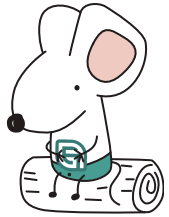
集萃药康
GemPharmatech



Pafah1b1-c.G661A基因突变小鼠模型制作方案

设计人：于佳
审核人：沈彦花
日期：2022年3月29日

Models To Defy Impossible 创新模型 创造可能

项目概览

Contents

一、项目名称：Pafah1b1-c.G661A

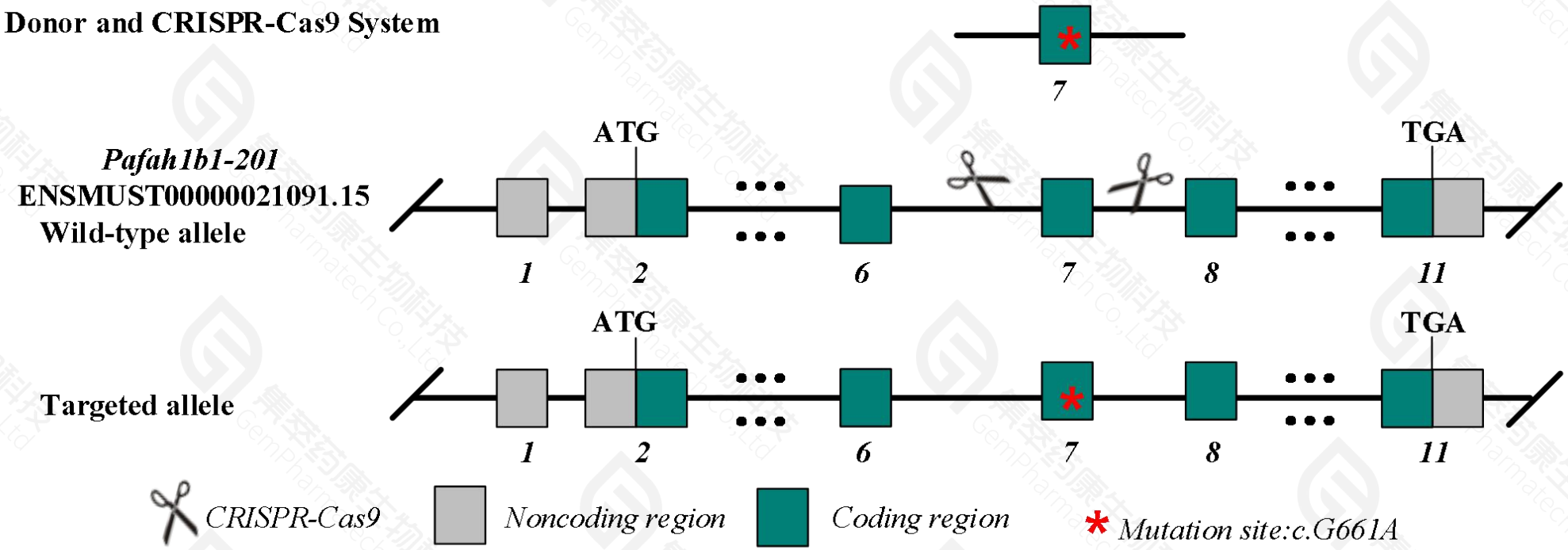
二、项目类型：Cas9-KI

三、遗传背景：C57BL/6JGpt

四、项目周期：5-8个月

敲入策略

此模型采用CRISPR-Cas9技术对*Pafah1b1*基因进行基因编辑，原理示意图如下：



技术路线

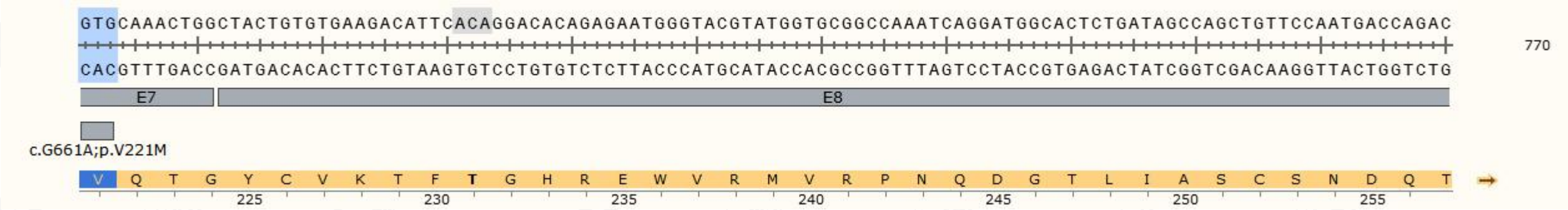
- Ensembl数据显示，鼠源*Pafah1b1*基因有5个转录本。
- 根据基因结构和客户要求，选择人源*PAFAH1B1-202*(ENST00000397195.10，NM_000430.4)，鼠源*Pafah1b1-201*(ENSMUST00000021091.15)转录本设计方案，即在鼠源 *Pafah1b1-201*(ENSMUST00000021091.15)转录本的exon 7制作c.G661A定点突变，对应的氨基酸由V突变成M。
- *Pafah1b1-201*转录本包含11个exons，翻译起始位点ATG位于exon 2， 翻译终止位点TGA位于exon 11，编码410 aa。
- 本项目采用CRISPR-Cas9技术对*Pafah1b1*基因进行修饰。简要过程如下：体外构建载体，将CRISPR-Cas9和Donor载体显微注射到C57BL/6JGpt小鼠的受精卵中，获得F0代小鼠。经PCR和测序验证正确的F0代阳性小鼠与C57BL/6JGpt小鼠交配获得可稳定遗传的F1代阳性小鼠模型。

风险提示

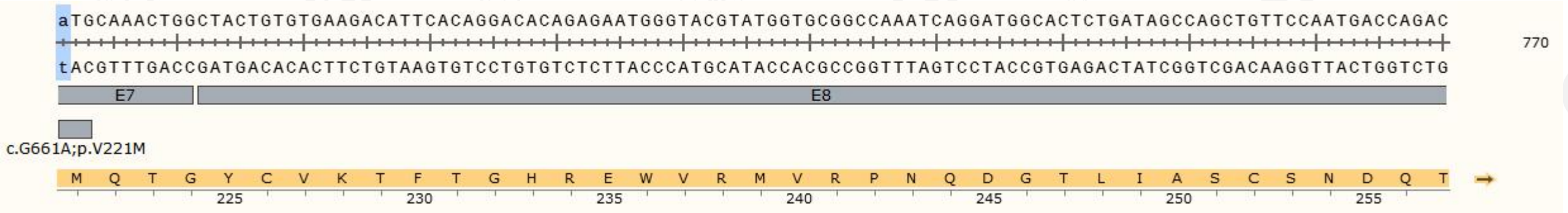
- 根据现有资料(MGI)，敲除*Pafah1b1*基因的纯合子小鼠胚胎期致死（E4.5）。
- Intron 6-7，intron 7-8可能分别引入2-4个氨基酸突变。
- 编码转录本204的3端不完整，本策略对这些转录本的影响未知。
- Intron 7-8只有718bp，本策略可能会影响*Pafah1b1*基因的剪接调控。
- *Pafah1b1*基因位于11 #染色体，如果将此基因突变小鼠与其他小鼠品系配繁，请避免两个基因位于同一染色体上，否则将无法得到双基因纯合阳性的小鼠后代。
- 本方案是根据现有数据库中的基因信息设计。由于基因转录和翻译过程复杂，在现有技术水平下不能全部预测。

突变位点

突变前



突变后



蓝色标记突变位点，c.G661A定点突变，对应的氨基酸由V突变成M。

鼠源PAFAH1B1蛋白

https://www.ncbi.nlm.nih.gov/CCDS/CcdsBrowse.cgi?REQUEST=CCDS&DATA=CCDS25035

MVLSQRQRDELNRAIADYLRSNGYEEAYSVFKKEAELDMNEELDKKYAGLLEKKWTSVIRLQKKVME
LESKLNEAKEEFTSGGPLGQKRDPKEWIPRPPEKYALSGHRSPVTRVIFHPVFSVMVSASEDATIKVWDY
ETGDFERTLKGHTDSVQDISFDHSGKLLASCSADMTIKLWDFQGFECIRTMHGHDHNVSSVAIMPNGDH
IVSASRDKTIKMWEVQTGYCVKTFTGHREWVRMVRPNQDGTLIASCSNDQTVRVWVVATKECKAELR
EHEHVVECISWAPESSYSSISEATGSETKKSGKPGPFLLSGSRDKTIKMWDVSTGMCLMTLVGHDNWVR
GVLFHSGGKFILSCADDKTLRVWDYKNKRCMKTLNAHEHFVTSLDFHKTAPYVVTGSVDQTVKVWEC
R*

标红：c.G661A;p.V221M

c.G661A突变型鼠源PAFAH1B1蛋白

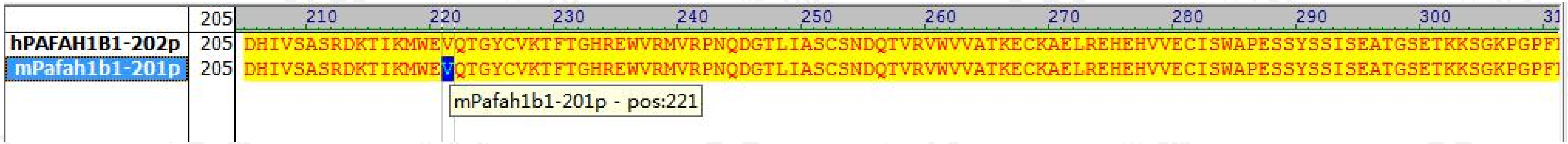
MVLSQRQRDELNRAIADYLRSNGYEEAYSVFKKEAELDMNEELDKKYAGLLEKKWTSVIRLQKKVME
LESKLNEAKEEFTSGGPLGQKRDPKEWIPRPPEKYALSGHRSPVTRVIFHPVFSVMVSASEDATIKVWDY
ETGDFERTLKGHTDSVQDISFDHSGKLLASCSADMTIKLWDFQGFECIRTMHGHDHNVSSVAIMPNGDH
IVSASRDKTIKMWEMQTGYCVKTFTGHREWVRMVRPNQDGTLIASCSNDQTVRVWVVATKECKAELR
EHEHVVECISWAPESSYSSISEATGSETKKSGKPGPFLLSGSRDKTIKMWDVSTGMCLMTLVGHDNWVR
GVLFHSGGKFILSCADDKTLRVWDYKNKRCMKTLNAHEHFVTSLDFHKTAPYVVTGSVDQTVKVWEC
R*

标红：c.G661A;p.V221M

人鼠PAFAH1B1蛋白同源性

```
                         1                                                                                                     100
hPAFAH1B1-202p     (1)  MVLSQRQRDELNRAIADYLRSNGYEEAYSVFKKEAELDVNEELDKKYAGLLEKKWTSVIRLQKKVMELESKLNEAKEEFTSGGPLGQKRDPKEWIPRPPE
mPafah1b1-201p     (1)  MVLSQRQRDELNRAIADYLRSNGYEEAYSVFKKEAELDMNEELDKKYAGLLEKKWTSVIRLQKKVMELESKLNEAKEEFTSGGPLGQKRDPKEWIPRPPE
     Consensus     (1)  MVLSQRQRDELNRAIADYLRSNGYEEAYSVFKKEAELDMNEELDKKYAGLLEKKWTSVIRLQKKVMELESKLNEAKEEFTSGGPLGQKRDPKEWIPRPPE
                         101                                                                                                   200
hPAFAH1B1-202p   (101)  KYALSGHRSPVTRVIFHPVFSVMVSASEDATIKVWDYETGDFERTLKGHTDSVQDISFDHSGKLLASCSADMTIKLWDFQGFECIRTMHGHDHNVSSVAI
mPafah1b1-201p   (101)  KYALSGHRSPVTRVIFHPVFSVMVSASEDATIKVWDYETGDFERTLKGHTDSVQDISFDHSGKLLASCSADMTIKLWDFQGFECIRTMHGHDHNVSSVAI
     Consensus   (101)  KYALSGHRSPVTRVIFHPVFSVMVSASEDATIKVWDYETGDFERTLKGHTDSVQDISFDHSGKLLASCSADMTIKLWDFQGFECIRTMHGHDHNVSSVAI
                         201                                                                                                   300
hPAFAH1B1-202p   (201)  MPNGDHIVSASRDKTIKMWEVQTGYCVKTFTGHREWVRMVRPNQDGTLIASCSNDQTVRVWVVATKECKAELREHEHVVECISWAPESSYSSISEATGSE
mPafah1b1-201p   (201)  MPNGDHIVSASRDKTIKMWEVQTGYCVKTFTGHREWVRMVRPNQDGTLIASCSNDQTVRVWVVATKECKAELREHEHVVECISWAPESSYSSISEATGSE
     Consensus   (201)  MPNGDHIVSASRDKTIKMWEVQTGYCVKTFTGHREWVRMVRPNQDGTLIASCSNDQTVRVWVVATKECKAELREHEHVVECISWAPESSYSSISEATGSE
                         301                                                                                                   400
hPAFAH1B1-202p   (301)  TKKSGKPGPFLLSGSRDKTIKMWDVSTGMCLMTLVGHDNWVRGVLFHSGGKFILSCADDKTLRVWDYKNKRCMKTLNAHEHFVTSLDFHKTAPYVVTGSV
mPafah1b1-201p   (301)  TKKSGKPGPFLLSGSRDKTIKMWDVSTGMCLMTLVGHDNWVRGVLFHSGGKFILSCADDKTLRVWDYKNKRCMKTLNAHEHFVTSLDFHKTAPYVVTGSV
     Consensus   (301)  TKKSGKPGPFLLSGSRDKTIKMWDVSTGMCLMTLVGHDNWVRGVLFHSGGKFILSCADDKTLRVWDYKNKRCMKTLNAHEHFVTSLDFHKTAPYVVTGSV
                         401     411
hPAFAH1B1-202p   (401)  DQTVKVWECR-
mPafah1b1-201p   (401)  DQTVKVWECR-
     Consensus   (401)  DQTVKVWECR
```

consensus positions: 99.8%   identity positions: 99.5%

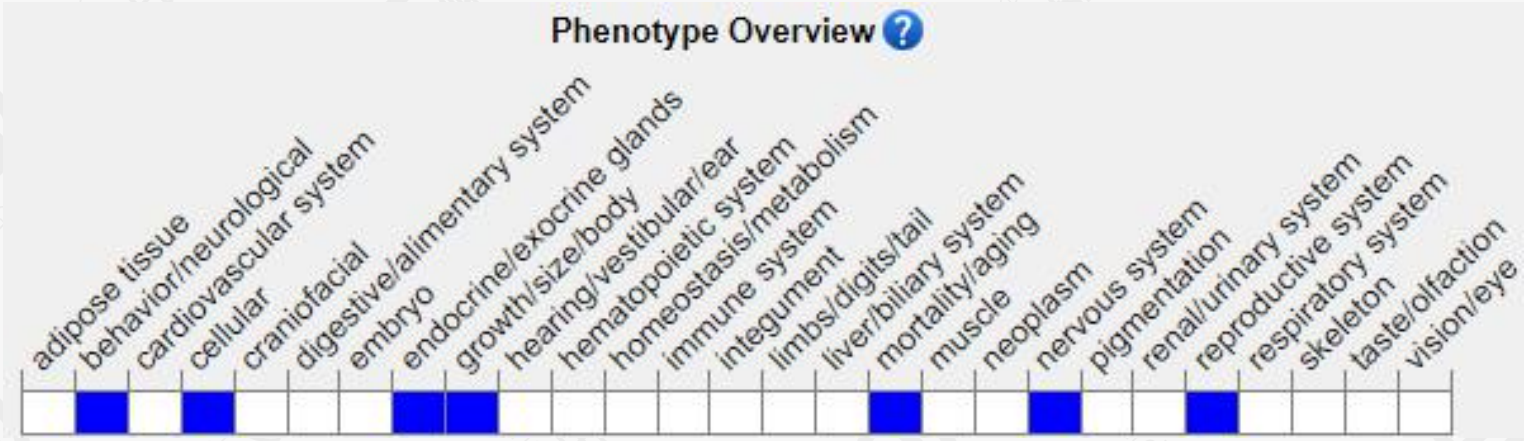


现有模型信息

网址链接：http://www.informatics.jax.org/allele/summary?markerId=MGI:109520&alleleType=Targeted

Allele Symbol Gene; Allele Name	Chr	Synonyms	Category	Abnormal Phenotypes Reported in these Systems	Human Disease Models
In(11Trp53;11Wnt3)8Brd inversion, Chr 11, Allan Bradley 8; inversion, Chr 11, Allan Bradley 8	11	In(11)8Brd, Inv(11)8Brd Trp53-Wnt3, Trp53-Wnt3 inversion	Targeted Involves 1200 genes (n-TQtto2, 4930406D14Rik, Gm11612...) **View all**	mortality/aging	
Pafah1b1 tm1Awb platelet-activating factor acetylhydrolase, isoform 1b, subunit 1; targeted mutation 1, Anthony Wynshaw-Boris	11	Lis1-, Lis-Neo, Pafah1b1 ex6neo-8, Pafah1b1-neo	Targeted (Null/knockout)	behavior, cellular, growth/size/body, mortality/aging, nervous system	lissencephaly (IDs) Miller-Dieker lissencephaly syndrome (IDs)
Pafah1b1 tm1c(EUCOMM)Hmgu platelet-activating factor acetylhydrolase, isoform 1b, subunit 1; targeted mutation 1c, Helmholtz Zentrum Muenchen GmbH	11		Targeted (Conditional ready)		
Pafah1b1 tm1Or platelet-activating factor acetylhydrolase, isoform 1b, subunit 1; targeted mutation 1, Orly Reiner	11	sLIS1	Targeted (Null/knockout)	cellular, mortality/aging, nervous system	lissencephaly (IDs) Miller-Dieker lissencephaly syndrome (IDs)
Pafah1b1 tm2.2Awb platelet-activating factor acetylhydrolase, isoform 1b, subunit 1; targeted mutation 2.2, Anthony Wynshaw-Boris	11	Pafah1b1-del, Pafah1b1 delex3-6	Targeted (Null/knockout)	mortality/aging, nervous system	lissencephaly (IDs) Miller-Dieker lissencephaly syndrome (IDs)
Pafah1b1 tm2Awb platelet-activating factor acetylhydrolase, isoform 1b, subunit 1; targeted mutation 2, Anthony Wynshaw-Boris	11	Lis1-loxP, Pafah1b1-loxP, Pafah1b1 loxPex3-6	Targeted (Conditional ready, Hypomorph)	growth/size/body, mortality/aging, nervous system	lissencephaly (IDs) Miller-Dieker lissencephaly syndrome (IDs)
Pafah1b1 tm1(NCOM)Mfgc platelet-activating factor acetylhydrolase, isoform 1b, subunit 1; targeted mutation 1, Mammalian Functional Genomics Centre	11		Targeted (Null/knockout, Reporter) **(Cell Line)**		
Pafah1b1 tm1a(EUCOMM)Hmgu platelet-activating factor acetylhydrolase, isoform 1b, subunit 1; targeted mutation 1a, Helmholtz Zentrum Muenchen GmbH	11		Targeted (Conditional ready, Null/knockout, Reporter) **(Cell Line)**		
Pafah1b1 tm1e(EUCOMM)Hmgu platelet-activating factor			Targeted		

MGI小鼠表型描述



Mutations at this locus result in neuronal migration defects. Homozygous null mutants die around implantation. Different allelic combinations show variable cortical, hippocampal and olfactory disorganization and impaired spatial learning and coordination.

http://www.informatics.jax.org/marker/MGI:109520

目的基因

基因名称	*Pafah1b1*
基因ID（NCBI）	18472
基因NCBI链接（NCBI）	https://www.ncbi.nlm.nih.gov/gene/18472
基因Ensembl链接	http://asia.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG00000020745;r=11:74564775-74615496
基因组位置	Chr 11

基因名称和位置（NCBI）

Pafah1b1 platelet-activating factor acetylhydrolase, isoform 1b, subunit 1 [*Mus musculus* (house mouse)]

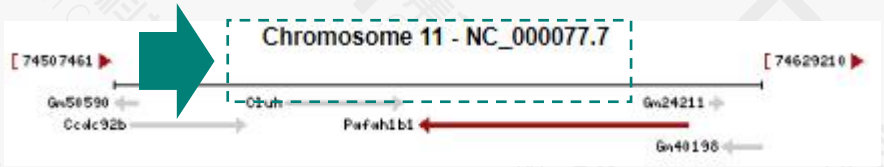
Gene ID: 18472, updated on 25-Jan-2022

Download Datasets

Summary

Official Symbol	Pafah1b1 provided by MGI
Official Full Name	platelet-activating factor acetylhydrolase, isoform 1b, subunit 1 provided by MGI
Primary source	MGI:MGI:109520
See related	Ensembl:ENSMUSG00000020745 AllianceGenome:MGI:109520
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	Lis1; Mdsh; LIS-1; Ms10u; Pafaha; MMS10-U
Summary	Enables identical protein binding activity; microtubule binding activity; and phosphoprotein binding activity. Involved in several processes, including cortical microtubule organization; establishment of planar polarity; and inner ear development. Acts upstream of or within several processes, including cytoskeleton organization; nervous system development; and transport along microtubule. Located in several cellular components, including centrosome; perinuclear region of cytoplasm; and stereocilium. Part of microtubule associated complex. Is expressed in several structures, including alimentary system; early conceptus; genitourinary system; nervous system; and sensory organ. Used to study Miller-Dieker lissencephaly syndrome and lissencephaly. Human ortholog(s) of this gene implicated in lissencephaly and lissencephaly 1. Orthologous to human PAFAH1B1 (platelet activating factor acetylhydrolase 1b regulatory subunit 1). [provided by Alliance of Genome Resources, Nov 2021]
Expression	Ubiquitous expression in CNS E18 (RPKM 47.2), cerebellum adult (RPKM 46.4) and 26 other tissues See more
Orthologs	human all

NEW **Try the new Gene table**
Try the new Transcript table

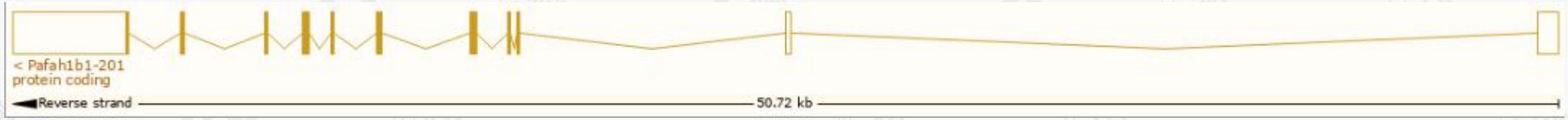


转录本信息（Ensembl）

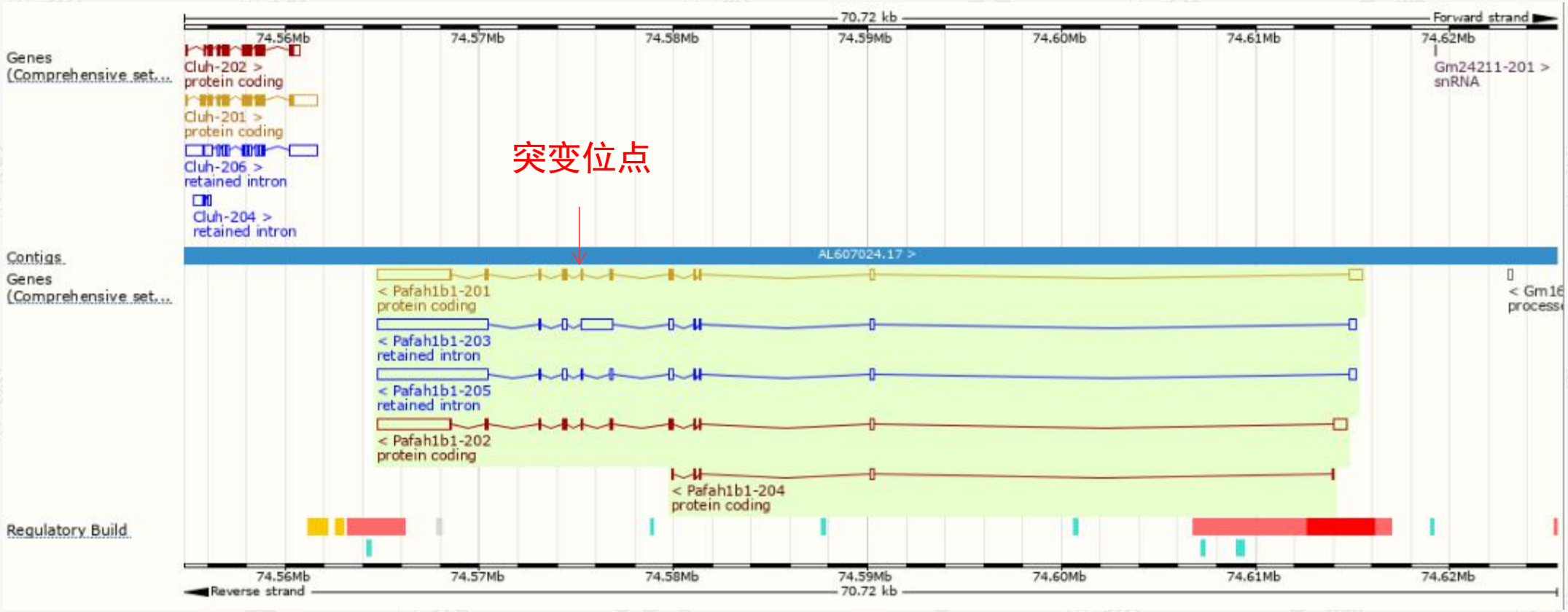
该基因有5个转录本，所有转录本信息如下图：

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000021091.15	Pafah1b1-201	5803	410aa	Protein coding	CCDS25035	P63005-1	GENCODE basic APPRIS P1 TSL:1
ENSMUST00000102520.9	Pafah1b1-202	5789	410aa	Protein coding	CCDS25035	P63005-1	GENCODE basic APPRIS P1 TSL:1
ENSMUST00000155493.2	Pafah1b1-204	525	97aa	Protein coding	-	Q5SW16	TSL:2 CDS 3' incomplete
ENSMUST00000126341.8	Pafah1b1-203	8570	No protein	Retained intron	-	-	TSL:2
ENSMUST00000156794.2	Pafah1b1-205	7165	No protein	Retained intron	-	-	TSL:2

该方案基于*Pafah1b1-201*(ENSMUST00000021091.15)转录本设计，该转录本11个exons，转录本长5803bp，编码410个氨基酸



基因位置分布


70.72 kb
Forward strand
74.56Mb
74.57Mb
74.58Mb
74.59Mb
74.60Mb
74.61Mb
74.62Mb
Genes
(Comprehensive set...
Cluh-202 >
protein coding
Cluh-201 >
protein coding
Cluh-206 >
retained intron
Cluh-204 >
retained intron
Gm24211-201 >
snRNA
突变位点
Contigs
AL607024.17 >
Genes
(Comprehensive set...
< Pafah1b1-201
protein coding
< Pafah1b1-203
retained intron
< Pafah1b1-205
retained intron
< Pafah1b1-202
protein coding
< Pafah1b1-204
protein coding
< Gm16
process
Regulatory Build
74.56Mb
74.57Mb
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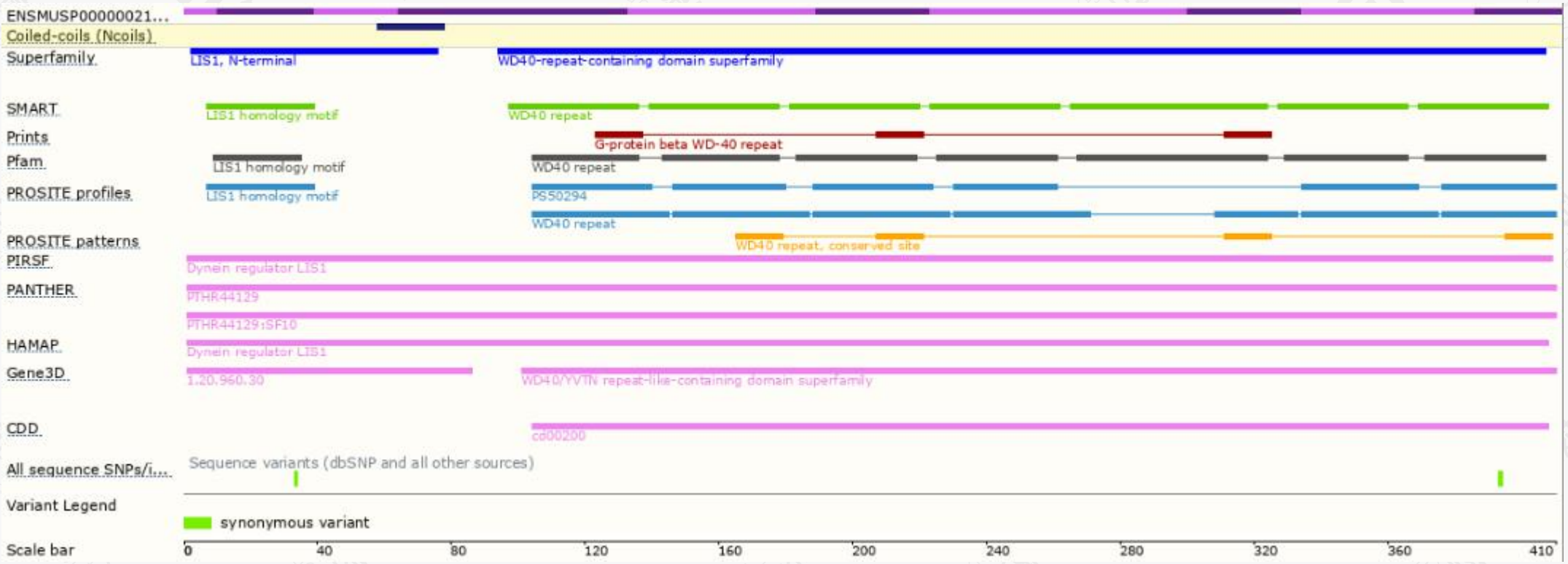
74.59Mb
74.60Mb
74.61Mb
74.62Mb
Reverse strand
70.72 kb

蛋白结构域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Superfamily
LIS1, N-terminal
WD40-repeat-containing domain superfamily
SMART
LIS1 homology motif
WD40 repeat
Prints
G-protein beta WD-40 repeat
Pfam
LIS1 homology motif
WD40 repeat
PROSITE profiles
LIS1 homology motif
PS50294
WD40 repeat
PROSITE patterns
WD40 repeat, conserved site
PIRSF
Dynein regulator LIS1
PANTHER
PTHR44129
PTHR44129:SF10
HAMAP
Dynein regulator LIS1
Gene3D
1.20.960.30
WD40/YVTN repeat-like-containing domain superfamily
CDD
cd00200
All sequence SNPs/i...
Sequence variants (dbSNP and all other sources)
Variant Legend
synonymous variant
Scale bar
0
40
80
120
160
200
240
280
320
360
410

目的基因

基因名称	*PAFAH1B1*
基因ID（NCBI）	5048
基因NCBI链接（NCBI）	https://www.ncbi.nlm.nih.gov/gene/5048
基因Ensembl链接	http://asia.ensembl.org/Homo_sapiens/Gene/Summary?g=ENSG00000007168;r=17:2593210-2685615
基因组位置	Chr 17

基因名称和位置（NCBI）

PAFAH1B1 platelet activating factor acetylhydrolase 1b regulatory subunit 1 [*Homo sapiens* (human)]

Download Datasets

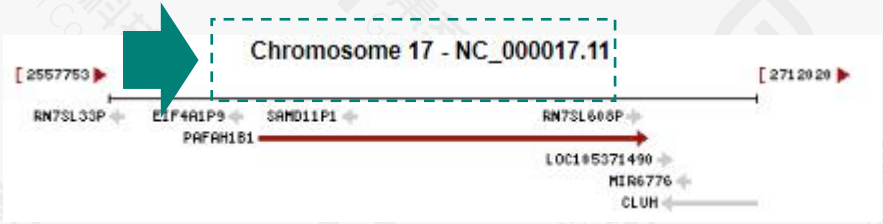
Gene ID: 5048, updated on 6-Mar-2022

Summary

Official Symbol	PAFAH1B1 provided by HGNC
Official Full Name	platelet activating factor acetylhydrolase 1b regulatory subunit 1 provided by HGNC
Primary source	HGNC:HGNC:8574
See related	Ensembl:ENSG00000007168 MIM:601545; AllianceGenome:HGNC:8574
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Homo sapiens
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as	MDS; LIS1; LIS2; MDCR; NudF; PAFAH
Summary	This locus was identified as encoding a gene that when mutated or lost caused the lissencephaly associated with Miller-Dieker lissencephaly syndrome. This gene encodes the non-catalytic alpha subunit of the intracellular Ib isoform of platelet-activating factor acteylhydrolase, a heterotrimeric enzyme that specifically catalyzes the removal of the acetyl group at the SN-2 position of platelet-activating factor (identified as 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine). Two other isoforms of intracellular platelet-activating factor acetylhydrolase exist: one composed of multiple subunits, the other, a single subunit. In addition, a single-subunit isoform of this enzyme is found in serum. [provided by RefSeq, Apr 2009]
Expression	Ubiquitous expression in brain (RPKM 59.5), testis (RPKM 32.6) and 25 other tissues See more
Orthologs	mouse all

NEW Try the new Gene table

Try the new Transcript table

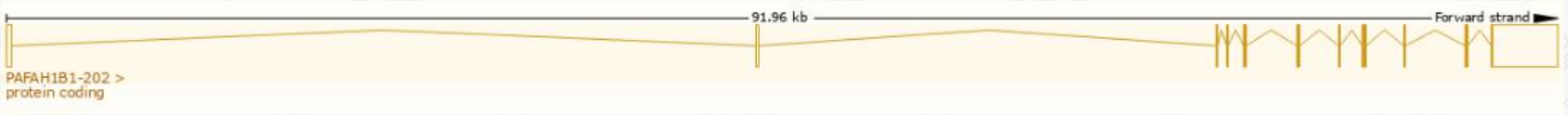


转录本信息（Ensembl）

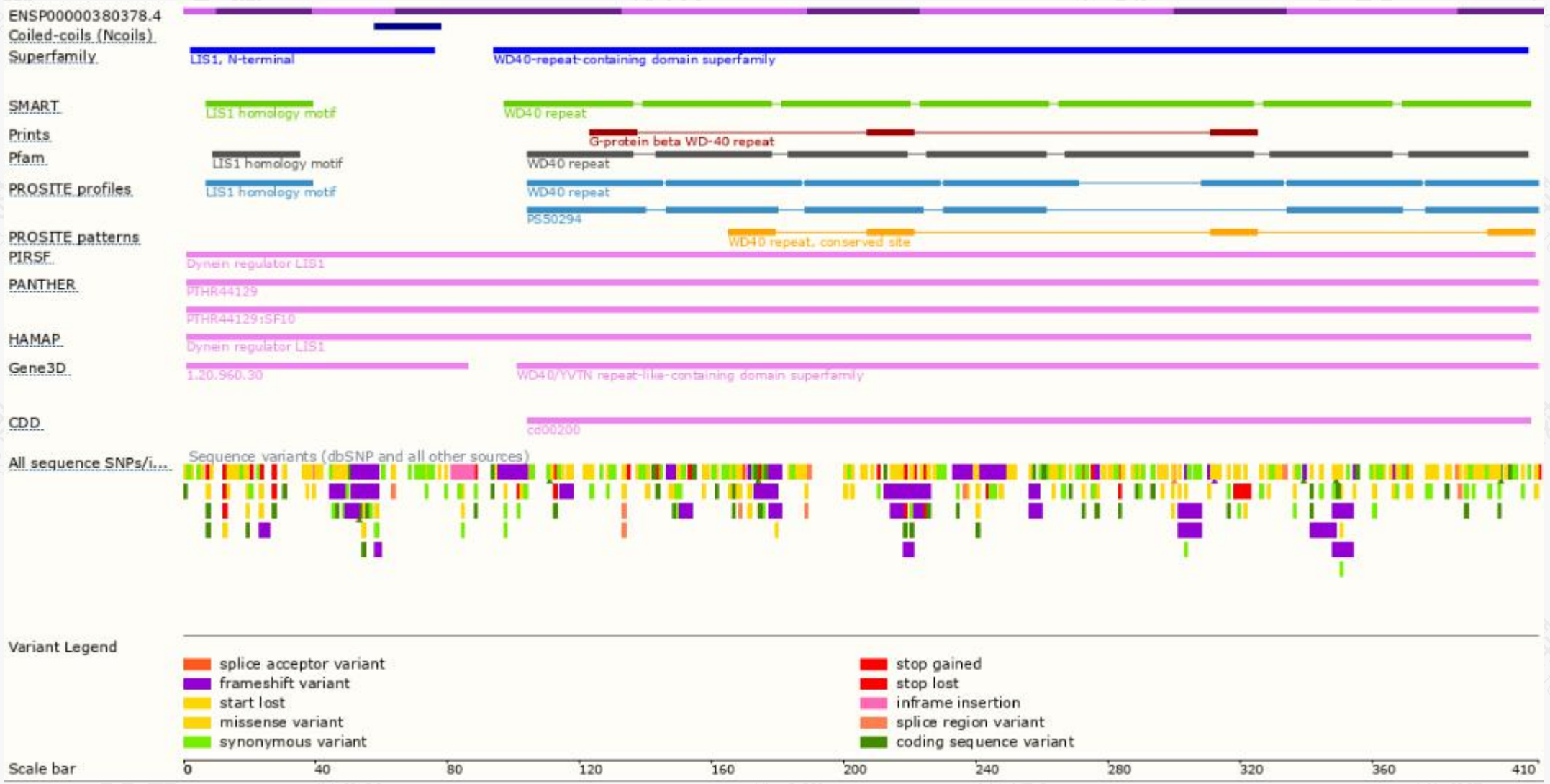
该基因有30个转录本，所有转录本信息如下图：

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	RefSeq Match	Flags
ENST00000676098.1	PAFAH1B1-226	5356	410aa	Protein coding	CCDS32528	P43034-1	-	GENCODE basic APPRIS P1
ENST00000676188.1	PAFAH1B1-227	5297	410aa	Protein coding	CCDS32528	P43034-1	-	GENCODE basic APPRIS P1
ENST00000674608.1	PAFAH1B1-214	5337	428aa	Protein coding	-	A0A6Q8PFU3	-	GENCODE basic
ENST00000676353.1	PAFAH1B1-229	5204	345aa	Protein coding	-	A0A6Q8PFT2	-	GENCODE basic
ENST00000674717.1	PAFAH1B1-215	5127	345aa	Protein coding	-	A0A6Q8PFT2	-	GENCODE basic
ENST00000574468.1	PAFAH1B1-208	1021	208aa	Protein coding	-	I3L3N5	-	TSL:2 CDS 5' incomplete
ENST00000576586.5	PAFAH1B1-211	557	82aa	Protein coding	-	I3L495	-	TSL:4 CDS 3' incomplete
ENST00000675764.1	PAFAH1B1-224	5384	47aa	Nonsense mediated decay	-	A0A6Q8PGF8	-	-
ENST00000676077.1	PAFAH1B1-225	4956	127aa	Nonsense mediated decay	-	A0A6Q8PH33	-	-
ENST00000675621.1	PAFAH1B1-223	1563	346aa	Nonsense mediated decay	-	A0A6Q8PG63	-	-
ENST00000570400.1	PAFAH1B1-203	661	14aa	Nonsense mediated decay	-	I3L384	-	TSL:3
ENST00000675385.1	PAFAH1B1-219	3544	No protein	Processed transcript	-	-	-	-
ENST00000572915.6	PAFAH1B1-206	1361	No protein	Processed transcript	-	-	-	TSL:1
ENST00000397193.7	PAFAH1B1-201	1277	No protein	Processed transcript	-	-	-	TSL:2
ENST00000675430.1	PAFAH1B1-221	957	No protein	Processed transcript	-	-	-	-
ENST00000575477.5	PAFAH1B1-210	694	No protein	Processed transcript	-	-	-	TSL:5
ENST00000574816.5	PAFAH1B1-209	662	No protein	Processed transcript	-	-	-	TSL:5
ENST00000574213.1	PAFAH1B1-207	634	No protein	Processed transcript	-	-	-	TSL:3
ENST00000675574.1	PAFAH1B1-222	8092	No protein	Retained intron	-	-	-	-
ENST00000571495.2	PAFAH1B1-205	6122	No protein	Retained intron	-	-	-	TSL:2
ENST00000610190.2	PAFAH1B1-213	4589	No protein	Retained intron	-	-	-	TSL:2
ENST00000676201.1	PAFAH1B1-228	1949	No protein	Retained intron	-	-	-	-
ENST00000676456.1	PAFAH1B1-230	1900	No protein	Retained intron	-	-	-	-
ENST00000675084.1	PAFAH1B1-216	762	No protein	Retained intron	-	-	-	-
ENST00000571289.1	PAFAH1B1-204	589	No protein	Retained intron	-	-	-	TSL:2
ENST00000609078.1	PAFAH1B1-212	570	No protein	Retained intron	-	-	-	TSL:4

该方案基于*PAFAH1B1-202*(ENST00000397195.10)转录本设计，该转录本11个exons，转录本长5589bp，编码410个氨基酸



蛋白结构域


ENSP00000380378.4
Coiled-coils (Ncoils)
Superfamily
LIS1, N-terminal
WD40-repeat-containing domain superfamily
SMART
LIS1 homology motif
WD40 repeat
Prints
G-protein beta WD-40 repeat
Pfam
LIS1 homology motif
WD40 repeat
PROSITE profiles
LIS1 homology motif
WD40 repeat
PS50294
PROSITE patterns
WD40 repeat, conserved site
PIRSF
Dynein regulator LIS1
PANTHER
PTHR44129
PTHR44129:SF10
HAMAP
Dynein regulator LIS1
Gene3D
1.20.960.30
WD40/YVTN repeat-like-containing domain superfamily
CDD
cd00200
All sequence SNPs/i...
Sequence variants (dbSNP and all other sources)
Variant Legend
splice acceptor variant
frameshift variant
start lost
missense variant
synonymous variant
stop gained
stop lost
inframe insertion
splice region variant
coding sequence variant
Scale bar
0
40
80
120
160
200
240
280
320
360
410

项目周期

制作周期合计：20~32周

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