

Amhr2 Cas9-KO Strategy

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Reviewer: Jiaojiao Yan

Design Date: 2023-6-15

Overview

Target Gene Name

• Amhr2

Project Type

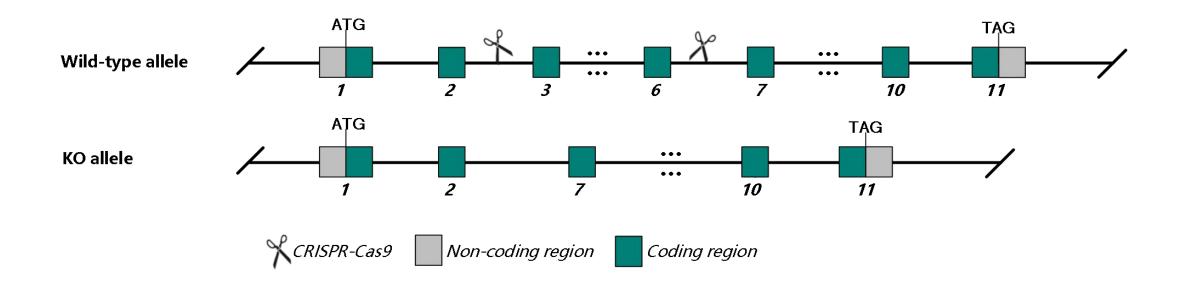
• Cas9-KO

Genetic Background

• C57BL/6JGpt



Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Amhr2 gene.

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Technical Information

- The *Amhr2* gene has 5 transcripts. According to the structure of *Amhr2* gene, exon 3-6 of *Amhr2*-201 (ENSMUST0000023809.11) is recommended as the knockout region. The region contains 608 bp of coding sequence. Knockout the region will result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Amhr2* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.



Gene Information

Amhr2 anti-Mullerian hormone type 2 receptor [Mus musculus (house mouse)]

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Gene ID: 110542, updated on 31-May-2023

Summary

Official Symbol	Amhr2 provided by MGI
Official Full Name	anti-Mullerian hormone type 2 receptor provided by MGI
Primary source	<u>MGI:MGI:105062</u>
See related	Ensembl:ENSMUSG0000023047 AllianceGenome:MGI:105062
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	C14; Mrii; Misiir; Misrii
Summary	Enables anti-Mullerian hormone receptor activity. Acts upstream of or within anti-Mullerian hormone signaling pathway; negative regulation of anti-Mullerian
	hormone signaling pathway; and sex differentiation. Predicted to be integral component of plasma membrane. Predicted to be part of activin receptor
	complex. Predicted to be active in plasma membrane. Is expressed in several structures, including early embryo; forebrain; genitourinary system; and nose.
	Used to study persistent Mullerian duct syndrome. Human ortholog(s) of this gene implicated in persistent Mullerian duct syndrome. Orthologous to human
	AMHR2 (anti-Mullerian hormone receptor type 2). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Biased expression in testis adult (RPKM 55.6) and ovary adult (RPKM 49.5) See more
Orthologs	human all
NEW	Try the new Gene table
	Try the new Transcript table
Genomic context	(*)
cation: 15 F3; 15 57	58 cM See Amhr2 in Genome Data Viewer
on count: 12	
on count. 12	

https://www.ncbi.nlm.nih.gov/gene/110542



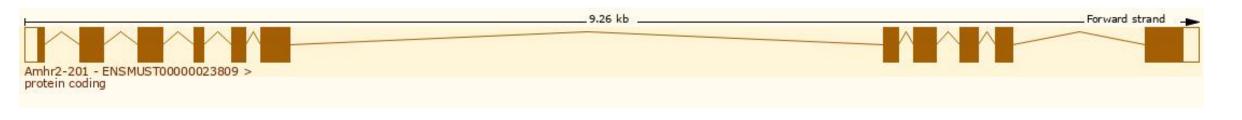
Transcript Information

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

Show/hide columns (1 hid	Filter									
Transcript ID 💧	Name 🔺	bp 🛊	Protein 🖕	Biotype 🖕	CCDS 🖕	UniProt Match	Flags			¢
ENSMUST0000023809.11	Amhr2-201	1938	<u>568aa</u>	Protein coding	<u>CCDS27882</u>	<u>Q8K592</u> &	Ensembl Canonical	GENCODE basic	APPRIS P1	TSL:1
ENSMUST00000161376.2	Amhr2-202	364	No protein	Retained intron		-	TSL:2			
ENSMUST00000162893.2	Amhr2-203	611	<u>190aa</u>	Protein coding		<u>F6V9X7</u> &	TSL:5 CDS 5' incomplete			
ENSMUST00000229278.2	Amhr2-204	1735	<u>448aa</u>	Protein coding		<u>A0A2R8W6H9</u> &	GENCODE basic			
ENSMUST00000229566.2	Amhr2-205	209	<u>61aa</u>	Protein coding		A0A2R8VHE2	CDS 3' incomplete			

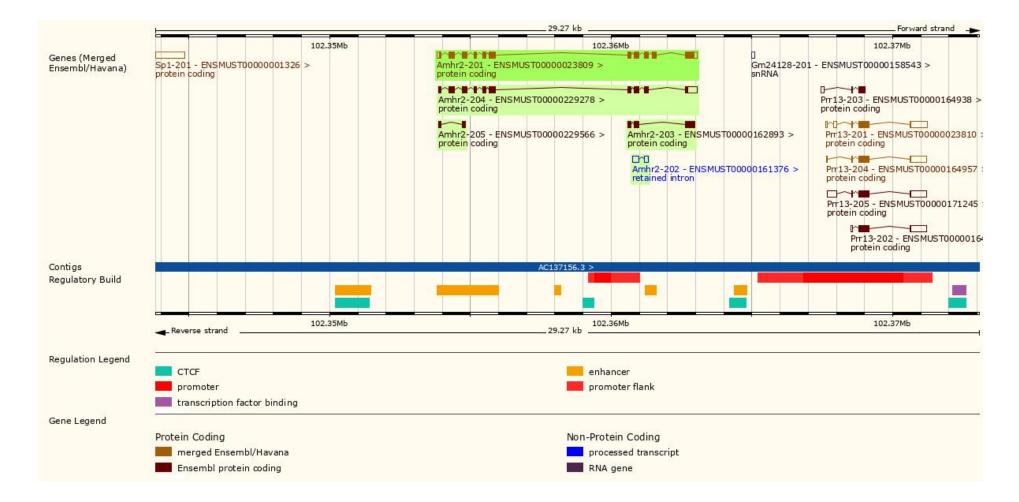
The strategy is based on the design of *Amhr2*-201 transcript, the transcription is shown below:



Source: http://asia.ensembl.org/

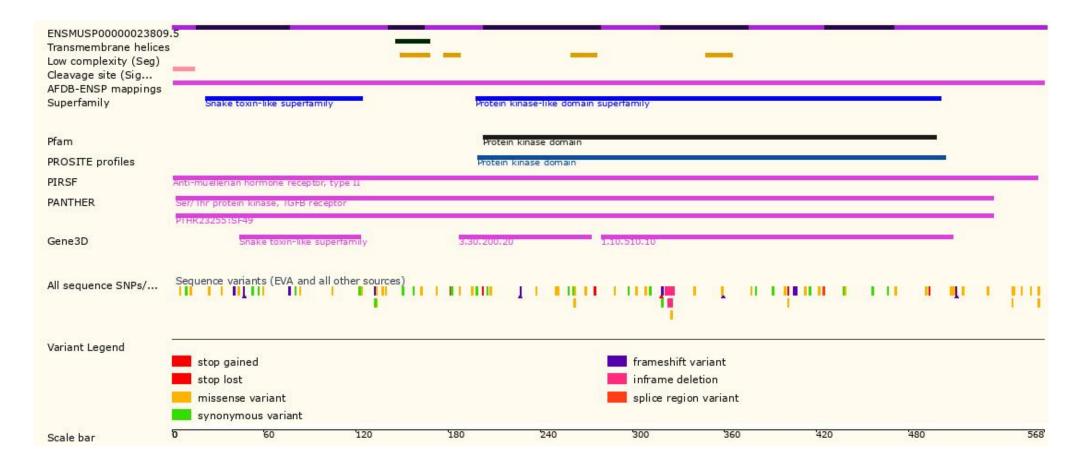
Genomic Information

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Source:http://asia.ensembl.org/

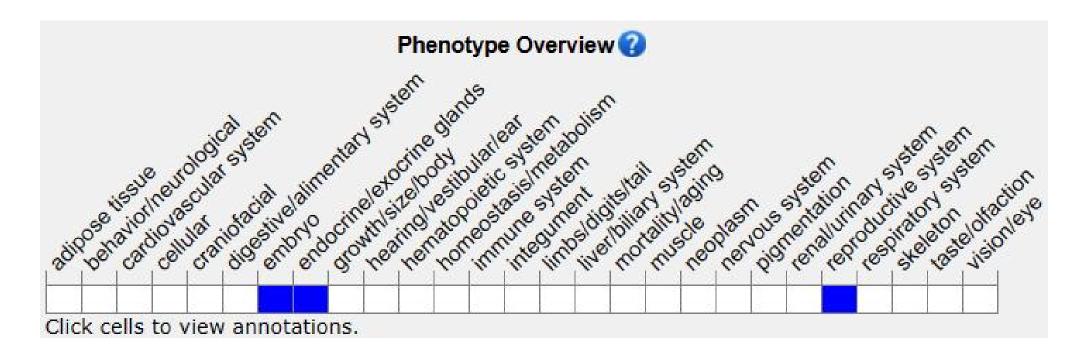
Protein Information



Source: : https://www.ensembl.org

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Mouse Phenotype Information (MGI)



Homozygous null mutant males have a complete male reproductive tract, but also a uterus and oviducts. Functional sperm are produced, but most males are infertile because female reproductive organs block sperm transfer.

Source: https://www.informatics.jax.org

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Important Information

- According to the existing MGI data, homozygous null mutant males have a complete male reproductive tract, but also a uterus and oviducts. Functional sperm are produced, but most males are infertile because female reproductive organs block sperm transfer.
- This stratergy may not affect *Amhr2*-202 and *Amhr2*-203 transcript.
- *Amhr2* is located on Chr 15. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

