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Models to Accelerate Innovation

Myh6-IRES-iCre Mouse Model Strategy
CRISPR-Cas9 technology

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Reviewer Daohua Xu

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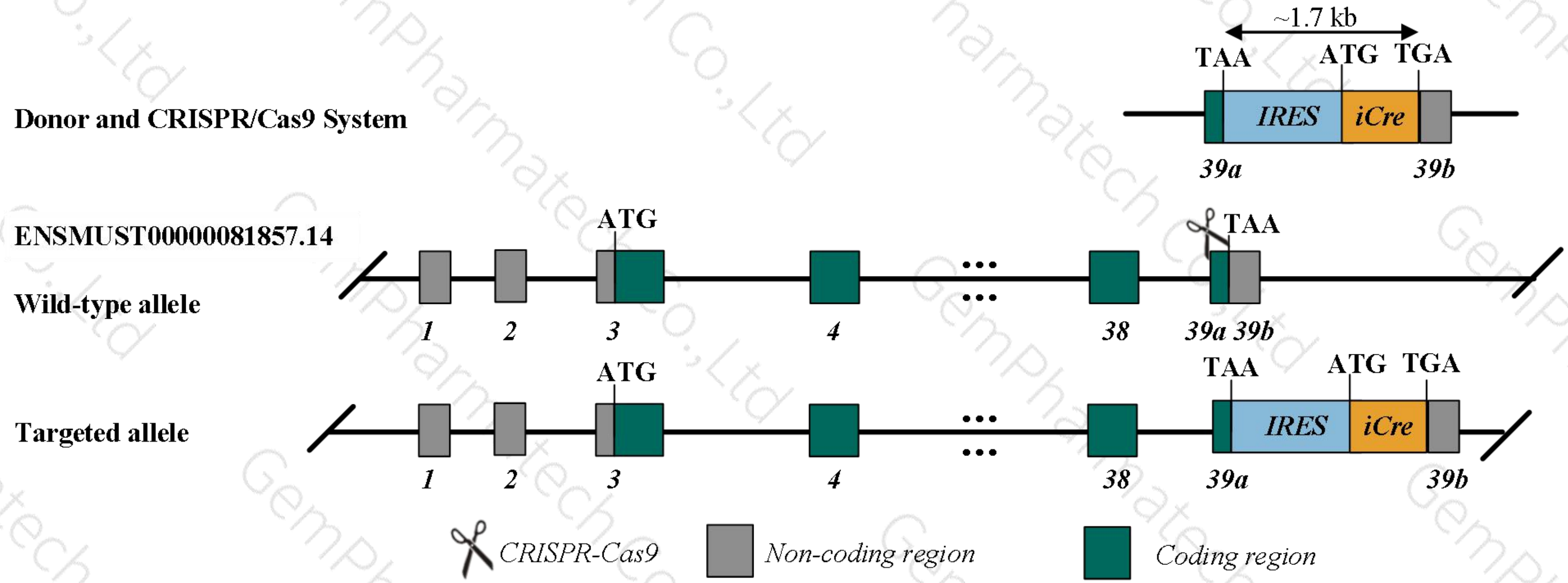
Project Overview

Project Name	***Myh6-IRES-iCre***
Project Type	**Cas9-KI**
Background	**C57BL/6JGpt**

Strategy

This model will use CRISPR-Cas9 technology to edit the mouse *Myh6* gene and the schematic diagram is as follows:



Technical Description

- The mouse *Myh6* gene has 7 transcripts.According to the structure of *Myh6* gene,*Myh6-201*(ENSMUST00000081857.14)transcript is selected for this strategy.The transcript of *Myh6-201* contains 39 exons,codes 1938 aa,the ATG is located in exon 3,and the TAA is located in exon 39.
- We constructed CRISPR/Cas9 system targeting mouse *Myh6* gene and donor vector,*IRES-iCre* will be introduced to near the TAA of mouse *Myh6* gene.The *iCre* will be expressed under the direction of endogenous regulatory mechanism.
- The project will use CRISPR/Cas9 technology to modify *Myh6* 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

Note

- According to the existing MGI data,mice homozygous for a knock-out allele exhibit embryonic lethality associated with heart defects whileheterozygotes show cardiac myofibrillar disarray, cardiac dysfunction and fibrosis. Mice heterozygous for different knock-in alleles may develop hypertrophic or dilated forms of cardiomyopathy.
- The IRES-linked genes will be transcripted together and then be translated two protein separately,but the downstream protein is lower than the upstream protein.
- The transcripts *Myh6-202* and *Myh6-203* are 3' incomplete, and the effect of *IRES-iCre* insertion on them is unknown.
- The insertion site is about 2.7 kb from the 3' end of the *Cmtm5* gene, and the fragment insertion might affect its function.
- The insertion site is within the intron 2-3 of gene *Gm49130*, and fragment insertion may be its function.
- Insertion of *IRES-iCre* may affect the regulation of the 3' end of the *Myh6* gene.
- There may be 2 to 4 bases mutation in exon 39 of *Myh6* gene in this strategy.
- The *Myh6* gene is located on the Chr14. 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 the genetic information available in existing databases. The insertion of foreign sequences between the coding region of a gene and its UTR may affect the biological processes such as gene expression and mRNA splicing. Under the current technology level, all risks cannot be predicted, and further experiments are recommended after verifying that the expression is consistent with expectations.

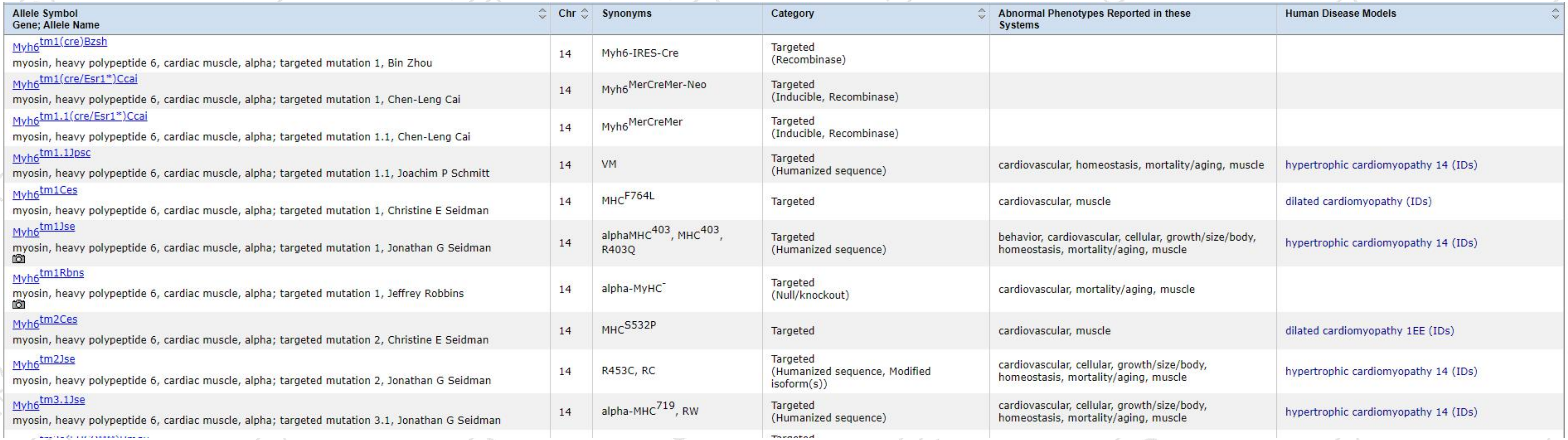
iCre Sequence (1056 bp)

Coding Sequence of Codon-Optimized Cre Gene[1].

ATGGTGCCCAAGAAGAAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGGATGCCACCTCT
GATGAAGTCAGGAAGAACCTGATGGACATGTTCAGGGACAGGCAGGCCTTCTCTGAACACACCTGGAAGATGCTCCTGTCTGTG
TGCAGATCCTGGGCTGCCTGGTGCAAGCTGAACAACAGGAAATGGTTCCCTGCTGAACCTGAGGATGTGAGGGACTACCTCCTG
TACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACAGCACCTGGGCCAGCTCAACATGCTGCACAGGAGATCTGGCCTG
CCTCGCCCTTCTGACTCCAATGCTGTGTCCCTGGTGATGAGGAGAATCAGAAAGGAGAATGTGGATGCTGGGGAGAGAGCCAAG
CAGGCCCTGGCCTTTGAACGCACTGACTTTGACCAAGTCAGATCCCTGATGGAGAACTCTGACAGATGCCAGGACATCAGGAAC
CTGGCCTTCCTGGGCATTGCCTACAACACCCTGCTGCGCATTGCCGAAATTGCCAGAATCAGAGTGAAGGACATCTCCCGCACCG
ATGGTGGGAGAATGCTGATCCACATTGGCAGGACCAAGACCCTGGTGTCCACAGCTGGTGTGGAGAAGGCCCTGTCCCTGGGG
TTACCAAGCTGGTGGAGAGATGGATCTCTGTGTCTGGTGTGGCTGATGACCCCAACAACTACCTGTTCTGCCGGGTCAGAAAGA
ATGGTGTGGCTGCCCCTTCTGCCACCTCCCAACTGTCCACCCGGGCCCTGGAAGGGATCTTTGAGGCCACCCACCGCCTGATCTA
TGGTGCCAAGGATGACTCTGGGCAGAGATACCTGGCCTGGTCTGGCCACTCTGCCAGAGTGGGTGCTGCCAGGGACATGGCCAG
GGCTGGTGTGTCCATCCCTGAAATCATGCAGGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCAGAAACCTGGAC
TCTGAGACTGGGGCCATGGTGAGGCTGCTCGAGGATGGGGACTGA

[1]Shimshek DR, Kim J, Hübner MR, Spergel DJ. Codon-improved Cre recombinase (iCre) expression in the mouse. Genesis.2002 Jan.32(1):19-26.

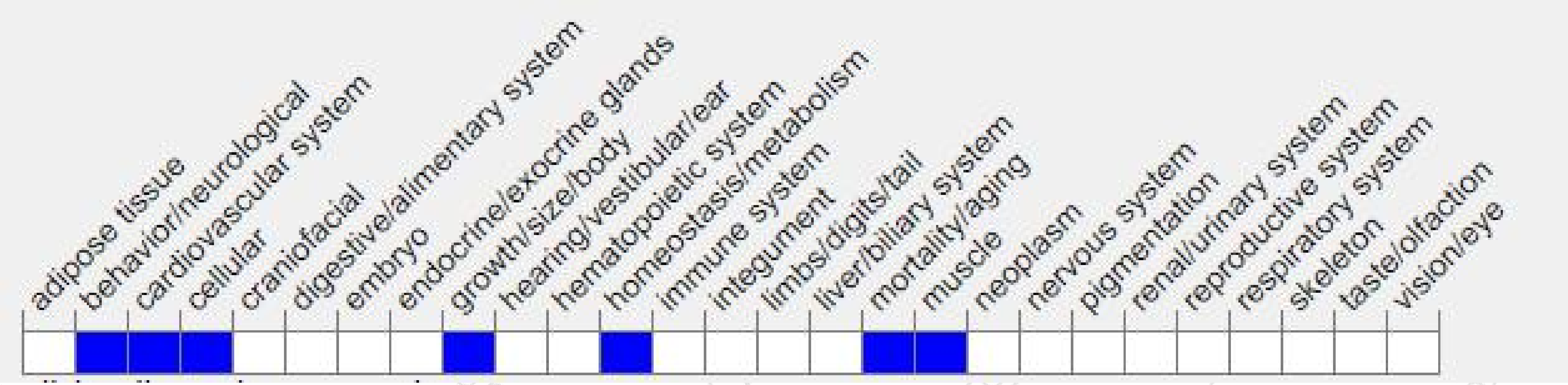
Existing model information



Allele Symbol Gene; Allele Name	Chr	Synonyms	Category	Abnormal Phenotypes Reported in these Systems	Human Disease Models
Myh6$^{tm1(cre)Bzsh}$ myosin, heavy polypeptide 6, cardiac muscle, alpha; targeted mutation 1, Bin Zhou	14	Myh6-IRES-Cre	Targeted (Recombinase)		
Myh6$^{tm1(cre/Esr1*)Ccai}$ myosin, heavy polypeptide 6, cardiac muscle, alpha; targeted mutation 1, Chen-Leng Cai	14	Myh6$^{MerCreMer-Neo}$	Targeted (Inducible, Recombinase)		
Myh6$^{tm1.1(cre/Esr1*)Ccai}$ myosin, heavy polypeptide 6, cardiac muscle, alpha; targeted mutation 1.1, Chen-Leng Cai	14	Myh6^{MerCreMer}	Targeted (Inducible, Recombinase)		
Myh6$^{tm1.1Jpsc}$ myosin, heavy polypeptide 6, cardiac muscle, alpha; targeted mutation 1.1, Joachim P Schmitt	14	VM	Targeted (Humanized sequence)	cardiovascular, homeostasis, mortality/aging, muscle	hypertrophic cardiomyopathy 14 (IDs)
Myh6^{tm1Ces} myosin, heavy polypeptide 6, cardiac muscle, alpha; targeted mutation 1, Christine E Seidman	14	MHC^{F764L}	Targeted	cardiovascular, muscle	dilated cardiomyopathy (IDs)
Myh6^{tm1Jse} myosin, heavy polypeptide 6, cardiac muscle, alpha; targeted mutation 1, Jonathan G Seidman 📷	14	alphaMHC^{403}, MHC^{403}, R403Q	Targeted (Humanized sequence)	behavior, cardiovascular, cellular, growth/size/body, homeostasis, mortality/aging, muscle	hypertrophic cardiomyopathy 14 (IDs)
Myh6^{tm1Rbns} myosin, heavy polypeptide 6, cardiac muscle, alpha; targeted mutation 1, Jeffrey Robbins 📷	14	alpha-MyHC^{-}	Targeted (Null/knockout)	cardiovascular, mortality/aging, muscle	
Myh6^{tm2Ces} myosin, heavy polypeptide 6, cardiac muscle, alpha; targeted mutation 2, Christine E Seidman	14	MHC^{S532P}	Targeted	cardiovascular, muscle	dilated cardiomyopathy 1EE (IDs)
Myh6^{tm2Jse} myosin, heavy polypeptide 6, cardiac muscle, alpha; targeted mutation 2, Jonathan G Seidman	14	R453C, RC	Targeted (Humanized sequence, Modified isoform(s))	cardiovascular, cellular, growth/size/body, homeostasis, mortality/aging, muscle	hypertrophic cardiomyopathy 14 (IDs)
Myh6$^{tm3.1Jse}$ myosin, heavy polypeptide 6, cardiac muscle, alpha; targeted mutation 3.1, Jonathan G Seidman	14	alpha-MHC^{719}, RW	Targeted (Humanized sequence)	cardiovascular, cellular, growth/size/body, homeostasis, mortality/aging, muscle	hypertrophic cardiomyopathy 14 (IDs)

Source: http://www.informatics.jax.org/allele/summary?markerId=MGI:97255&alleleType=Targeted

Mouse Phenotype Information (MGI)



adipose tissue	behavior/neurological	cardiovascular system	cellular	craniofacial	digestive/alimentary system	embryo	endocrine/exocrine glands	growth/size/body	hearing/vestibular/ear	hematopoietic system	homeostasis/metabolism	immune system	integument	limbs/digits/tail	liver/biliary system	mortality/aging	muscle	neoplasm	nervous system	pigmentation	renal/urinary system	reproductive system	respiratory system	skeleton	taste/olfaction	vision/eye
	■	■	■					■			■					■	■									

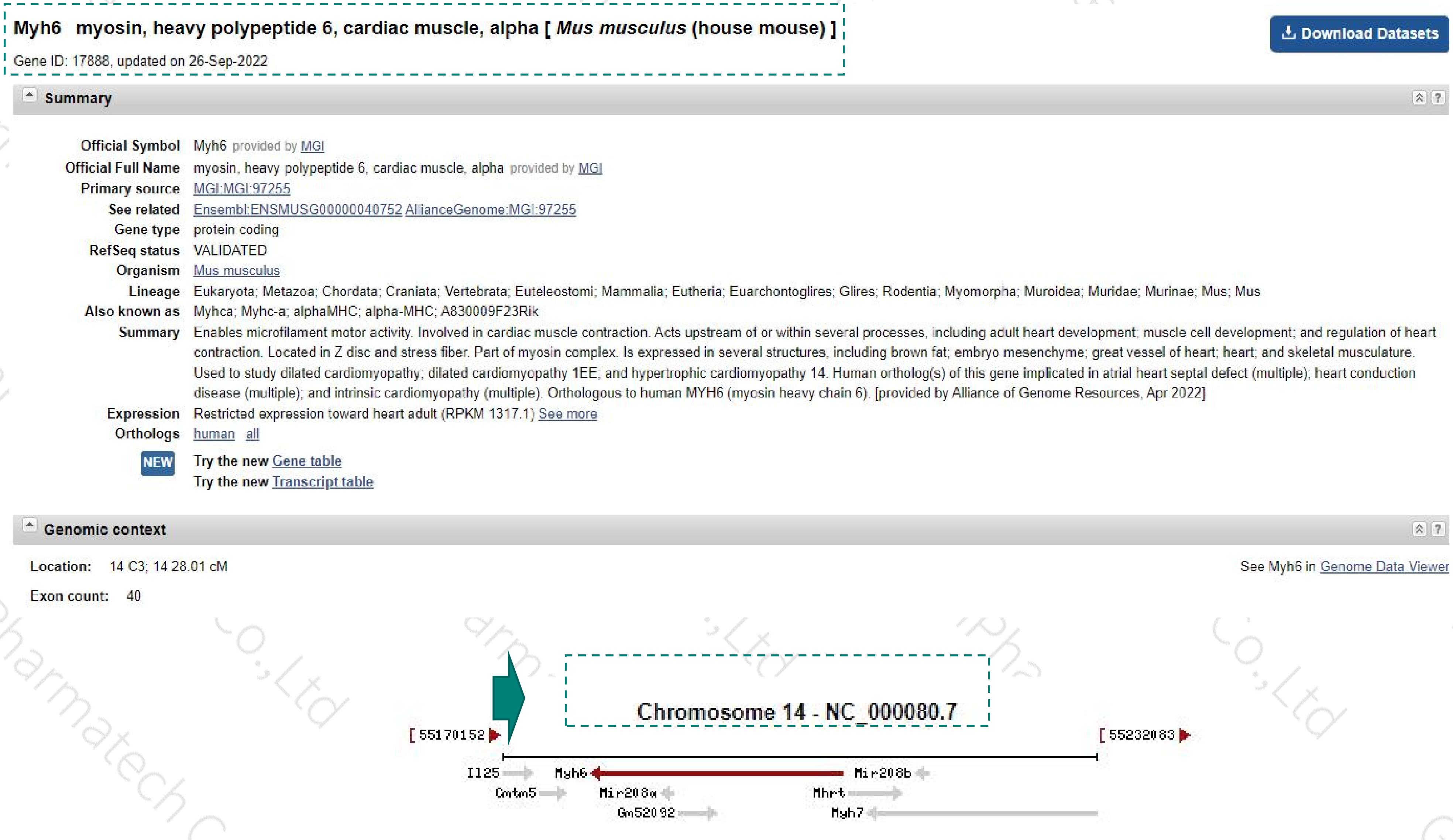
Mice homozygous for a knock-out allele exhibit embryonic lethality associated with heart defects while heterozygotes show cardiac myofibrillar disarray, cardiac dysfunction and fibrosis. Mice heterozygous for different knock-in alleles may develop hypertrophic or dilated forms of cardiomyopathy.

Source: http://www.informatics.jax.org/marker/MGI:97255

Target Gene

Gene name	mouse *Myh6*
Gene ID (NCBI)	17888
Gene link (NCBI)	https://www.ncbi.nlm.nih.gov/gene/17888
Gene link (Ensembl)	http://uswest.ensembl.org/Mus_musculus/Gene/Summary?db=core;g=ENSMUSG00000040752;r=14:55179378-55204384
Chromosome location	Chr14

Gene Information (NCBI)



Myh6 myosin, heavy polypeptide 6, cardiac muscle, alpha [*Mus musculus* (house mouse)]

Gene ID: 17888, updated on 26-Sep-2022

Summary

Official Symbol	Myh6 provided by MGI
Official Full Name	myosin, heavy polypeptide 6, cardiac muscle, alpha provided by MGI
Primary source	MGI:MGI:97255
See related	Ensembl:ENSMUSG00000040752 AllianceGenome:MGI:97255
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	Myhca; Myhc-a; alphaMHC; alpha-MHC; A830009F23Rik
Summary	Enables microfilament motor activity. Involved in cardiac muscle contraction. Acts upstream of or within several processes, including adult heart development; muscle cell development; and regulation of heart contraction. Located in Z disc and stress fiber. Part of myosin complex. Is expressed in several structures, including brown fat; embryo mesenchyme; great vessel of heart; heart; and skeletal musculature. Used to study dilated cardiomyopathy; dilated cardiomyopathy 1EE; and hypertrophic cardiomyopathy 14. Human ortholog(s) of this gene implicated in atrial heart septal defect (multiple); heart conduction disease (multiple); and intrinsic cardiomyopathy (multiple). Orthologous to human MYH6 (myosin heavy chain 6). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Restricted expression toward heart adult (RPKM 1317.1) See more
Orthologs	human all
NEW	**Try the new** Gene table **Try the new** Transcript table

Genomic context

Location: 14 C3; 14 28.01 cM

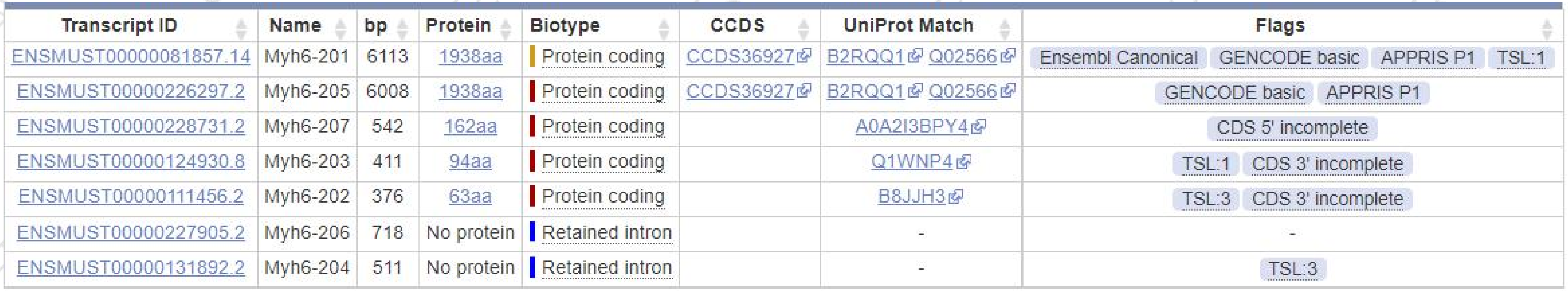
Exon count: 40

See Myh6 in Genome Data Viewer

Chromosome 14 - NC_000080.7

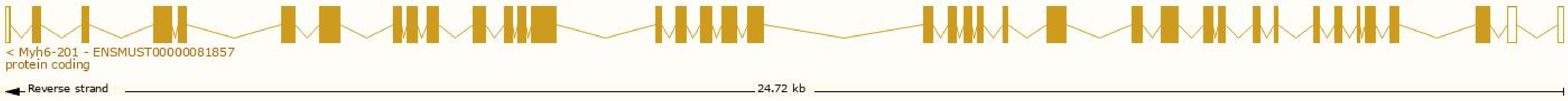
Transcript Information (Ensembl)

The gene has 7 transcripts, as shown below:

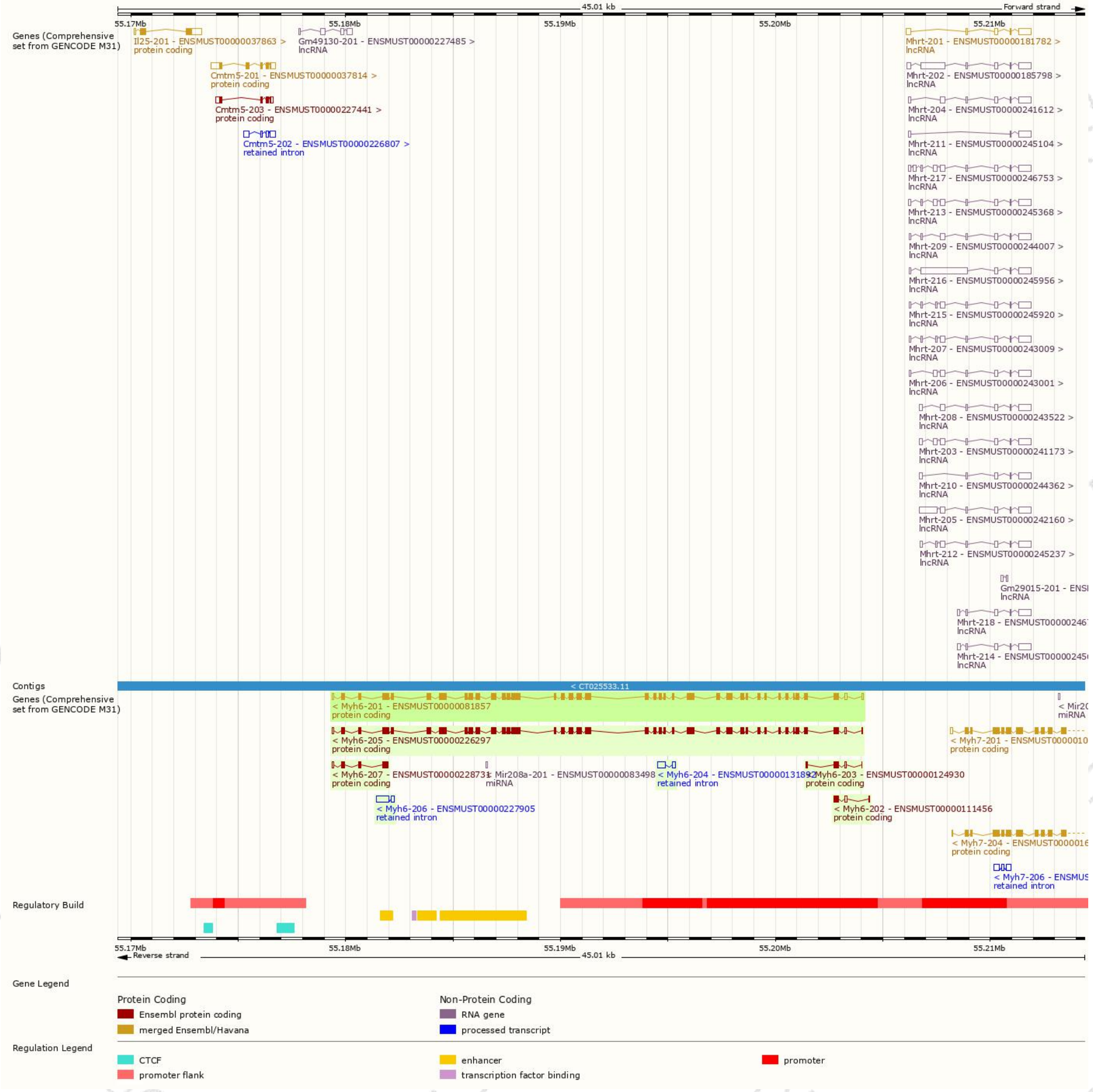


Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000081857.14	Myh6-201	6113	1938aa	Protein coding	CCDS36927	B2RQQ1 Q02566	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000226297.2	Myh6-205	6008	1938aa	Protein coding	CCDS36927	B2RQQ1 Q02566	GENCODE basic APPRIS P1
ENSMUST00000228731.2	Myh6-207	542	162aa	Protein coding		A0A2I3BPY4	CDS 5' incomplete
ENSMUST00000124930.8	Myh6-203	411	94aa	Protein coding		Q1WNP4	TSL:1 CDS 3' incomplete
ENSMUST00000111456.2	Myh6-202	376	63aa	Protein coding		B8JJH3	TSL:3 CDS 3' incomplete
ENSMUST00000227905.2	Myh6-206	718	No protein	Retained intron		-	-
ENSMUST00000131892.2	Myh6-204	511	No protein	Retained intron		-	TSL:3

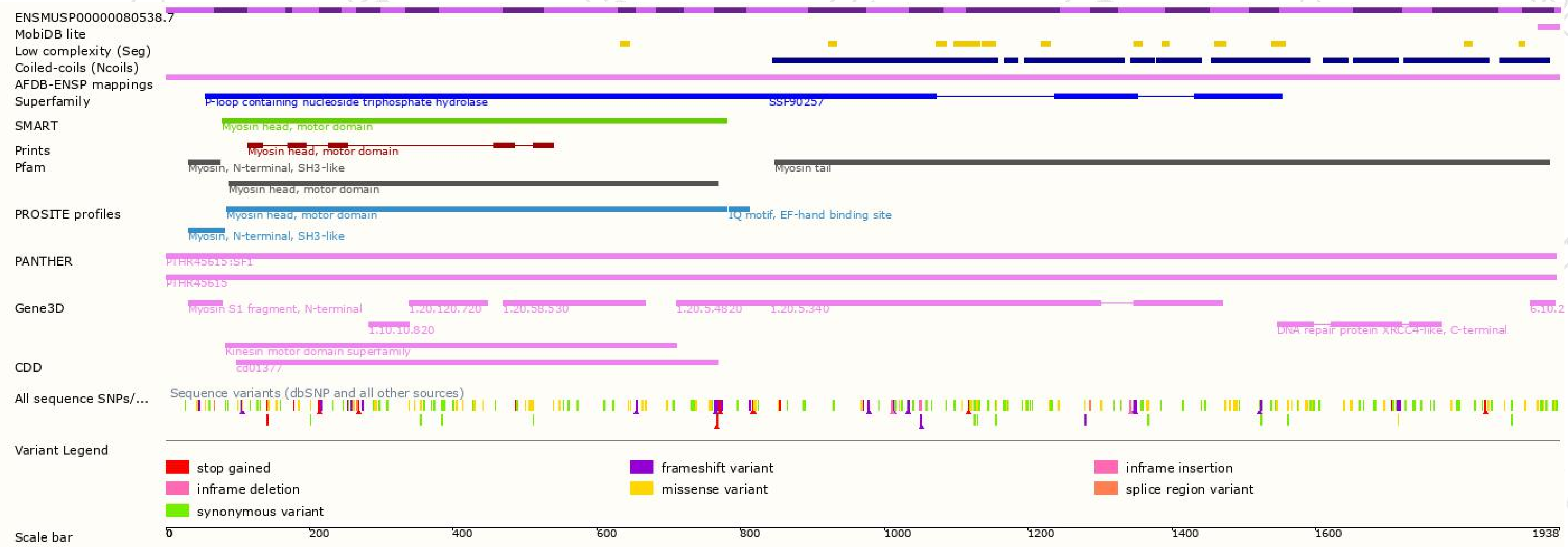
The strategy is based on *Myh6-201*(ENSMUST00000081857.14), which contains 39 exons, is 6113 bps long, and encodes 1938 amino acids.



Genomic Information



Protein domain



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