

# E4f1 Cas9-KO Strategy

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Reviewer: Jia Yu

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## **Project Overview**



**Project Name** 

E4f1

**Project type** 

Cas9-KO

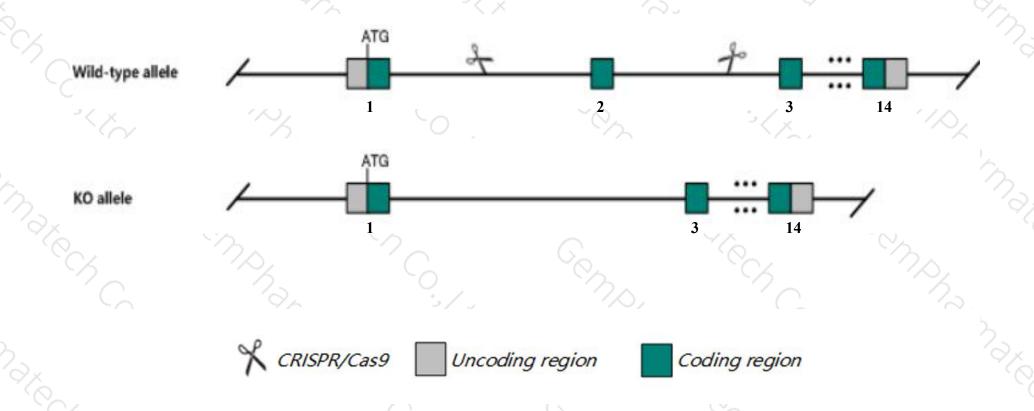
Strain background

C57BL/6JGpt

## **Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *E4f1* gene. The schematic diagram is as follows:



### **Technical routes**



- $\Rightarrow$  The *E4f1* gene has 8 transcripts. According to the structure of *E4f1* gene, exon2 of *E4f1-201* (ENSMUST00000056032.8) transcript is recommended as the knockout region. The region contains 152bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *E4f1* 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 null mice display early embryonic lethality with mitotic progression failure and increased apoptosis.
- $\gt$  The *E4f1* gene is located on the Chr17. 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)



#### E4f1 E4F transcription factor 1 [Mus musculus (house mouse)]

Gene ID: 13560, updated on 13-Mar-2020

#### Summary

☆ ?

Official Symbol E4f1 provided by MGI

Official Full Name E4F transcription factor 1 provided by MGI

Primary source MGI:MGI:109530

See related Ensembl: ENSMUSG00000024137

Gene type protein coding
RefSeq status REVIEWED

Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as p120E4F, phi-AP3

Summary This gene encodes a member of the GLI-Kruppel zinc finger family. The encoded protein is likely to be multi-functional, with both adenovirus

E1A-regulated transcription factor and ubiquitin E3 ligase activities, including roles in cell cycle regulation and the ubiquitination of p53.

Alternative splicing results in multiple transcript variants. [provided by RefSeq, Sep 2014]

Expression Ubiquitous expression in ovary adult (RPKM 25.6), thymus adult (RPKM 24.6) and 28 other tissuesSee more

Orthologs human all

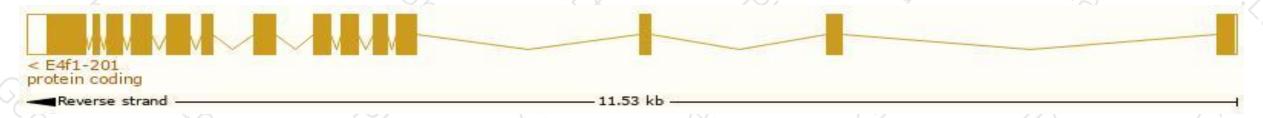
## Transcript information (Ensembl)



The gene has 8 transcripts, all transcripts are shown below:

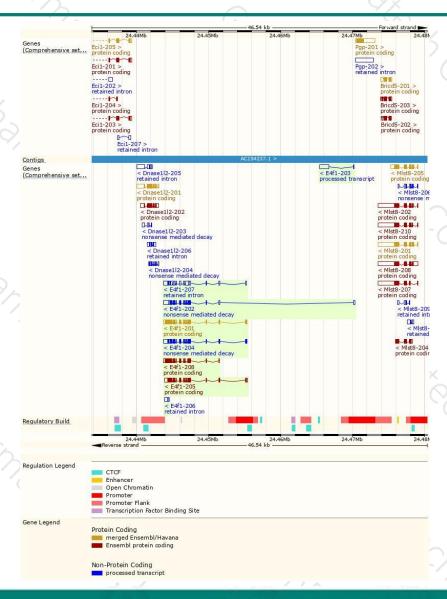
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
E4f1-201	ENSMUST00000056032.8	2574	782aa	Protein coding	CCDS28481	Q8CCE9	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P2
E4f1-205	ENSMUST00000226941.1	2642	684aa	Protein coding	-	Q8CCE9	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 ALT2
E4f1-208	ENSMUST00000228882.1	2226	<u>675aa</u>	Protein coding	21	A0A2I3BR50	CDS 5' incomplete
E4f1-202	ENSMUST00000226654.1	2674	427aa	Nonsense mediated decay	25	A0A2I3BRE9	
E4f1-204	ENSMUST00000226754.1	2552	586aa	Nonsense mediated decay	5.0	A0A2I3BPE3	
E4f1-203	ENSMUST00000226743.1	1377	No protein	Processed transcript			
E4f1-207	ENSMUST00000227293.1	2708	No protein	Retained intron	21	-	
E4f1-206	ENSMUST00000227241.1	556	No protein	Retained intron	24	82	

The strategy is based on the design of E4f1-201 transcript, the transcription is shown below:



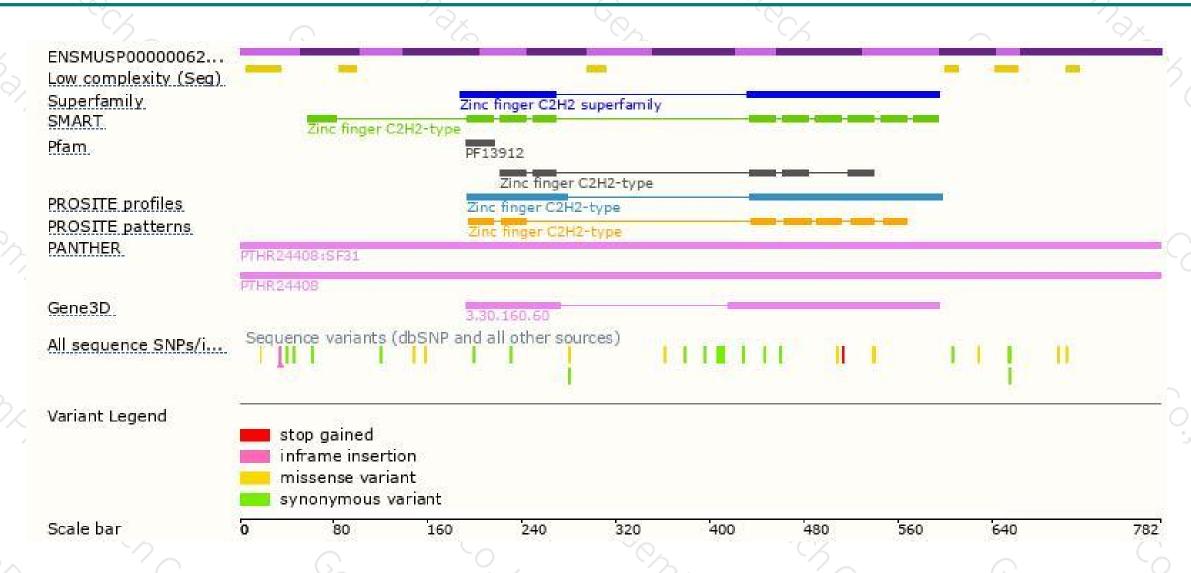
### Genomic location distribution





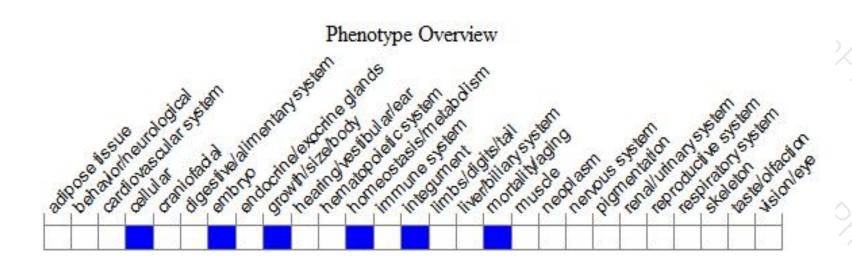
### Protein domain





## 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 display early embryonic lethality with mitotic progression failure and increased apoptosis.



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





