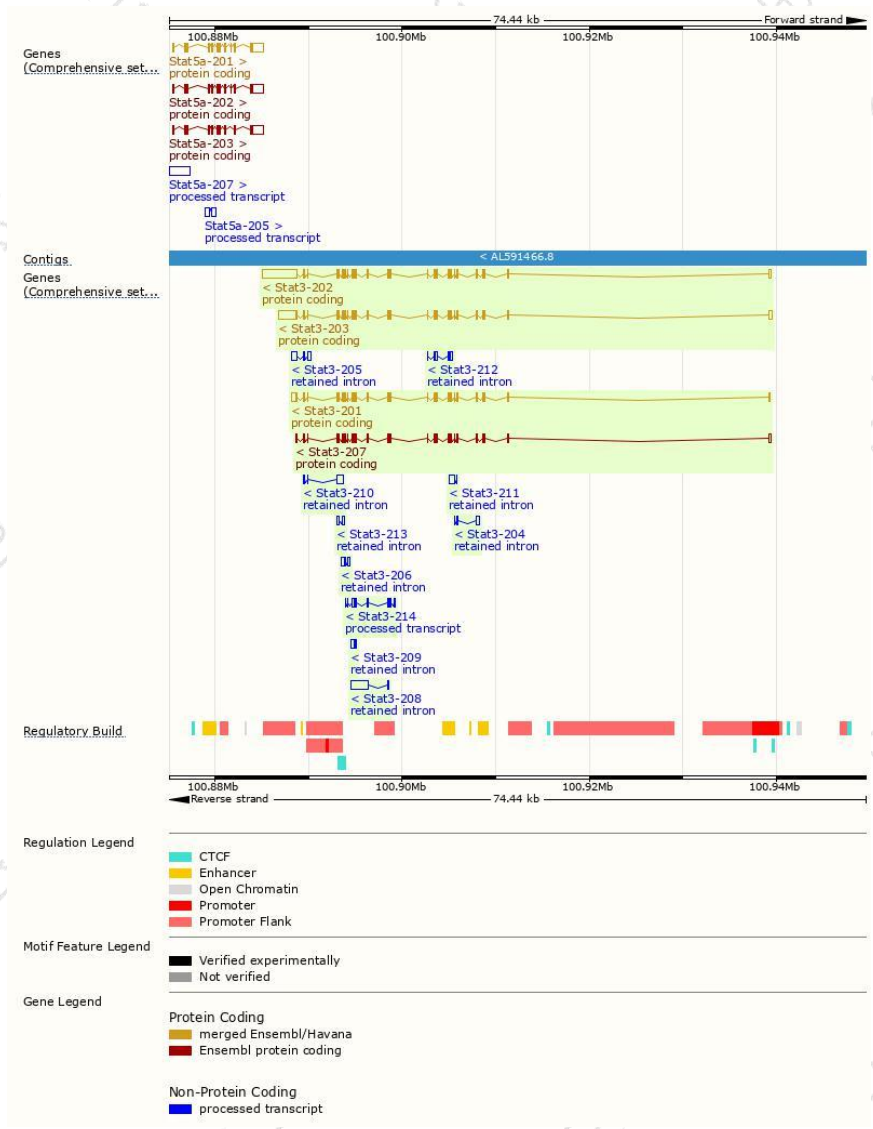
Protein domain
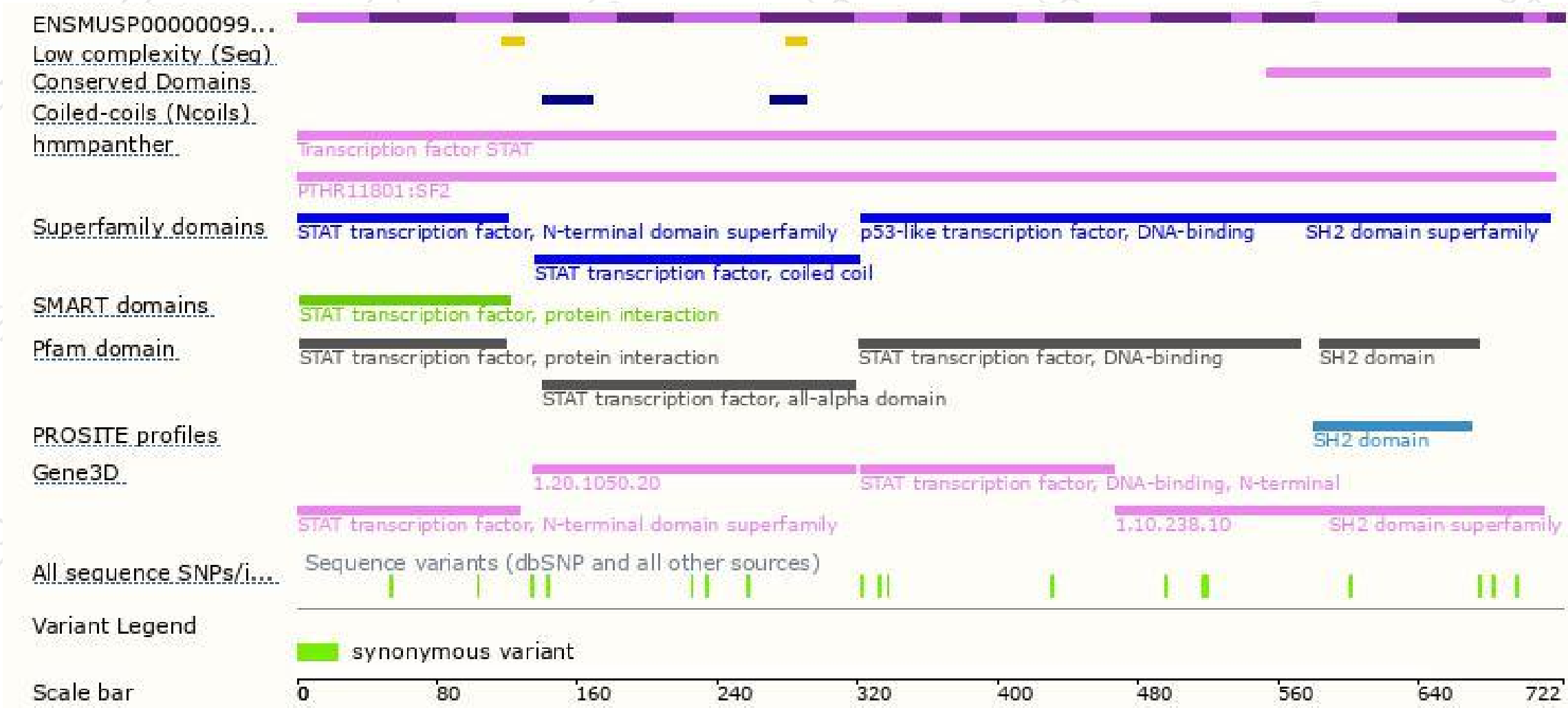
Mouse phenotype description(MGI)

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 die at embryonic day 6.5-7.5. Conditional, tissue specific mutants are variably viable and show diverse defects including obesity, diabetes, thermal dysregulation and infertility.

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