

# Mbd3 Cas9-CKO Strategy

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# Overview

## Target Gene Name

- Mbd3

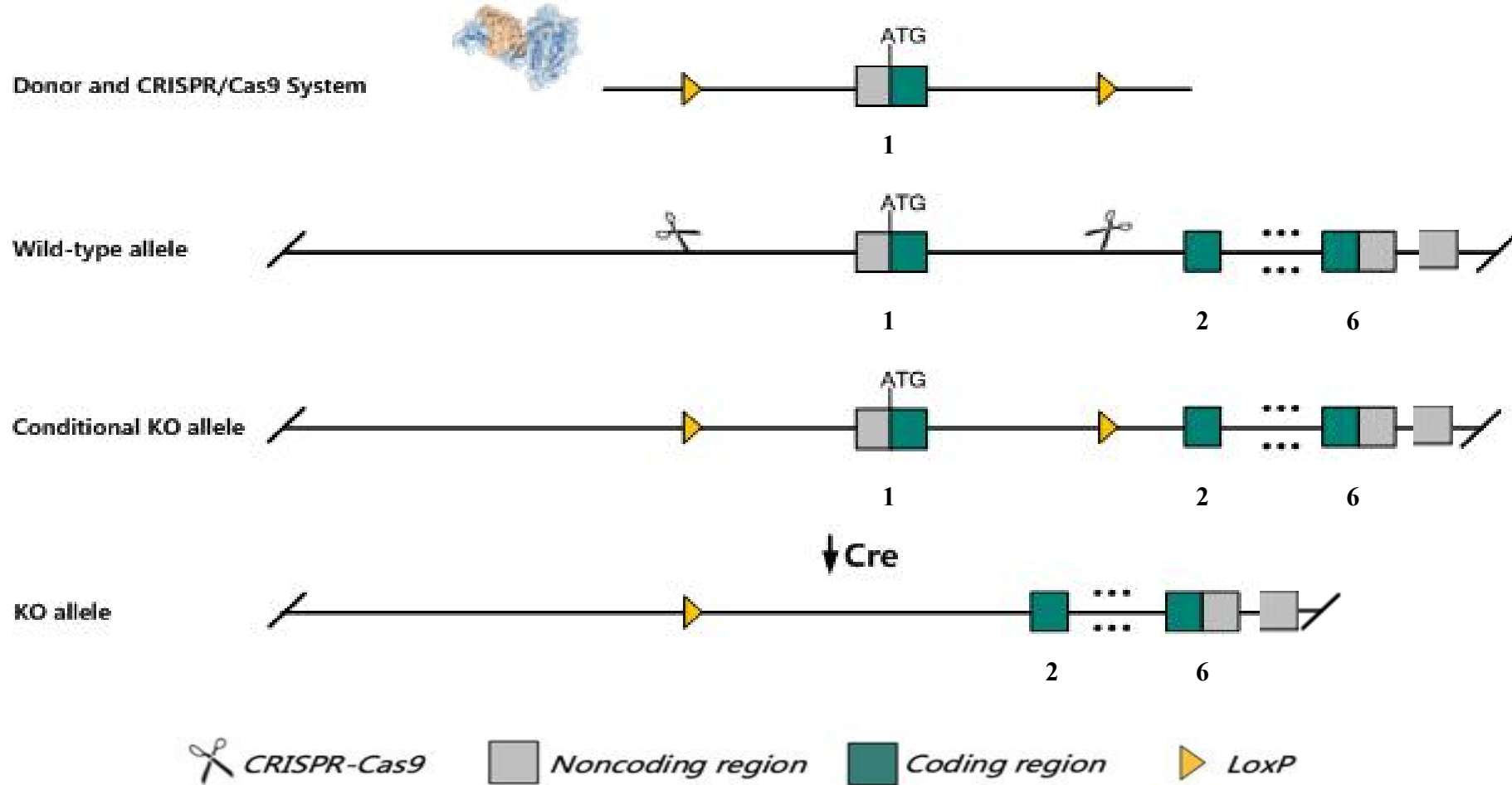
## Project Type

- Cas9-CKO

## Genetic Background

- C57BL/6JGpt

# Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Mbd3* gene.

# Technical Information

- The *Mbd3* gene has 6 transcripts. According to the structure of *Mbd3* gene, exon 1 of *Mbd3*-201 (ENSMUST00000092295.10) transcript is recommended as the knockout region. The region contains start codon ATG. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Mbd3* 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

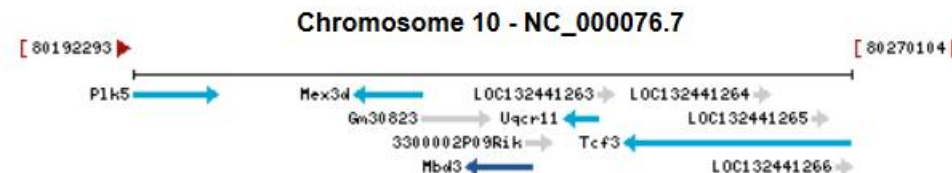
**Mbd3 methyl-CpG binding domain protein 3 [ *Mus musculus* (house mouse) ]**

[Download Datasets](#)

Gene ID: 17192, updated on 5-Sep-2025

## Summary

<b>Official Symbol</b>	Mbd3 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	methyl-CpG binding domain protein 3 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1333812</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000035478</a> <a href="#">AllianceGenome:MGI:1333812</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	REVIEWED
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Summary</b>	This gene encodes a member of the MBD family of nuclear proteins that contain a methyl-CpG binding domain (MBD). The encoded protein is a component of the nucleosome remodeling and histone deacetylation (NuRD) complex. Deletion of this gene causes embryonic lethality in mice. Embryonic stem cells lacking the encoded protein are severely compromised in their ability to differentiate and fail to commit to developmental lineages in the absence of leukemia inhibitory factor. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Apr 2015]
<b>Expression</b>	Ubiquitous expression in CNS E11.5 (RPKM 83.1), ovary adult (RPKM 79.9) and 28 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>



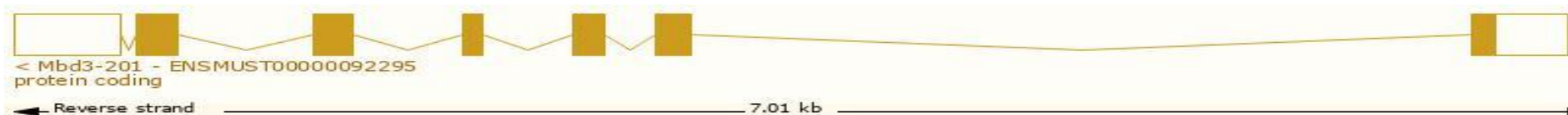
Source: <https://www.ncbi.nlm.nih.gov/>

# Transcript Information

The gene has 6 transcripts, all transcripts are shown below:

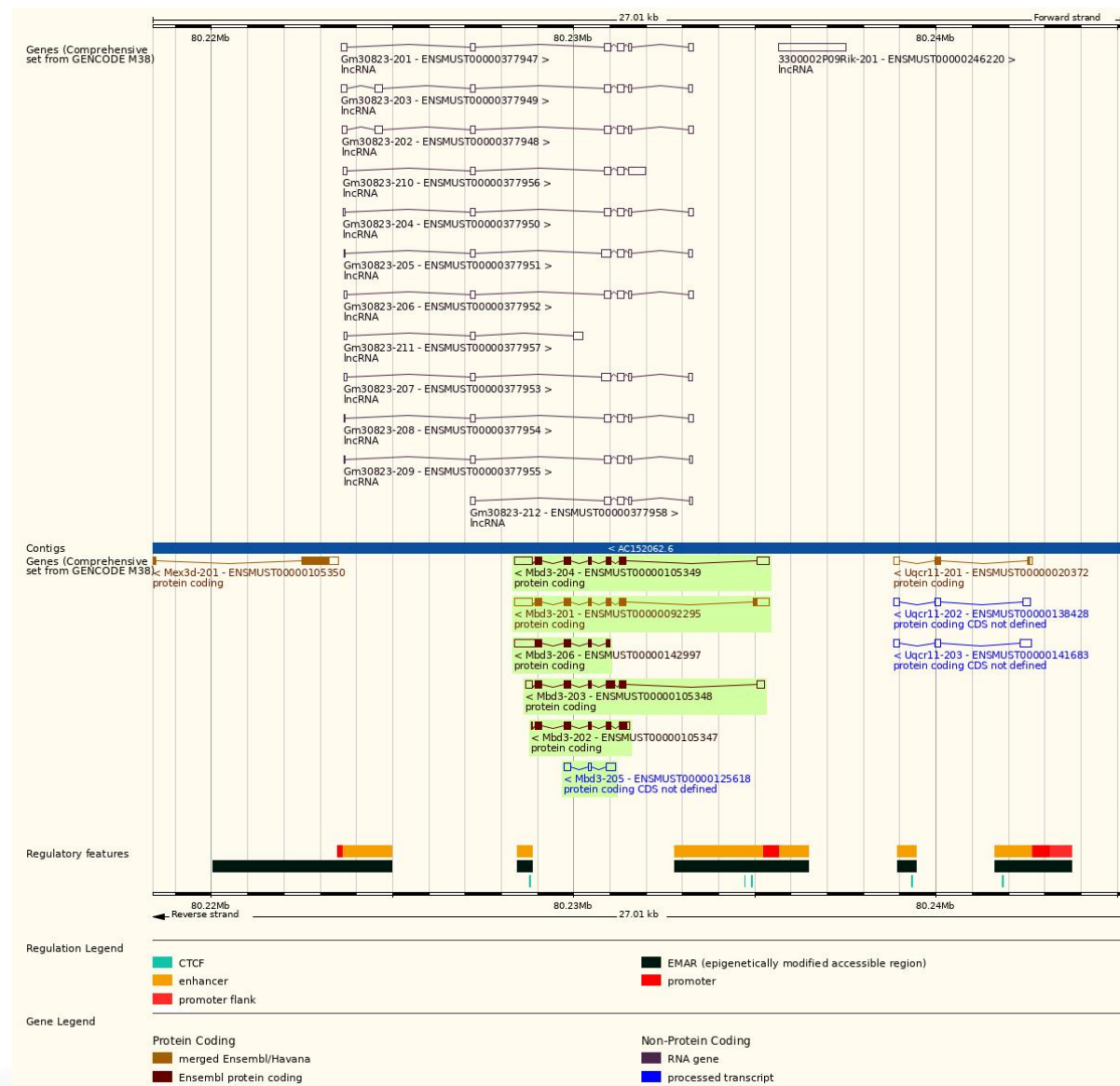
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
<a href="#">ENSMUST00000092295.10</a>	Mbd3-201	1662	<a href="#">285aa</a>	Protein coding	<a href="#">CCDS24020</a>	<a href="#">Q9Z2D8-1</a>	Ensembl Canonical Gencode Primary Gencode Basic APPRIS P1 TSL:1
<a href="#">ENSMUST00000105349.8</a>	Mbd3-204	1566	<a href="#">253aa</a>	Protein coding	<a href="#">CCDS78860</a>	<a href="#">Q9Z2D8-2</a>	Gencode Basic TSL:1
<a href="#">ENSMUST00000105348.8</a>	Mbd3-203	1222	<a href="#">281aa</a>	Protein coding		<a href="#">D3YTR4</a>	Gencode Basic TSL:5
<a href="#">ENSMUST00000142997.8</a>	Mbd3-206	1118	<a href="#">185aa</a>	Protein coding		<a href="#">F6WZH1</a>	TSL:2 CDS 5' incomplete
<a href="#">ENSMUST00000105347.2</a>	Mbd3-202	914	<a href="#">261aa</a>	Protein coding		<a href="#">D3YTR5</a>	Gencode Basic TSL:2
<a href="#">ENSMUST00000125618.2</a>	Mbd3-205	530	No protein	Protein coding CDS not defined		-	TSL:3

The strategy is based on the design of *Mbd3-201* transcript, the transcription is shown below:

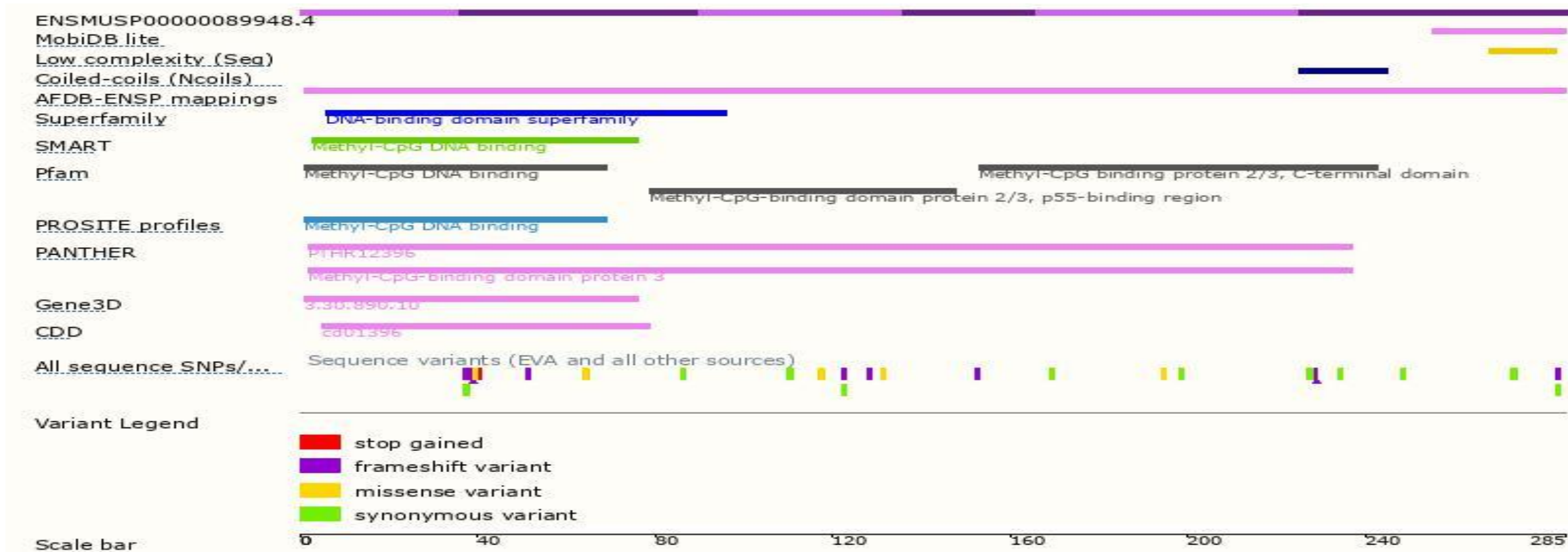


Source: <https://www.ensembl.org>

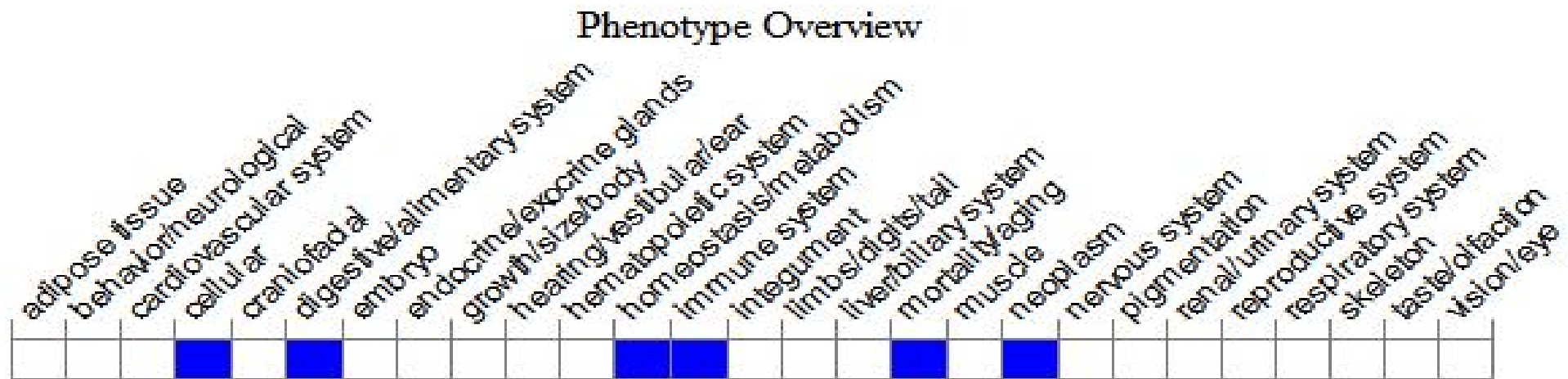
# Genomic Information



# Protein Information



# Mouse Phenotype Information (MGI)



- Mice homozygous for disruptions in this gene experience deficiencies as embryos around implantation and die before birth.

# Reference

This allele, in which exon 1 is flanked by loxP sites, was generated in embryonic stem (ES) cells heterozygous for Mbd3tm1Bh using a negative selection approach. First, a loxP-flanked hygromycin/thymidine kinase (hyg/tk) cassette was introduced into the wild-type allele upstream of exon 1. The ES cells were re-targeted with a vector having loxP sites flanking exon 1; two rounds of exposure to 6-thioguanine selected for cells in which the hyg/tk-tagged exon 1 had been replaced by the floxed exon. Immunoblot analysis of nuclear extracts of ES cells heterozygous for this floxed allele and the null Mbd3tm1Bh allele using antibody to MBD3 demonstrated expression of both normal protein isoforms, one of which (referred to as Mbda) contains the entire methyl-CpG-binding domain (MBD) and the other (Mbdb) lacking the N-terminal half of the MBD, encoded by exon 1. Following Cre recombinase-mediated excision of exon 1, neither isoform is detected.

## METHODS

**Cell culture and transfection.** Culture of embryonic stem cells and differentiation by retinoic acid was previously described<sup>25</sup>. Construction of *Mbd3*-null embryonic stem cells was performed as follows: a hygromycin–thymidine kinase cassette was integrated adjacent to exon 1 on the wild-type *Mbd3* allele of heterozygote M3β6C embryonic stem cells<sup>4</sup> (see Supplementary Information Fig. S1a). Cells were subsequently transfected with a floxed exon 1 construct, plated out at  $5 \times 10^4$ ,  $10^5$ ,  $2 \times 10^5$  and  $5 \times 10^5$  cells in a 150-mm dish, and 2.5 μM 6-thioguanine (Sigma–Aldrich, Gillingham, UK) was added to the media after 5–6 days. Surviving colonies were picked and expanded in the continued presence of selection. Clones were recovered in which the hygromycin–thymidine kinase-marked exon 1 had been replaced by the floxed exon 1 using homologous recombination and were referred to as *Mbd3*<sup>Flox/-</sup>. This protocol also allowed the recovery of clones in which no wild-type allele of *Mbd3* was detectable by Southern blotting (see Supplementary Information Fig. S1b). RT-PCR (data not shown) and western blotting (Fig. 1a) of nuclear extracts from two independent clones verified that these cell lines expressed no *Mbd3* transcript or protein, and are truly *Mbd3* null (*Mbd3*<sup>-/-</sup>). Two independent clones were found to be karyotypically normal (data not shown). We suggest that these cell lines have arisen through loss of the wild-type *Mbd3* allele (in this case containing the hygromycin–thymidine kinase insertion) and duplication of the βgeo containing (that is, null) allele, resulting in a homozygous null cell line. Subsequently, another null line was made by Cre-mediated deletion in an embryonic stem line in which the entire *Mbd3* gene is flanked by LoxP sites (see Supplementary Information, Fig. S1c, d). No differences in phenotype were detected between the *Mbd3*<sup>-/-</sup> line used in this study and the Cre-deleted line *Mbd3*<sup>Δ/-</sup>.

[1] Kaji K, et al., The NuRD component Mbd3 is required for pluripotency of embryonic stem cells. Nat Cell Biol. 2006 Mar;8(3):285-92.

# Important Information

- Based the data of MGI, mice homozygous for disruptions in this gene experience deficiencies as embryos around implantation and die before birth.
- The flox region will contain partial structure of the *3300002P09Rik* gene, and its impact on the gene's function remains unknown.
- The effect of this model on *Mbd3-202* and *Mbd3-206* transcripts is unknown, and there may be residual protein products from both transcripts.
- The *Uqcr11* and *Gm30823* genes are close to the knockout region, and this model may affect the functions of these two genes.
- *Mbd3* is located on Chr10. 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.