Rspo1 Cas9-CKO Strategy

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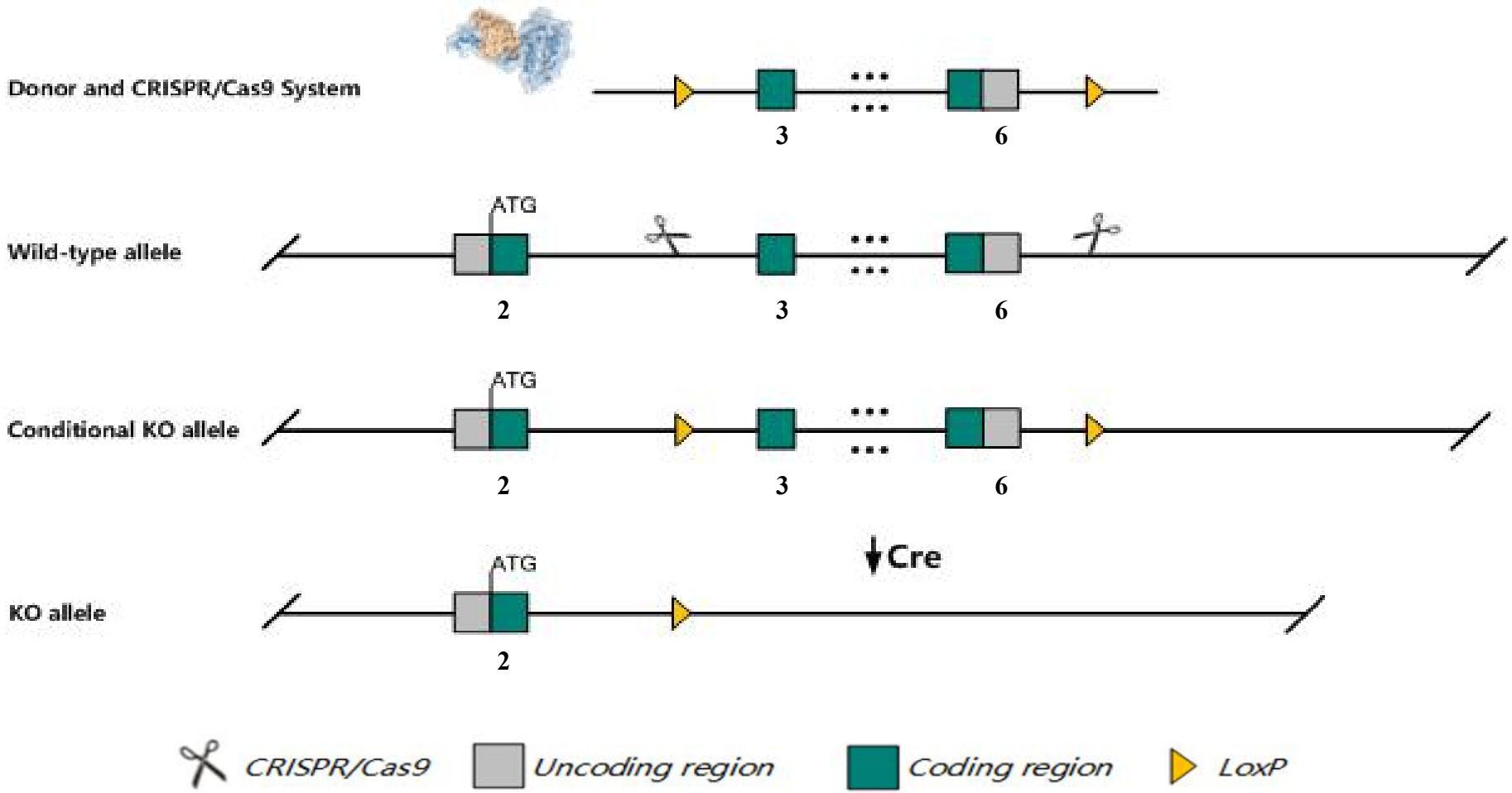
Reviewer	**Huan Fan**
Design Date:	**2020-4-10**

Project Overview

Project Name	***Rspo1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rspo1* gene. The schematic diagram is as follows:



Technical routes

The *Rspo1* gene has 2 transcripts. According to the structure of *Rspo1* gene, exon3-exon6 of *Rspo1-201* (ENSMUST00000030687.7) transcript is recommended as the knockout region.The region contains most coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Rspo1* 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 and cell types.

Notice

According to the existing MGI data,homozygous inactivation of this gene leads to abnormal ovarian development and masculinized features including pseudohermaphroditism in genital ducts, depletion of fetal oocytes, male-like vascularization, and ectopic testosterone production in the ovaries.

The *Rspo1* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information NCBI

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Rspo1 R-spondin 1 [Mus musculus (house mouse)]

Gene ID: 192199, updated on 13-Mar-2020

Summary

Official Symbol	Rspo1 provided by MGI
Official Full Name	R-spondin 1 provided by MGI
Primary source	MGI:MGI:2183426
See related	Ensembl:ENSMUSG00000028871
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	R-spondin, Rspondin
Expression	Broad expression in ovary adult (RPKM 13.8), CNS E11.5 (RPKM 10.5) and 19 other tissues See more
Orthologs	human all

Transcript information Ensembl

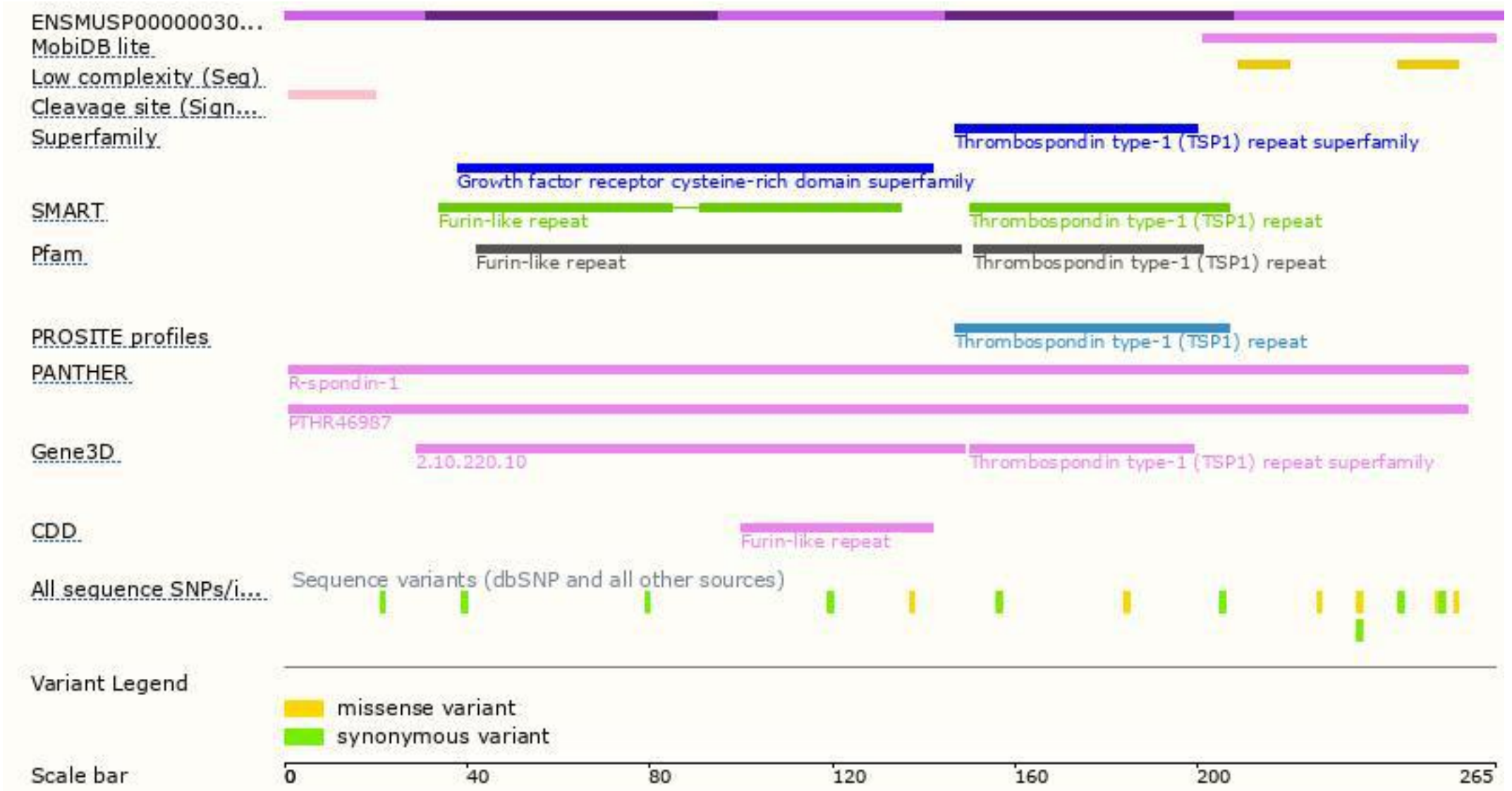
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rspo1-201	ENSMUST00000030687.7	1834	265aa	Protein coding	CCDS18633	B1ASC1	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Rspo1-202	ENSMUST00000140698.1	446	No protein	Processed transcript	-	-	TSL:3

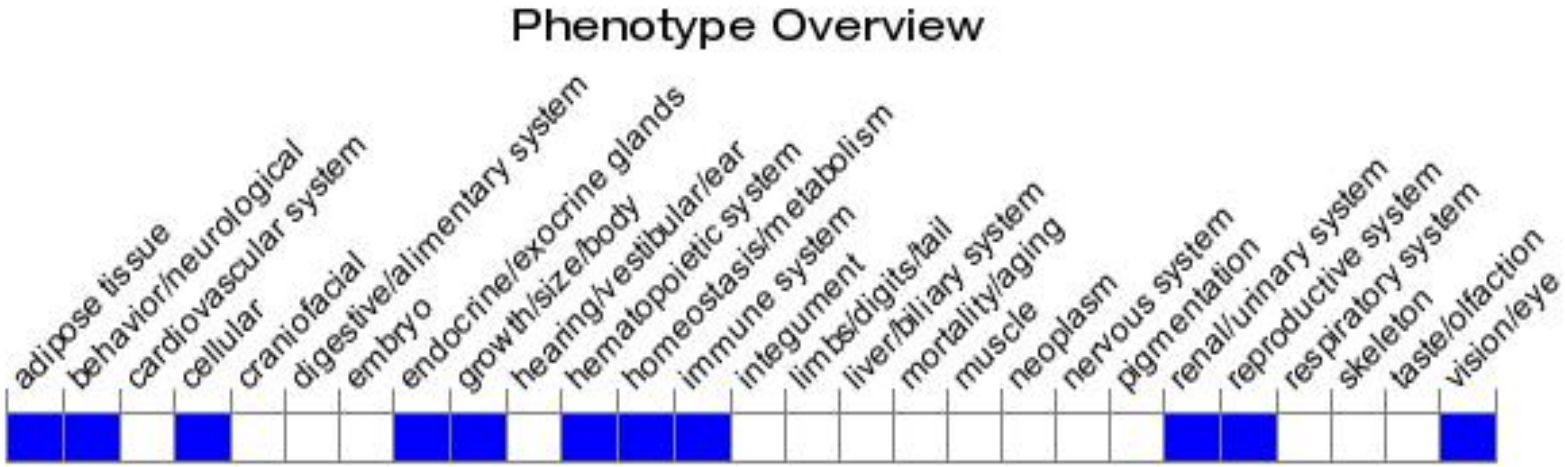
The strategy is based on the design of *Rspo1-201* transcript,the transcription is shown below

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/).

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