Ebf4 Cas9-CKO Strategy

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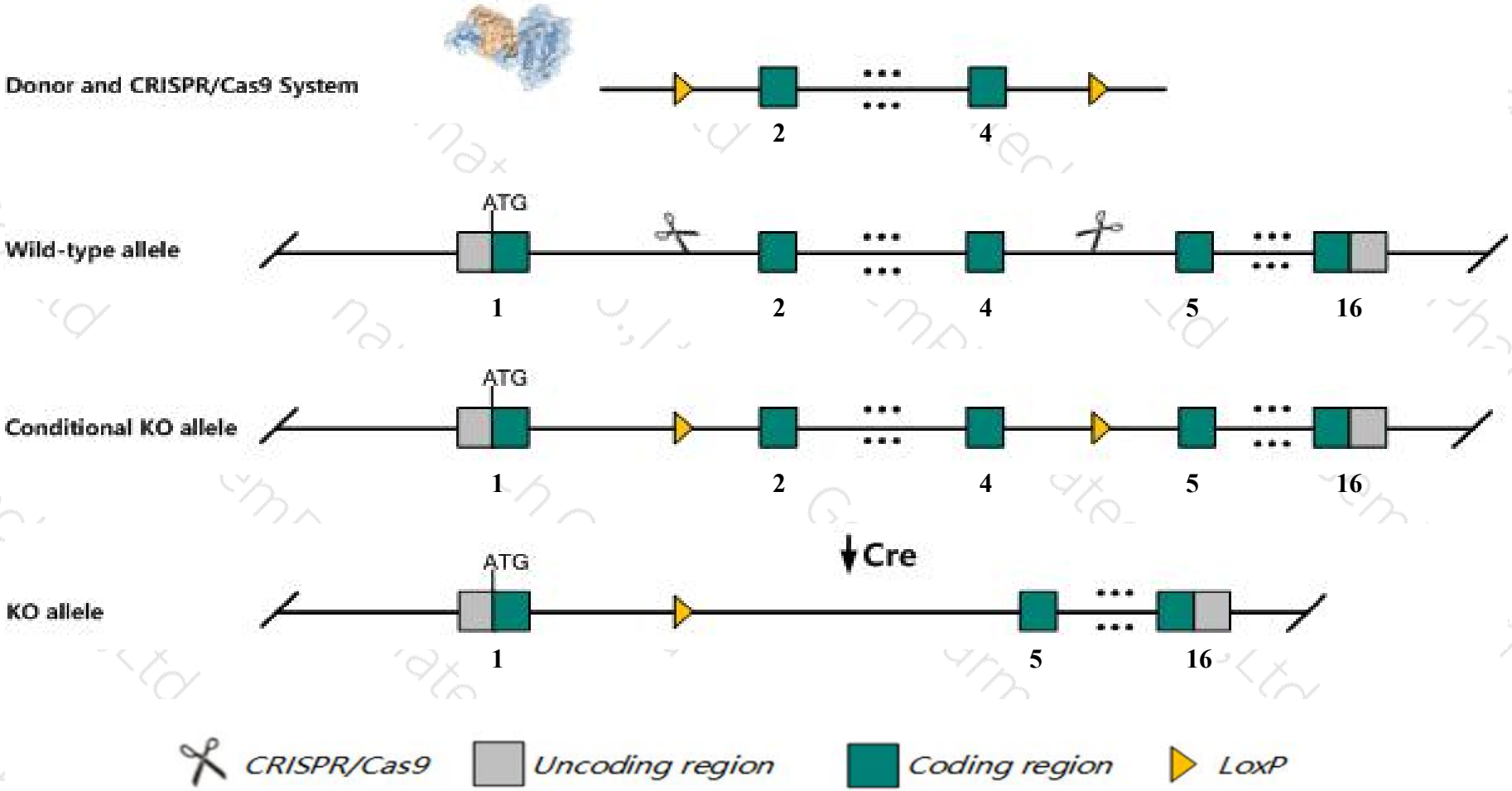
Project Overview

Project Name	***Ebf4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ebf4* gene. The schematic diagram is as follows:



Technical routes

- The *Ebf4* gene has 6 transcripts. According to the structure of *Ebf4* gene, exon2-exon4 of *Ebf4-201* (ENSMUST00000110286.7) transcript is recommended as the knockout region.The region contains 277bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ebf4* 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

➢ The *Ebf4* gene is located on the Chr2. 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）

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Ebf4 early B cell factor 4 [Mus musculus (house mouse)]

Gene ID: 228598, updated on 13-Mar-2020

Summary

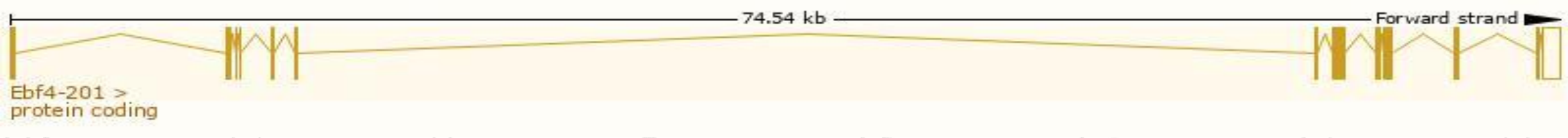
Official Symbol	Ebf4 provided by MGI
Official Full Name	early B cell factor 4 provided by MGI
Primary source	MGI:MGI:2385972
See related	Ensembl:ENSMUSG00000053552
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Ebf3, O/E-4, Olf-1
Expression	Broad expression in ovary adult (RPKM 14.4), limb E14.5 (RPKM 10.1) and 18 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

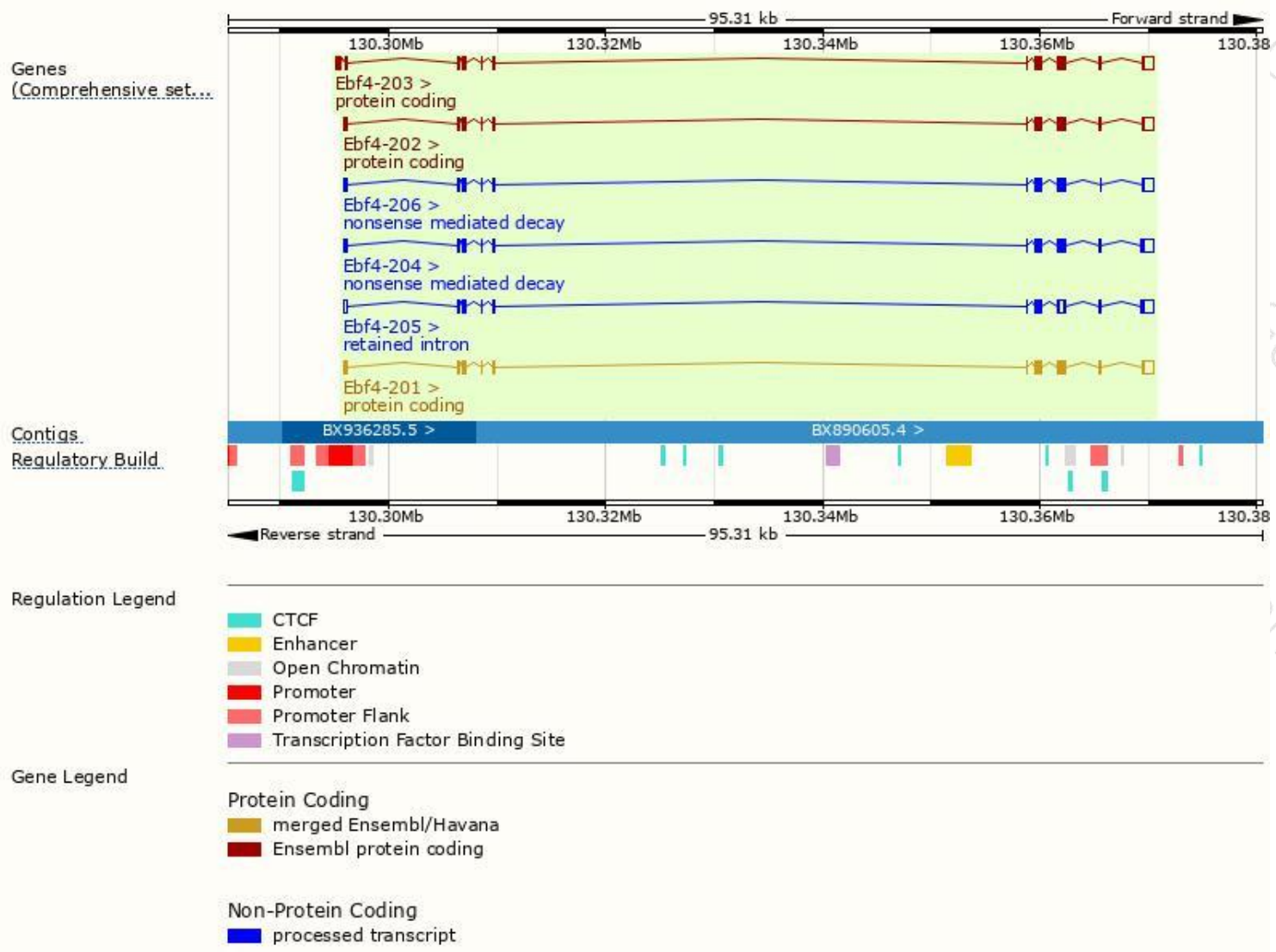
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ebf4-201	ENSMUST00000110286.7	2670	599aa	Protein coding	CCDS50709	B2RQX3 Q8K4J2	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 P1
Ebf4-203	ENSMUST00000110288.8	2982	696aa	Protein coding	-	A2BI80	TSL:5 GENCODE basic
Ebf4-202	ENSMUST00000110287.1	2580	569aa	Protein coding	-	A2BI84	TSL:5 GENCODE basic
Ebf4-204	ENSMUST00000126740.7	2740	582aa	Nonsense mediated decay	-	Q8K4J2	TSL:2
Ebf4-206	ENSMUST00000140169.7	2554	541aa	Nonsense mediated decay	-	Q8K4J2	TSL:2
Ebf4-205	ENSMUST00000134728.7	2835	No protein	Retained intron	-	-	TSL:2

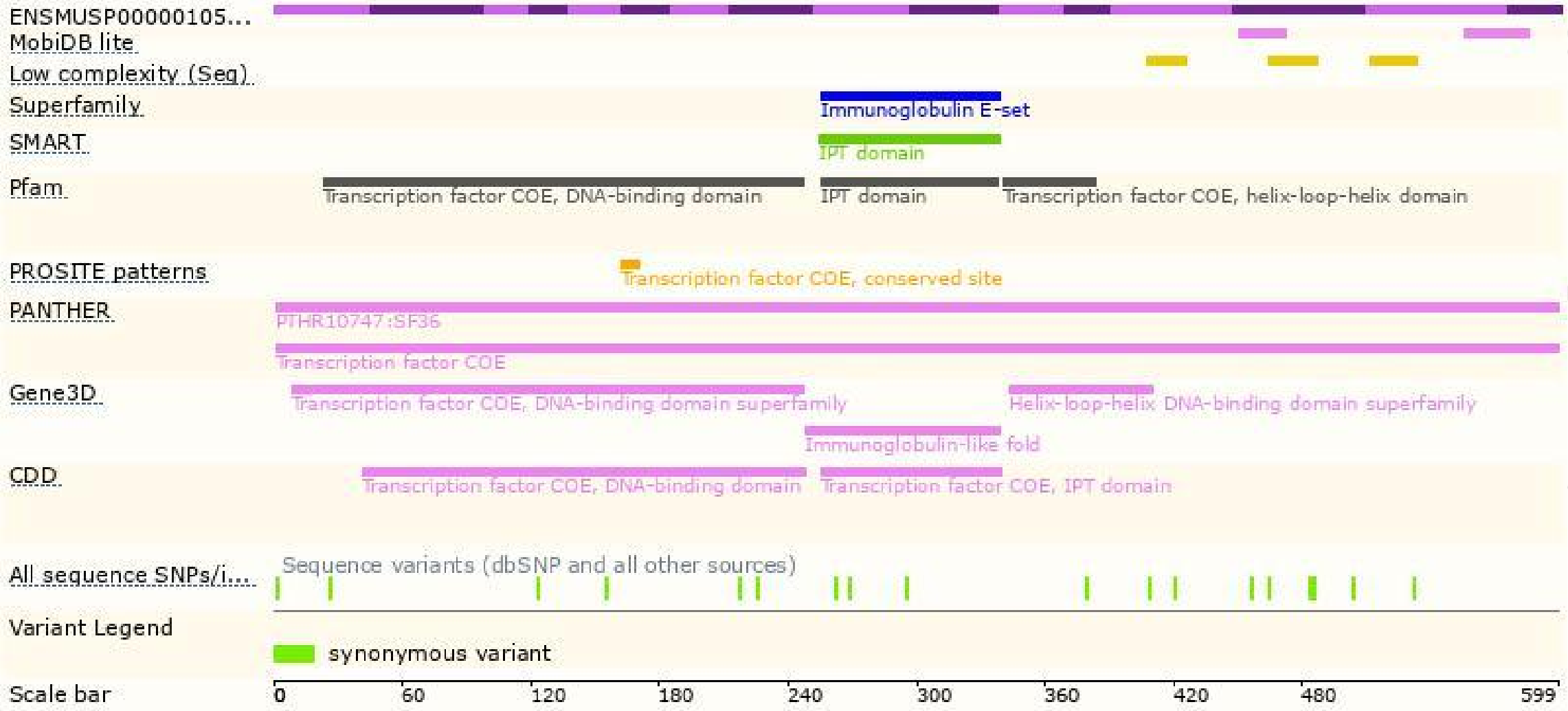
The strategy is based on the design of *Ebf4-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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