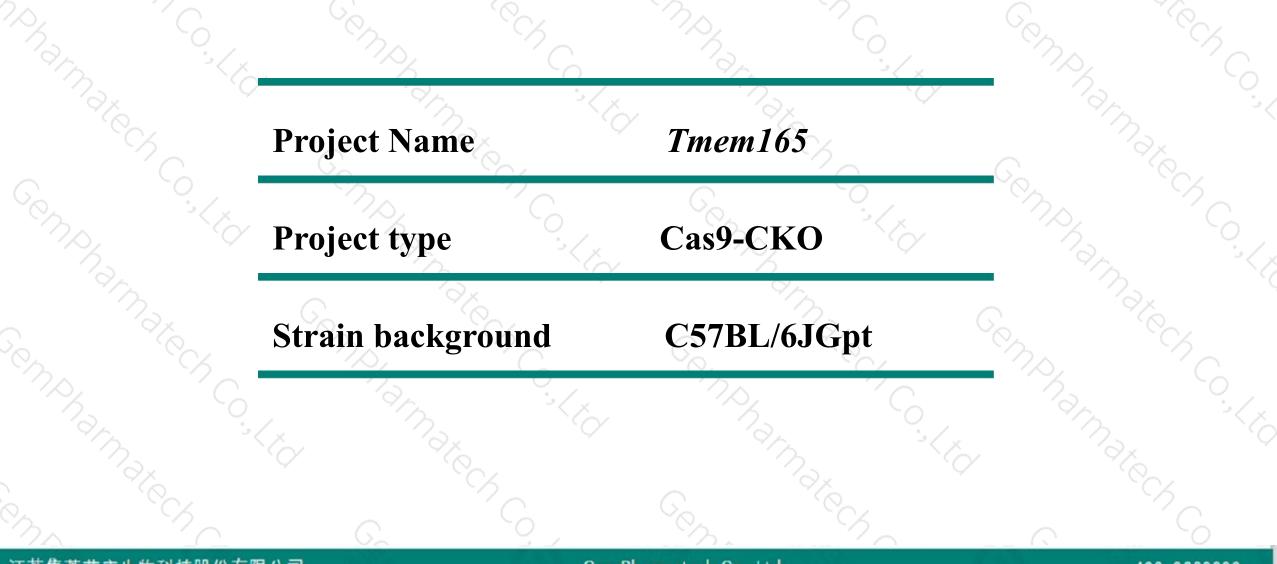


Tmem165 Cas9-CKO Strategy

Designer: Reviewer: Design Date: JiaYu Xiaojing Li 2020-3-4

Project Overview





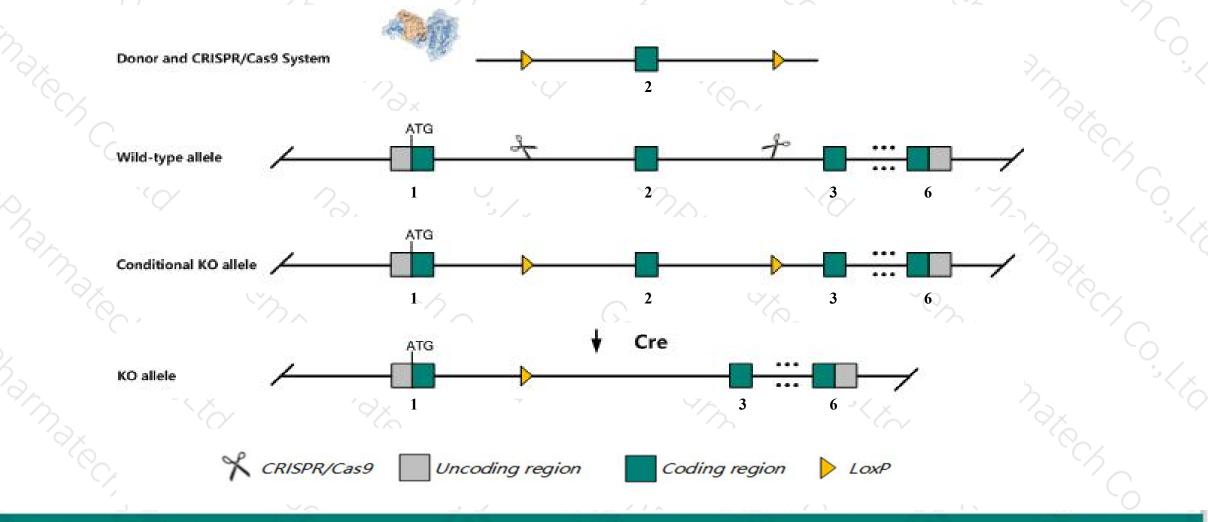
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Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Tmem165* gene. The schematic diagram is as follows:



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The *Tmem165* gene has 5 transcripts. According to the structure of *Tmem165* gene, exon2 of *Tmem165-201* (ENSMUST00000031144.13) transcript is recommended as the knockout region. The region contains 226bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Tmem165* 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.



- The Tmem165 gene is located on the Chr5. 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)



\$?

Tmem165 transmembrane protein 165 [Mus musculus (house mouse)]

Gene ID: 21982, updated on 31-Jan-2019

Summary

Official Symbol	Tmem165 provided by MGI
Official Full Name	transmembrane protein 165 provided by MGI
Primary source	MGI:MGI:894407
See related	Ensembl:ENSMUSG0000029234
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	AV026557, Tpardl, Tparl, pFT27
Expression	Ubiquitous expression in placenta adult (RPKM 60.9), limb E14.5 (RPKM 51.0) and 28 other tissues See more
Orthologs	human all

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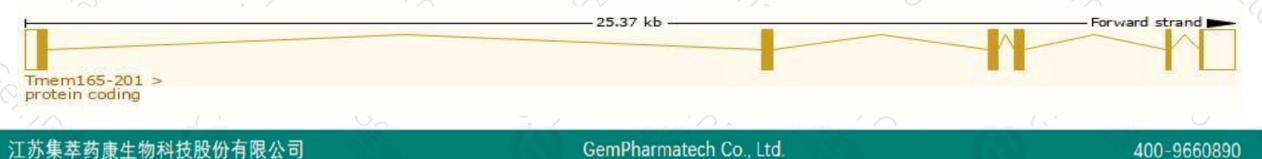
Transcript information (Ensembl)



The gene has 5 transcripts, all transcripts are shown below:

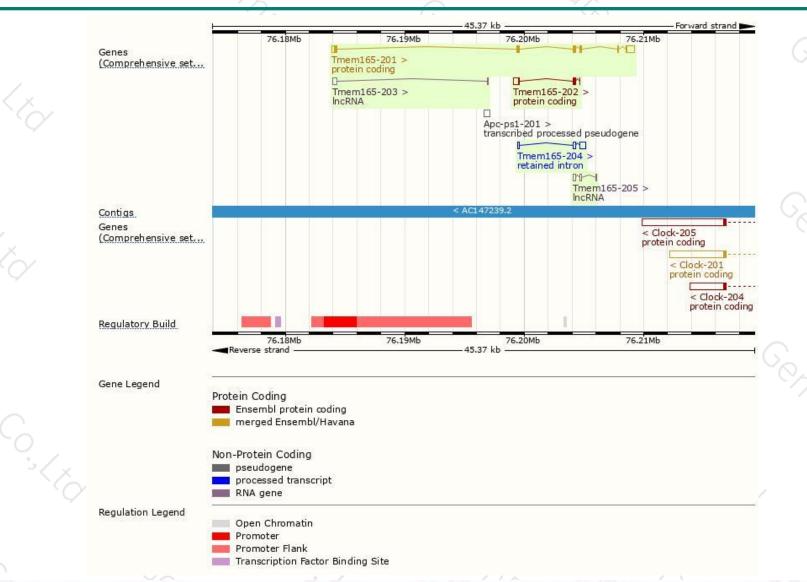
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tmem165-201	ENSMUST0000031144.13	1897	<u>323aa</u>	Protein coding	CCDS19359	P52875	TSL:1 GENCODE basic APPRIS P1
Tmem165-202	ENSMUST00000130842.1	649	<u>90aa</u>	Protein coding	-8	D3YV67	CDS 3' incomplete TSL:1
Tmem165-204	ENSMUST00000138544.1	821	No protein	Retained intron		2	TSL:1
Tmem165-203	ENSMUST00000136424.1	462	No protein	IncRNA	20 	- 1	TSL:3
Tmem165-205	ENSMUST00000153633.1	408	No protein	IncRNA	-		TSL:3

The strategy is based on the design of *Tmem165-201* transcript, The transcription is shown below



Genomic location distribution





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Protein domain



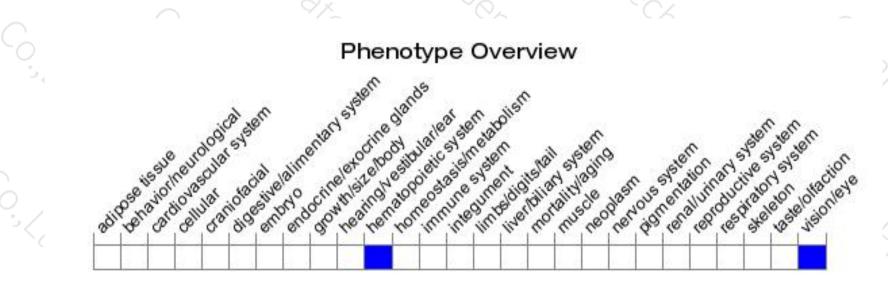


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Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).



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



