

# Mga Cas9-KO Strategy

Designer:Qiong Zhou

# **Project Overview**



Project Name Mga

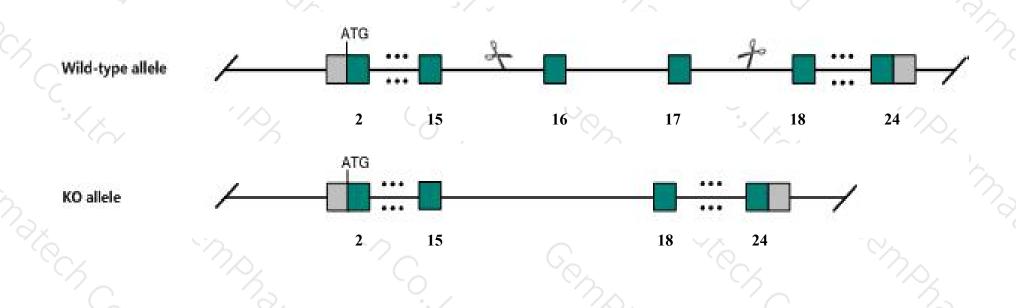
Project type Cas9-KO

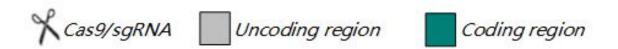
Strain background C57BL/6J

# **Knockout strategy**



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





### **Technical routes**



- ➤ The *Mga* gene has 15 transcripts. According to the structure of *Mga* gene, exon16-exon17 of *Mga-204*(ENSMUST00000110774.7) transcript is recommended as the knockout region. The region contains 1760bp coding sequence Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Mga* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

### **Notice**



- ➤ According to the existing MGI data, Embryos homozygous for a gene trap allele die shortly after implantation due to defective development of the inner cell mass (ICM) and the epiblast. ICM derivatives fail to develop past E4.5 and show increased apoptosis but no change in cell proliferation.
- ➤ The Mga gene is located on the Chr2. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

### Gene information (NCBI)



#### Mga MAX gene associated [Mus musculus (house mouse)]

Gene ID: 29808, updated on 31-Jan-2019

#### Summary

↑ ?

Official Symbol Mga provided by MGI

Official Full Name MAX gene associated provided by MGI

Primary source MGI:MGI:1352483

See related Ensembl:ENSMUSG00000033943

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 AV312082, C130042M01Rik, C80739, D030062C11Rik, Mad5

Expression Ubiquitous expression in limb E14.5 (RPKM 3.0), CNS E11.5 (RPKM 2.8) and 26 other tissuesSee more

Orthologs <u>human all</u>

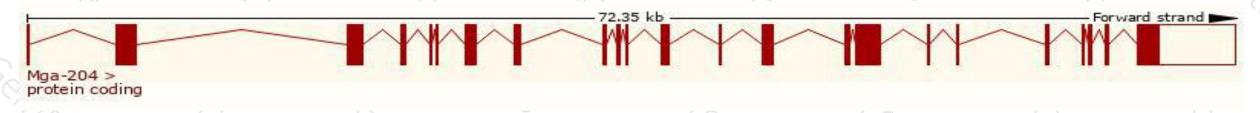
# Transcript information (Ensembl)



#### The gene has 15 transcripts, all transcripts are shown below:

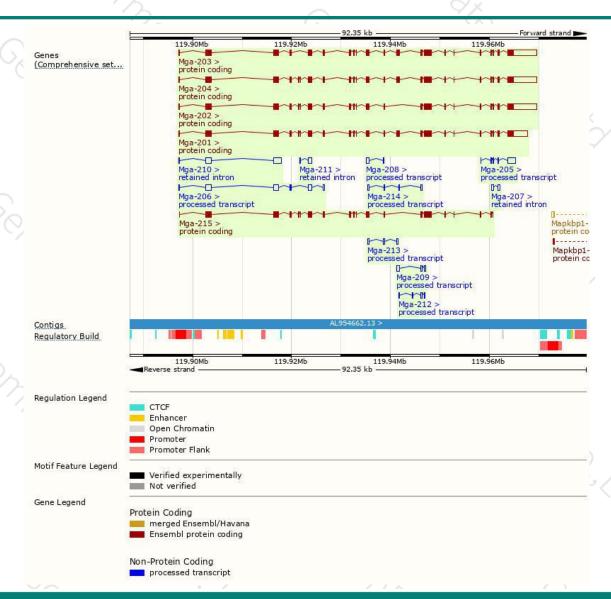
Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
ENSMUST00000110774.7	13931	3042aa	Protein coding	CCDS38208	E9QLG3	TSL:5 GENCODE basic APPRIS P3	
ENSMUST00000079934.11	13304	2833aa	Protein coding	CCDS50675	H7BX50	TSL:5 GENCODE basic APPRIS ALT2	
ENSMUST00000110773.8	13577	2924aa	Protein coding	140	A2AWL7	TSL:5 GENCODE basic APPRIS ALT2	
ENSMUST00000046717.12	11982	3003aa	Protein coding	750	A2AWL7	TSL:5 GENCODE basic APPRIS ALT2	
ENSMUST00000156510.1	6995	2295aa	Protein coding	-	A2AWL7	CDS 3' incomplete TSL:1	
ENSMUST00000129405.1	3317	No protein	Processed transcript	-	#d	TSL:1	
ENSMUST00000122889.1	2110	No protein	Processed transcript	120	20	TSL:1	
ENSMUST00000138851.7	1264	No protein	Processed transcript	750	20	TSL:1	
ENSMUST00000156074.1	864	No protein	Processed transcript	-	54	TSL:3	
ENSMUST00000132054.1	739	No protein	Processed transcript		-ts	TSL:3	
ENSMUST00000152148.1	739	No protein	Processed transcript	120	20	TSL:3	
ENSMUST00000151074.1	649	No protein	Processed transcript	750	20	TSL:3	
ENSMUST00000141776.7	2763	No protein	Retained intron		-	TSL:1	
ENSMUST00000131396.1	985	No protein	Retained intron	-	<del>-</del> 11	TSL:2	
ENSMUST00000150742.1	825	No protein	Retained intron	020	20	TSL:2	
	ENSMUST00000110774.7  ENSMUST00000079934.11  ENSMUST000000110773.8  ENSMUST00000046717.12  ENSMUST00000156510.1  ENSMUST00000129405.1  ENSMUST0000012989.1  ENSMUST00000138851.7  ENSMUST00000136074.1  ENSMUST00000132054.1  ENSMUST00000152148.1  ENSMUST00000151074.1  ENSMUST00000141776.7  ENSMUST00000131396.1	ENSMUST00000110774.7 13931 ENSMUST00000079934.11 13304 ENSMUST000000110773.8 13577 ENSMUST00000046717.12 11982 ENSMUST00000156510.1 6995 ENSMUST00000129405.1 3317 ENSMUST00000122889.1 2110 ENSMUST00000138851.7 1264 ENSMUST00000138851.7 1264 ENSMUST00000156074.1 864 ENSMUST0000015074.1 739 ENSMUST00000152148.1 739 ENSMUST00000151074.1 649 ENSMUST00000141776.7 2763 ENSMUST00000131396.1 985	ENSMUST00000110774.7         13931         3042aa           ENSMUST00000079934.11         13304         2833aa           ENSMUST000000110773.8         13577         2924aa           ENSMUST00000046717.12         11982         3003aa           ENSMUST00000156510.1         6995         2295aa           ENSMUST00000129405.1         3317         No protein           ENSMUST00000122889.1         2110         No protein           ENSMUST00000138851.7         1264         No protein           ENSMUST00000156074.1         864         No protein           ENSMUST00000152148.1         739         No protein           ENSMUST00000151074.1         649         No protein           ENSMUST00000141776.7         2763         No protein           ENSMUST00000131396.1         985         No protein	ENSMUST00000110774.7         13931         3042aa         Protein coding           ENSMUST00000079934.11         13304         2833aa         Protein coding           ENSMUST00000110773.8         13577         2924aa         Protein coding           ENSMUST00000046717.12         11982         3003aa         Protein coding           ENSMUST00000156510.1         6995         2295aa         Protein coding           ENSMUST00000129405.1         3317         No protein         Processed transcript           ENSMUST00000122889.1         2110         No protein         Processed transcript           ENSMUST00000138851.7         1264         No protein         Processed transcript           ENSMUST00000156074.1         864         No protein         Processed transcript           ENSMUST00000132054.1         739         No protein         Processed transcript           ENSMUST00000152148.1         739         No protein         Processed transcript           ENSMUST00000141776.7         2763         No protein         Retained intron           ENSMUST00000131396.1         985         No protein         Retained intron	ENSMUST00000110774.7         13931         3042aa         Protein coding         CCDS38208           ENSMUST00000079934.11         13304         2833aa         Protein coding         CCDS50675           ENSMUST00000110773.8         13577         2924aa         Protein coding         -           ENSMUST00000046717.12         11982         3003aa         Protein coding         -           ENSMUST00000156510.1         6995         2295aa         Protein coding         -           ENSMUST00000129405.1         3317         No protein         Processed transcript         -           ENSMUST00000122869.1         2110         No protein         Processed transcript         -           ENSMUST00000138851.7         1264         No protein         Processed transcript         -           ENSMUST00000156074.1         864         No protein         Processed transcript         -           ENSMUST00000152148.1         739         No protein         Processed transcript         -           ENSMUST00000151074.1         649         No protein         Processed transcript         -           ENSMUST00000141776.7         2763         No protein         Retained intron         -           ENSMUST00000131396.1         985         No protein         Reta	ENSMUST00000110774.7         13931         3042aa         Protein coding         CCDS38208         E9OLG3           ENSMUST00000079934.11         13304         2833aa         Protein coding         CCDS50675         H7BX50           ENSMUST00000110773.8         13577         2924aa         Protein coding         -         A2AWL7           ENSMUST00000046717.12         11982         3003aa         Protein coding         -         A2AWL7           ENSMUST00000129405.1         6995         2295aa         Protein coding         -         A2AWL7           ENSMUST00000129405.1         3317         No protein         Processed transcript         -         -           ENSMUST00000122889.1         2110         No protein         Processed transcript         -         -           ENSMUST00000138851.7         1264         No protein         Processed transcript         -         -           ENSMUST00000156074.1         864         No protein         Processed transcript         -         -           ENSMUST00000152148.1         739         No protein         Processed transcript         -         -           ENSMUST00000151074.1         649         No protein         Retained intron         -         -           ENSMUST00000131396.1<	

The strategy is based on the design of Mga-204 transcript, The transcription is shown below



### Genomic location distribution





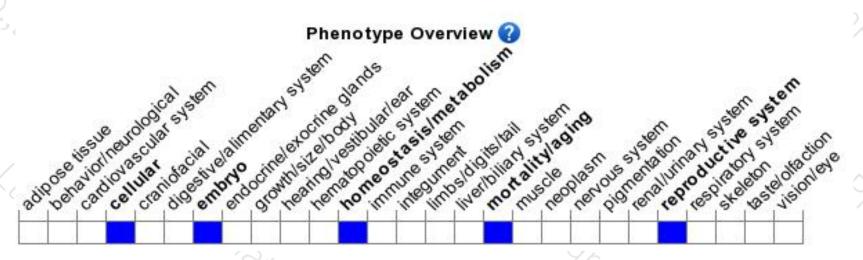
### Protein domain





## 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, Embryos homozygous for a gene trap allele die shortly after implantation due to defective development of the inner cell mass (ICM) and the epiblast. ICM derivatives fail to develop past E4.5 and show increased apoptosis but no change in cell proliferation.



If you have any questions, you are welcome to inquire.

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