Ercc4 Cas9-CKO Strategy

Designer: Huimin Su

Reviewer: Ruirui Zhang

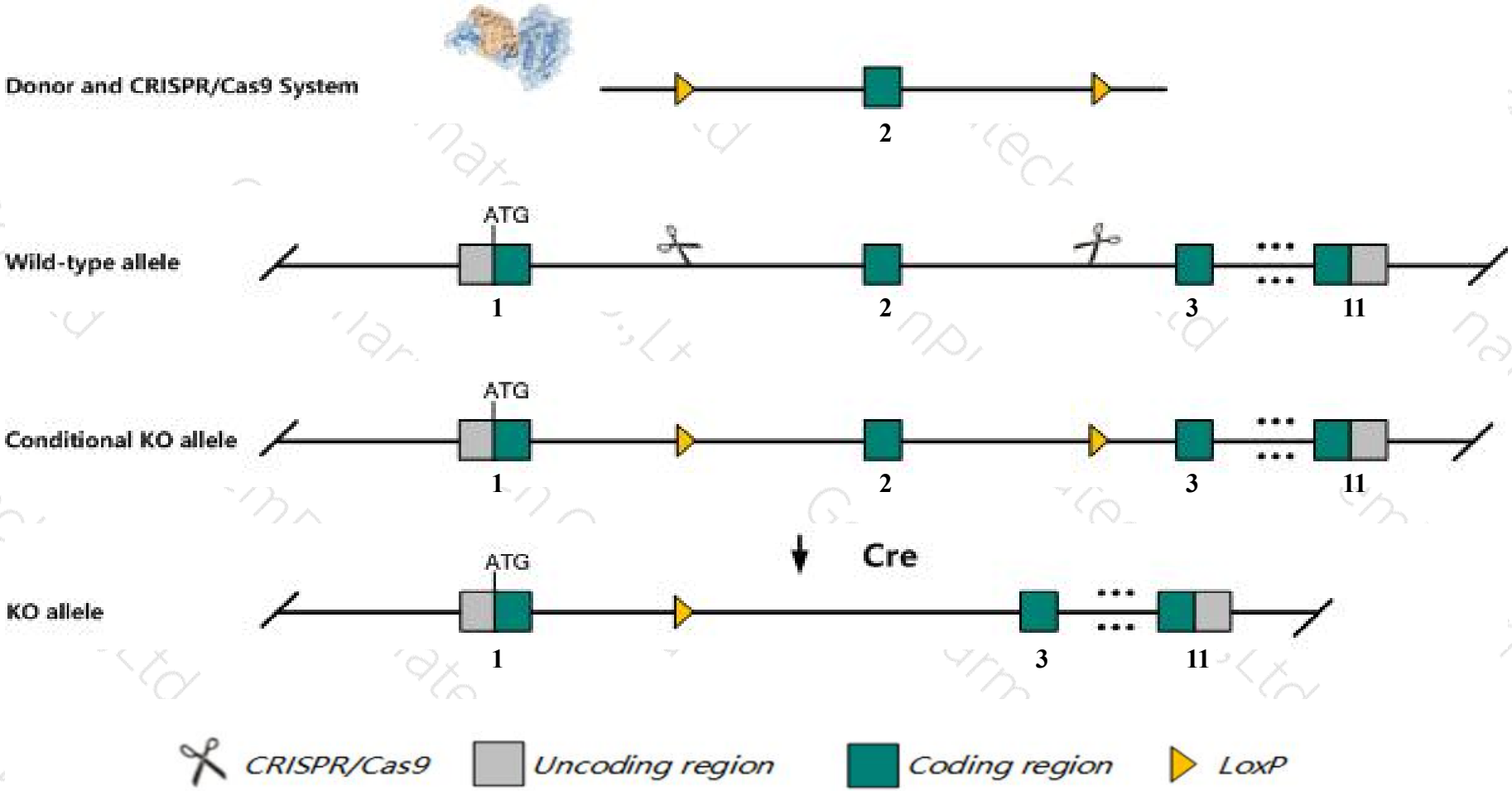
Design Date: 2020/2/13

Project Overview

Project Name	***Ercc4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ercc4* gene. The schematic diagram is as follows:



Technical routes

- The *Ercc4* gene has 5 transcripts. According to the structure of *Ercc4* gene, exon2 of *Ercc4-201* (ENSMUST00000023206.13) transcript is recommended as the knockout region.The region contains 181bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ercc4* 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,Homozygous null mice show impaired growth and do not survive longer than several weeks of age. Cultutred cells obtained from mutant mice were shown to be hypersensitive to UV irradiation.

➢ The *Ercc4* gene is located on the Chr16. 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）

集萃药康 GemPharmatech

Ercc4 excision repair cross-complementing rodent repair deficiency, complementation group 4 [*Mus musculus* (house mouse)]

Gene ID: 50505, updated on 10-Oct-2019

Summary

Official Symbol	Ercc4 provided by MGI
Official Full Name	excision repair cross-complementing rodent repair deficiency, complementation group 4 provided by MGI
Primary source	MGI:MGI:1354163
See related	Ensembl:ENSMUSG00000022545
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	Xpf; AI606920
Expression	Ubiquitous expression in testis adult (RPKM 8.4), ovary adult (RPKM 5.2) and 28 other tissues See more
Orthologs	human all

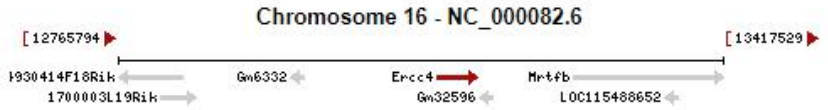
Genomic context

Location: 16; 16 A1

See Ercc4 in Genome Data Viewer

Exon count: 11

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	16	NC_000082.6 (13109366..13152009)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	16	NC_000082.5 (13109829..13152102)

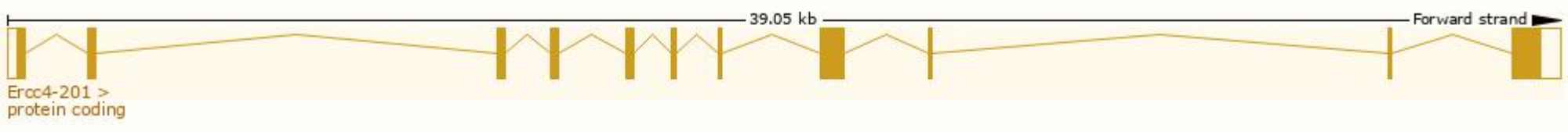


Transcript information（Ensembl）

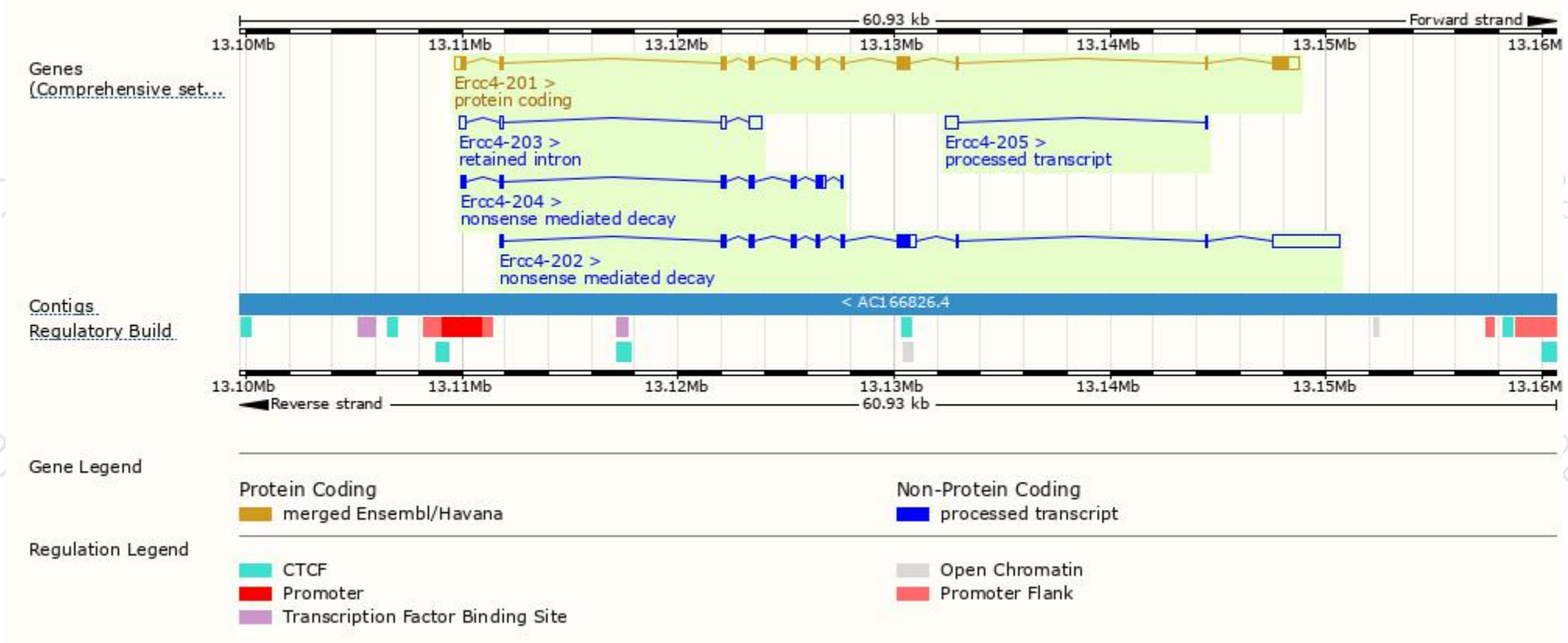
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ercc4-201	ENSMUST00000023206.13	3480	917aa	Protein coding	CCDS37256	Q9QZD4	TSL:1 GENCODE basic APPRIS P1
Ercc4-202	ENSMUST00000129049.1	5130	519aa	Nonsense mediated decay	-	F7A043	CDS 5' incomplete TSL:1
Ercc4-204	ENSMUST00000141024.7	1455	393aa	Nonsense mediated decay	-	D6RHP2	TSL:1
Ercc4-205	ENSMUST00000156393.1	651	No protein	Processed transcript	-	-	TSL:3
Ercc4-203	ENSMUST00000138527.1	1118	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Ercc4-201* transcript,The transcription is shown below

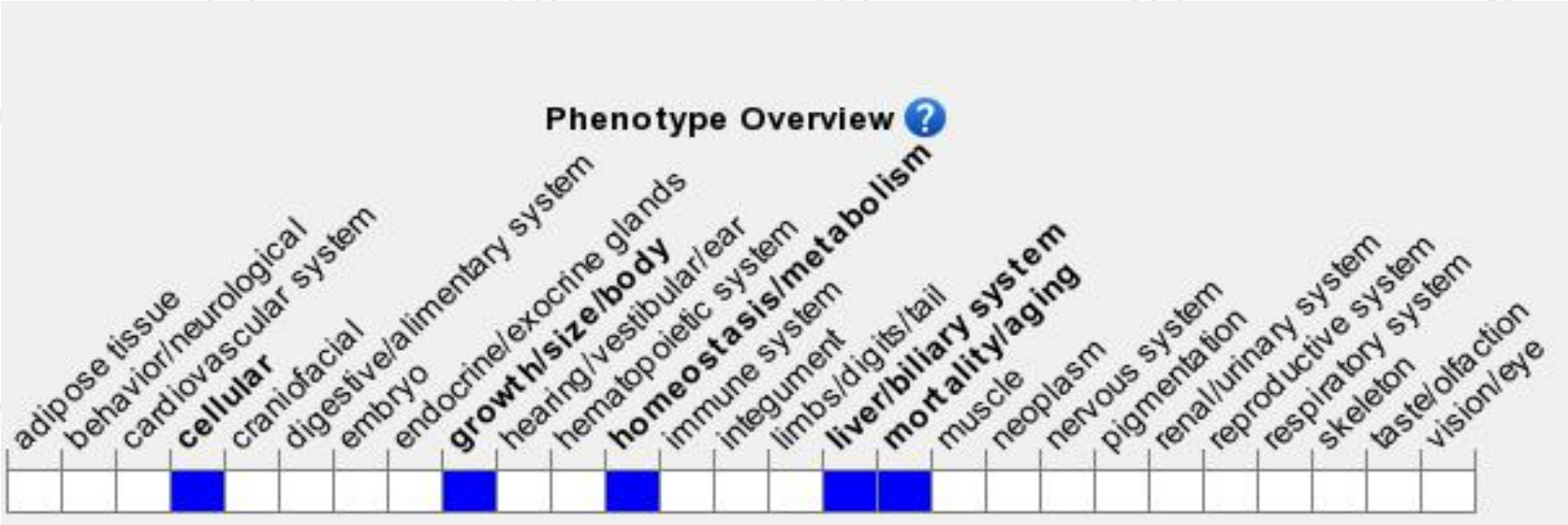


Genomic location distribution



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,Homozygous null mice show impaired growth and do not survive longer than several weeks of age. Cultutred cells obtained from mutant mice were shown to be hypersensitive to UV irradiation.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd