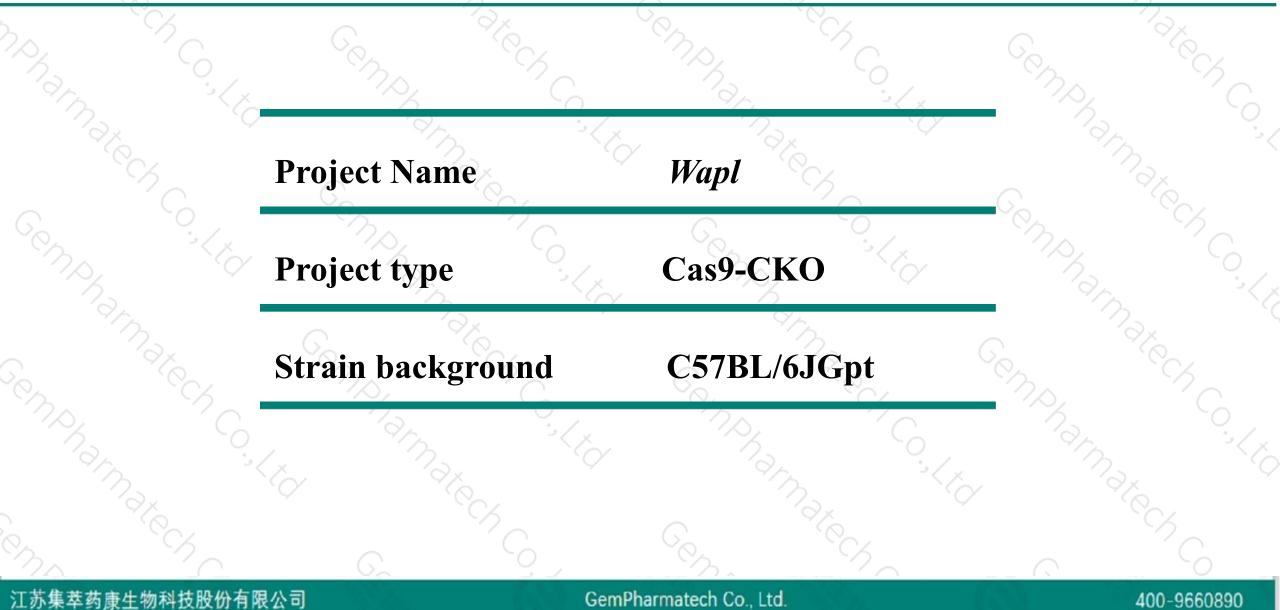


Wapl Cas9-CKO Strategy

Designer: Reviewer: Design Date: JiaYu Xiaojing Li 2019-8-28

Project Overview



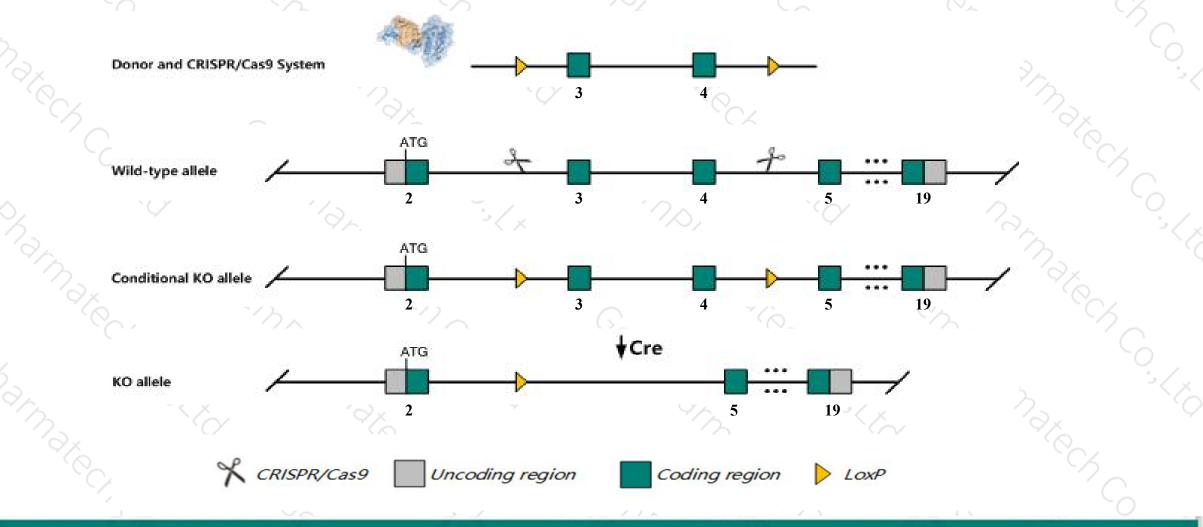


Conditional Knockout strategy



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



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The Wapl gene has 7 transcripts. According to the structure of Wapl gene, exon3-exon4 of Wapl-201 (ENSMUST00000048263.13) transcript is recommended as the knockout region. The region contains 1172bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Wapl* 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, Mice homozygous for a targeted allele exhibit prenatal lethality.
- The Wapl gene is located on the Chr14. 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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Wapl WAPL cohesin release factor [Mus musculus (house mouse)]

Gene ID: 218914, updated on 31-Jan-2019

Summary

- Official SymbolWap provided by MGIOfficial Full NameWAPL cohesin release factor provided by
MGIPrimary sourceMGI:MGI:2675859See relatedEnsembi:ENSMUSG0000041408Gene typeprotein codingRefSeq statusREVIEWEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Murinae; Mus; MusAlso known asA530089A20Rik, BC037674, DIF-2, FOE, WapalSummaryStudies suggest that the protein encoded by this gene is important for the release of cohesin from chromatin. This gene product is thought to
be essential for development, and reduced expression of this gene in cells causes defects in chromatin structure. High levels of expression of
this gene in cells causes defects in chromatin structure. High levels of expression of
 - be essential for development, and reduced expression of this gene in cells causes defects in chromatin structure. High levels of expression of the human ortholog of this gene are observed in cervical cancers, and expression of the human ortholog of this gene in mice results in tumor formation. Alternative splicing results in multiple transcript variants encoding different protein isoforms. [provided by RefSeq, Aug 2014]
 - Expression Ubiquitous expression in liver E14 (RPKM 13.4), placenta adult (RPKM 11.9) and 28 other tissues See more

Orthologs human all

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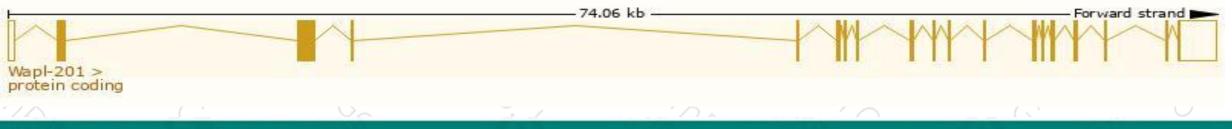
Transcript information (Ensembl)



The gene has 7 transcripts, all transcripts are shown below:

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Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags			
ENSMUST00000048263.13 6351 1200aa Protein coding		CCDS36880	<u>Q65Z40</u>	TSL:1 GENCODE basic APPRIS P3					
ENSMUST0000090027.10	4185	<u>1194aa</u>	Protein coding	CCDS79296	<u>B7ZP47</u>	TSL:5 GENCODE basic APPRIS ALT2			
ENSMUST00000169910.7	4066	<u>1200aa</u>	Protein coding	CCDS36880	<u>Q65Z40</u>	TSL:1 GENCODE basic APPRIS P3			
ENSMUST00000151285.7	1820	<u>430aa</u>	Protein coding	2	F6YCH1	CDS 5' incomplete TSL:5			
ENSMUST00000174559.2	479	<u>160aa</u>	Protein coding		G3UXQ2	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5			
ENSMUST00000111895.7	2688	No protein	Processed transcript	-	1963	TSL:1			
ENSMUST00000172658.1	382	No protein	Processed transcript	2	(12)	TSL:3			
	ENSMUST0000048263.13 ENSMUST00000090027.10 ENSMUST00000169910.7 ENSMUST00000151285.7 ENSMUST00000174559.2 ENSMUST00000111895.7	ENSMUST0000048263.13 6351 ENSMUST00000090027.10 4185 ENSMUST00000169910.7 4066 ENSMUST00000151285.7 1820 ENSMUST00000174559.2 479 ENSMUST00000111895.7 2688	ENSMUST0000048263.13 6351 1200aa ENSMUST00000090027.10 4185 1194aa ENSMUST00000169910.7 4066 1200aa ENSMUST00000151285.7 1820 430aa ENSMUST00000174559.2 479 160aa ENSMUST00000111895.7 2688 No protein	ENSMUST00000048263.1363511200aaProtein codingENSMUST0000090027.1041851194aaProtein codingENSMUST00000169910.740661200aaProtein codingENSMUST00000151285.71820430aaProtein codingENSMUST00000174559.2479160aaProtein codingENSMUST00000111895.72688No proteinProcessed transcript	ENSMUST00000048263.1363511200aaProtein codingCCDS36880ENSMUST00000090027.1041851194aaProtein codingCCDS79296ENSMUST00000169910.740661200aaProtein codingCCDS36880ENSMUST00000151285.71820430aaProtein coding-ENSMUST00000174559.2479160aaProtein coding-ENSMUST00000111895.72688No proteinProtein coding-	ENSMUST0000048263.13 6351 1200aa Protein coding CCDS36880 065Z40 ENSMUST00000048263.13 4185 1194aa Protein coding CCDS36800 065Z40 ENSMUST00000169910.7 4066 1200aa Protein coding CCDS36800 065Z40 ENSMUST0000151285.7 1820 430aa Protein coding CCDS36800 66YCH1 ENSMUST0000174559.2 479 160aa Protein coding G3UXQ2 ENSMUST00000111895.7 2688 No protein Protein coding			

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

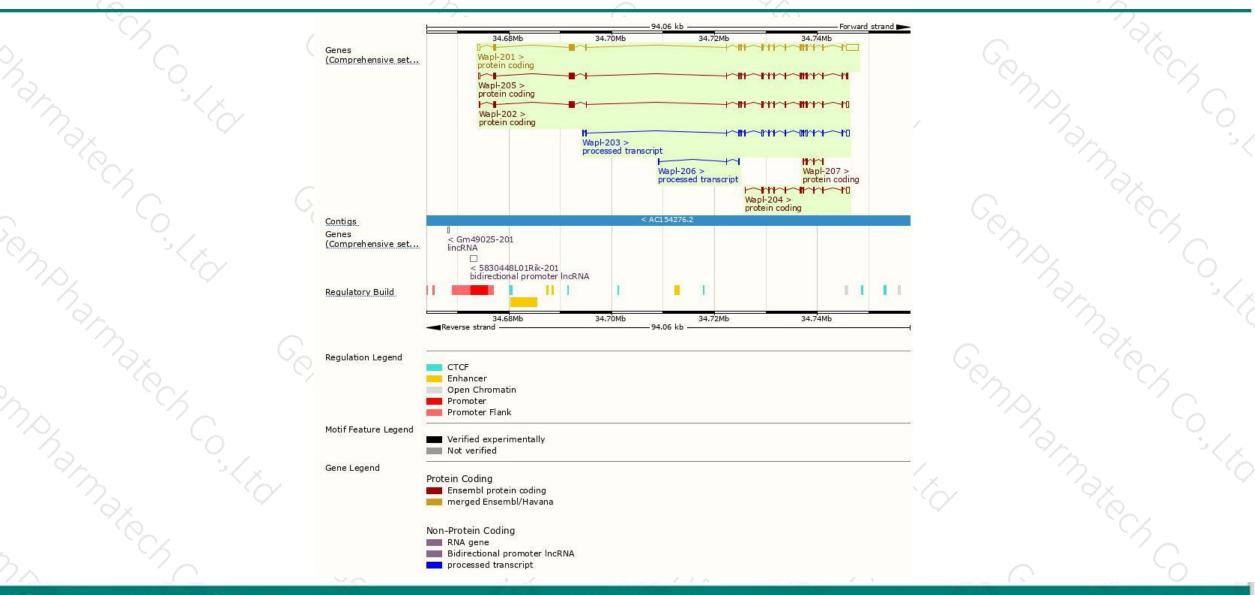


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Genomic location distribution



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Protein domain

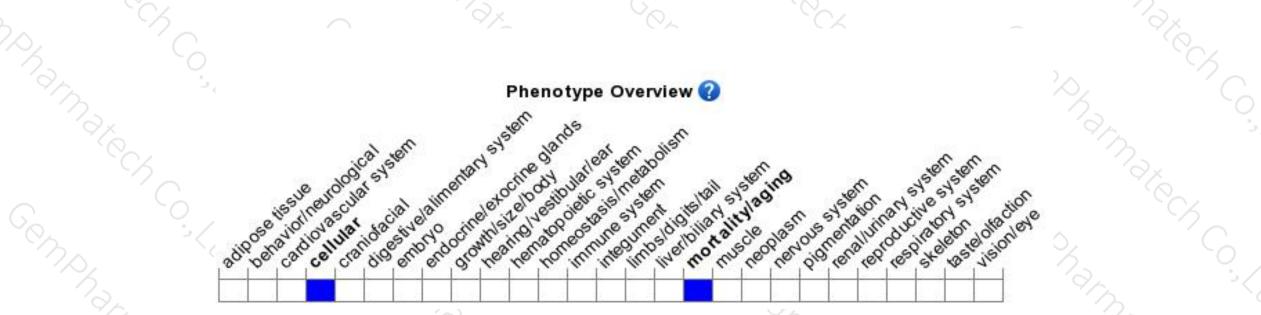
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Mouse phenotype description(MGI)





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, Mice homozygous for a targeted allele exhibit prenatal lethality.



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



