

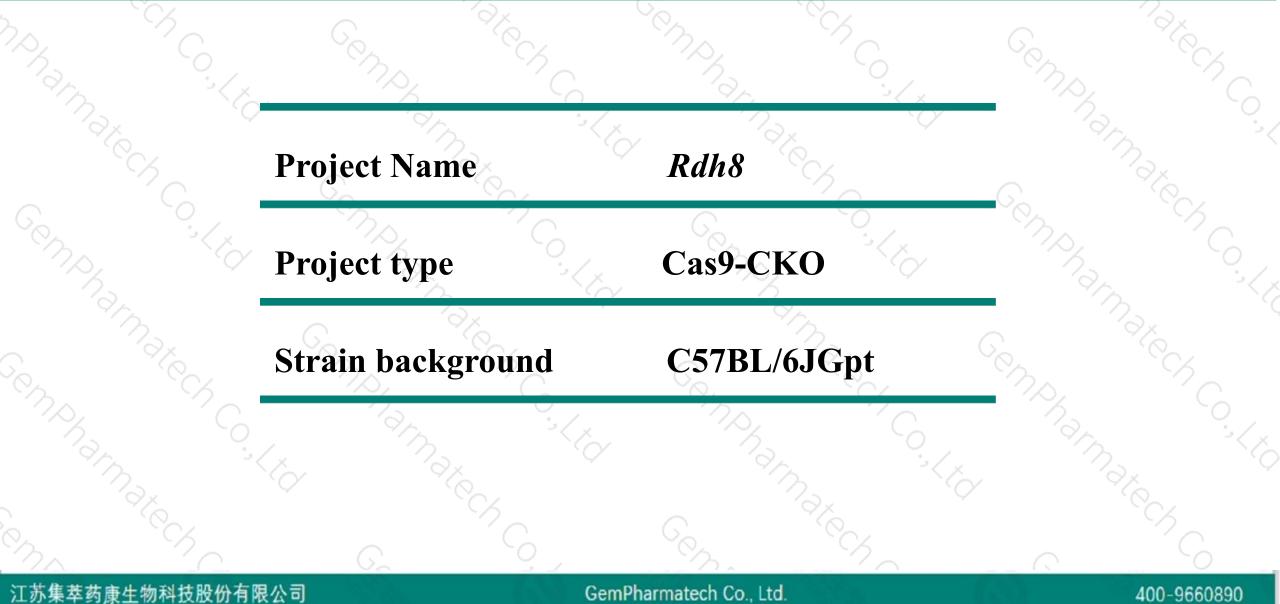
Rdh8 Cas9-CKO Strategy

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Designer:Xueting Zhang Reviewer:Yanhua Shen Date:2020-1-19

Project Overview

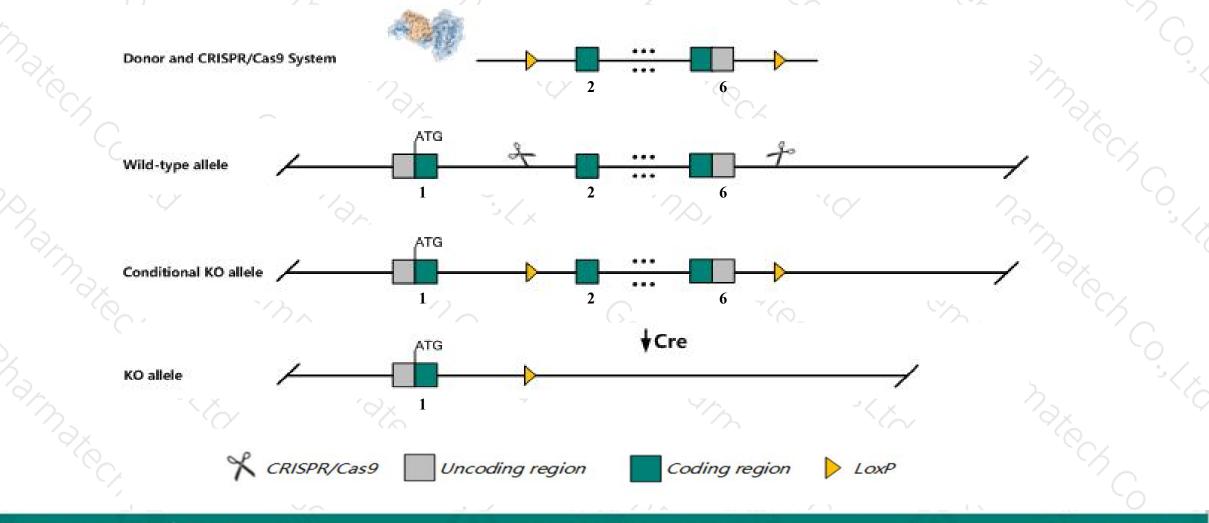




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Rdh8* gene. The schematic diagram is as follows:



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- The *Rdh8* gene has 1 transcript. According to the structure of *Rdh8* gene, exon2-exon6 of *Rdh8-201* (ENSMUST0000066387.5) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rdh8* 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.

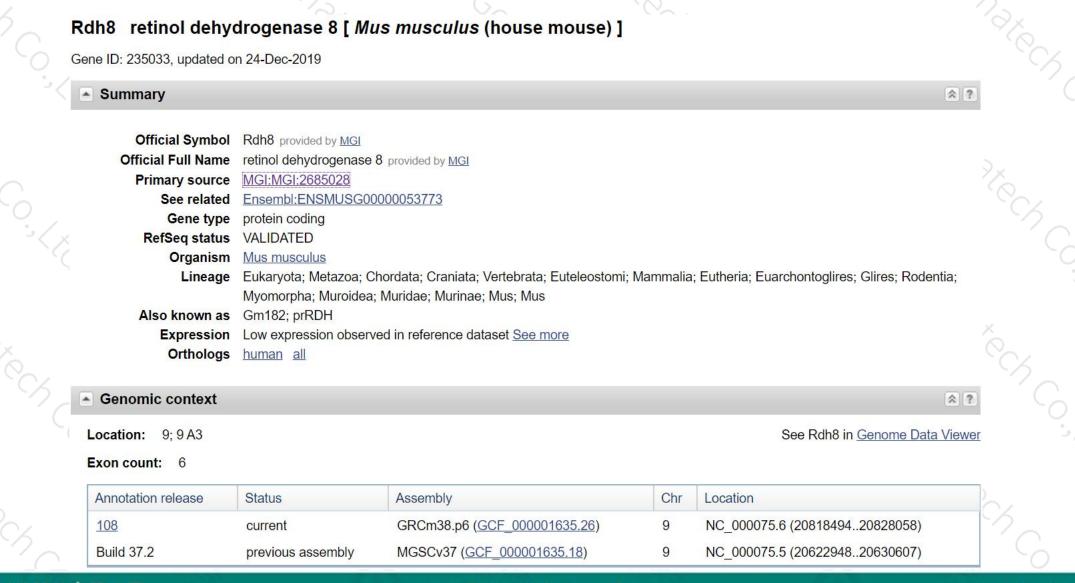


> According to the existing MGI data, Homozygous null mice are viable and fertile but display delayed dark adaptation.

- ➤The floxed region is near to the N-terminal of Col5a3 gene, this strategy may influence the regulatory function of the N-terminal of Col5a3 gene.
- The *Rdh8* gene is located on the Chr9. 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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Transcript information (Ensembl)



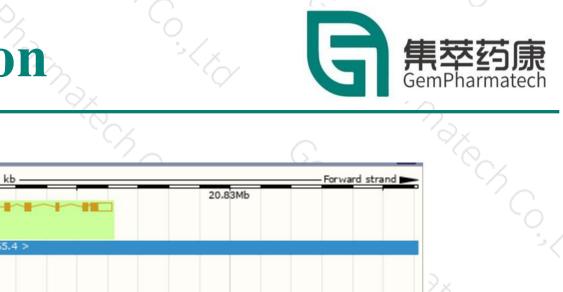
The gene has 1 transcript, and the transcript is shown below:

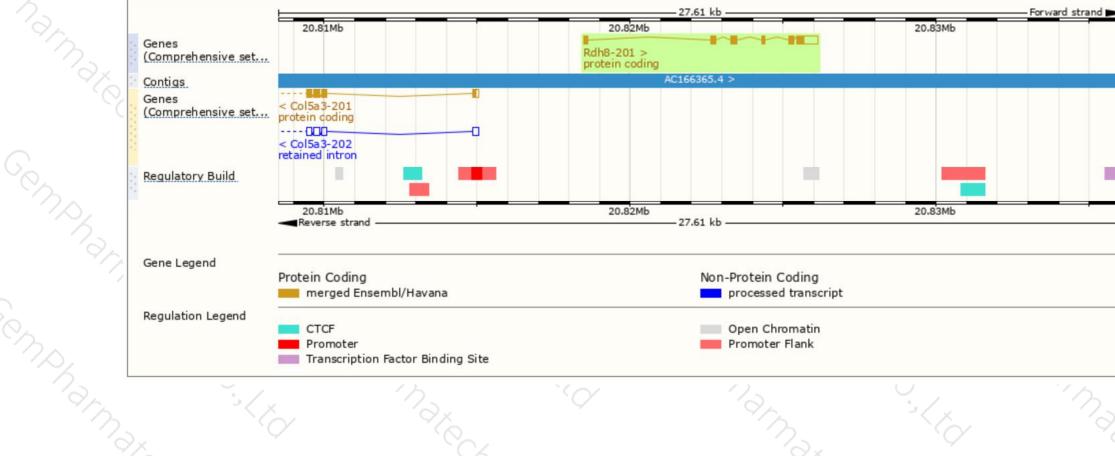
Name 💧	Transcript ID 💧	bp 💧	Protein 🖕	Biotype 🖕	CCDS 🍦	UniProt 🖕		Flags	\$
Rdh8-201	ENSMUST0000066387.5	1405	<u>317aa</u>	Protein coding	<u>CCDS22884</u> &	<u>D3Z6W3</u> &	TSL:2	GENCODE basic	APPRIS P1

The strategy is based on the design of *Rdh8-201* transcript, The transcription is shown below



Genomic location distribution





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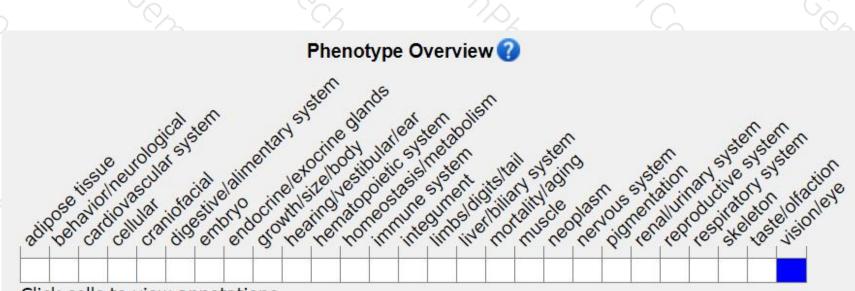
Protein domain



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Mouse phenotype description(MGI)





Click cells to view annotations.

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 are viable and fertile but display delayed dark adaptation.



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



