

# *Acta2* Cas9-CKO Strategy

Designer: Lixin Lv

# Project Overview

**Project Name**

*Acta2*

**Project type**

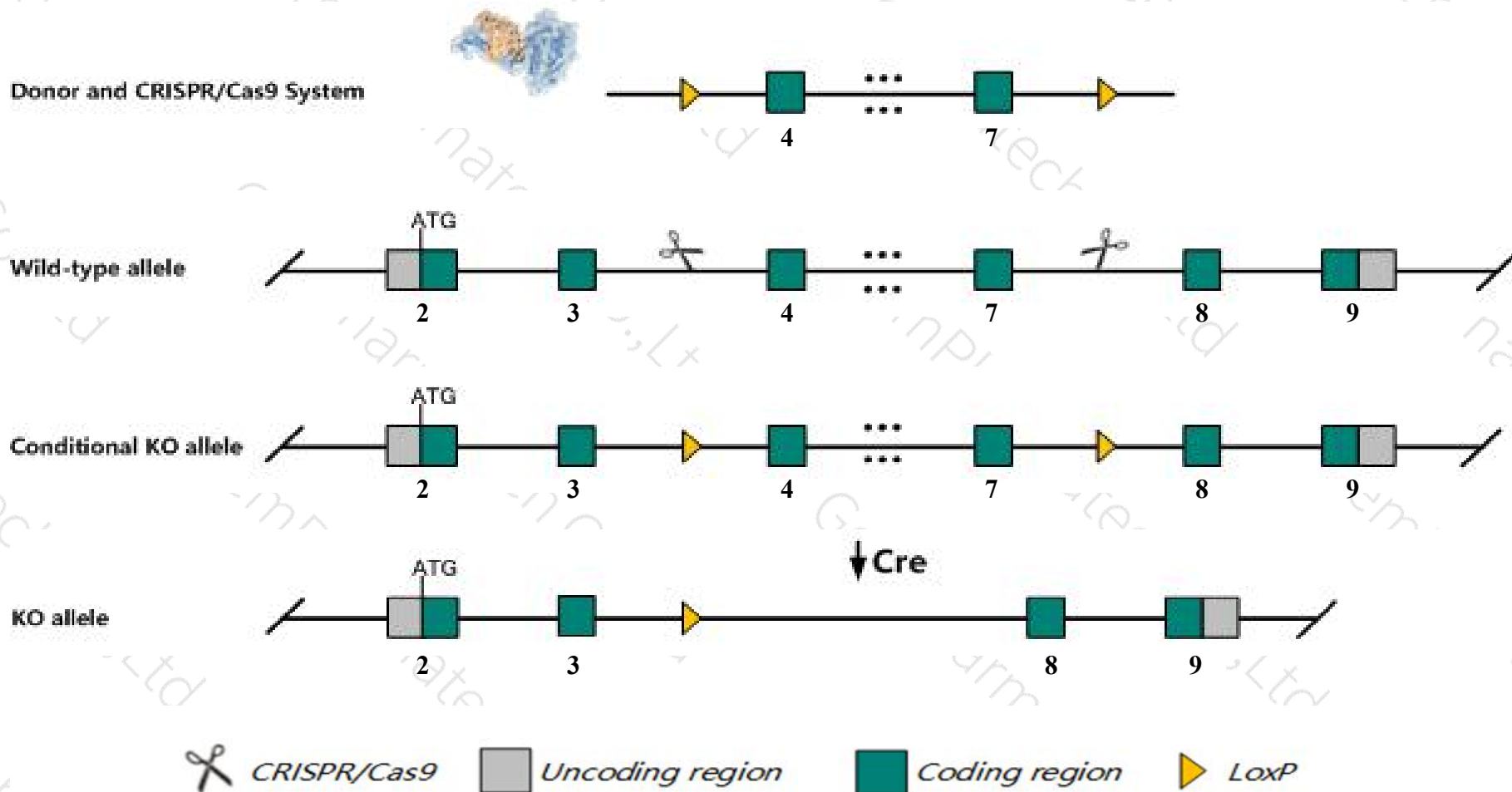
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Acta2* gene. The schematic diagram is as follows:



# Technical routes

- The *Acta2* gene has 2 transcripts. According to the structure of *Acta2* gene, exon4-exon7 of *Acta2-201* (ENSMUST00000039631.9) transcript is recommended as the knockout region. The region contains 550bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Acta2* 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.

- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit impaired vascular contractility and blood pressure homeostasis, increased blood-retina barrier permeability, and reduced retinal cone and rod function.
- The *Acta2* gene is located on the Chr19. 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)

## Acta2 actin, alpha 2, smooth muscle, aorta [Mus musculus (house mouse)]

Gene ID: 11475, updated on 9-Apr-2019

### Summary



**Official Symbol** Acta2 provided by [MGI](#)

**Official Full Name** actin, alpha 2, smooth muscle, aorta provided by [MGI](#)

**Primary source** [MGI:MGI:87909](#)

**See related** [Ensembl:ENSMUSG00000035783](#)

**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** 0610041G09Rik, Actvs, SMAalpha, SMalphaA, a-SMA, alphaSMA

**Summary** The protein encoded by this gene belongs to the actin family of proteins, which are highly conserved proteins that play a role in cell motility, structure and integrity. Alpha, beta and gamma actin isoforms have been identified, with alpha actins being a major constituent of the contractile apparatus, while beta and gamma actins are involved in the regulation of cell motility. This actin is an alpha actin that is found in skeletal muscle. [provided by RefSeq, Sep 2015]

**Expression** Biased expression in bladder adult (RPKM 2693.2), ovary adult (RPKM 563.6) and 10 other tissues [See more](#)

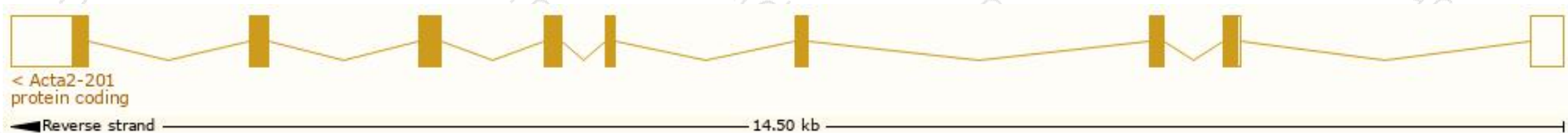
**Orthologs** [human](#) [all](#)

# Transcript information (Ensembl)

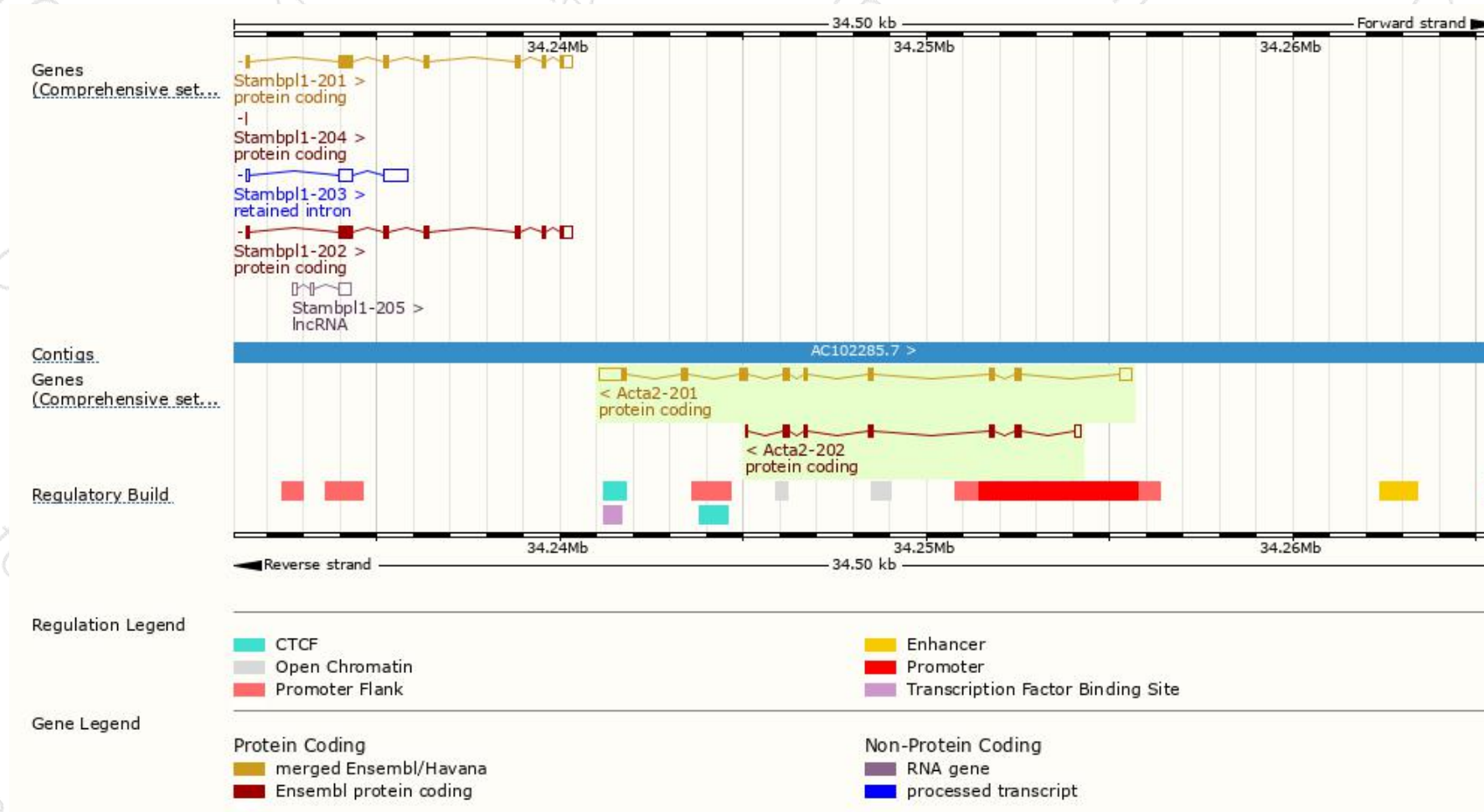
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Acta2-201	<a href="#">ENSMUST00000039631.9</a>	2035	<a href="#">377aa</a>	Protein coding	<a href="#">CCDS29757</a>	<a href="#">P62737</a>	TSL:1 GENCODE basic APPRIS P1
Acta2-202	<a href="#">ENSMUST00000238147.1</a>	826	<a href="#">214aa</a>	Protein coding	-	-	CDS 3' incomplete

The strategy is based on the design of *Acta2-201* transcript,The transcription is shown below



# Genomic location distribution

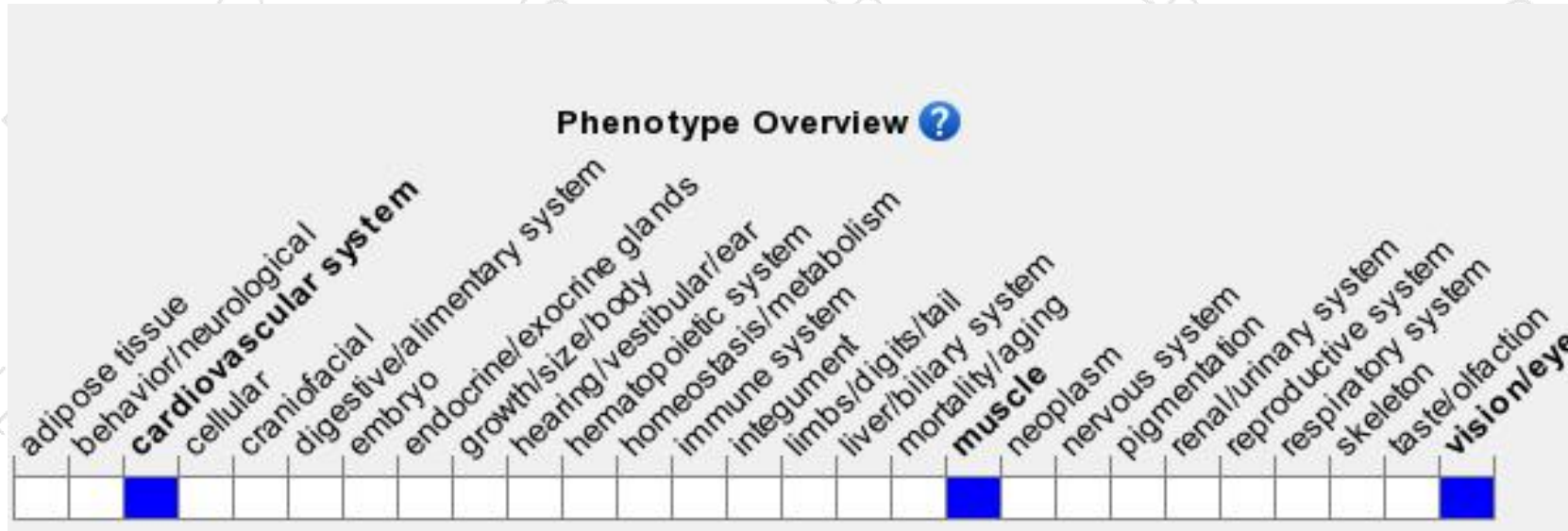




# Protein domain



# 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, Mice homozygous for a knock-out allele exhibit impaired vascular contractility and blood pressure homeostasis, increased blood-retina barrier permeability, and reduced retinal cone and rod function.

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

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