Crhr2 Cas9-KO Strategy

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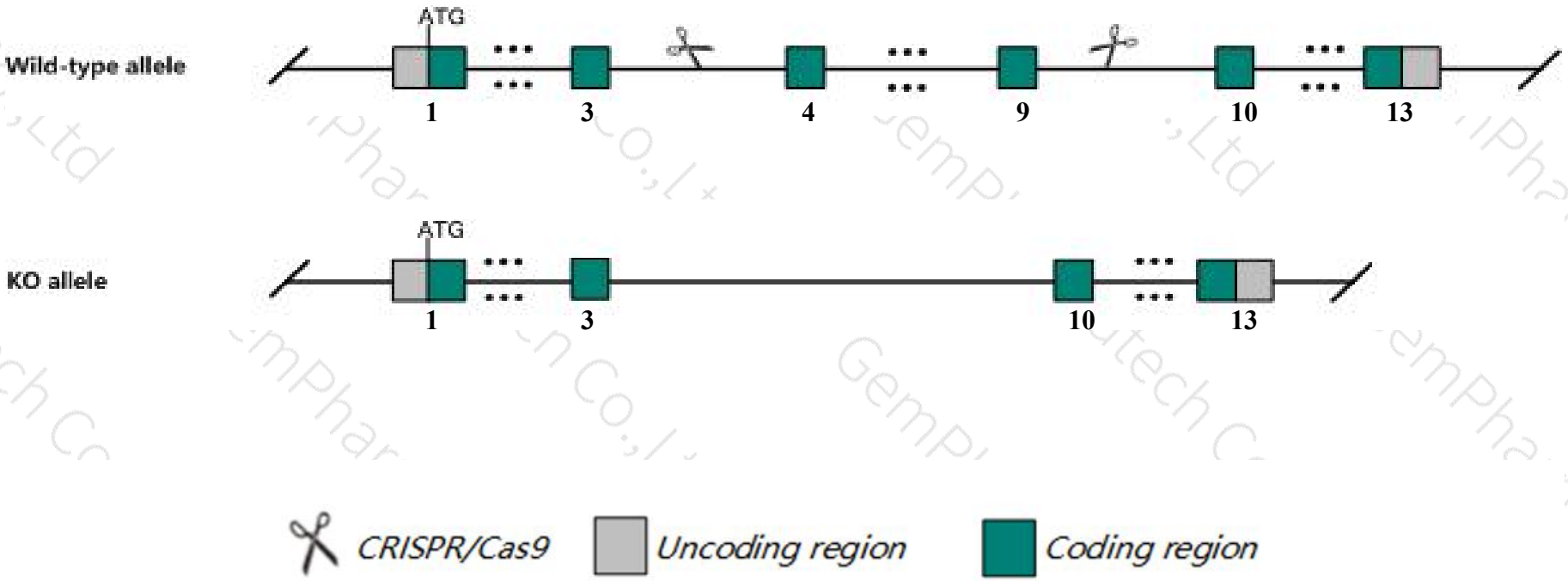
Design Date: 2019-8-5

Project Overview

Project Name	***Crhr2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Crhr2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Crhr2* gene has 9 transcripts. According to the structure of *Crhr2* gene, exon4-exon9 of *Crhr2-208* (ENSMUST00000212633.1) transcript is recommended as the knockout region.The region contains 602bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Crhr2* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

Notice

➢ According to the existing MGI data,Homozygous inactivation of this gene may result in hypersensitivity to stress, increased anxiety-like behavior, abnormal homeostatic responses to challenges of increased dietary fat and cold, and cardiovascular abnormalities, including hypertension and decreased cardiac contractility.

➢ The *Crhr2* gene is located on the Chr6. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Crhr2 corticotropin releasing hormone receptor 2 [Mus musculus (house mouse)]

Gene ID: 12922, updated on 12-Mar-2019

Summary

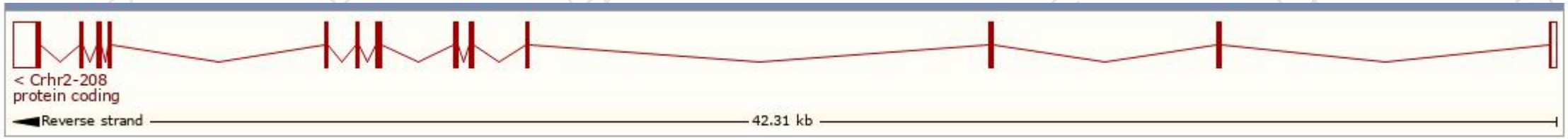
Official Symbol	Crhr2 provided by MGI
Official Full Name	corticotropin releasing hormone receptor 2 provided by MGI
Primary source	MGI:MGI:894312
See related	Ensembl:ENSMUSG00000003476
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CRF-R2, CRFR2alpha, CRFR2beta, Crfr2
Expression	Biased expression in heart adult (RPKM 14.2), mammary gland adult (RPKM 3.1) and 2 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

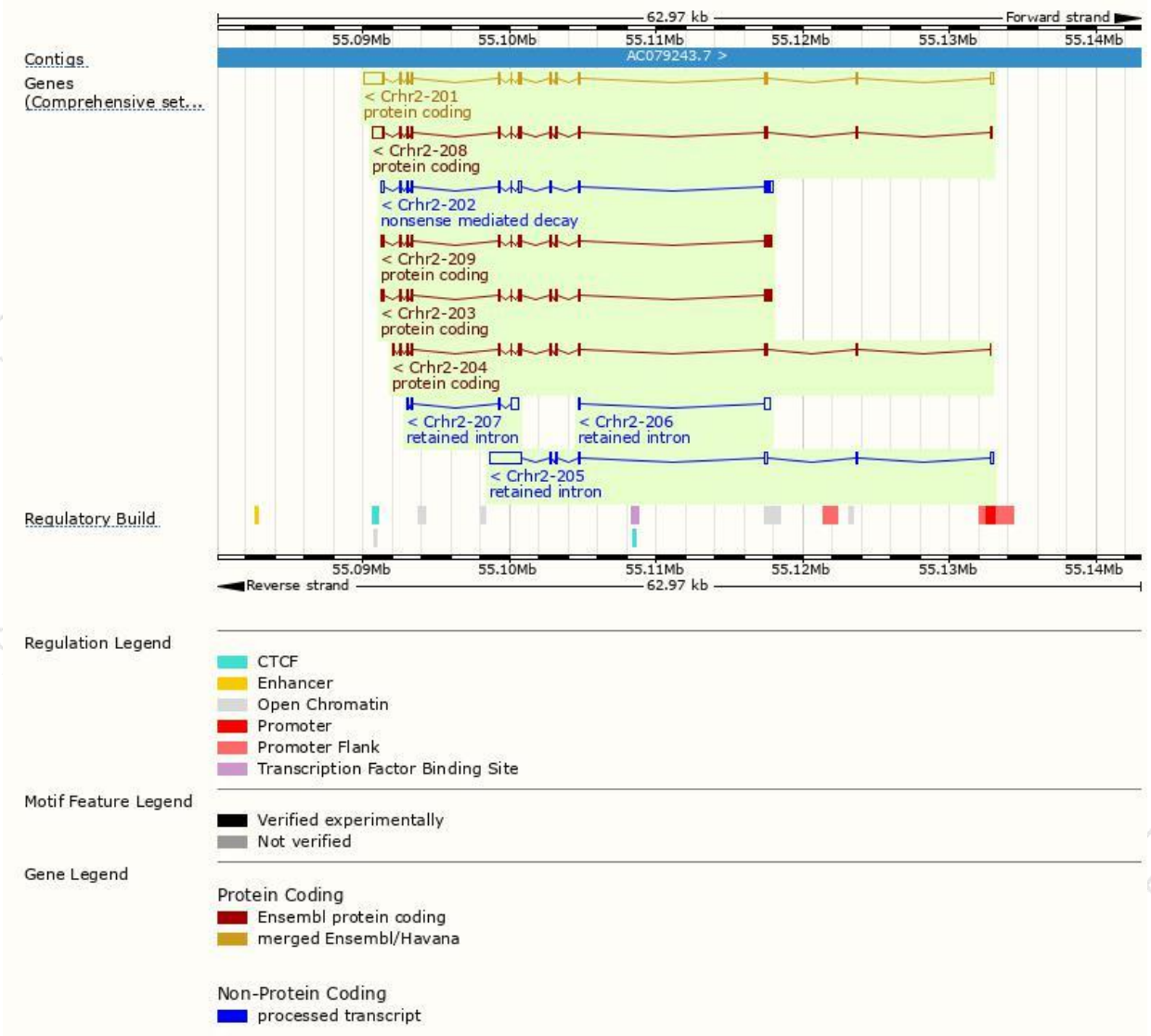
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Crhr2-201	ENSMUST00000003568.14	2695	430aa	Protein coding	CCDS20162	Q3UQP0	TSL:1 GENCODE basic
Crhr2-208	ENSMUST00000212633.1	2041	431aa	Protein coding	CCDS85053	A0A1D5RLK7	TSL:1 GENCODE basic
Crhr2-209	ENSMUST00000213026.1	1443	411aa	Protein coding	CCDS85052	Q60748	TSL:1 GENCODE basic APPRIS P2
Crhr2-203	ENSMUST00000114374.8	1440	410aa	Protein coding	-	E9QNK5	TSL:5 GENCODE basic APPRIS ALT1
Crhr2-204	ENSMUST00000164012.2	1263	420aa	Protein coding	-	E9PXI8	TSL:1 GENCODE basic
Crhr2-202	ENSMUST00000095898.9	1432	143aa	Nonsense mediated decay	-	Q5ERJ2	TSL:2
Crhr2-205	ENSMUST00000166954.1	2924	No protein	Retained intron	-	-	TSL:1
Crhr2-207	ENSMUST00000204117.1	794	No protein	Retained intron	-	-	TSL:5
Crhr2-206	ENSMUST00000169777.1	425	No protein	Retained intron	-	-	TSL:5

The strategy is based on the design of *Crhr2-208* transcript,The transcription is shown below

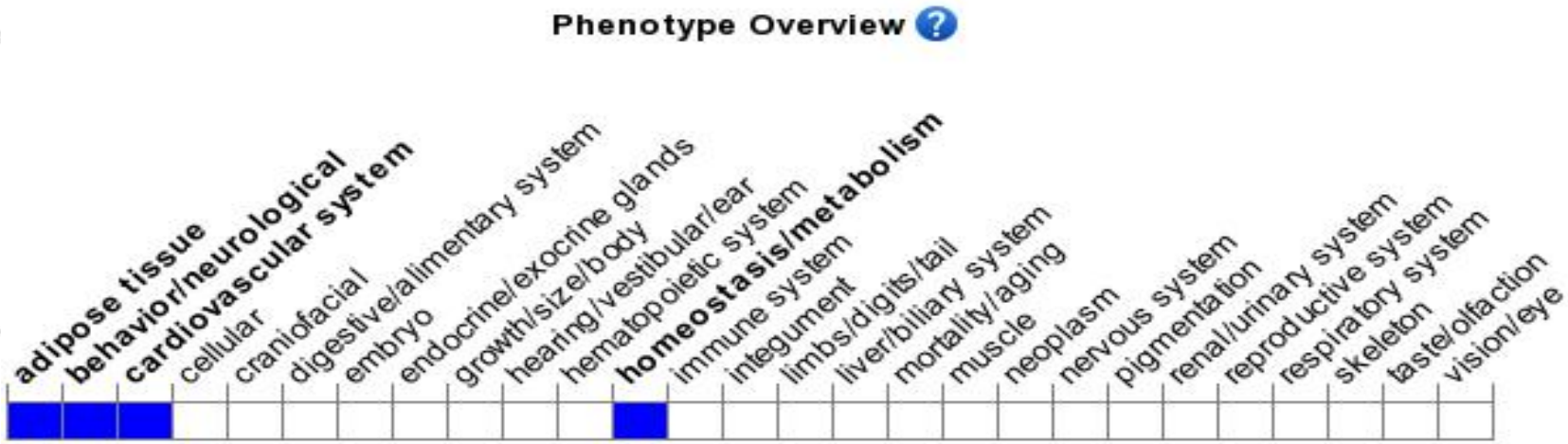


Genomic location distribution



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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