Amh-P2A-iCre Cas9-KI Strategy

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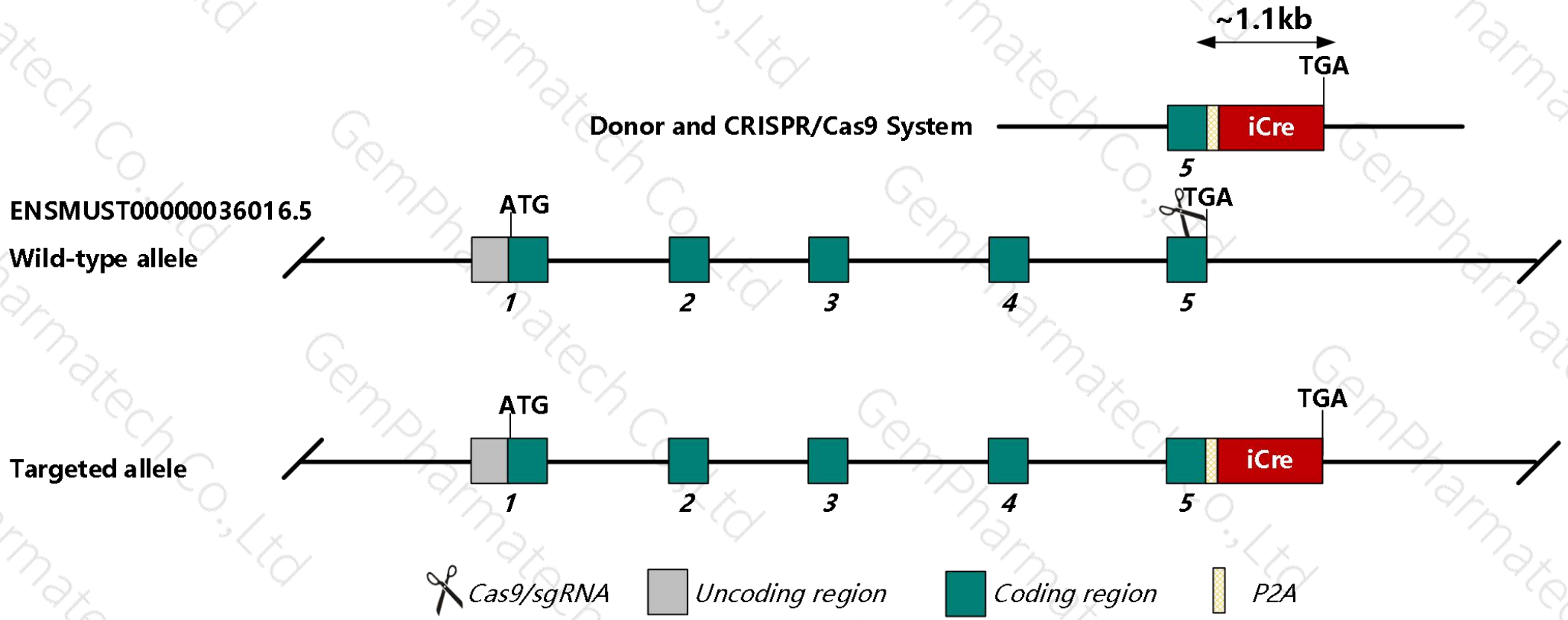
Reviewer:	**Jia Yu**
Design Date:	**2019-08-09**

Project Overview

Project Name	***Amh-P2A-iCre***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

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



Technical routes

- The *Amh* gene has 1 transcript.According to the structure of *Amh* gene, *Amh-201*(ENSMUST00000036016.5) is selected for presentation of the recommended strategy.

- *Amh-201* gene has 5 exons, with the ATG start codon in exon1 and TGA stop codon in exon5.

- We make *Amh-P2A-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near stop coding(TGA) of *Amh* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in *P2A-iCre* before stop coding(TGA) of *Amh* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, homozygous null mutant males have a complete male reproductive tract and functional sperm, but also uterus and oviducts. Most are infertile due to female organs blocking sperm transfer. Females are fertile with enlarged ovaries and atypical follicles.
- According to the existing JAX data, Cre is expressed specifically in testis Sertoli cells.
- Insertion of iCre may affect the regulation of the 3' end of the *Amh* gene.
- There will be 1 to 2 amino acid synonymous mutation in exon5 of *Amh* gene in this strategy.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- The insertion site is about 0.8 kb from the C-terminus of the *Jsrp1* gene, which may affect the regulation of the C-terminus of *Jsrp1* gene.
- The *Amh* gene is located on the Chr10. If the knockin 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

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Amh anti-Mullerian hormone [*Mus musculus* (house mouse)]

Gene ID: 11705, updated on 30-Jul-2019

Summary

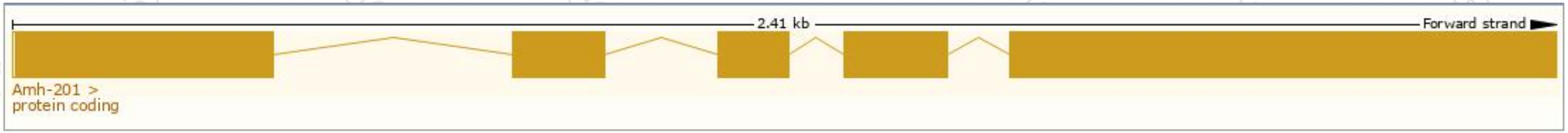
Official Symbol	Amh provided by MGI
Official Full Name	anti-Mullerian hormone provided by MGI
Primary source	MGI:MGI:88006
See related	Ensembl:ENSMUSG00000035262
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	MIS
Summary	This gene encodes a secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Ligands of this family bind various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression. The encoded preproprotein is proteolytically processed to generate N- and C-terminal cleavage products that homodimerize and associate to form a biologically active noncovalent complex. This complex binds to the anti-Mullerian hormone receptor type 2 and causes the regression of Mullerian ducts in the male embryo that would otherwise differentiate into the uterus and fallopian tubes. This protein also plays a role in Leydig cell differentiation and function and follicular development in adult females. Homozygous knockout male mice develop female reproductive organs and are often sterile, while homozygous knockout female mice exhibit premature depletion of primordial follicles. [provided by RefSeq, Jul 2016]
Expression	Biased expression in ovary adult (RPKM 123.7) and stomach adult (RPKM 9.9) See more
Orthologs	human all

Transcript information （Ensembl）

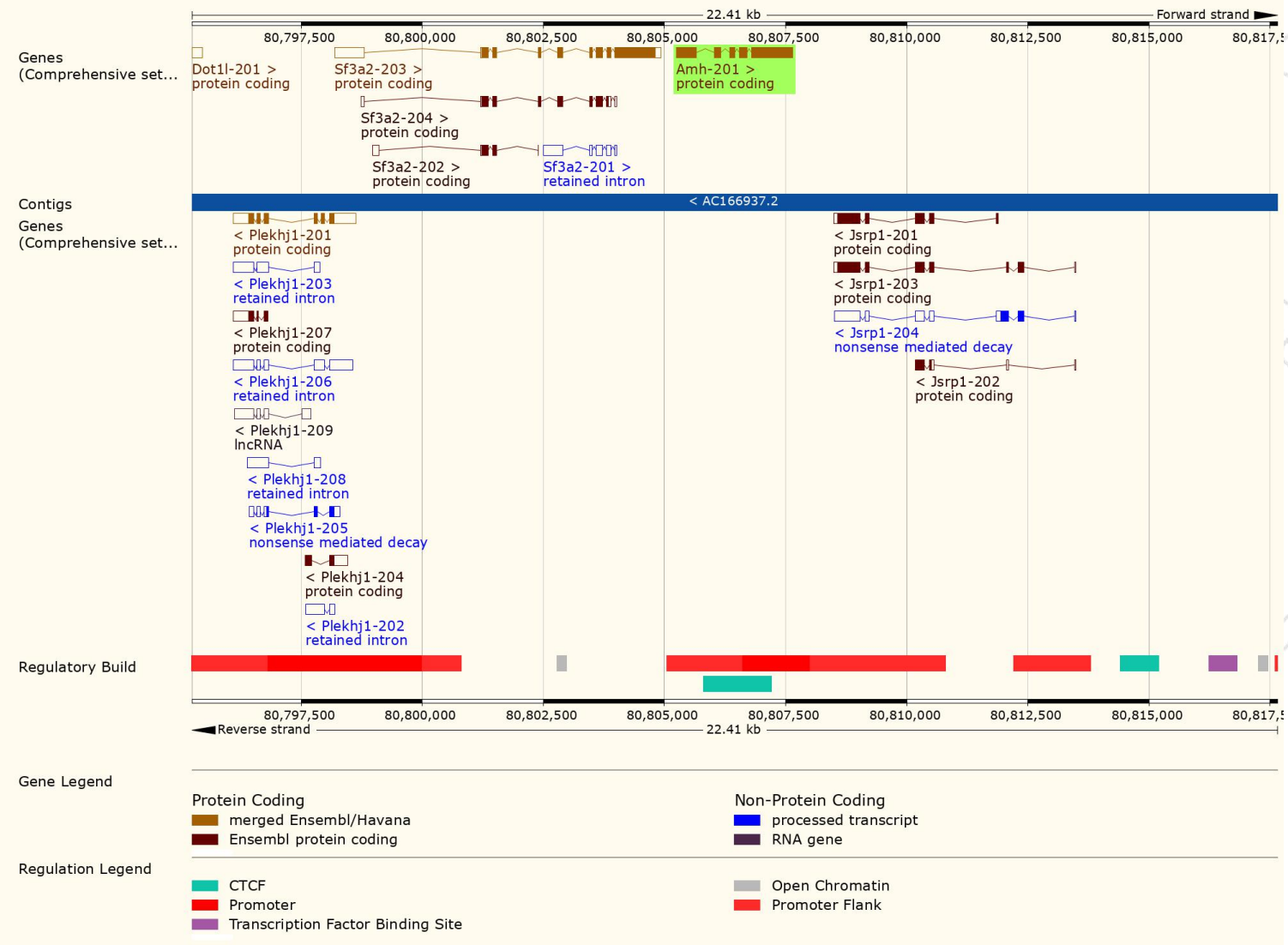
The gene has 1 transcript, and the transcript is shown below：

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Amh-201	ENSMUST00000036016.5	1670	554aa	Protein coding	CCDS24033	Q5EC55	TSL:1 GENCODE basic APPRIS P1

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



Genomic location distribution



Protein domain

Targeted Progress (from JAX)

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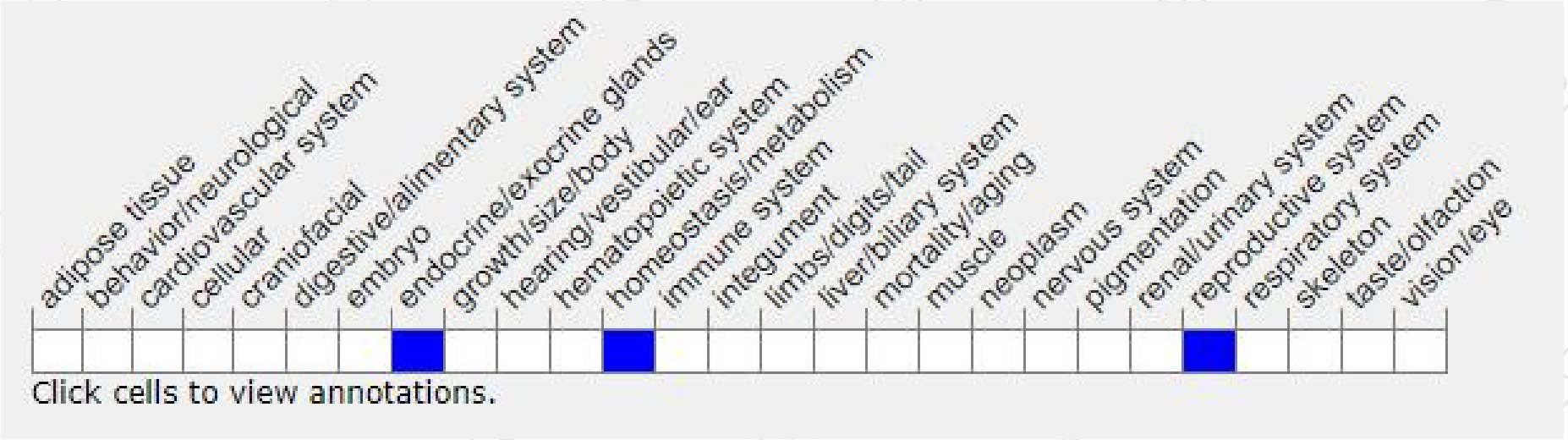
Allele Symbol: Tg(Amh-cre)8815Reb MGI

Allele Name	transgene insertion 8815, Robert E Braun
Allele Type	Transgenic (Recombinase-expressing)
Allele Synonym(s)	
Gene Symbol and Name	Tg(Amh-cre)8815Reb MGI, transgene insertion 8815, Robert E Braun
Gene Synonym(s)	
Promoter	*Amh*, anti-Mullerian hormone, mouse, laboratory
Expressed Gene	*cre*, cre recombinase, bacteriophage P1
Site of Expression	Cre is expressed specifically in testis Sertoli cells.
Strain of Origin	FVB/N
Chromosome	UN
Molecular Note	The promoter region of the Amh gene was fused, at the AUG start codon of exon 1, to a cre cDNA. Intron 1 of the Amh gene was left fused to the 3' end of the cre sequence.
Mutations Made By	Robert Braun, The Jackson Laboratory

https://www.jax.org/strain/007915

Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/) .

Homozygous null mutant males have a complete male reproductive tract and functional sperm, but also uterus and oviducts. Most are infertile due to female organs blocking sperm transfer. Females are fertile with enlarged ovaries and atypical follicles.

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

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