

***Etv4* Cas9-CKO Strategy**

Designer:Fengjuan Wang

Reviewer:Shilei Zhu

Design Date:2020-2-26

Project Overview

Project Name

Etv4

Project type

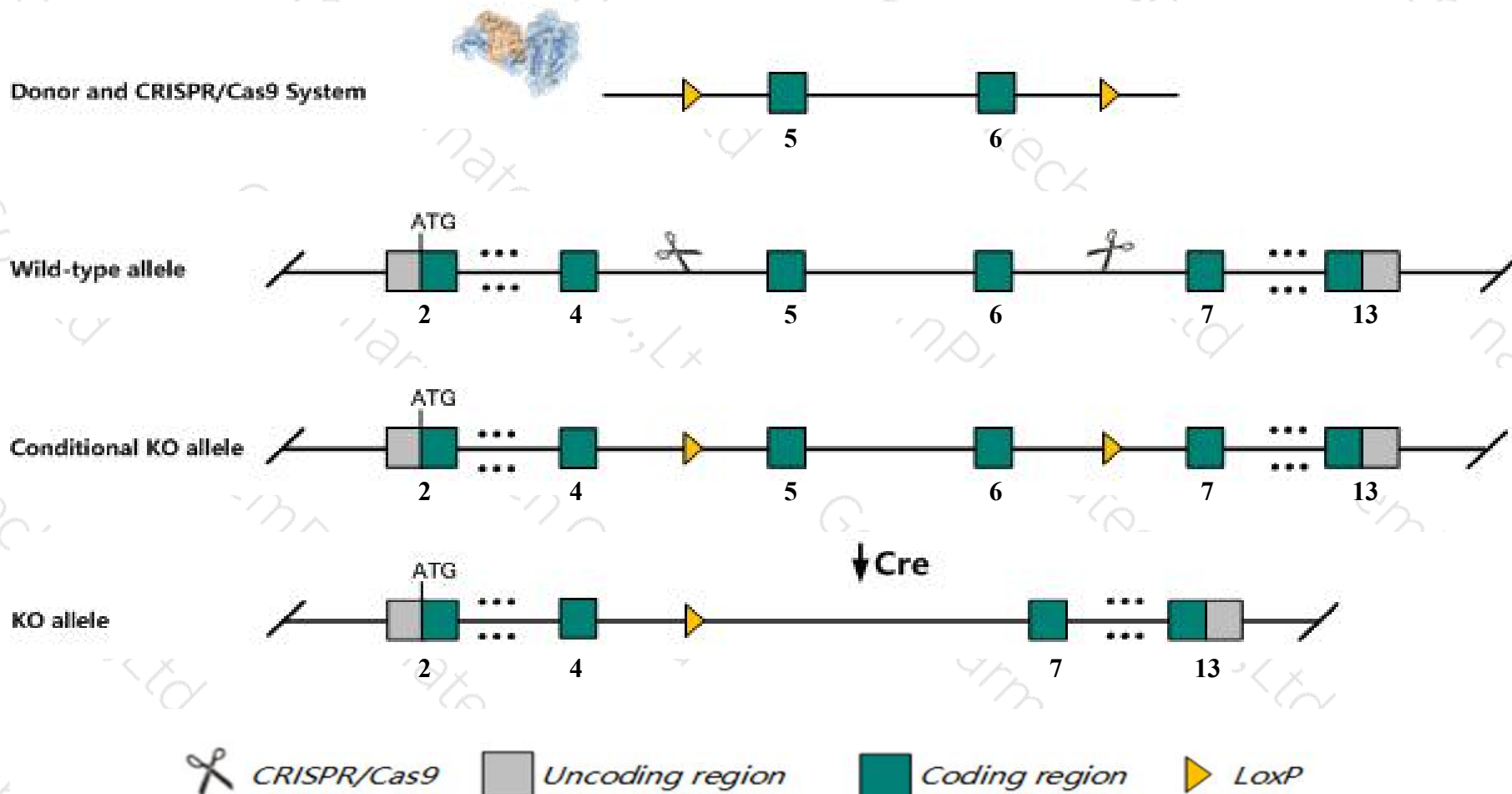
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Etv4* gene. The schematic diagram is as follows:



- The *Etv4* gene has 12 transcripts. According to the structure of *Etv4* gene, exon5-exon6 of *Etv4-201* (ENSMUST00000017868.6) 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 *Etv4* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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, Homozygotes for targeted null mutations exhibit male infertility due to failure to ejaculate, impaired branching of motor neurons, and abnormal mammary gland terminal differentiation.
- The *Etv4* 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)

Etv4 ets variant 4 [Mus musculus (house mouse)]

Gene ID: 18612, updated on 31-Jan-2019

Summary



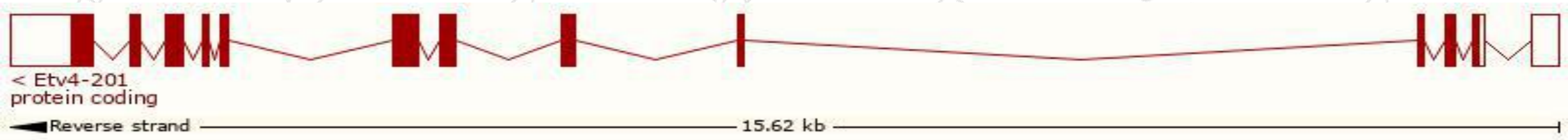
Official Symbol	Etv4 provided by MGI
Official Full Name	ets variant 4 provided by MGI
Primary source	MGI:MGI:99423
See related	Ensembl:ENSMUSG00000017724
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW414408, Pea-3, Pea3
Expression	Biased expression in genital fat pad adult (RPKM 15.6), colon adult (RPKM 7.7) and 13 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

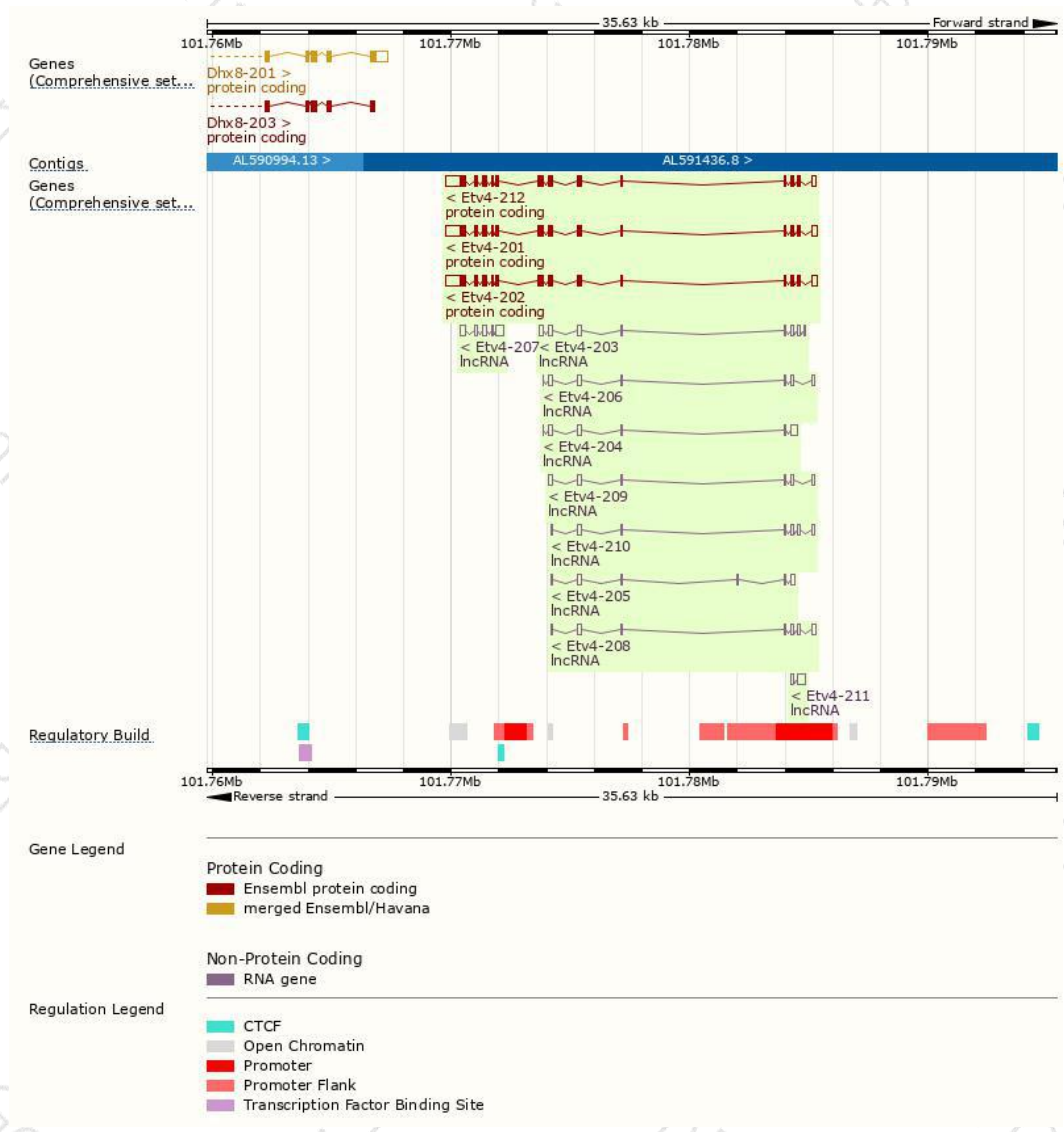
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Etv4-201	ENSMUST00000017868.6	2395	486aa	Protein coding	CCDS83903	A2A5C3	TSL:5 GENCODE basic APPRIS ALT 1
Etv4-202	ENSMUST000000107176.7	2377	480aa	Protein coding	CCDS83902	A2A5C2	TSL:1 GENCODE basic
Etv4-212	ENSMUST000000164750.7	2336	485aa	Protein coding	CCDS48937	P28322	TSL:1 GENCODE basic APPRIS P3
Etv4-207	ENSMUST000000132040.1	882	No protein	lncRNA	-	-	TSL:1
Etv4-203	ENSMUST000000129160.7	857	No protein	lncRNA	-	-	TSL:5
Etv4-204	ENSMUST000000129995.7	672	No protein	lncRNA	-	-	TSL:2
Etv4-206	ENSMUST000000131862.7	668	No protein	lncRNA	-	-	TSL:5
Etv4-210	ENSMUST000000149099.7	662	No protein	lncRNA	-	-	TSL:3
Etv4-208	ENSMUST000000137179.1	654	No protein	lncRNA	-	-	TSL:5
Etv4-209	ENSMUST000000140970.7	606	No protein	lncRNA	-	-	TSL:5
Etv4-205	ENSMUST000000131117.7	542	No protein	lncRNA	-	-	TSL:5
Etv4-211	ENSMUST000000154512.1	400	No protein	lncRNA	-	-	TSL:3

The strategy is based on the design of *Etv4-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain

ENSMUSP00000017...

[MobiDB lite](#)

[Low complexity \(Seq\)](#)

[Superfamily](#)

[SMART](#)

[Prints](#)

[Pfam](#)

PEA3-type ETS-domain transcription factor, N-terminal

Winged helix DNA-binding domain superl

Ets domain

Ets domain

Ets domain

[PROSITE profiles](#)

[PROSITE patterns](#)

Ets domain

Ets domain

Ets domain

[PANTHER](#)

PTHR11849:SF181

PTHR11849

[Gene3D](#)

Winged helix-like DNA-binding domain superfa

[All sequence SNPs/i...](#)

Sequence variants (dbSNP and all other sources)

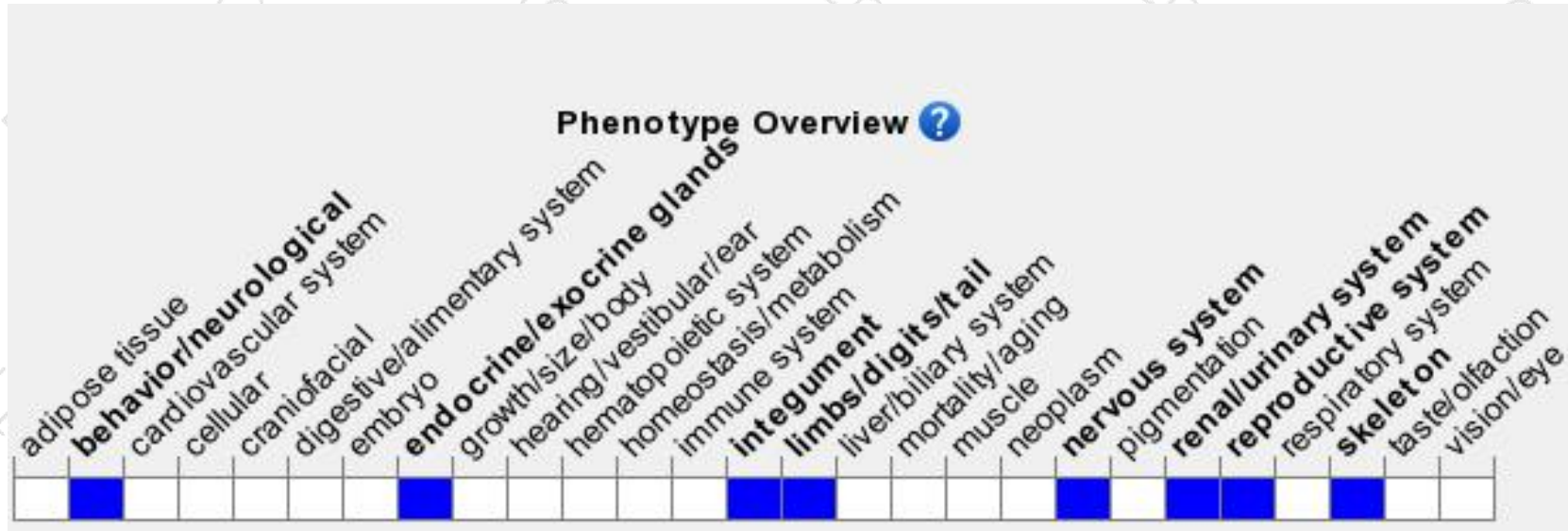
[Variant Legend](#)

 missense variant
 synonymous variant

[Scale bar](#)

0 60 120 180 240 300 360 420 486

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, Homozygotes for targeted null mutations exhibit male infertility due to failure to ejaculate, impaired branching of motor neurons, and abnormal mammary gland terminal differentiation.

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

Tel: 400-9660890

