

Wdpcp Cas9-CKO Strategy

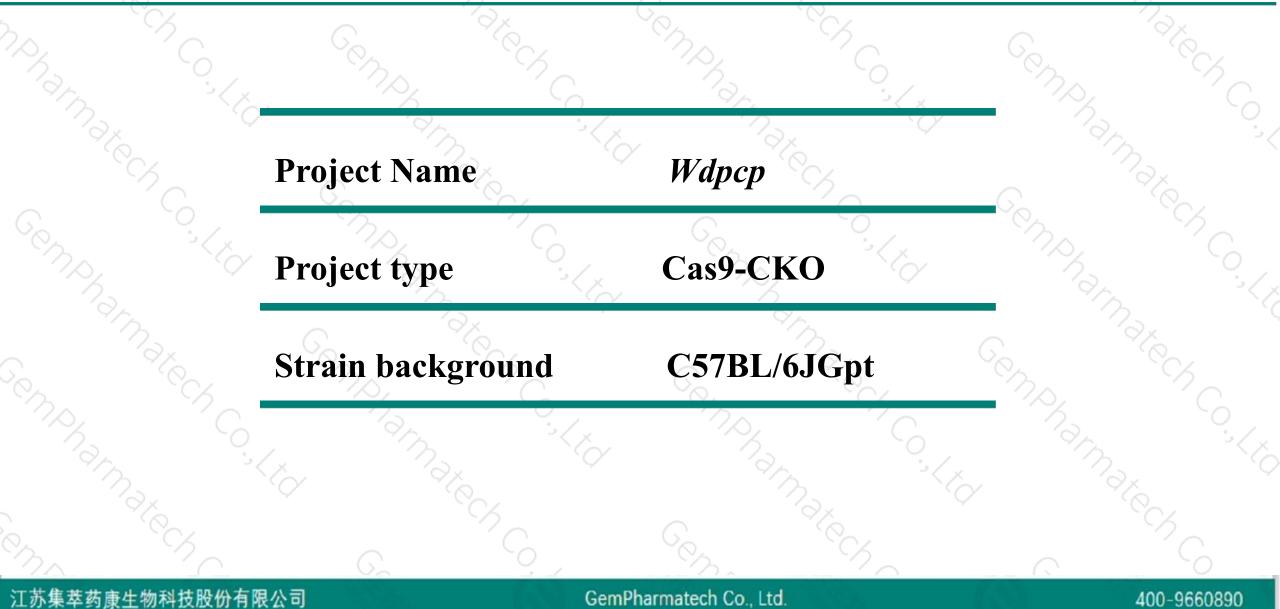
Designer: Reviewer:

Design Date:

Huan Wang Huan Fan 2020-4-23

Project Overview

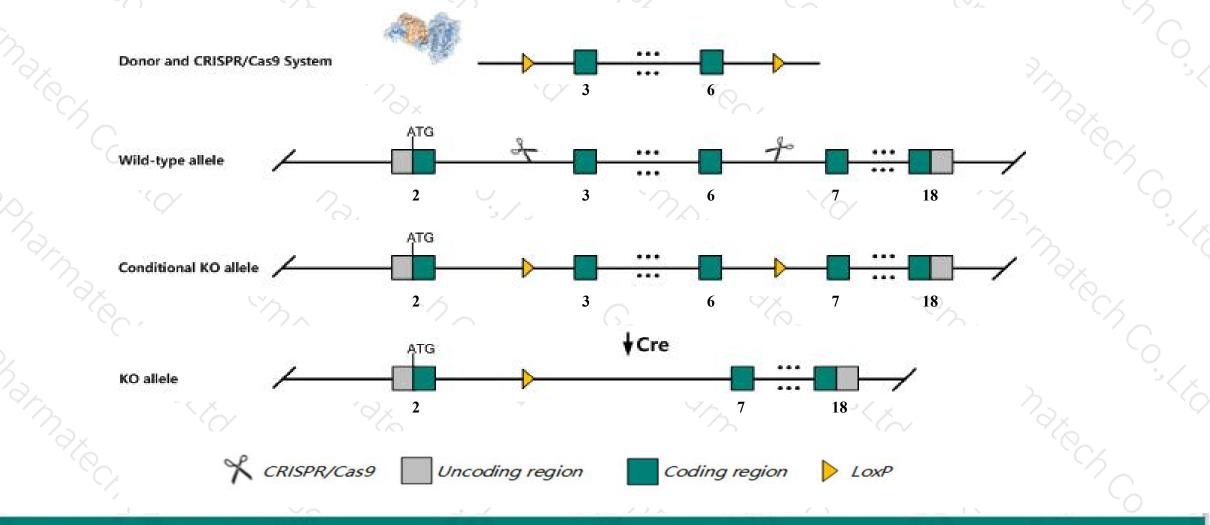




Conditional Knockout strategy



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



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The Wdpcp gene has 6 transcripts. According to the structure of Wdpcp gene, exon3-exon6 of Wdpcp-201 (ENSMUST0000020568.9) transcript is recommended as the knockout region. The region contains 230bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Wdpcp* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 null mutation display ciliogenesis defects, anophthalmia, cysts in multiple tissues, central polydactyly, duplex kidney, and septation defects in the outflow tract and cloaca.
- The Wdpcp gene is located on the Chr11. 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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Wdpcp WD repeat containing planar cell polarity effector [Mus musculus (house mouse)]

Gene ID: 216560, updated on 13-Mar-2020

Summary

Official Symbol	Wdpcp provided by MGI
Official Full Name	WD repeat containing planar cell polarity effector provided by MGI
Primary source	MGI:MGI:2144467
See related	Ensembl:ENSMUSG0000020319
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	AV249152, Frtz, homolog-13
Expression	Broad expression in cerebellum adult (RPKM 9.9), testis adult (RPKM 7.2) and 24 other tissues See more
Orthologs	human all

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Transcript information (Ensembl)



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The gene has 6 transcripts, all transcripts are shown below:

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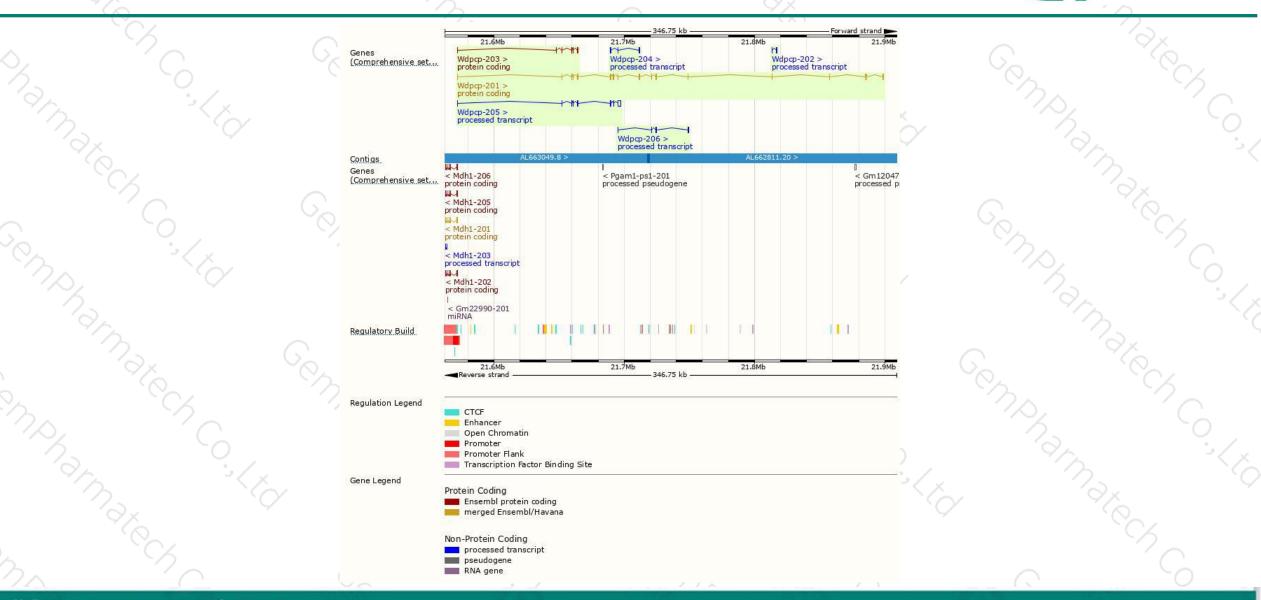
lame	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
pcp-201 EN	<u>ISMUST00000020568.9</u>	2792	<u>722aa</u>	Protein coding	CCDS24467	B1ATJ7 Q8C456	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS
pcp-203 EN	NSMUST00000131135.2	505	<u>97aa</u>	Protein coding		B1ATQ4	CDS 3' incomplete TSL:3
pcp-205 EN	SMUST00000149757.1	3020	No protein	Processed transcript	(12)	1940	TSL:1
ocp-206 EN	SMUST00000156624.1	963	No protein	Processed transcript	120	1020	TSL1
0cp-204 EN	SMUST00000143608.1	741	No protein	Processed transcript		151	TSL:2
0cp-202 EN	SMUST00000128794.1	480	No protein	Processed transcript	1943	1-1	TSL:3
pcp-202 EN	NSMUST00000128794.1	480	No protein	Processed transcript	(m.)	(19)	TSL:3

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

		Forw	- Forward strand				
Wdpcp-201 > protein coding	_(10 ×			

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Genomic location distribution



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Protein domain

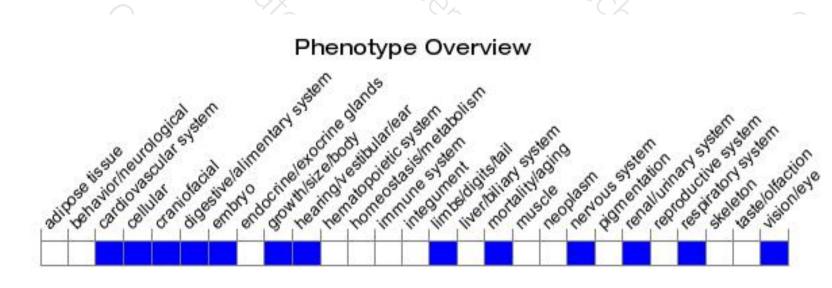
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	ENSMUSP00000020 MobiDB lite Low complexity (Seg) Coiled-coils (Ncoils) Superfamily			WD40)-repeat-containing d	omain superfamil			~~~ ~<		
2	<u>Pfam</u>	WD repeat-containing and planar cell polarity effector protein Fritz									
S.	PANTHER	WD repeat-containing a	nd planar cell pola	rity effector prote	in Fritz						
	Gene3D	WD40/YVTN repeat-like-containing domain superfamily									
	All sequence SNPs/i	Sequence variants (c	bSNP and all ot	ner sources)	i al	e du	1.10	11,00-1			
2012	Variant Legend	inframe insertio inframe deletion missense variar synonymous va	ו ול								
	Scale bar	0 80	160	240 3	320 400	480	560	640 722			
	ALC CH				No.) ~		°° C			
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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 null mutation display ciliogenesis defects, anophthalmia, cysts in multiple tissues, central polydactyly, duplex kidney, and septation defects in the outflow tract and cloaca.

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



