Polr2a Cas9-CKO Strategy

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Design Date: 2019-11-22

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Project Overview

Project Name	***Polr2a***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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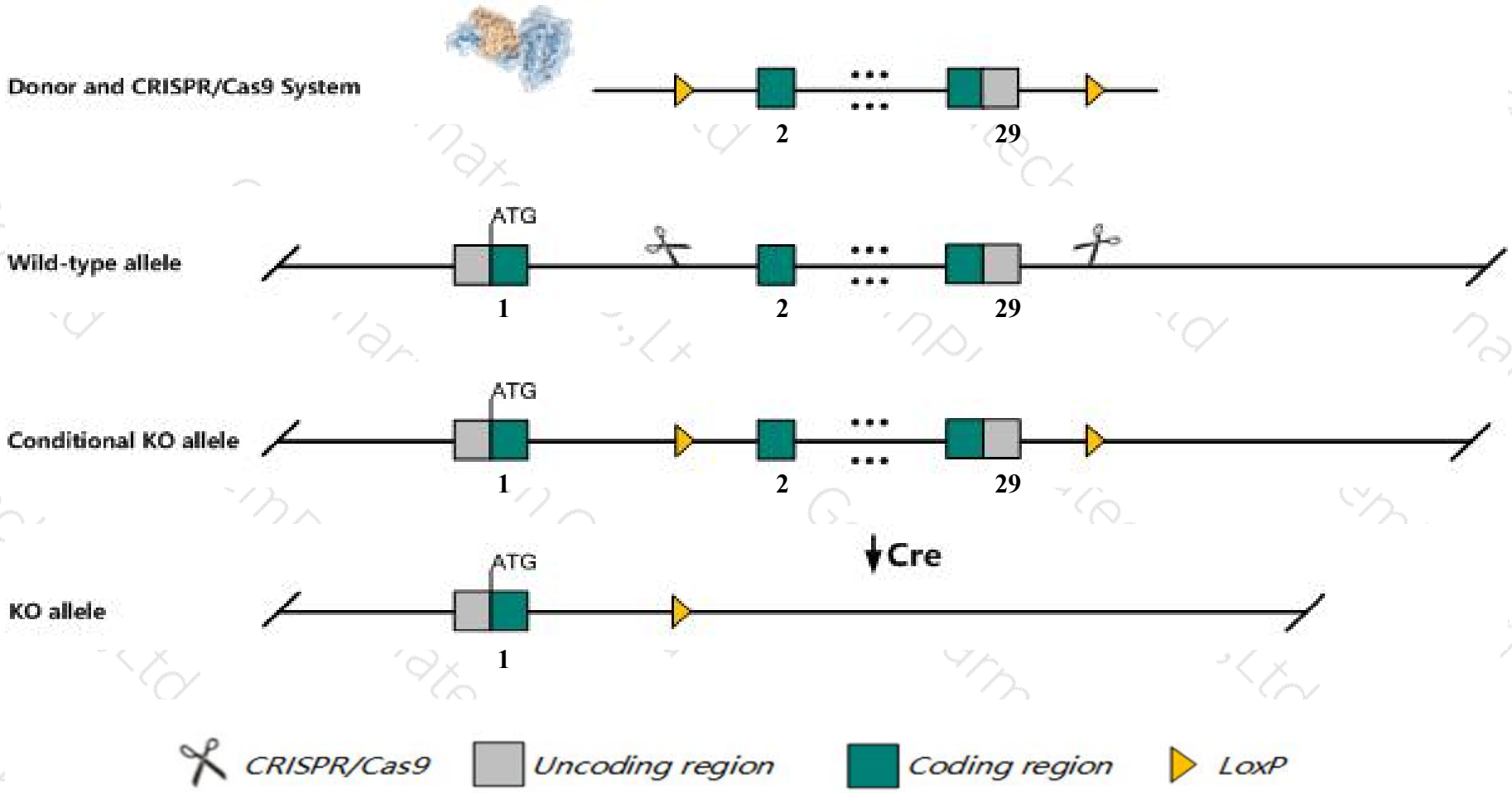
This model will use CRISPR/Cas9 technology to edit the *Polr2a* gene. The schematic diagram is as follows:



Technical routes

- The *Polr2a* gene has 4 transcripts. According to the structure of *Polr2a* gene, exon2-exon29 of *Polr2a-201* (ENSMUST00000058470.15) 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 *Polr2a* 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 a reporter allele show prenatal lethality. Homozygotes for a small deletion in the C-terminal domain are viable, fertile and developmentally normal. Homozygotes for a larger deletion show reduced fetal size and partial postnatal lethality; survivors are small but otherwise normal.

- The *Polr2a* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Polr2a polymerase (RNA) II (DNA directed) polypeptide A [Mus musculus (house mouse)]

Gene ID: 20020, updated on 5-Mar-2019

Summary

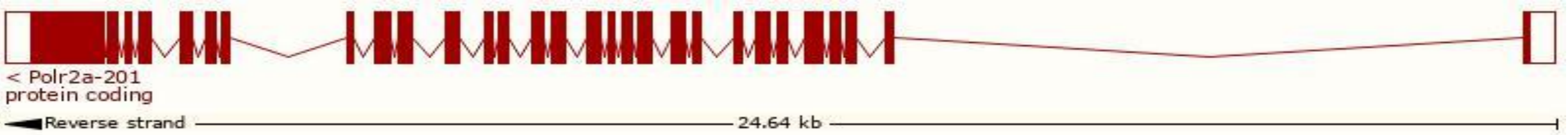
Official Symbol	Polr2a provided by MGI
Official Full Name	polymerase (RNA) II (DNA directed) polypeptide A provided by MGI
Primary source	MGI:MGI:98086
See related	Ensembl:ENSMUSG00000005198
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	220kDa, Rpb1, Rpo2-1
Expression	Ubiquitous expression in thymus adult (RPKM 39.8), limb E14.5 (RPKM 30.7) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

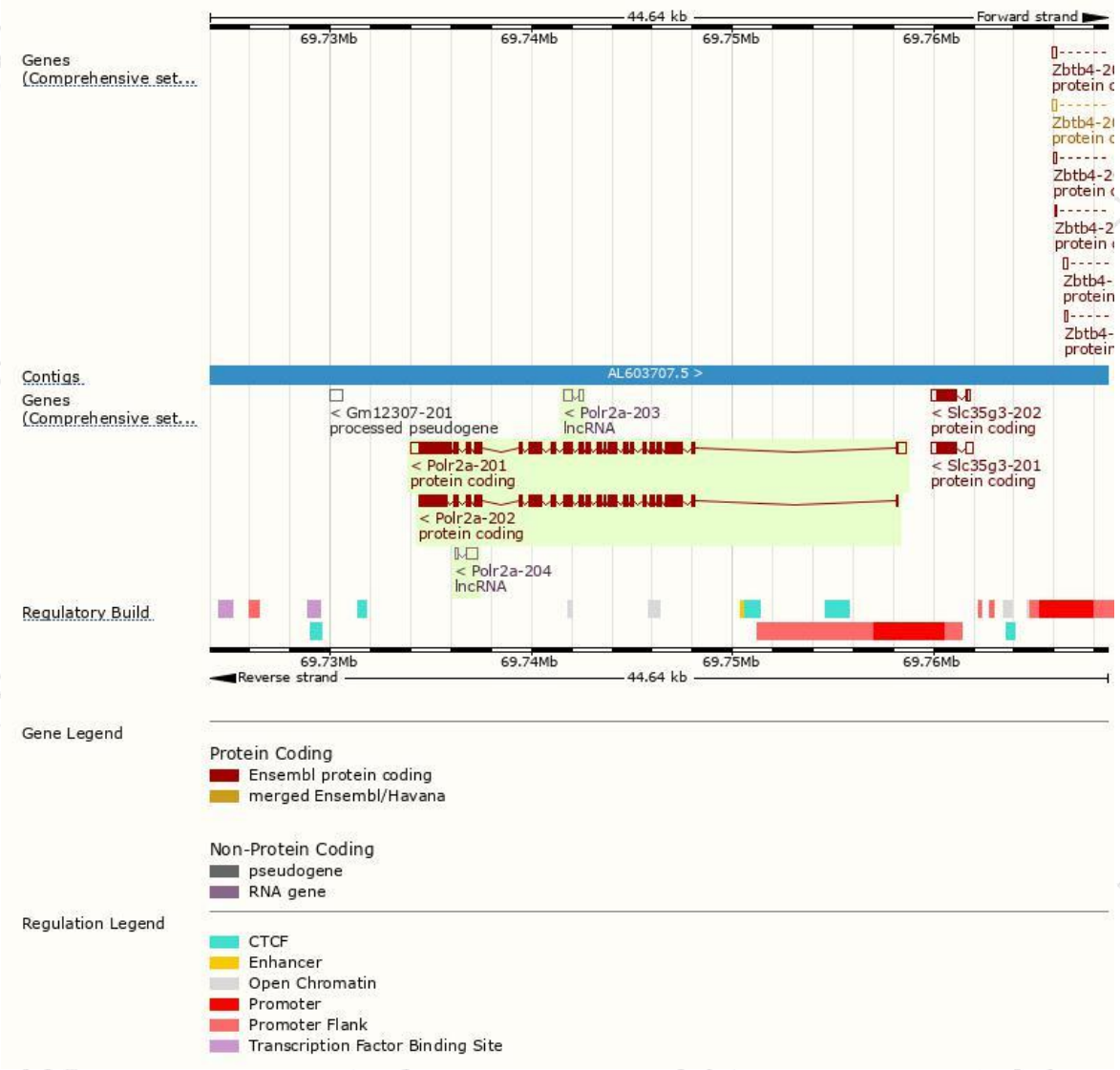
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Polr2a-201	ENSMUST00000058470.15	6740	1970aa	Protein coding	CCDS70217	P08775	TSL:1 GENCODE basic APPRIS P1
Polr2a-202	ENSMUST00000071213.3	5799	1932aa	Protein coding	-	A0A0R4J0V5	TSL:5 GENCODE basic
Polr2a-204	ENSMUST00000156588.1	689	No protein	lncRNA	-	-	TSL:3
Polr2a-203	ENSMUST00000151586.1	586	No protein	lncRNA	-	-	TSL:2

The strategy is based on the design of *Polr2a-201* transcript,The transcription is shown below

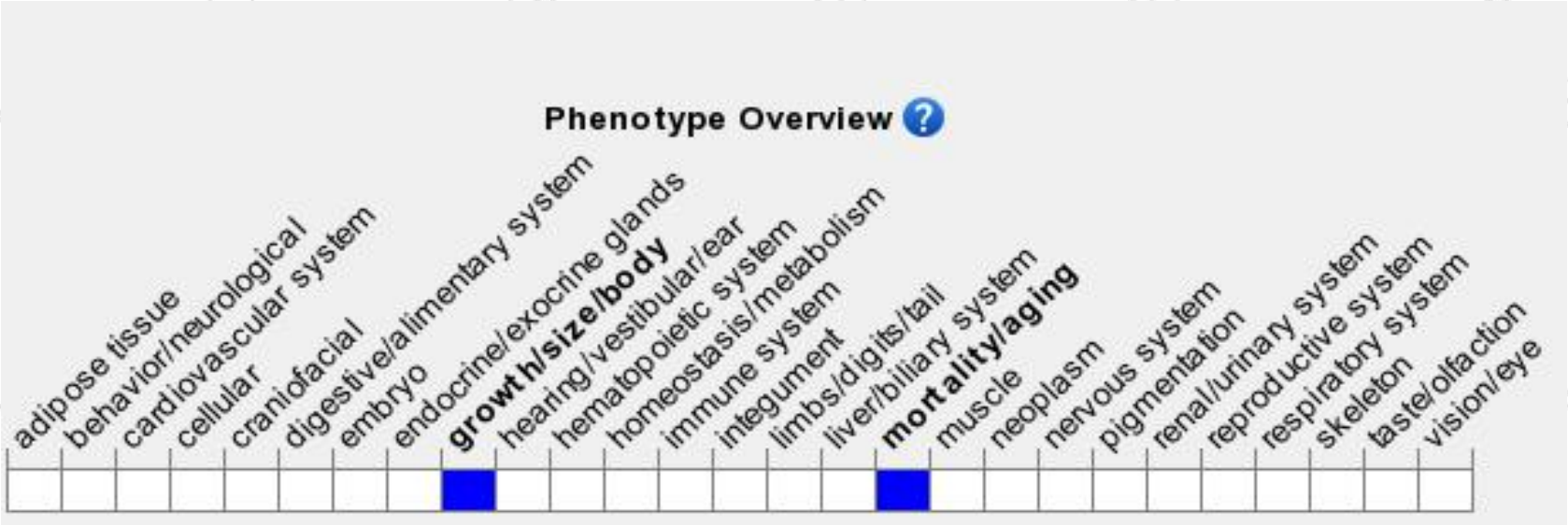


Genomic location distribution



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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