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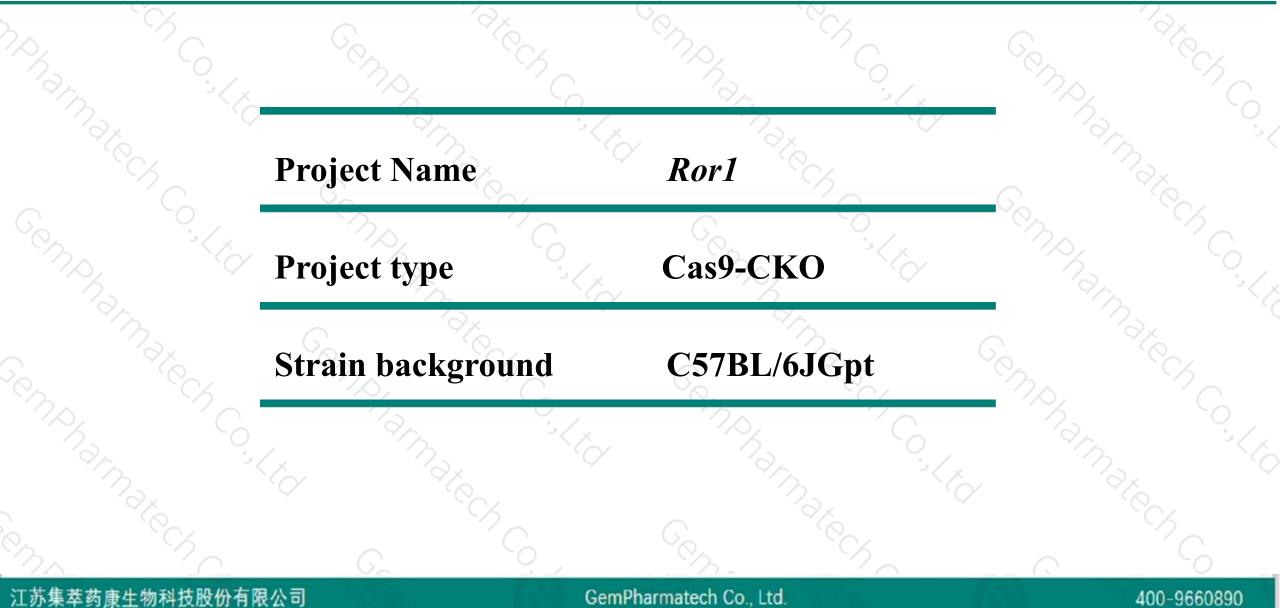
Ror1 Cas9-CKO Strategy

ripharma

Designer: Jinling Wang Reviewer: Shilei Zhu Date: 2019/11/11

Project Overview

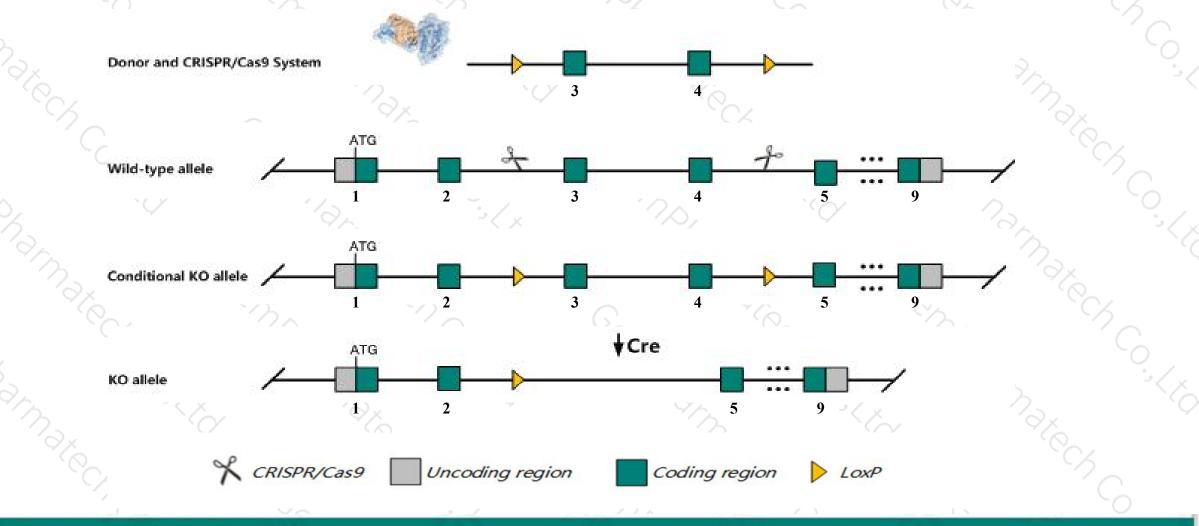




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Ror1* gene. The schematic diagram is as follows:



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The Ror1 gene has 1 transcript. According to the structure of Ror1 gene, exon3-exon4 of Ror1-201 (ENSMUST00000039630.5) transcript is recommended as the knockout region. The region contains 319bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Ror1* 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 some disruptions in this gene die within the first day after birth from respiratory defects.
- The *Ror1* gene is located on the Chr4. 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)



Gene ID: 26563	updated o	on 26-Feb-2019
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- Summary

Official Symbol	Ror1 provided by MGI						
Official Full Name	receptor tyrosine kinase-like orphan receptor 1 provided by MGI						
Primary source	MGI:MGI:1347520						
See related	Ensembl:ENSMUSG0000035305						
Gene type	protein coding						
RefSeq status	REVIEWED						
Organism	Mus musculus						
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;						
	Muroidea; Muridae; Murinae; Mus; Mus						
Also known as	2810404D04Rik, Ntrkr1						
Summary	This gene encodes a receptor tyrosine kinase that has been implicated in nervous system development, specifically in the maintenance of						
	neural progenitor cell fate, neurite extension and synapse formation. The encoded protein, likely a pseudokinase that lacks catalytic activity,						
	may also regulate adipogenesis. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Aug 2015]						
Expression	Broad expression in bladder adult (RPKM 2.2), limb E14.5 (RPKM 1.7) and 23 other tissues See more						
Orthologs	human all						

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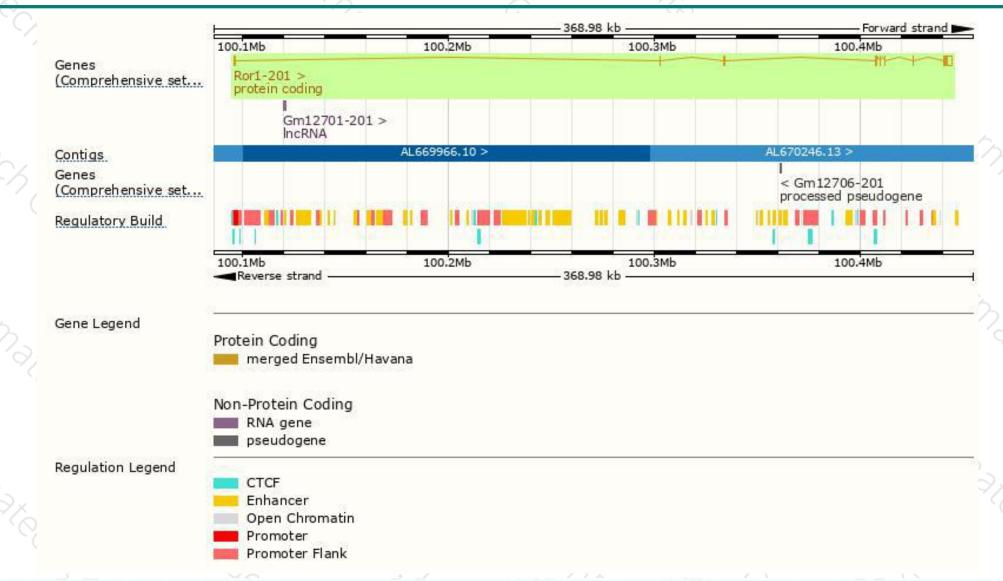
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The gene has 1 transcript, and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
or1-201	ENSMUST0000039630.5	5762 <u>937aa</u>		Protein coding	<u>CCDS18389</u> <u>Q9Z139</u>		TSL:1 GENCODE basic APPRIS P1	
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e strateg	y is based on the design o	f Rorl-	201 trans	cript,The transc	cription is sho	wn below		
					<i>D</i> .			
				348.98 kb —		1	Forward strand	
10								
1-201 >								
ein coding								

Genomic location distribution





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Protein domain



Č.	ENSMUSP00000048	
10	Transmembrane heli MobiDB lite	
	Low complexity (Seg)	Kringle-like fold Protein kinase-like domain superfamily Immunoglobulin-like domain superfamily Immunoglobulin subtype Immunoglobulin subtype 2 Immunoglobulin 1-set PR00018 Kringle Serine-threonine/tyrosine-protein kinase, catalytic domain
	Cleavage site (Sign	$\gamma_{\rm c} = \gamma_{\rm c}$
	Superfamily	Kringle-like fold Protein kinase-like domain superfamily
		Immunoglobulin-like domain superfamily
	SMART	Immunoglobulin subtype Kringle
		Immunoglobulin subtype 2
	Prints	PR00018 Serine-threonine/tyrosine-protein kinase, catalytic dom
	Pfam	Immunoglobulin I-set Kringle Serine-threonine/tyrosine-protein kinase, catalytic domain
		Frizzled domain
2/4	PROSITE profiles	Frizzled domain Kringle Protein kinase domain
		Immunoglobulin-like domain
	PROSITE patterns	Kringle, conserved site Tyrosine-protein kinase, active site
		Immunoglobulin-like domain Kringle, conserved site Tyrosine-protein kinase, receptor ROR PTHR24416
	PIRSF	Tyrosine-protein kinase, receptor ROR
	PANTHER	PTHR24416
		PTHR24416;SF134
\sim	Gene3D	Immunoglobulin-like fold Kringle superfamily 3.30.200.20 1.10.510.10
		Frizzled cysteine-rich domain superfamily
í C	CDD	cd00096 cd07467 Kringle cd05090
		$\gamma_{r_{2}} = \gamma_{r_{2}}$
* ***	All sequence SNPs/i	Sequence variants (dbSNP and all other sources)
		$\gamma $
	Variant Legend	missense variant
		synonymous variant
10	Scale bar	0 80 160 240 320 400 480 560 640 720 800 937

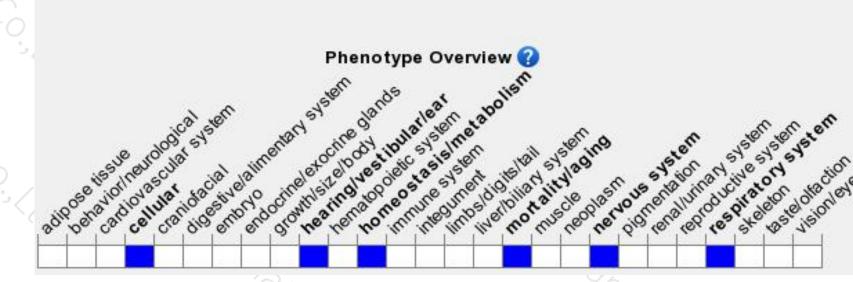
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Mouse phenotype description(MGI)





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 some disruptions in this gene die within the first day after birth from respiratory defects.



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



