



Cckar Cas9-CKO Strategy

Designer:

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Design Date:

2019-7-17

Project Overview

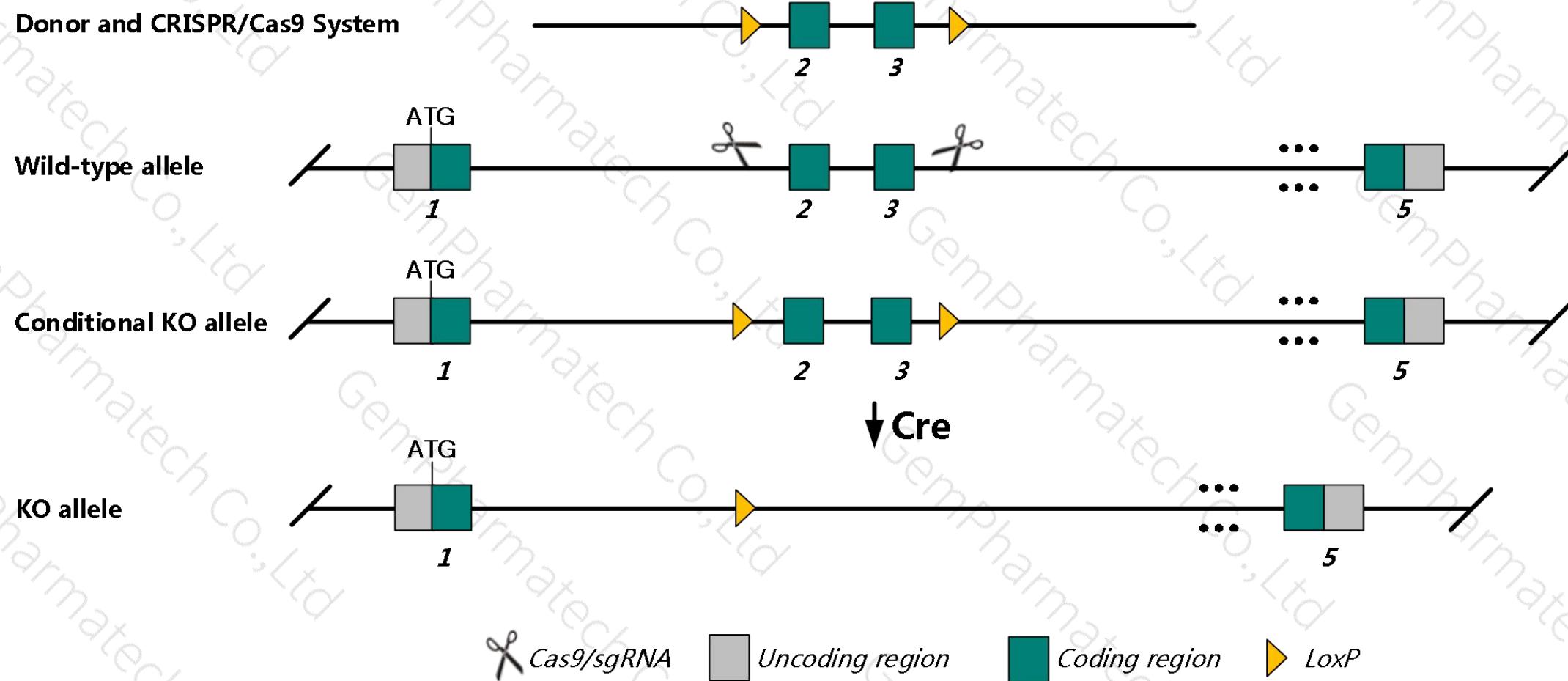
Project Name**Cckar**

Project type**Cas9-CKO**

Strain background**C57BL/6JGpt**

Conditional Knockout strategy

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



Technical routes

- The *Cckar* gene has 3 transcripts. According to the structure of *Cckar* gene, exon2-exon3 of *Cckar-201* (ENSMUST00000031093.4) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cckar* 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.



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Notice

- According to the existing MGI data, Homozygous mutant mice cannot regulate core body temperature in response to changes in ambient temperature.
- The *Cckar* gene is located on the Chr5. 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)

Cckar cholecystokinin A receptor [Mus musculus (house mouse)]

Gene ID: 12425, updated on 2-Apr-2019

Summary



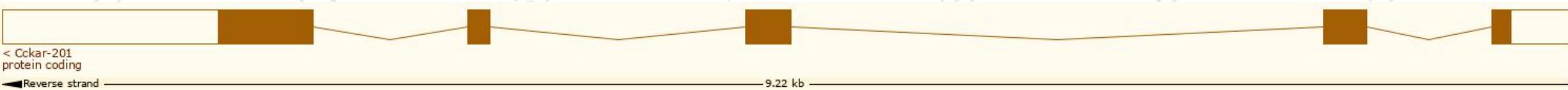
Official Symbol	Cckar provided by MGI
Official Full Name	cholecystokinin A receptor provided by MGI
Primary source	MGI:MGU99478
See related	Ensembl:ENSMUSG000000029193
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW106902
Expression	Biased expression in lung adult (RPKM 14.7), kidney adult (RPKM 12.1) and 4 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

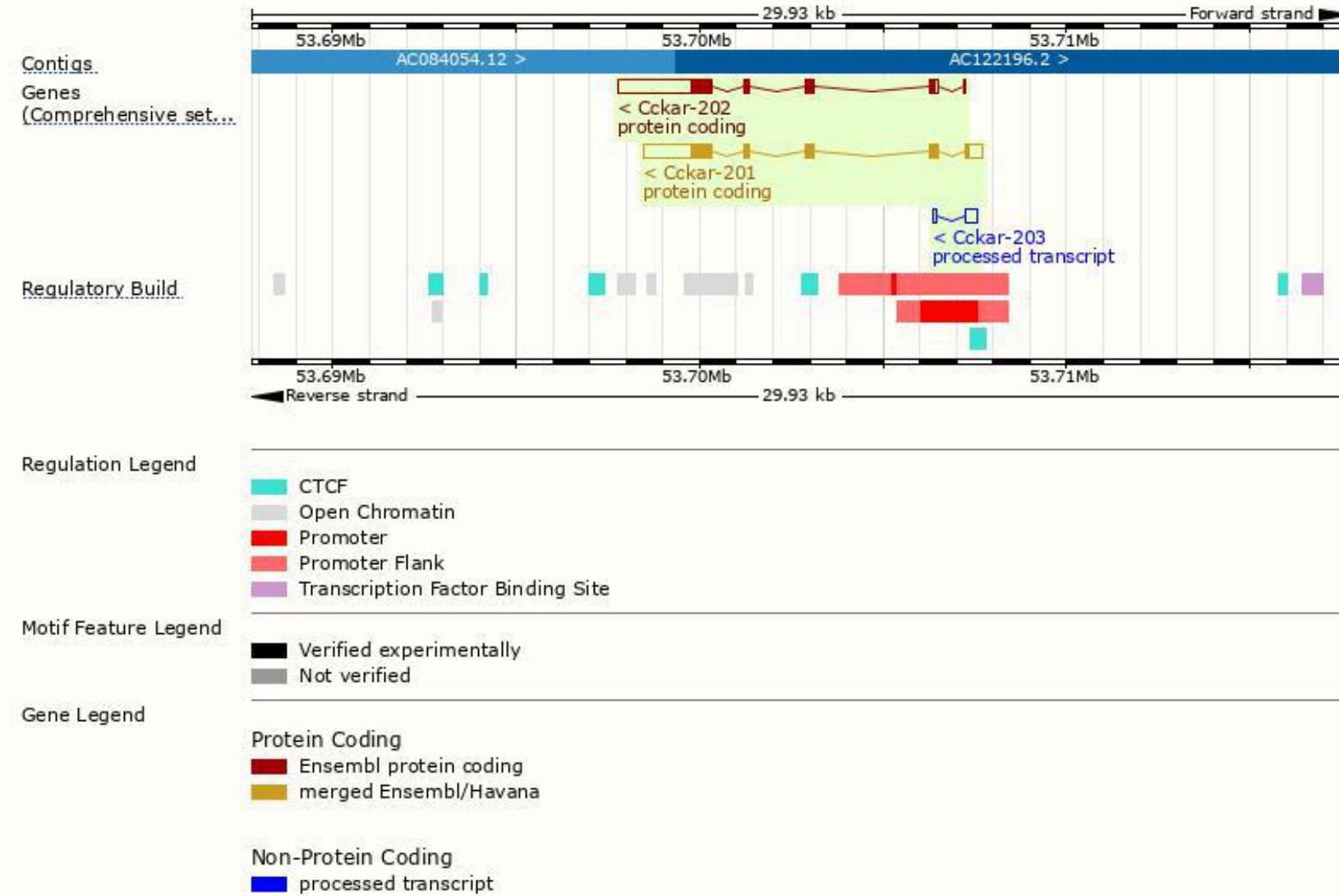
The gene has 3 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cckar-202	ENSMUST00000200691.3	3230	365aa	Protein coding	CCDS84881	Q3TPL0	TSL:1 GENCODE basic
Cckar-201	ENSMUST00000031093.4	2930	436aa	Protein coding	CCDS19293	O08786	TSL:1 GENCODE basic APPRIS P1
Cckar-203	ENSMUST00000202946.1	424	No protein	Processed transcript	-	-	TSL:3

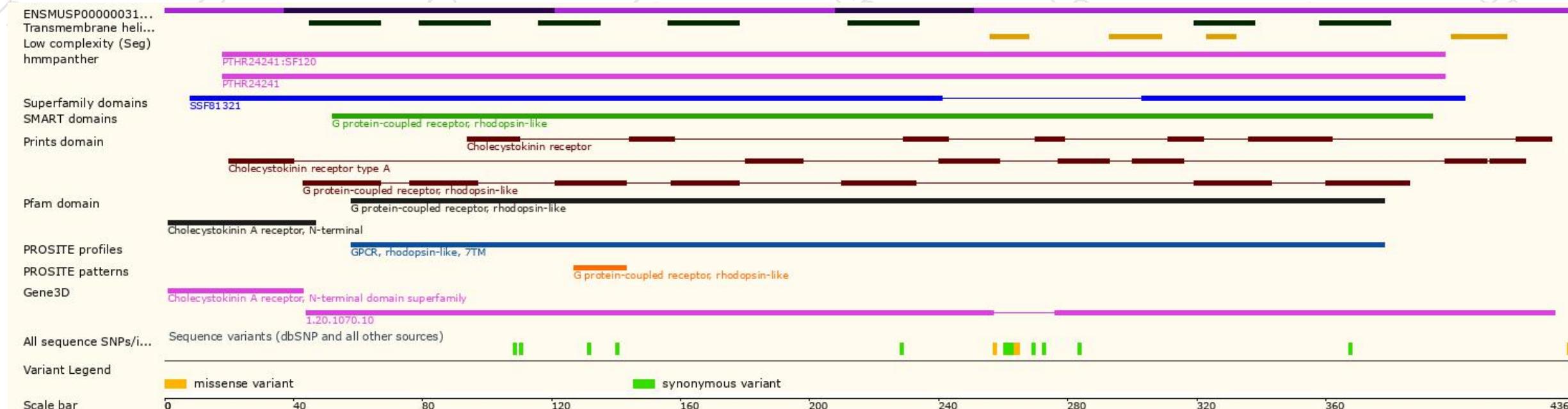
The strategy is based on the design of *Cckar-201* transcript, The transcription is shown below



Genomic location distribution



Protein domain





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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/>).

According to the existing MGI data, Homozygous mutant mice cannot regulate core body temperature in response to changes in ambient temperature.



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

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