# Aldh111-P2A-CreERT2 Cas9-KI Strategy

Designer Reviewer

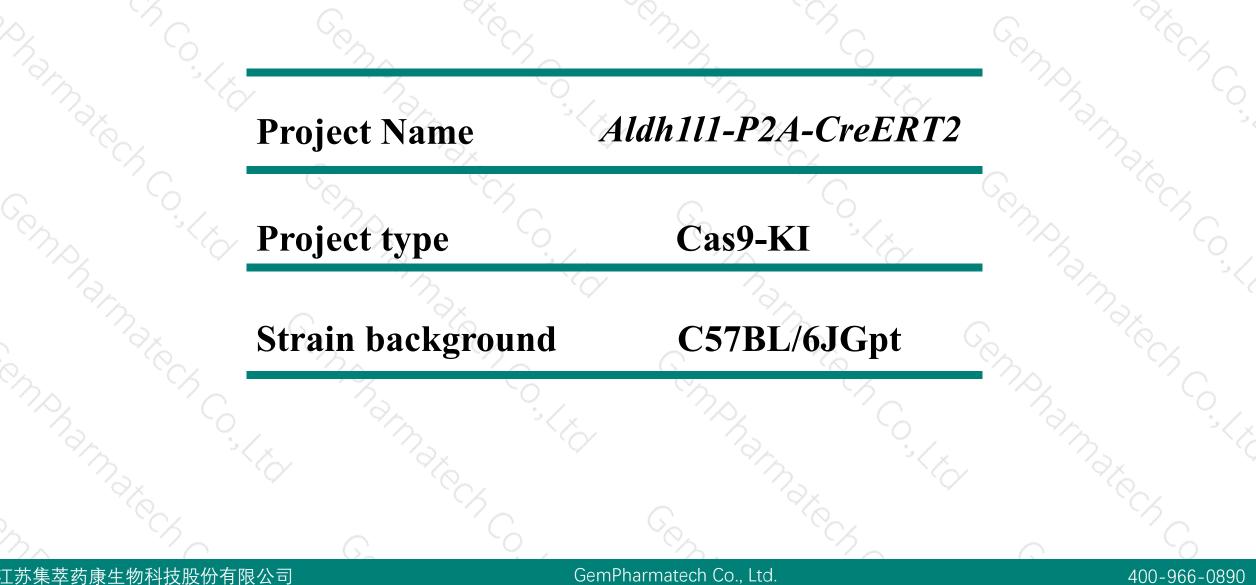
**Design Date** 

Qiong Zhou Ruirui Zhang

2019-8-13

### **Project Overview**



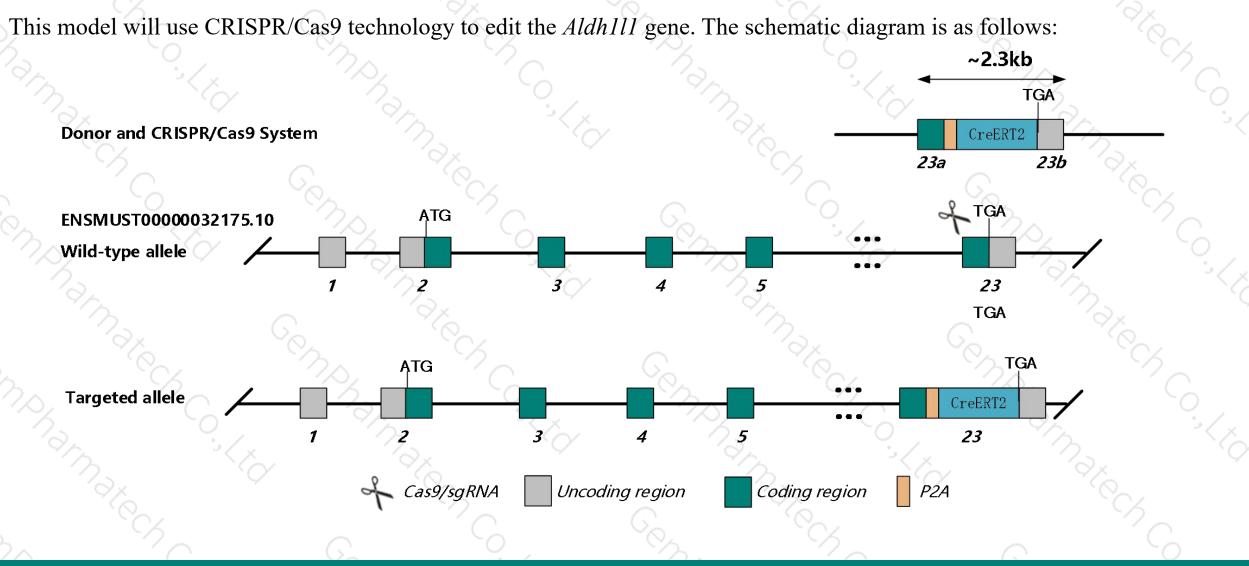


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### **Knockin strategy**





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- The Aldh111 gene has 7 transcripts. According to the structure of Aldh111 gene, Aldh111-201(ENSMUST00000032175.10) is selected for presentation of the recommended strategy.
- > Aldh111-201 gene has 23 exons, with the ATG start codon at exon2 and TGA stop codon at exon23.
  - We make *Aldh111-P2A-CreERT2* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be coinjected into zygotes. sgRNA direct Cas9 endonuclease cleavage near stop codon(TGA) of *Aldh111* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in P2A-iCre before stop codon(TGA) of *Aldh111* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

### Notice



- > According to the existing references, CreERT2-mediated recombination is expressed in the vast majority of astrocytes.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- The insertion of P2A-CreERT2 may affect the 5-terminal regulation of downstream *Slc41a3* gene.
- ➤ Insertion of CreERT2 may affect the regulation of the 3' end of the *Aldh111* gene.
- The Aldh111 gene is located on the Chr6. If the knockin 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.

# Gene information (NCBI)



#### Aldh1l1 aldehyde dehydrogenase 1 family, member L1 [ Mus musculus (house mouse) ]

Gene ID: 107747, updated on 12-Aug-2019

#### Summary

Official Symb	Aldh111 provided by MGI
Official Full Nam	e aldehyde dehydrogenase 1 family, member L1 provided by MGI
Primary source	e <u>MGI:MGI:1340024</u>
See relate	d Ensembl:ENSMUSG0000030088
Gene typ	e protein coding
RefSeq statu	IS VALIDATED
Organis	m <u>Mus musculus</u>
Lineag	e Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae;
	Murinae; Mus; Mus
Also known a	s FDH; Fthfd; Neut2; 1810048F20Rik
Expressio	n Biased expression in liver adult (RPKM 382.3), kidney adult (RPKM 107.4) and 7 other tissues See more
Ortholog	s <u>human</u> all
Sal pro	$\sim$

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# **Transcript information (Ensembl)**

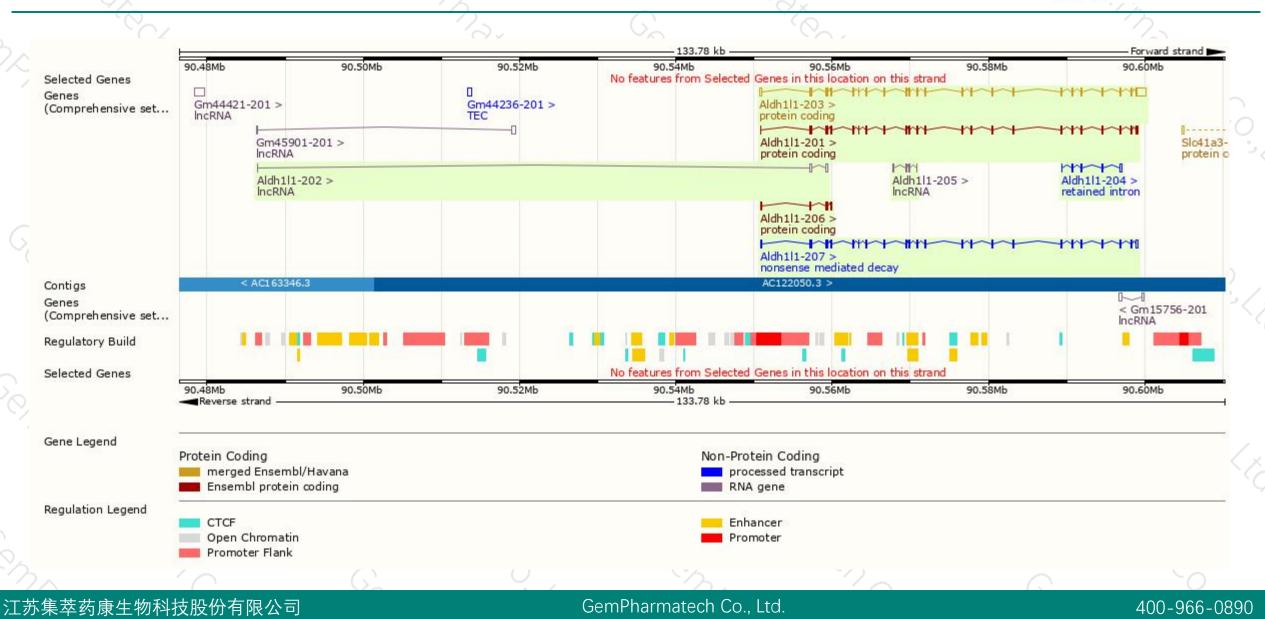


Name 🍦	Transcript ID	bp 🖕	Protein 🖕	Biotype 💧	CCDS 🖕	UniProt v	Flags
Aldh111-203	ENSMUST00000130418.7	4175	<u>902aa</u>	Protein coding	<u>CCDS20361</u> &	Q8R0Y6	TSL:1 GENCODE basic APPRIS P1
Aldh111-201	ENSMUST0000032175.10	3052	<u>902aa</u>	Protein coding	<u>CCDS20361</u> &	<u>Q8R0Y6</u> &	TSL:1 GENCODE basic APPRIS P1
Aldh111-207	ENSMUST00000204796.2	3025	<u>359aa</u>	Nonsense mediated decay	10 <b>7</b> 01	<u>A0A0N4SW56</u> 교	TSL:5
Aldh111-206	ENSMUST00000203111.1	556	<u>135aa</u>	Protein coding	-	A0A0N4SVT2	CDS 3' incomplete TSL:5
Aldh111-204	ENSMUST00000137669.1	653	No protein	Retained intron	10 <b>7</b> 01	2	TSL:3
Aldh111-205	ENSMUST00000152436.1	472	No protein	IncRNA	-	-	TSL:3
Aldh111-202	ENSMUST00000127199.1	400	No protein	IncRNA	100	-	TSL:3

The strategy is based on the design of *Aldh111-201* transcript, The transcription is shown below:

Aldh111-201 > protein coding

### **Genomic location distribution**



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### **Protein domain**



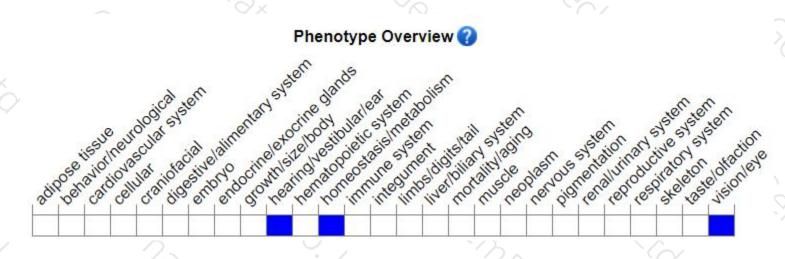
PIRSF 10-formyltetrahydrofolate dehydrog enase   PANTHER PTHR11699   PTHR11699:SF120 PTHR11699:SF120   Gene3D 3.40.50.170 ACP-like superfamily Aldehyde dehydrogenase, N-terminal	800 902
Pfam Formyl transferase, N-terminal Formyl transferase, C-terminal domain superfamily Formyl transferase, C-terminal Aldehyde dehydrogenase domain   PROSITE profiles PROSITE patterns Phosphopantetheine binding ACP domain   PRSF 10-formyl tetrahydrofelate dehydrogenase Aldehyde dehydrogenase, N-terminal   PIRSF 10-formyl tetrahydrofelate dehydrogenase Aldehyde dehydrogenase, N-terminal   Gene3D 3.40.50.170 ACP-like superfamily Aldehyde dehydrogenase, N-terminal   CDD cd08647 cd08703 cd07140   All sequence SNPs/i Sequence variants (dbSNP and all other sources) Inter sources) Inter sources)	
Pfam   Formyl transferase, N-terminal   Formyl transferase-like, C-terminal domain superfamily     PROSITE profiles   Promyl transferase, C-terminal   Aldehyde dehydrogenase domain     PROSITE profiles   Phosphopantetheine binding ACP domain     PROSITE patterns   Phosphoribosylglycinamide formyltransferase, active site   Aldehyde dehydrogenase     PIRSF   10-formyltetrahydrofolate dehydrogenase   Aldehyde dehydrogenase, N-terminal     PITHR116599   PTHR116599   ACP-like superfamily   Aldehyde dehydrogenase, N-terminal     Addehyde dehydrogenase, C-terminal domain superfamily   Aldehyde dehydrogenase, N-terminal   Aldehyde dehydrogenase, N-terminal	
Pfam Formyl transferase, N-terminal Formyl transferase-like, C-terminal domain superfamily   PROSITE profiles Formyl transferase, C-terminal Aldehyde dehydrogenase domain   PROSITE profiles Phosphonibosylglycinamide formyltransferase, active site Aldehyde dehydrogenase domain   PIRSF 10-formyltetrahydrofolate dehydrog enase Aldehyde of enase   PANTHER PTHR11699 THR11699   PTHR11699:SF120 PTHR11699	drogenase, C-terminal
Pfam Formyl transferase, N-terminal Formyl transferase-like, C-terminal domain superfamily Formyl transferase, C-terminal Aldehyde dehydrogenase   PROSITE profiles PROSITE patterns Phosphoribosylglycinamide formyltransferase, active site Aldehyde dehydrogenase domain   PIRSF 10-formyltetrahydrofolate dehydrogenase Informyltetrahydrofolate dehydrogenase	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Pfam Formyl transferase, N-terminal Formyl transferase-like, C-terminal domain superfamily   PROSITE profiles PROSITE patterns Aldehyde dehydrogenase domain   PROSITE patterns Phosphoribosylglycinamide formyltransferase, active site Aldehyde dehydrogenase domain	
Aldehyde/histidinol dehydrogenase Formyl transferase-like, C-terminal domain superfamily Formyl transferase, C-terminal Aldehyde dehydrogenase domain	dehydrogenase, cysteine active site drogenase, glutamic acid active site
Aldehyde/histidinol dehydrogenase	
ENSMUSP00000032 Low complexity (Seg)	2

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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/marker/MGI:1340024).

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



