

Sar1b Cas9-CKO Strategy

Designer:

Reviewer:

Design Date:

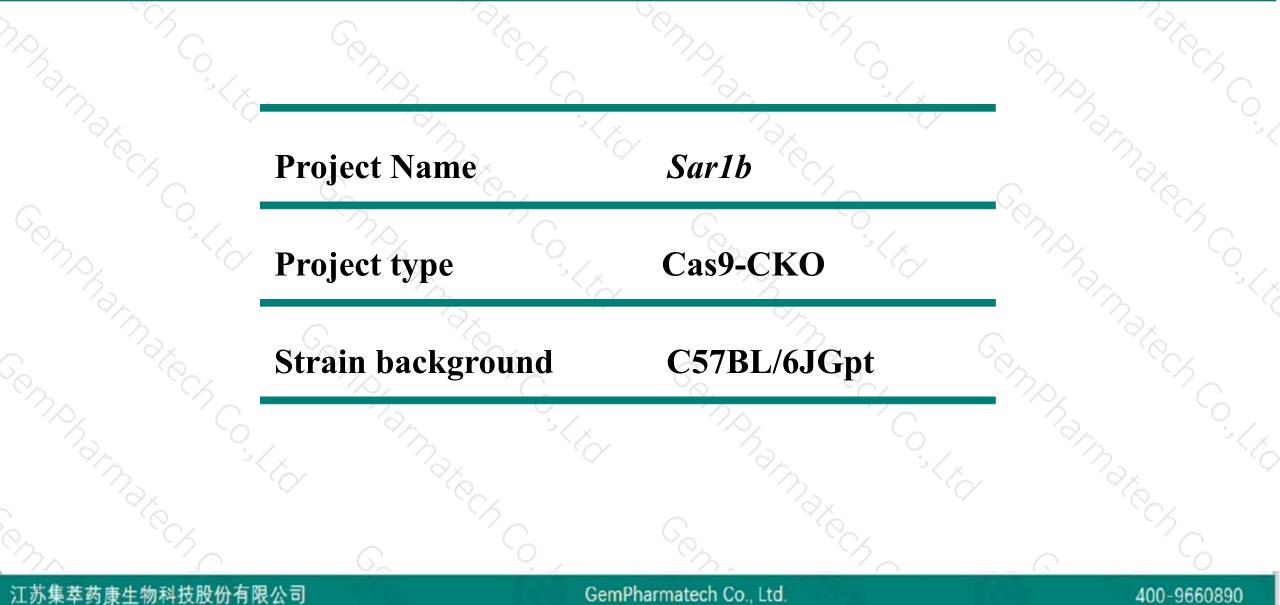
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2020-4-8

Project Overview



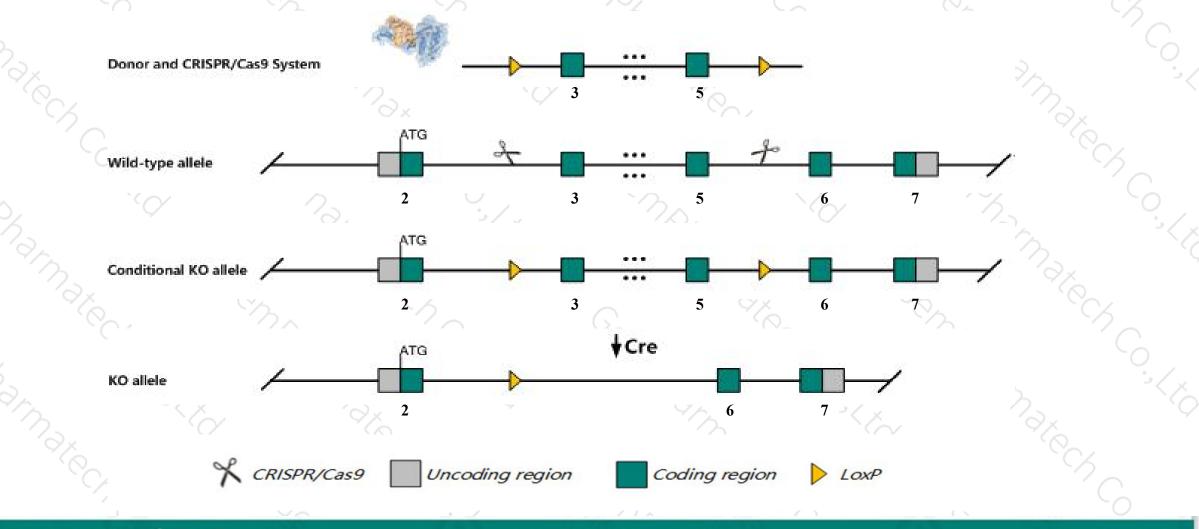


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the Sar1b gene. The schematic diagram is as follows:



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The Sar1b gene has 2 transcripts. According to the structure of Sar1b gene, exon3-exon5 of Sar1b-201 (ENSMUST0000020653.5) transcript is recommended as the knockout region. The region contains 290bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify Sar1b 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.



- The Sar1b 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)



< ?

Sar1b secretion associated Ras related GTPase 1B [Mus musculus (house mouse)]

Gene ID: 66397, updated on 13-Mar-2020

Summary

| Official Symbol | Sar1b provided by MGI |
|----------------------|--|
| Official Full Name | secretion associated Ras related GTPase 1B provided by MGI |
| Primary source | MGI:MGI:1913647 |
| See related | Ensembl:ENSMUSG0000020386 |
| 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 | 2310075M17Rik, 2900019l22Rik, CMRD, Sara1b, Sara2, Sarb |
| Expression | Ubiquitous expression in liver E18 (RPKM 51.6), bladder adult (RPKM 37.1) and 25 other tissues See more |
| Orthologs | human all |

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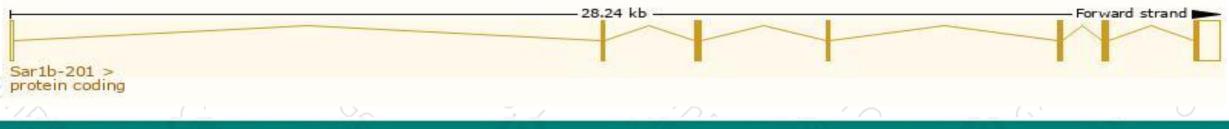
Transcript information (Ensembl)



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

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags | | | | | | | |
|-----------|----------------------|------|--------------|----------------------|-----------|---------|--|--|--|--|--|--|--|--|
| Sar1b-201 | ENSMUST00000020653.5 | 1201 | <u>198aa</u> | Protein coding | CCDS24661 | | STRET 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 P | | | | | | | |
| Sar1b-202 | ENSMUST00000136363.1 | 377 | No protein | Processed transcript | 100 | ÷ . | TSL:3 | | | | | | | |
| | | | | | | | | | | | | | | |

The strategy is based on the design of Sar1b-201 transcript, The transcription is shown below



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Genomic location distribution







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Protein domain



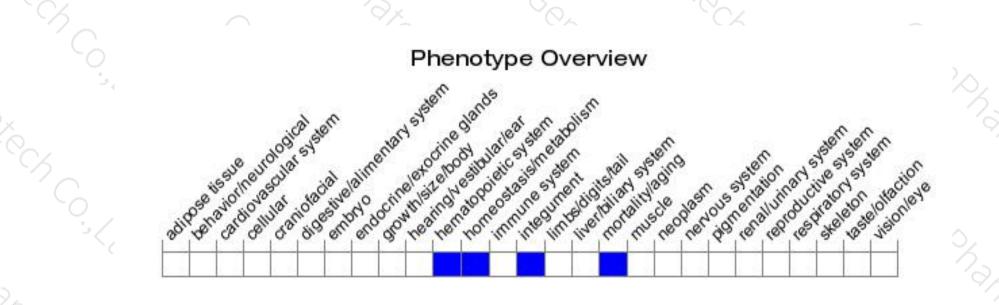
| 3 | ENSMUSP00000020 TIGRFAM | Si Si | mall GTP-binding | protein doma | ain | | | | | | |
|---|----------------------------|---|------------------|----------------|--------------|-------|-----|-----|-----|-----|----------|
| ð | Superfamily | P-la | op containing nu | cleoside triph | osphate hydr | olase | | | | | ~ 0 |
| | SMART | SM00177 | | | | | | | | - | |
| | | SM00178 | | _ | | | | | | | |
| | Prints | | Small GTPase su | perfamily, AR | F/SAR type | 8 | | | | | |
| | <u>Pfam</u> | Small GTP | ase superfamily, | ARF/SAR typ | e | | | | | | |
| | PROSITE profiles | Smal | GTPase superfa | mily, SAR1-ty | pe | | | | | | 9.7 |
| | PANTHER | PTHR45684:SF20 | | | | | | | | | |
| | | PTHR45684 | | | | | | | | | |
| | Gene3D | 3.40.50.300 | | | | | | | | | |
| | CDD | cd00879 | | | | | | | | | |
| 2 | All sequence SNPs/i | Sequence variants (dbSNP and all other sources) | | | | | | | | | |
| | 25 25 | | | | | | | | | | |
| | Variant Legend | synonymo | us variant | | | | | | | | |
| | Scale bar | 0 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 198 | |
| | | | | | | | | | | | |
| | | | | and the second | | | 1 | | | | |

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Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).



If you have any questions, you are welcome to inquire. Tel: 400-9660890



