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# Aldh7a1 Cas9-CKO Strategy

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# Overview

## Target Gene Name

- Aldh7a1

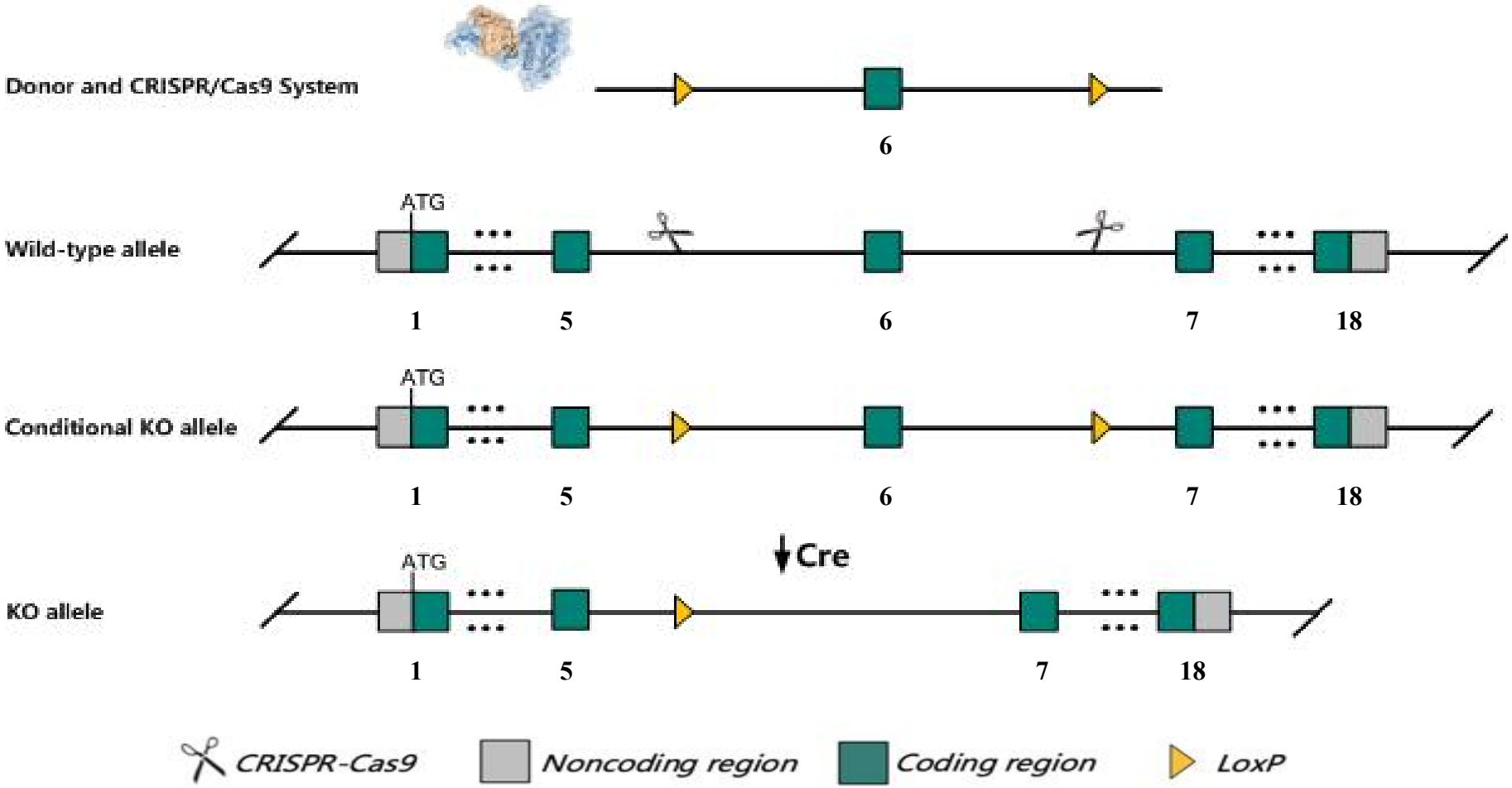
## Project Type

- Cas9-CKO

## Genetic Background

- C57BL/6JGpt

# Strain Strategy




Schematic representation of CRISPR-Cas9 engineering used to edit the *Aldh7a1* gene.

# Technical Information

- The *Aldh7a1* gene has 9 transcripts. According to the structure of *Aldh7a1* gene, exon6 of *Aldh7a1*-201 (ENSMUST00000066208.13) transcript is recommended as the knockout region. The region contains 133bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Aldh7a1* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.

# Gene Information

**Aldh7a1 aldehyde dehydrogenase family 7, member A1 [Mus musculus (house mouse)]**

Gene ID: 110695, updated on 19-Jan-2021

**Summary**

| | |
|---|---|
| **Official Symbol** | Aldh7a1 provided by MGI |
| **Official Full Name** | aldehyde dehydrogenase family 7, member A1 provided by MGI |
| **Primary source** | MGI:MGI:108186 |
| **See related** | Ensembl:ENSMUSG00000053644 |
| **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** | Atq, Atq1, D18Wsu181, D18Wsu181e |
| **Expression** | Broad expression in placenta adult (RPKM 56.9), liver adult (RPKM 51.3) and 23 other tissues See more |
| **Orthologs** | human all |

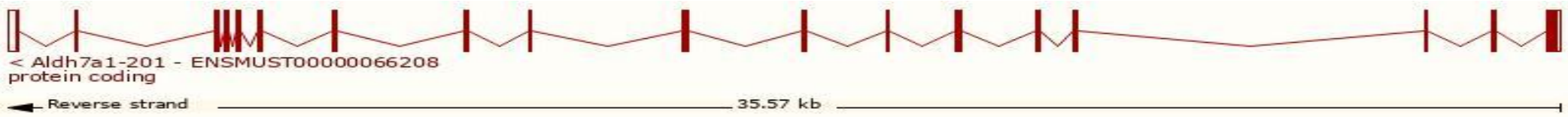
Source: https://www.ncbi.nlm.nih.gov/

# Transcript Information

The gene has 9 transcripts,all transcripts are shown below:

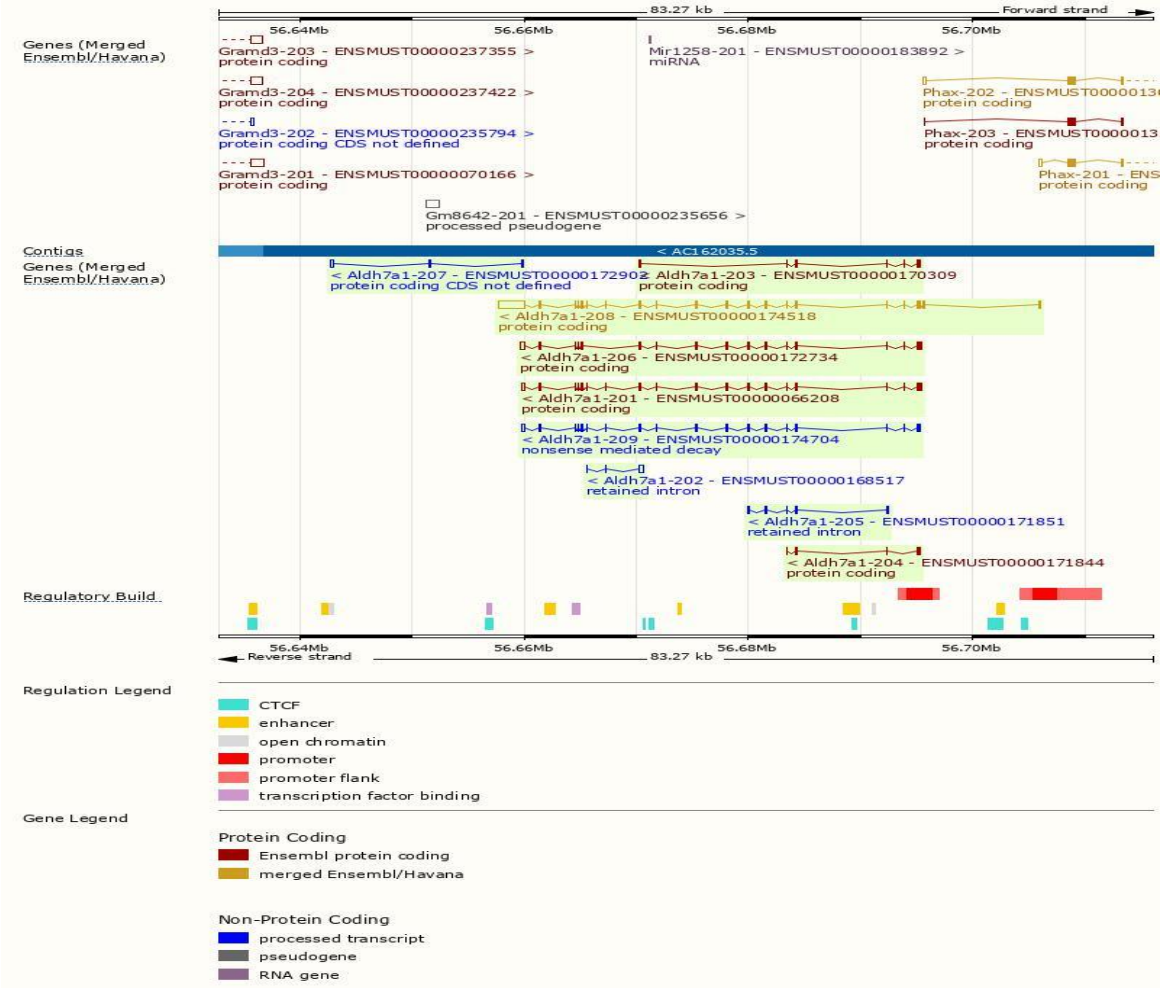
| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|---|---|---|---|---|---|---|---|
| **Aldh7a1-208** | ENSMUST00000174518.8 | 4131 | 511aa | Protein coding | CCDS50291 | | TSL:1 , GENCODE basic , APPRIS P1 , |
| **Aldh7a1-201** | ENSMUST00000066208.13 | 1914 | 539aa | Protein coding | CCDS29258 | | TSL:1 , GENCODE basic , |
| **Aldh7a1-206** | ENSMUST00000172734.8 | 1880 | 475aa | Protein coding | - | | TSL:5 , GENCODE basic , |
| **Aldh7a1-203** | ENSMUST00000170309.8 | 483 | 161aa | Protein coding | - | | CDS 5' and 3' incomplete , TSL:3 , |
| **Aldh7a1-204** | ENSMUST00000171844.3 | 365 | 121aa | Protein coding | - | | CDS 5' and 3' incomplete , TSL:3 , |
| **Aldh7a1-209** | ENSMUST00000174704.8 | 1789 | 321aa | Nonsense mediated decay | - | | CDS 5' incomplete , TSL:5 , |
| **Aldh7a1-207** | ENSMUST00000172902.2 | 588 | No protein | Processed transcript | - | | TSL:3 , |
| **Aldh7a1-205** | ENSMUST00000171851.2 | 568 | No protein | Retained intron | - | | TSL:2 , |
| **Aldh7a1-202** | ENSMUST00000168517.2 | 555 | No protein | Retained intron | - | | TSL:1 , |

The strategy is based on the design of *Aldh7a1*-201 transcript, the transcription is shown below:




Source: https://www.ensembl.org

# Genomic Information




Source: : https://www.ensembl.org

# Protein Information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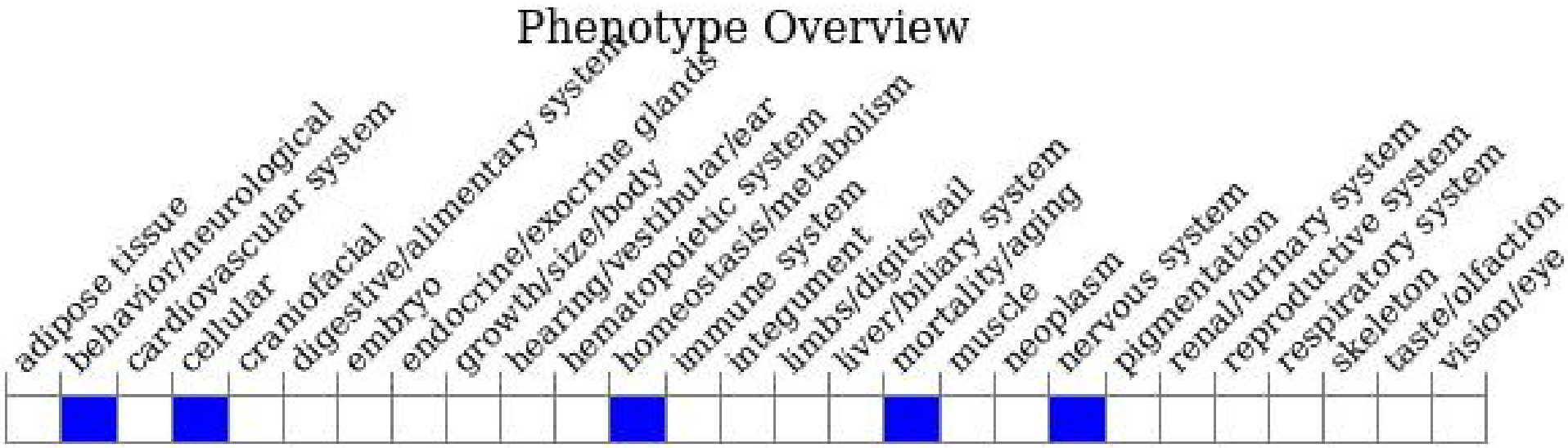
Source: : https://www.ensembl.org

# Mouse Phenotype Information (MGI)




- Normal phenotype is seen in one allele that disrupts the gene. Another knock-out allele shows high levels of upstream lysine metabolites in the brain and liver, abnormal amino acid metabolism, increased oxidative stress, and high lysine/low pyridoxine diet-induced seizures.

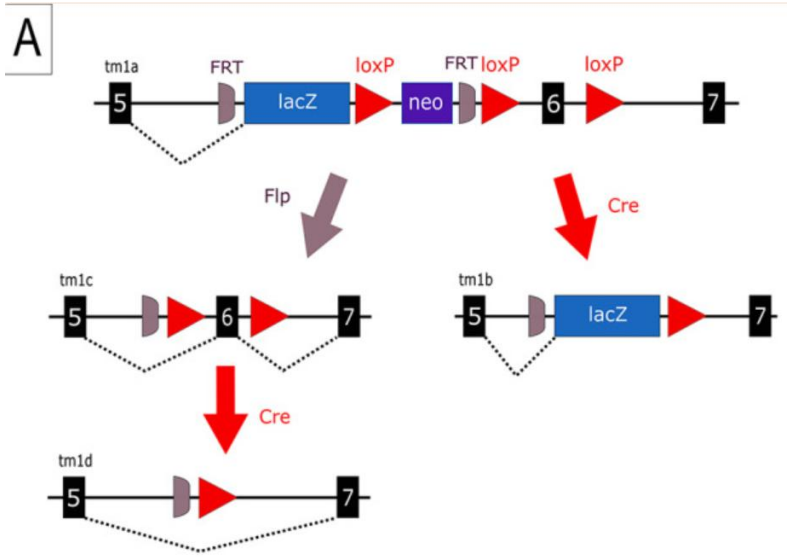
Source: https://www.informatics.jax.org

# Important Information

- According to the existing MGI data,normal phenotype is seen in one allele that disrupts the gene. Another knock-out allele shows high levels of upstream lysine metabolites in the brain and liver, abnormal amino acid metabolism, increased oxidative stress, and high lysine/low pyridoxine diet-induced seizures.
- The effect on transcript *Aldh7a1*-203&204 is unknown.
- *Aldh7a1* is located on Chr18. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.

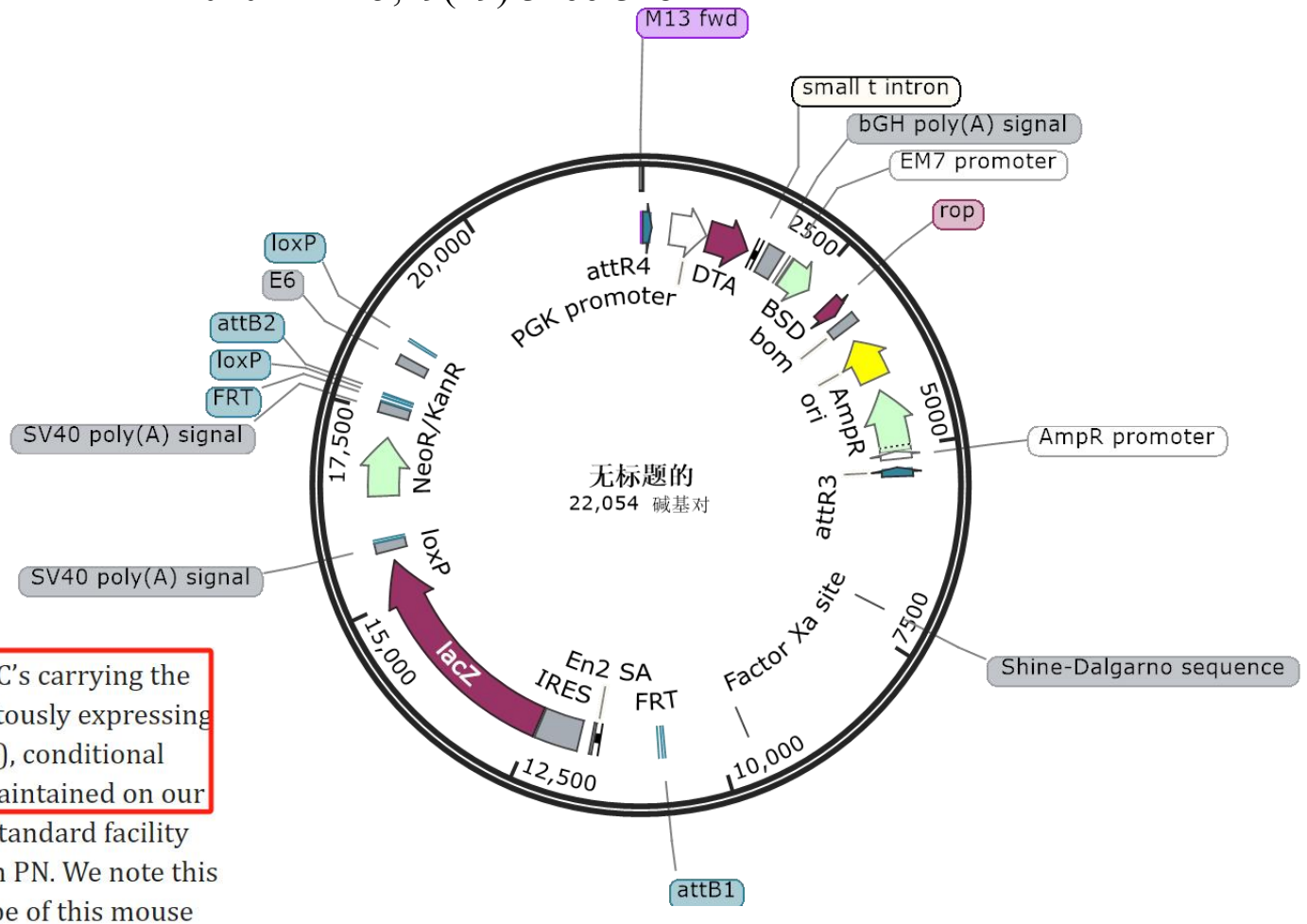
# Reference

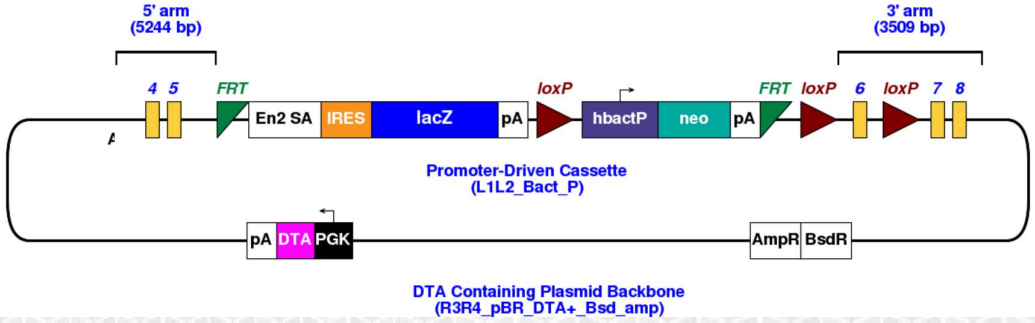
Al-Shekaili HH, et al., A novel mouse model for pyridoxine-dependent epilepsy due to antiquitin deficiency. Hum Mol Genet. 2020 Nov 25;29(19):3266-3284




Establishing *Aldh7a1*-targeted strains

We generated three *Aldh7a1*-targeted strains of mice by blastocyst injection of ESC's carrying the *Aldh7a1*$^{tm1a(EUCOMM)Hmgu}$ allele followed by sequential crossings with mice ubiquitously expressing Cre recombinase or FLPo recombinase, respectively: reporter-tagged (tm1b allele), conditional floxed (tm1c allele) and constitutive KO (tm1d allele) mice (Fig. 2A). Mice were maintained on our facility's standard chow (referred to as 'regular' diet hereafter in reference to its standard facility use and independent of nutrient content), which contains 0.9% lysine and 18 ppm PN. We note this up front as diet is subsequently shown to have a significant effect on the phenotype of this mouse model (Figs 6–8).

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