Apbal Cas9-CKO Strategy matech Co. 1 to Rondhamater Co-ty

Designer: Enphamaten C. It

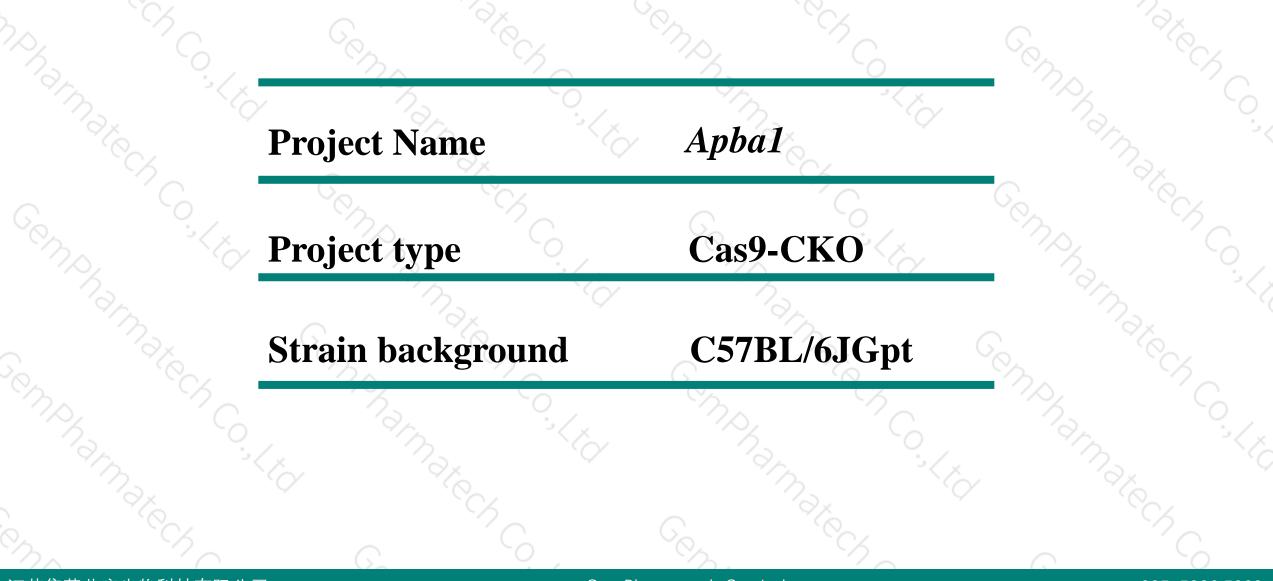
Daohua Xu

Cenphamatech.

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Project Overview

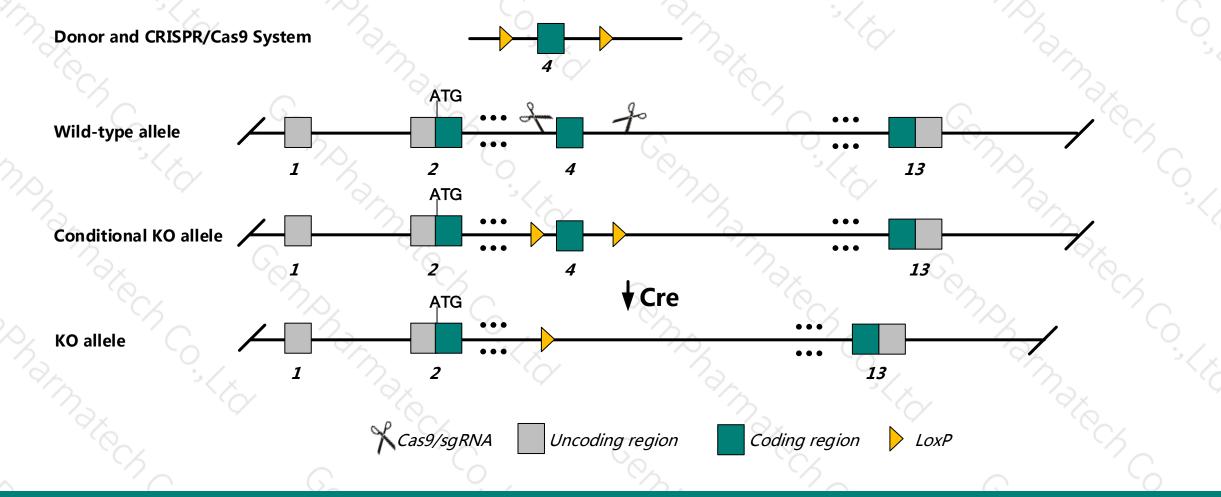




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Apba1 gene. The schematic diagram is as follows:



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- The Apba1 gene has 2 transcripts. According to the structure of Apba1 gene, exon4 of Apba1-201 transcript is recommended as the knockout region. The region contains 40bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Apba1* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.

- The Apba1 gene is located on the Chr19. 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Notice

Gene information (NCBI)



				$\gamma_{\mathcal{D}_{\mathcal{I}}}$			
dr.	Apba1 amyloid be	ta (A4) precursor pro	otein binding, fami	ly A, member 1 [M	lus musculus (hou	se mouse)]	
- n.	Gene ID: 319924, updated or	n 7-May-2019					
× (6	Summary						* ?
	Official Symbol	Apba1 provided by MGI					
\bigcirc	-	amyloid beta (A4) precursor	protein binding, family A, m	ember 1 provided by MGI			
	Primary source						
	See related	Ensembl:ENSMUSG000002	24897				
	Gene type	protein coding					
25	RefSeq status	VALIDATED					
$\langle \forall \rangle$	Organism	Mus musculus					
	Lineage	Eukaryota; Metazoa; Chorda		teleostomi; Mammalia; Euth	eria; Euarchontoglires; Gliro	es; Rodentia; Myomorpha;	
\sim		Muroidea; Muridae; Murinae;					
52		X11; Mint; Mint1; Lin-10; Urop					
	-	Broad expression in cortex a	dult (RPKM 21.6), cerebellu	um adult (RPKM 21.4) and 1	7 other tissues <u>See more</u>		
$\gamma \sim$	Orthologs	<u>human</u> <u>all</u>					
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## **Transcript information (Ensembl)**



#### The gene has 2 transcripts, and all transcripts are shown below :

2	Show/hide columns (1 hidden)							Filter		
	Name 🍦 🛛 Transcript ID 🍦 bp		bp 🍦	Protein 🖕	Biotype	CCDS 🖕	UniProt 🖕	Flags		
	Apba1-201	ENSMUST0000025830.8	6620	<u>842aa</u>	Protein coding	<u>CCDS50407</u> &	<u>B2RUJ5</u> ₽	TSL:1 GENCODE basic	APPRIS P2	
	Apba1-202	ENSMUST00000237688.1	3174	<u>452aa</u>	Protein coding	-	-	GENCODE basic APPF	RIS ALT2	

The strategy is based on the design of Apba1-201 transcript, The transcription is shown below

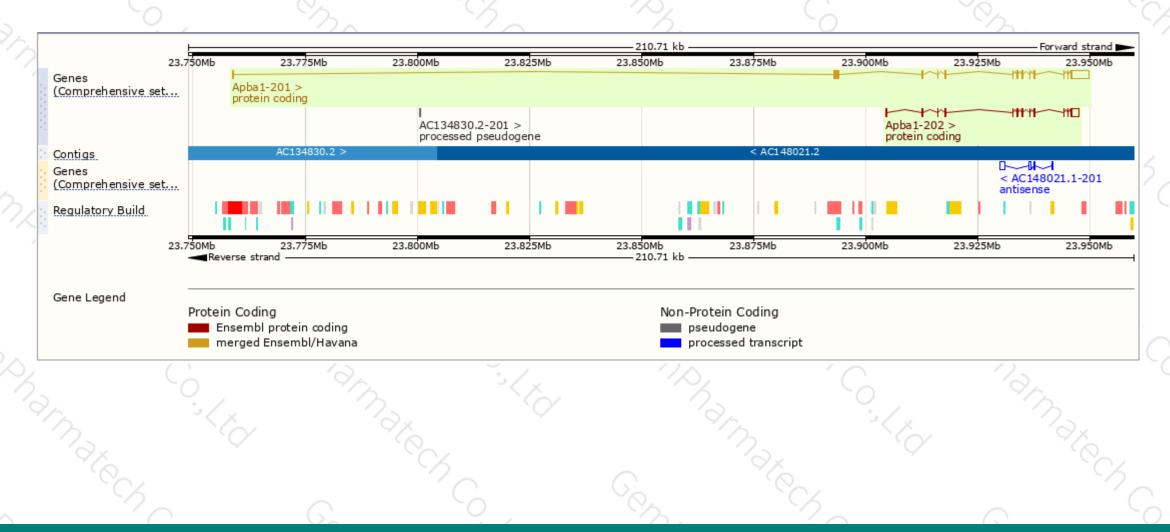
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### **Genomic location distribution**





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## **Protein domain**



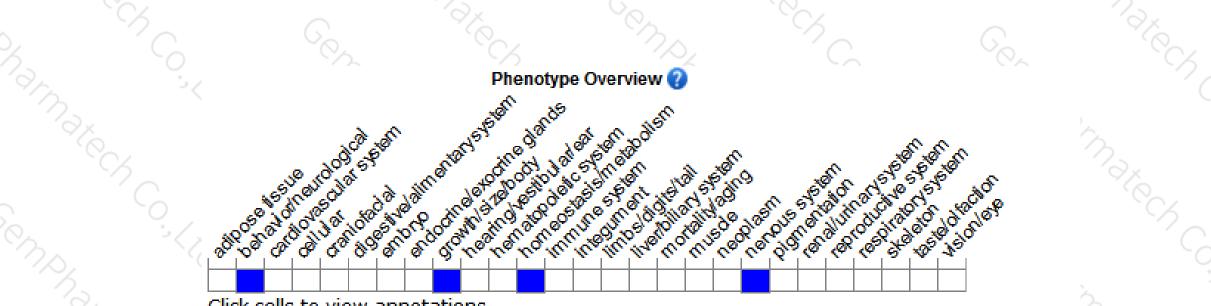
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6	ENSMUSP00000025										
	MobiDB lite Low complexity (Seg)		_								<u> </u>
	Conserved Domains										
	hmmpanther		cursor protein-binding far	nily A member 1							
		PTHR12345									
	Superfamily domains					SSF50729		PDZ superfa	amily		
	SMART domains					PTB/PI domain		PDZ	domain	5	
	Pfam domain					PTB/PI domai	'n	PDZ d	lomain	[	
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	Gene3D					PH-like domain :		2.30.42			- '> {
	All sequence SNPs/i	Sequence variant:	s (dbSNP and all other s	sources)		L I	1 11 1	1.1.1		П	
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## Mouse phenotype description(MGI)





Click cells to view annotations.

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, Animals carrying a homozygous mutation of this gene have reduced body

size.

If you have any questions, you are welcome to inquire. Tel: 025-5864 1534





