

# Hecw1 Cas9-CKO Strategy

Designer: Xingkai Xiao

Reviewer: Xiangli Bian

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#### Overview

#### **Target Gene Name**

• Hecw1

#### **Project Type**

• Cas9-CKO

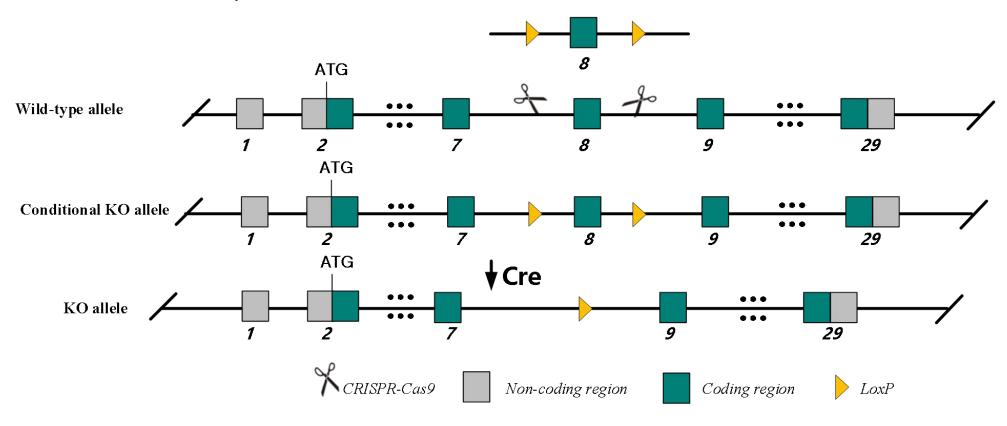
#### Genetic Background

• C57BL/6JGpt

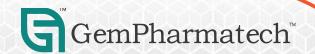


# Strain Strategy

Donor and CRISPR-Cas9 System



Schematic representation of CRISPR-Cas9 engineering used to edit the *Hecw1* gene.



#### **Technical Information**

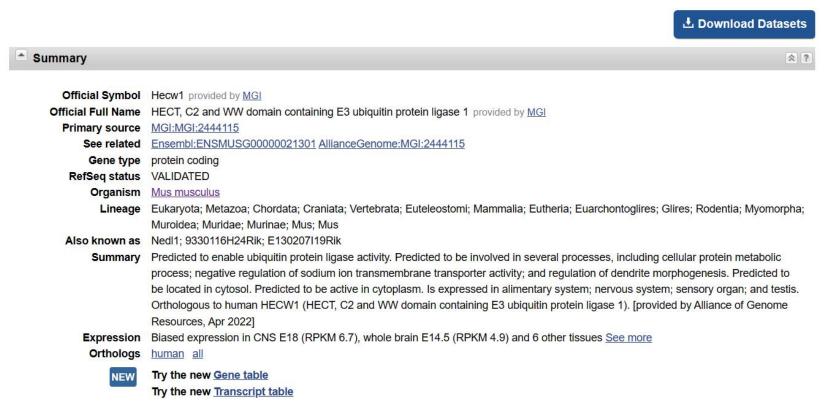
- The *Hecw1* gene has 10 transcripts. According to the structure of *Hecw1* gene, exon8 of *Hecw1*-201 (ENSMUST00000110516.3) transcript is recommended as the knockout region. The region contains 143 bp of coding sequences. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Hecw1* 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 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.
- 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.



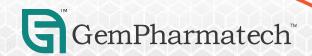
#### Gene Information

Hecw1 HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1 [ Mus musculus (house mouse) ]

Gene ID: 94253, updated on 28-Nov-2023



Source: https://www.ncbi.nlm.nih.gov/

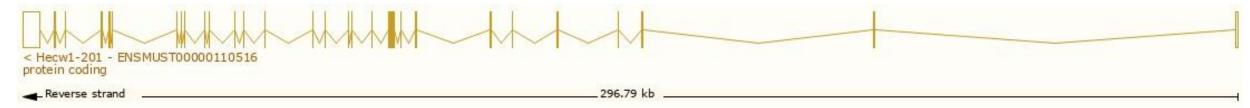


## Transcript Information

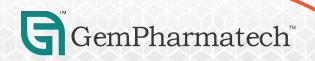
The gene has 10 transcripts, all transcripts are shown below:

Transcript ID		Name	bp 🌲	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST0000011051	6.3	Hecw1-201	9460	1604aa	Protein coding	CCDS49207₺	Q8K4P8-1₺	Ensembl Canonical GENCODE basic APPRIS P1 TSL:
ENSMUST0000022071	8.2	Hecw1-202	6124	1177aa	Protein coding		A0A1Y7VJ05醛	TSL:1 CDS 5' incomplete
ENSMUST0000022127	4.2	Hecw1-203	404	<u>74aa</u>	Protein coding		A0A1Y7VJB7@	TSL:3   CDS 3' incomplete
ENSMUST0000022186	3.2	Hecw1-204	3372	No protein	Retained intron		2	TSL:1
ENSMUST0000022224	1.2	Hecw1-205	3696	No protein	Retained intron		2	TSL:1
ENSMUST0000022290	4.2	Hecw1-206	1357	No protein	Retained intron		7	TSL:1
ENSMUST0000022318	9.2	Hecw1-207	650	132aa	Protein coding		A0A1Y7VJ41@	TSL:3   CDS 3' incomplete
ENSMUST0000022328	3.2	Hecw1-208	2554	No protein	Retained intron		a	TSL:1
ENSMUST0000022331	7.2	Hecw1-209	1864	No protein	Retained intron		=	TSL:1
ENSMUST0000022355	0.2	Hecw1-210	523	23aa	Protein coding		A0A1Y7VMF2년	TSL:2 CDS 3' incomplete

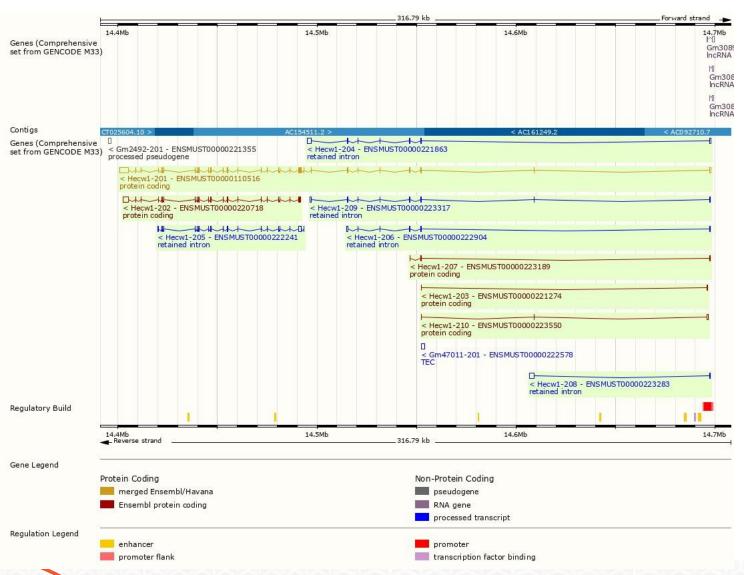
The strategy is based on the design of *Hecw1*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org



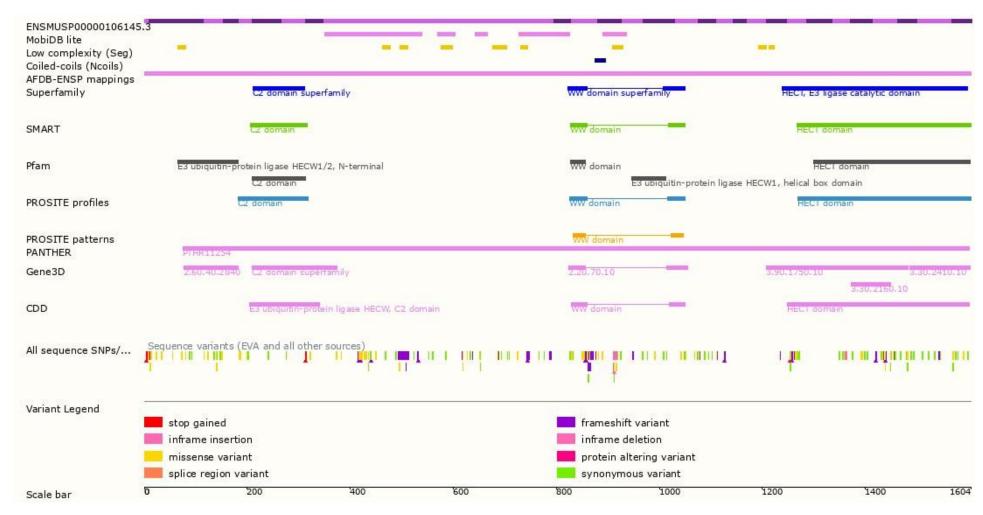
#### Genomic Information





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

#### **Protein Information**





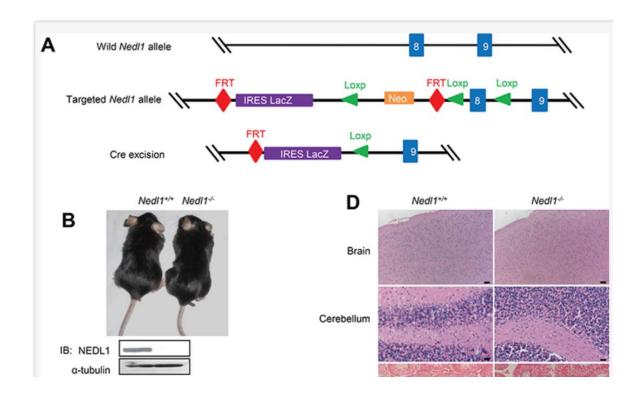
Source: : https://www.ensembl.org

### Important Information

- There will be 267 amino acids left at the N-terminus of *Hecw1*, the risk is unknown.
- The 5' of *Hecw1*-202 and 3' of *Hecw1*-203, 207 and 210 is incomplete, the effect is unknown. The knockout region do not affect the transcript *Hecw1*-205,206 and 208, the risk is unknown.
- *Hecw1* is located on Chr13. 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 risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.



#### Reference



• NEDL2 regulates enteric nervous system and kidney development in its Nedd8 ligase activity-dependent manner.2016; 7:31440-31453. https://doi.org/10.18632/oncotarget.8951

