GemPharmatech™

Plekhg2 Cas9-CKO Strategy

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Design Date: 2024-3-19

Overview

Target Gene Name

- Plekhg2

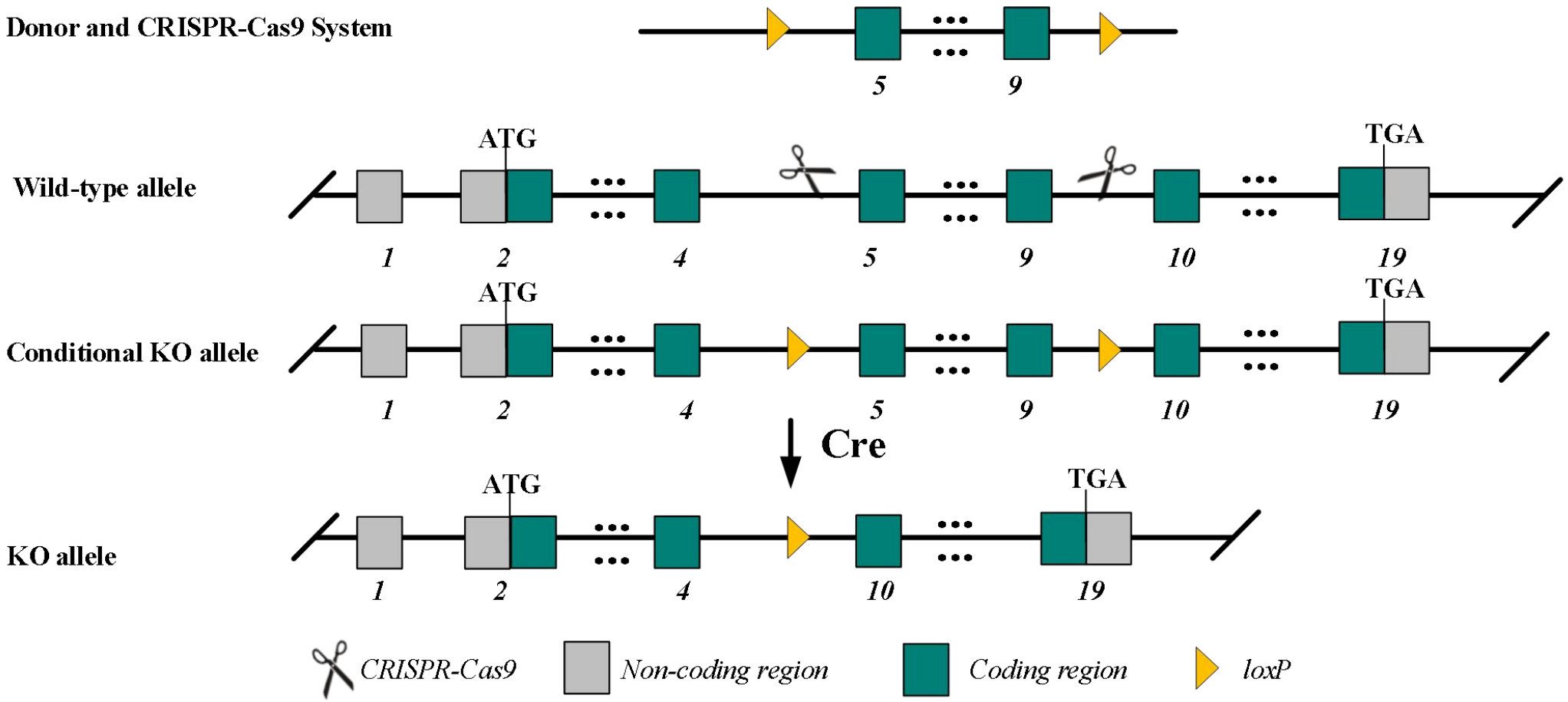
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Plekhg2* gene.

Technical Information

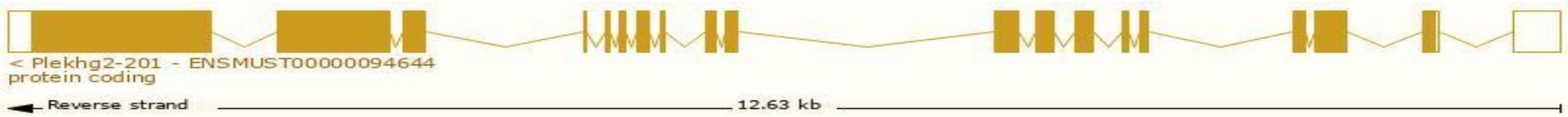
- The *Plekhg2* gene has 10 transcripts. According to the structure of *Plekhg2* gene, exon 5-exon 9 of *Plekhg2*-201 (ENSMUST00000094644.11) transcript is recommended as the knockout region. The region contains 604 bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Plekhg2* 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.

Transcript Information

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

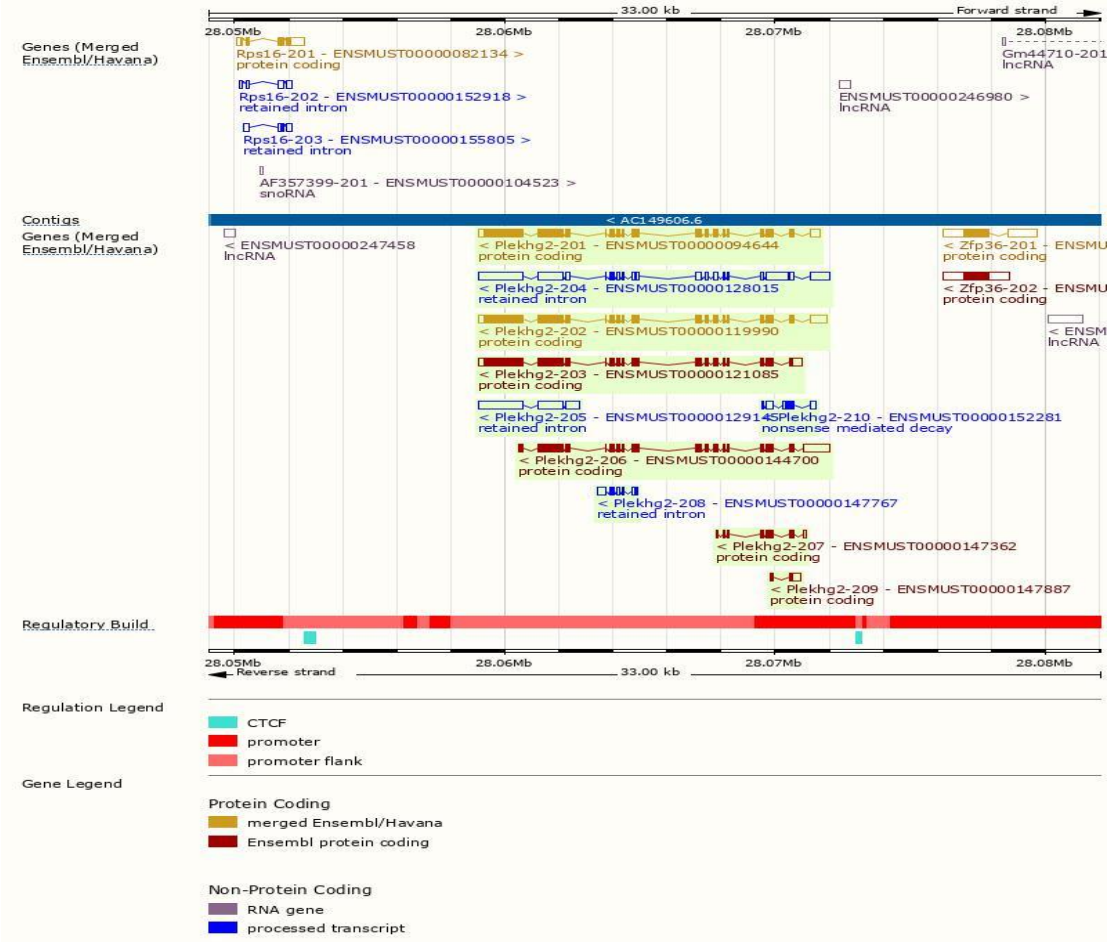
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000121085.8	Plekhg2-203	4649	1365aa	Protein coding		D3Z5N8	Ensembl Canonical GENCODE basic APPRIS ALT2 TSL:2
ENSMUST00000094644.11	Plekhg2-201	4623	1341aa	Protein coding	CCDS21040	E9QKB6	GENCODE basic APPRIS P4 TSL:1
ENSMUST00000119990.8	Plekhg2-202	4880	1340aa	Protein coding	CCDS52162	G5E8T4	GENCODE basic APPRIS ALT2 TSL:1
ENSMUST00000144700.8	Plekhg2-206	3721	912aa	Protein coding		A0A0R4J1S3	TSL:1 CDS 3' incomplete
ENSMUST00000147362.8	Plekhg2-207	729	205aa	Protein coding		D3YY99	TSL:3 CDS 3' incomplete
ENSMUST00000147887.2	Plekhg2-209	476	63aa	Protein coding		D3Z1B1	TSL:3 CDS 3' incomplete
ENSMUST00000152281.2	Plekhg2-210	858	56aa	Nonsense mediated decay		D6RFC7	TSL:5
ENSMUST00000128015.8	Plekhg2-204	5511	No protein	Retained intron		-	TSL:2
ENSMUST00000129145.8	Plekhg2-205	3074	No protein	Retained intron		-	TSL:2
ENSMUST00000147767.2	Plekhg2-208	661	No protein	Retained intron		-	TSL:3

The strategy is based on the design of *Plekhg2*-201 transcript, the transcription is shown below:



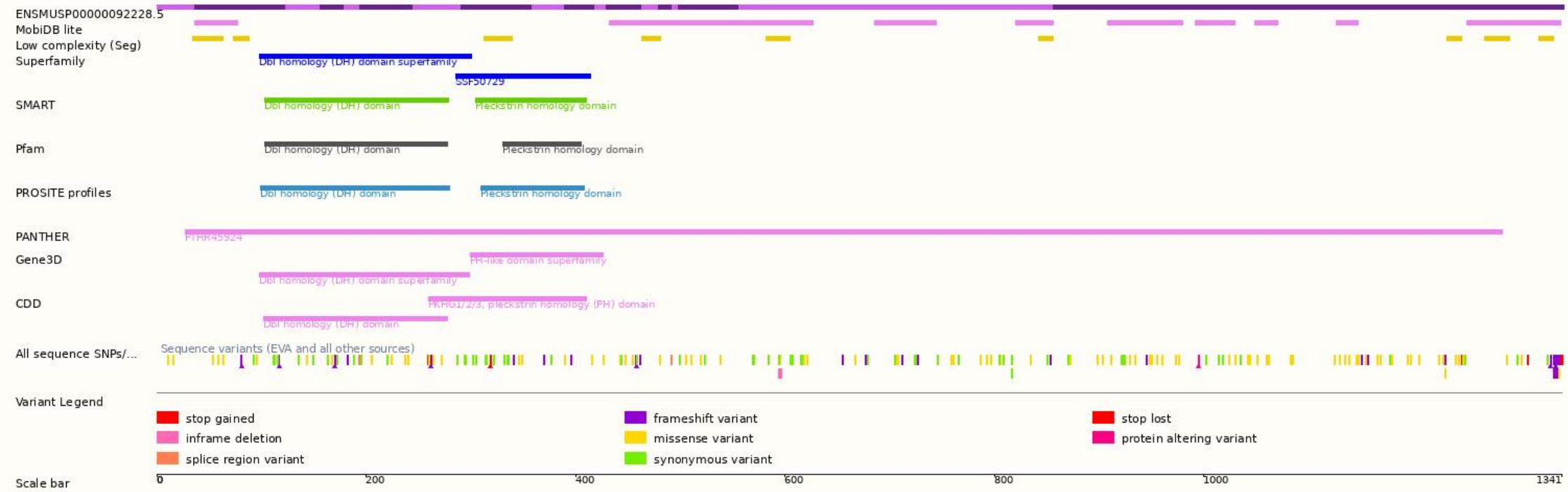
Source: https://www.ensembl.org

Genomic Information



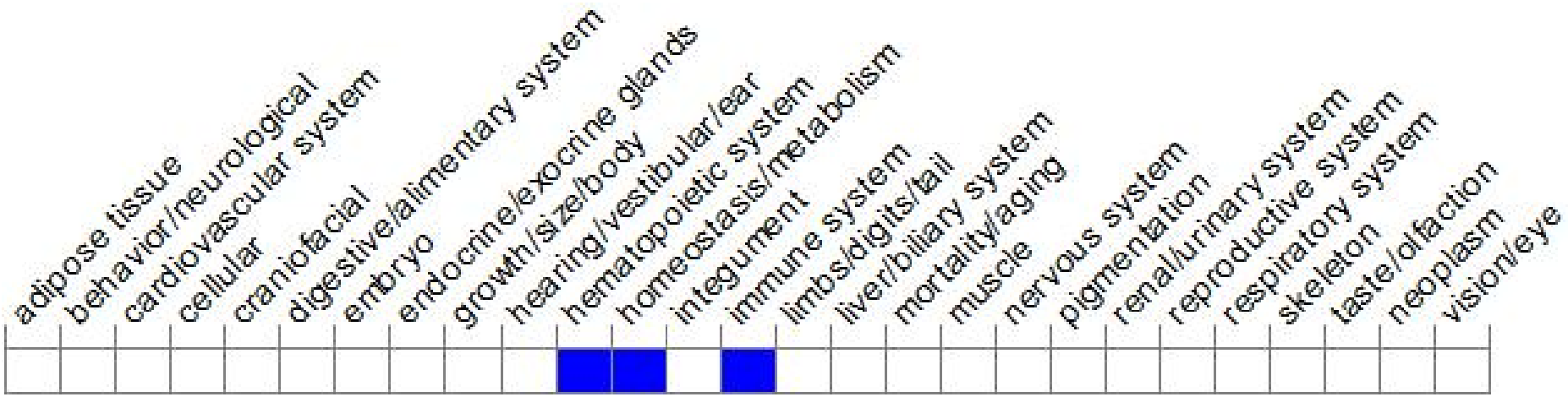
Source: : https://www.ensembl.org

Protein Information



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

Mouse Phenotype Information (MGI)



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

Important Information

- After cross cre, 156 amino acids remained at the N-terminus of this strategy, with unknown effects.
- This strategy may affect the 5-terminal regulatory function of *Gm57221* and *Gm44710*.
- The effect of this strategy on transcripts *Plekhg2*-207 and *Plekhg2*-209 is unknown.
- *Plekhg2* is located on Chr7. 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.