



(19) 대한민국특허청(KR)
(12) 공개특허공보(A)

(11) 공개번호 10-2017-0042625
(43) 공개일자 2017년04월19일

- (51) 국제특허분류(Int. Cl.)
A61K 31/7105 (2006.01) A61K 31/713 (2006.01)
A61K 45/06 (2006.01) C12N 15/113 (2010.01)
- (52) CPC특허분류
A61K 31/7105 (2013.01)
A61K 31/713 (2013.01)
- (21) 출원번호 10-2017-7005777
- (22) 출원일자(국제) 2015년08월04일
심사청구일자 없음
- (85) 번역문제출일자 2017년02월28일
- (86) 국제출원번호 PCT/US2015/043571
- (87) 국제공개번호 WO 2016/022536
국제공개일자 2016년02월11일
- (30) 우선권주장
62/033,018 2014년08월04일 미국(US)
- (71) 출원인
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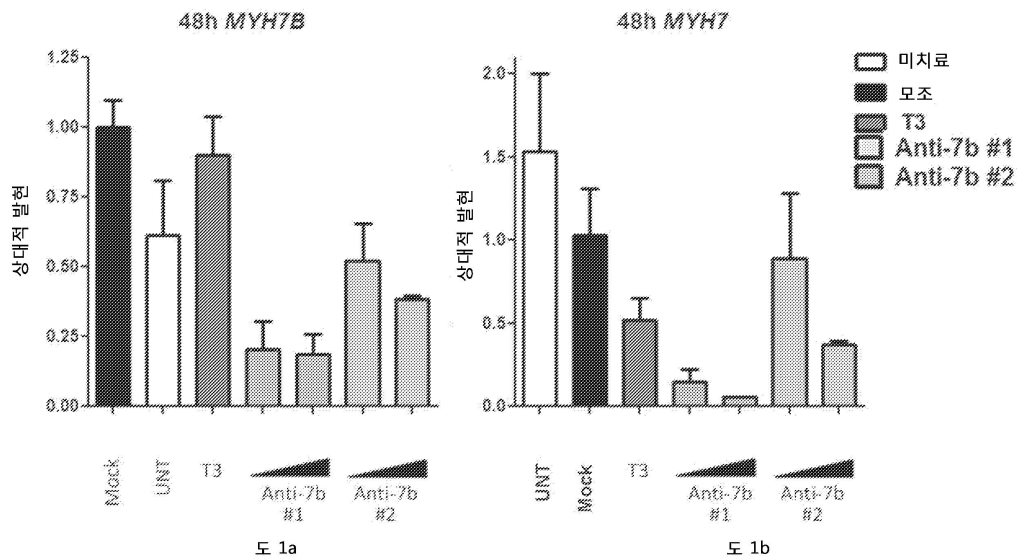
전체 청구항 수 : 총 41 항

(54) 발명의 명칭 MYH7B의 억제제 및 이의 용도

(57) 요약

본 발명은 MYH7B의 핵산 억제제 및 이의 조성물에 관한 것이다. 본 발명은 또한 MYH7B의 억제제를 대상에게 투여함으로써 심장비대증, 심근경색증 및 심부전증과 같은 심장 질환을 치료 또는 예방하는 방법을 제공한다. 본 발명은 MYH7B의 억제제를 대상에게 투여함으로써 대상의 심장 세포에서 β -MHC의 발현 또는 활성을 조절하는 방법을 추가로 제공한다.

대표도 - 도1



(52) CPC특허분류

A61K 45/06 (2013.01)

C12N 15/113 (2013.01)

A61K 2300/00 (2013.01)

C12N 2310/321 (2013.01)

C12N 2310/3521 (2013.01)

명세서

청구범위

청구항 1

MYH7B의 안티센스 올리고뉴클레오타이드 억제제로서, 안티센스 올리고뉴클레오타이드 억제제는 8 내지 18개 뉴클레오타이드의 길이를 가지며, 상기 안티센스 올리고뉴클레오타이드의 서열은 SEQ ID NO: 6의 서열에 실질적으로 상보적인 안티센스 올리고뉴클레오타이드 억제제.

청구항 2

제 1 항에 있어서,

안티센스 올리고뉴클레오타이드의 서열은 뉴클레오타이드 4300-4335로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 안티센스 올리고뉴클레오타이드 억제제.

청구항 3

제 2 항에 있어서,

안티센스 올리고뉴클레오타이드는 12 내지 18개 뉴클레오타이드의 길이를 가지며, 안티센스 올리고뉴클레오타이드의 서열은 뉴클레오타이드 4300-4317로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 안티센스 올리고뉴클레오타이드 억제제.

청구항 4

제 2 항에 있어서,

안티센스 올리고뉴클레오타이드는 12 내지 18개 뉴클레오타이드의 길이를 가지며, 안티센스 올리고뉴클레오타이드의 서열은 뉴클레오타이드 4316-4333으로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 안티센스 올리고뉴클레오타이드 억제제.

청구항 5

제 3 항에 있어서,

안티센스 올리고뉴클레오타이드는 14개 뉴클레오타이드의 길이를 가지며, 안티센스 올리고뉴클레오타이드의 서열은 뉴클레오타이드 4302-4315로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 안티센스 올리고뉴클레오타이드 억제제.

청구항 6

제 4 항에 있어서,

안티센스 올리고뉴클레오타이드는 14개 뉴클레오타이드의 길이를 가지며, 안티센스 올리고뉴클레오타이드의 서열은 뉴클레오타이드 4318-4331로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 안티센스 올리고뉴클레오타이드 억제제.

청구항 7

제 1 항에 있어서,

안티센스 올리고뉴클레오타이드는 적어도 하나의 변형 뉴클레오타이드를 함유하는 안티센스 올리고뉴클레오타이드 억제제.

청구항 8

제 7 항에 있어서,

상기 변형 뉴클레오타이드는 당, 염기 및/또는 주쇄 변형을 포함하는 안티센스 올리고뉴클레오타이드 억제제.

청구항 9

제 7 항에 있어서,

상기 변형 뉴클레오타이드는 잠금 뉴클레오타이드인 안티센스 올리고뉴클레오타이드 억제제.

청구항 10

제 1 항에 있어서,

상기 안티센스 올리고뉴클레오타이드는 1개 내지 6개 잠금 뉴클레오타이드를 함유하는 안티센스 올리고뉴클레오타이드 억제제.

청구항 11

제 1 항에 있어서,

상기 안티센스 올리고뉴클레오타이드는 5' 말단에 적어도 3개의 잠금 뉴클레오타이드를 함유하는 안티센스 올리고뉴클레오타이드 억제제.

청구항 12

제 1 항에 있어서,

상기 안티센스 올리고뉴클레오타이드는 3' 말단에 적어도 3개의 잠금 뉴클레오타이드를 함유하는 안티센스 올리고뉴클레오타이드 억제제.

청구항 13

제 11 항에 있어서,

5' 말단에 있는 적어도 3개의 잠금 뉴클레오타이드는 리보뉴클레오타이드인 안티센스 올리고뉴클레오타이드 억제제.

청구항 14

제 12 항에 있어서,

3' 말단에 있는 적어도 3개의 잠금 뉴클레오타이드는 리보뉴클레오타이드인 안티센스 올리고뉴클레오타이드 억제제.

청구항 15

제 1 항에 있어서,

상기 안티센스 올리고뉴클레오타이드는 적어도 하나의 데옥시리보뉴클레오타이드를 함유하는 안티센스 올리고뉴클레오타이드 억제제.

청구항 16

제 15 항에 있어서,

상기 안티센스 올리고뉴클레오타이드는 2 내지 8개의 데옥시리보뉴클레오타이드를 함유하는 안티센스 올리고뉴클레오타이드 억제제.

청구항 17

제 8 항에 있어서,

당 변형은 2'-O, 4'-C 메틸렌 가교, 2'-O, 4'-C 에틸렌 가교, 2'-CH₂-NH-CH₂-4' 가교, 2'-데옥시, 2'-O-알킬 및 2'-할로 변형으로 이루어진 그룹으로부터 선택되는 안티센스 올리고뉴클레오타이드 억제제.

청구항 18

제 8 항에 있어서,

주쇄 변형은 포스포로티오에이트 결합인 안티센스 올리고뉴클레오타이드 억제제.

청구항 19

제 18 항에 있어서,

상기 안티센스 올리고뉴클레오타이드는 둘 이상의 포스포로티오에이트 결합을 함유하는 안티센스 올리고뉴클레오타이드 억제제.

청구항 20

제 19 항에 있어서,

상기 안티센스 올리고뉴클레오타이드는 완전히 포스포로티오에이트 결합되는 안티센스 올리고뉴클레오타이드 억제제.

청구항 21

제 7 항에 있어서,

상기 변형 뉴클레오타이드는 5'-메틸 시티딘인 안티센스 올리고뉴클레오타이드 억제제.

청구항 22

제 1 항에 있어서,

상기 안티센스 올리고뉴클레오타이드는 표 1-5로부터 선택된 서열을 포함하는 안티센스 올리고뉴클레오타이드 억제제.

청구항 23

제 1 항에 있어서,

상기 안티센스 올리고뉴클레오타이드는 5'-lTs1Ts1GsdAsdTsdCsdTsdTsdGsdGsdCs1Cs1Ts1C-3'(SEQ ID NO: 146) 또는 5'-lCs1Ts1GsdCsdAsdTsdCsdCsdTs1Cs1Cs1A-3'(SEQ ID NO: 148)의 서열을 포함하는 안티센스 올리고뉴클레오타이드 억제제.

청구항 24

제 1 항 내지 제 23 항 중 어느 한 항의 MYH7B의 안티센스 올리고뉴클레오타이드 억제제 및 약학적으로 허용가능한 부형제를 포함하는 약학적 조성물.

청구항 25

제 24 항에 있어서,

제 2 치료제를 더 포함하며, 제 2 치료제는 miR-208a, miR-208b, miR-499, miR-15a, miR-15b, miR-16, miR-195 또는 이의 혼합물의 안티센스 올리고뉴클레오타이드 억제제인 약학적 조성물.

청구항 26

제 1 항의 안티센스 올리고뉴클레오타이드 억제제를 대상에게 투여하는 단계를 포함하여, 필요한 대상의 병적 심근비대증, 심근경색증 또는 심부전증을 치료 또는 예방하는 방법.

청구항 27

제 26 항에 있어서,

병적 심근비대증은 비대성 심근병증인 방법.

청구항 28

제 26 항에 있어서,

대상은 병적 심근비대증의 위험이 있는 것인 방법.

청구항 29

제 28 항에 있어서,

위험이 있는 대상은 베타 마이오신 중쇄 유전자에서 돌연변이를 갖는 것인 방법.

청구항 30

제 26 항에 있어서,

MYH7B의 발현 또는 활성은 억제제의 투여 후 대상의 심장 세포에서 감소되는 것인 방법.

청구항 31

제 26 항에 있어서,

베타 마이오신 중쇄의 발현은 억제제의 투여 후 대상의 심장 세포에서 감소되는 것인 방법.

청구항 32

제 26 항에 있어서,

대상의 심장 세포에서 miR-499의 발현은 억제제의 투여 후 통계적으로 상이하지 않은 것인 방법.

청구항 33

제 26 항에 있어서,

안티센스 올리고뉴클레오티드는 5'-lTs1Ts1GsdAsdTsdCsdTsdTsdGsdGsdCs1Cs1Ts1C-3'(SEQ ID NO: 146) 또는 5'-lCs1Ts1GsdCsdAsdGsdCsdTsdCsdCsdTs1Cs1Cs1A-3'(SEQ ID NO: 148)의 서열을 포함하는 것인 방법.

청구항 34

제 26 항에 있어서,

제 2 심장 치료제를 투여하는 단계를 더 포함하는 것인 방법.

청구항 35

제 34 항에 있어서,

제 2 심장 치료제는 miR-208a, miR-208b, miR-499, miR-15a, miR-15b, miR-16, miR-195 또는 이의 조합의 안티센스 올리고뉴클레오티드 억제제인 방법.

청구항 36

제 26 항에 있어서,

대상은 인간인 방법.

청구항 37

MYH7B의 억제제를 대상에게 투여하는 단계를 포함하여, 필요한 대상의 병적 심근비대증, 심근경색증 또는 심부전증을 치료 또는 예방하는 방법.

청구항 38

제 37 항에 있어서,

MYH7B의 억제제는 안티센스 올리고뉴클레오티드, 앵타머, 리보자임, 소형 간섭 RNA 또는 소형 헤어핀 RNA로부터 선택되는 핵산 억제제인 방법.

청구항 39

제 38 항에 있어서,

핵산 억제제는 약 10 내지 약 30개 뉴클레오티드의 이중 가닥 영역을 포함하는 소형 간섭 RNA 또는 소형 헤어핀 RNA이며, 상기 이중 가닥 영역은 (i) 인간 *Myh7b* 유전자의 서열과 적어도 70% 동일한 서열을 갖는 제 1 RNA 가닥 및 (ii) 제 1 RNA 가닥에 부분적으로, 실질적으로 또는 완전히 상보적인 제 2 RNA 가닥을 포함하는 것인 방법.

청구항 40

제 39 항에 있어서,

제 1 RNA 가닥은 5'-GAGGCCAAGATCAA-3'(SEQ ID NO: 4)의 서열과 적어도 70% 동일한 서열을 갖는 것인 방법.

청구항 41

제 39 항에 있어서,

제 1 RNA 가닥은 5'-TGGAGGAGCTGCAG-3'(SEQ ID NO: 5)의 서열과 적어도 70% 동일한 서열을 갖는 것인 방법.

발명의 설명

기술 분야

[0001] 본 발명은 마이오신 중쇄 유전자, MYH7B의 억제제 및 이의 조성물에 관한 것이다. 본 발명은 또한 MYH7B의 억제제를 투여함으로써 심장비대증, 심근경색증 및 심부전증과 같은 심장 질환을 치료 또는 예방하는 방법을 제공한다. 특히, 본 발명은 MYH7B의 핵산 억제제 및 이의 조성물, 및 필요한 대상에서 MYH7B의 발현 또는 활성을 억제함으로써 심장 질환을 치료 또는 예방하는 방법을 개시한다.

배경 기술

[0002] 심장 질환은 미국에서 사망의 주원인이며 전세계 수백만 명의 사람들에게 큰 건강 위협을 제공한다. 비대성 심근병증, 심근경색증 및 심부전증을 포함하는 다양한 유형의 심장 질환으로 고통받는 환자를 진단, 치료 및 지원하는 비용은 매우 높으며 의료 시스템에 심각한 부담을 준다.

[0003] 비대성 심근병증(HCM)은 불시의 돌발성 심장사의 중요한 원인이며 심장 마비가 발병하기 전에 종종 무증상이기 때문에 특별한 관심의 대상이다. HCM은 심실 기능에 영향을 미치고 심부정맥을 유발할 수 있는 심근 세포의 두꺼워짐을 특징으로 한다. HCM은 여러 가지 염색체 유전자에서 우성 돌연변이로 인해 발생하는 심장의 주요 유전 질환이며, 염색체 유전자 중 8개가 광범위하게 연구되고 있다(Teekakirikul et al., "Hypertrophic cardiomyopathy: translating cellular cross talk into therapeutics," J. Cell. Biol., 2012, 199(3), 417-421). HCM에서의 이러한 우성 돌연변이 중, 약 40%는 β -마이오신 유전자, MYH7의 미스센스 돌연변이이다. 유전학적 및 약리학적 연구는 이 우성 대립형질의 발현 감소가 매우 적지만 강력한 표현형 효과를 나타낼 수 있다는 것을 입증하였다(Jiang et al., "Allele-specific silencing of mutant MYH6 transcripts in mice suppresses hypertrophic cardiomyopathy," Science, 2013, 342(6154), 111-114).

[0004] 마이오신은 심장과 골격근 세포의 주요 수축 단백질이다. 심장 근육 수축은 두 마이오신 중쇄(MHC) 단백질, α -MHC (MYH6) 및 β -MHC (MYH7)의 발현과 상대적 비율에 따라 결정된다. 설치류에서, α -MHC인 속근 MHC는 성인 심장에서 지배적인 마이오신 아이소폼인 반면, β -MHC인 지근 MHC는 성장하는 심장에서 주로 발현되고 출생 후 하향조절된다(Morkin, E., "Control of cardiac myosin heavy chain gene expression," Microsc. Res. Tech., 2000, 50, 522-531). 반면, 인간의 심장에서, β -MHC 아이소폼이 크게 발현되어 α -MHC 아이소폼은 전체 심실성 MHC의 8% 미만을 차지한다(Miyata et al., "Myosin heavy chain isoform expression in the failing and nonfailing human heart," Circ. Res., 2000, 86(4):386-90). 여러 종에서 알파- 및 베타-마이오신의 발현 차이와 관계없이, 연구는 베타-마이오신의 발현은 사람, 쥐 및 토끼를 포함하는 이들 종의 심장 질환에서 상향조절되는 것을 나타낸다. 예를 들어, 부전성 성체 생쥐 심장에서, 정상적으로 지배적인 알파-MHC에서 베타-MHC로의 이동이 종종 관찰된다(Harada et al., Circulation, 1999, 100, 2093-2099). 유사하게, 쥐에서 울혈성 심부전은 베타-마이오신의 증가된 발현 및 알파-MHC의 감소된 발현과 관련이 있다. 설치류 연구와 일치하여, 베타-

마이오신 발현은 부전성 인간 심장에서 상향조절되었고 알파-마이오신 발현은 현저히 하향조절되었다(Miyata et al., "Myosin heavy chain isoform expression in the failing and nonfailing human heart," Circ. Res., 2000, 86(4):386-90). 이 연구는 알파- 및 베타-마이오신의 발현이 종에 의존적임에도 불구하고, 베타-마이오신의 발현을 하향 조절하는 것이 다양한 종에서 심장 보호 역할을 하는 것으로 나타낸다. 예를 들어, β -MHC 발현의 증가를 둔화시키고 α -MHC 발현을 증가시키는 것은 토끼에서 심장 보호적인 것으로 나타났다(James et al., "Forced expression of alpha-myosin heavy chain in the rabbit ventricle results in cardioprotection under cardiomyopathic conditions," Circulation, 2005, 111(18), 2339-2346).

[0005] α -MHC (MYH6), β -MHC (MYH7) 및 관련 마이오신인 MYH7B를 암호화하는 유전자는 또한 각각 인트론성 miRNA의 패밀리에 miR-208a, miR-208b 및 miR-499를 암호화한다. 이 세 가지 miRNA는 서열 상동성을 공유하며 "MyomiRs"라고 불린다(van Rooij et al., "A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance," Dev. Cell, 2009, 17, 662-673). MyomiR은 병적인 심장 리모델링, 근육 마이오신 함량, 근섬유 정체성 및 근 수축을 제어하는 것으로 나타났다(Liu and Olson, "MicroRNA regulatory networks in cardiovascular development," Dev. Cell, 2010, 510-525).

[0006] 심장 및 근육 발달에 있어서 α -MHC, β -MHC 및 myomiR(miR-208a, miR-208b 및 miR-499)의 역할이 광범위하게 연구되었지만 세 번째 마이오신인 MYH7B의 역할은 크게 알려지지 않았다. MYH7B 유전자는 골격근, 심장 및 뇌에서 시냅스 구조와 기능을 조절하는 뇌 세포의 하부세트에서 발현된다(Yeung et al., "Myh7b/miR-499 gene expression is transcriptionally regulated by MRFs and Eos," Nucleic Acids Res., 2012, 40(15):7303-18). 최근 보고는 MYH7B 단백질은 슬로우 토닉(slow-tonic) 섬유에 해당하는 외안근 및 근방추의 낭섬유에서 작은 섬유 집단으로 탐지되는 것을 제안한다(Rossi et al., "Two novel/ancient myosins in mammalian skeletal muscles: MYH14/7b and MYH15 are expressed in extraocular muscles and muscle spindles", J Physiol., Jan. 15, 2010; 588(Pt 2):353-64). 그러나, 인간의 심장에서 MYH7B 유전자는 비생산적 스플라이싱을 거쳐 기능성 MYH7B 단백질을 암호화할 수 없는 RNA를 생성한다고 생각된다(Bell et al., "Uncoupling of expression of an intronic microRNA and its myosin host gene by exon skipping," Mol. Cell. Biol., 30, 1937-1945). 흥미롭게도, 심장 세포에서 MYH7B mRNA의 비생산적인 스플라이싱은 인트론성 miR-499의 변형된 발현과 관련이 없었다. 따라서, 본 발명에 이르기까지, MYH7B는 심장 세포에서 miR-499의 비 기능성 운반체로 생각되었다(Gerald Dorn, II, "MicroRNAs: redefining mechanisms in cardiac disease," J. Cardiovasc. Pharmacol., 2010, 56(6), 589-595). 또한, miR-499와 달리 MYH7B는 심장 및 골격근 생물학에서 중요한 역할을 하지 않는 것으로 생각된다.

발명의 내용

해결하려는 과제

[0007] 본 발명은 상기한 문제를 해결하는 것을 목적으로 한다.

과제의 해결 수단

[0008] 본 발명은 부분적으로 MYH7B가 심장 세포에서 β -MHC의 발현을 조절하여 심장 수축성 및 스트레스-유도 심장 리모델링을 조절한다는 놀라운 발견에 기초한다. 특히, MYH7B의 억제제는 심장 세포에서 β -MHC mRNA 및 단백질의 하향 조절을 초래한다. 따라서, 한 실시태양에서, 본 발명은 MYH7B 억제제, 이의 조성물, 및 MYH7B의 억제제를 대상에게 투여하는 단계를 포함하여 필요한 대상에서 β -MHC의 발현 및/또는 활성을 조절하는 방법을 제공한다. 한 실시태양에서, 대상에 대한 MYH7B의 억제제의 투여는 투여 후 대상의 심장 세포에서 MYH7B의 발현 또는 활성을 하향 조절한다. 한 실시태양에서, 대상에 대한 MYH7B의 억제제의 투여는 투여 후 대상의 심장 세포에서 β -MHC(MYH7)의 발현 또는 활성을 하향 조절한다. 다른 실시태양에서, 대상에 대한 MYH7B의 억제제를 투여는 투여 후 대상의 심장 세포에서 α -MHC(MYH6)의 발현 또는 활성을 상향 조절한다. 또 다른 실시태양에서, 대상에 대한 MYH7B의 억제제의 투여는 마이오신에 "전환(switch)"이 유도되어 β -MHC의 발현 또는 활성이 하향 조절되고 α -MHC의 발현 또는 활성이 투여 후 대상에서 상향 조절된다. 한 실시태양에서, 대상에 대한 MYH7B의 억제제의 투여는 대상의 심장 세포에서 miR-208a, miR-208b 또는 miR-499의 발현 또는 활성을 변화시키지 않는다.

[0009] 또 다른 실시태양에서, 본 발명은 대상에게 MYH7B의 억제제를 투여하는 단계를 포함하여 병적 심장비대증, 심근 경색증, 심부전증 또는 비대성 심근병증과 같은 심장 질환을 치료 또는 예방하기 위한 조성물 및 방법을 제공한다. 한 실시태양에 있어서, 심장 질환을 갖는 대상은 건강한 대상과 비교하여 심장 세포에서 β -MHC의 증가된

수준을 가졌다. 다른 실시태양에서, 심장 질환을 갖는 대상은 건강한 대상과 비교하여 심장 세포에서 β -MHC/ α -MHC의 변형된 비율을 가진다. 한 실시태양에서, 심장 질환을 갖는 대상에 대한 MYH7B의 억제제의 투여는 투여 후 대상의 심장 세포에서 MYH7B의 발현 또는 활성 및/또는 MYH7의 발현 또는 활성을 억제한다. 다른 실시태양에서, 심장 질환을 갖는 대상에 대한 MYH7B 억제제를 투여는 투여 후 대상의 심장 세포에서 β -MHC/ α -MHC 비율을 완전히 또는 부분적으로 정상 수준으로 회복한다. 한 실시태양에서, 심장 질환은 심근비대증, 심근경색증, 심부전증 또는 비대성 심근병증이다.

- [0010] 한 실시태양에서, MYH7B의 억제제는 안티센스 올리고뉴클레오타이드, 앵타머, 리보자임, 소형 간섭 RNA(siRNA) 또는 소형 헤어핀 RNA(shRNA)로부터 선택되는 핵산 억제제이다. 다른 실시태양에서, MYH7B의 억제제는 MYH7B 단백질에 특이적으로 결합하는 항체 또는 이의 결합 단편이다. 특정 실시태양에서, MYH7B의 억제제는 MYH7B 암호화 서열에 적어도 부분적으로 상보적인 서열을 포함하는 안티센스 올리고뉴클레오타이드이다. 본 발명의 조성물 및 방법에 사용되는 안티센스 올리고뉴클레오타이드는 약 6 내지 약 22개 뉴클레오타이드의 길이를 가질 수 있다. 일부 실시태양에서, 안티센스 올리고뉴클레오타이드는 당, 주쇄 및/또는 염기 변형과 같은 하나 이상의 화학적 변형을 포함한다.
- [0011] 한 실시태양에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제는 8 내지 18개 뉴클레오타이드의 길이를 가지며, 안티센스 올리고뉴클레오타이드의 서열은 SEQ ID NO: 6의 서열에 실질적으로 상보적이다.
- [0012] 일부 실시태양에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제는 8 내지 18개 뉴클레오타이드의 길이를 가지며, 안티센스 올리고뉴클레오타이드의 서열은 뉴클레오타이드 4300-4335로부터 SEQ ID NO: 6의 서열에 실질적으로 상보적이다.
- [0013] 일부 다른 실시태양에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제는 12 내지 18개 뉴클레오타이드의 길이를 가지며, 안티센스 올리고뉴클레오타이드의 서열은 뉴클레오타이드 4300-4317로부터 SEQ ID NO: 6의 서열에 실질적으로 상보적이다.
- [0014] 또 다른 실시태양에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제는 12 내지 18개 뉴클레오타이드의 길이를 가지며, 안티센스 올리고뉴클레오타이드의 서열은 뉴클레오타이드 4316-4333으로부터 SEQ ID NO: 6의 서열에 실질적으로 상보적이다.
- [0015] 한 실시태양에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제는 14개 뉴클레오타이드의 길이를 가지며, 안티센스 올리고뉴클레오타이드의 서열은 뉴클레오타이드 4302-4315로부터 SEQ ID NO: 6의 서열에 실질적으로 상보적이다.
- [0016] 다른 실시태양에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제는 14개 뉴클레오타이드의 길이를 가지며, 안티센스 올리고뉴클레오타이드의 서열은 뉴클레오타이드 4318-4331로부터 SEQ ID NO: 6의 서열에 실질적으로 상보적이다.
- [0017] 일부 실시태양에서, 본 발명의 안티센스 올리고뉴클레오타이드 억제제는 적어도 하나의 변형 뉴클레오타이드를 함유한다. 변형 뉴클레오타이드는 당, 염기 및/또는 주쇄 변형을 포함할 수 있다.
- [0018] 한 실시태양에서, 변형 뉴클레오타이드는 잠금 뉴클레오타이드이다. 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 1개 내지 6개의 잠금 뉴클레오타이드를 함유한다. 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제의 5' 말단의 적어도 처음 3개의 뉴클레오타이드는 잠금 뉴클레오타이드이다. 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제의 3' 말단의 적어도 처음 3개의 뉴클레오타이드는 잠금 뉴클레오타이드이다.
- [0019] 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제의 5' 말단의 적어도 처음 3개 뉴클레오타이드는 잠금 또는 비 잠금 리보뉴클레오타이드이다. 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제의 3' 말단의 적어도 처음 3개 뉴클레오타이드는 잠금 또는 비 잠금 리보뉴클레오타이드이다.
- [0020] 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 적어도 하나의 데옥시리보뉴클레오타이드를 함유한다. 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 2 내지 8개의 데옥시리보뉴클레오타이드를 함유한다.
- [0021] 다양한 실시태양에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제는 2'-O, 4'-C 메틸렌 가교, 2'-O, 4'-C 에틸렌 가교, 2'-CH₂-NH-CH₂-4' 가교, 2'-데옥시, 2'-O-알킬 및 2'-할로 변형으로 이루어진 그룹으로부터 선택된 당 변형을 포함할 수 있다.
- [0022] 다양한 실시태양에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제는 주쇄 변형을 포함할 수 있다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 적어도 하나의 포스포로티오에이트 결합을 함유한다. 다른 실시

태양에서, 안티센스 올리고뉴클레오타이드 억제제는 2개 이상의 포스포로티오에이트 결합을 함유한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오타이드는 완전히 포스포로티오에이트 결합된다.

[0023] 한 실시태양에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제는 링커를 통해 연결된 펩타이드 또는 당 모이어티를 더 포함한다. 링커는 3'-티올-변형-C₃-다이설파이드 링커, (C₆)₂ 다이설파이드 링커 또는 1-3 포스포다이에스터 결합일 수 있다.

[0024] 특정 실시태양에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제는 표 1-5로부터 선택된 서열을 포함한다.

[0025] 한 실시태양에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제는 5'-1TslTslGsdAsdTsdCsdTsdTsdGsdGsdCs1Cs1TslC-3'(SEQ ID NO: 146) 또는 5'-1Cs1TslGsdCsdAsdTsdCsdTsdCsdTslCslCs1A-3'(SEQ ID NO: 148)의 서열을 포함한다.

[0026] 본 발명은 또한 MYH7B의 억제제 및 약학적으로 허용가능한 부형제를 포함하는 약학적 조성물을 제공한다. 한 실시태양에서, MYH7B의 억제제는 안티센스 올리고뉴클레오타이드이다. 한 양태에서, 약학적 조성물은 miR-208a, miR-208b, miR-499, miR-15a, miR-15b, miR-16, miR-195 또는 이의 혼합물의 안티센스 올리고뉴클레오타이드 억제제인 제 2 치료제를 더 포함한다.

[0027] 본 발명은 MYH7B의 안티센스 올리고뉴클레오타이드 억제제를 대상에게 투여하는 단계를 포함하여, 필요한 대상의 병적 심근비대증, 심근경색증 또는 심부전증을 치료 또는 예방하는 방법을 추가로 제공한다. 한 실시태양에서, 병적 심근비대증은 비대성 심근병증이다. 일부 실시태양에서, 본 발명에 따른 방법은 miR-208a, miR-208b, miR-499, miR-15a, miR-15b, miR-16, miR-195, 또는 이들의 조합의 안티센스 올리고뉴클레오타이드 억제제와 같은 제 2 심장 치료제를 포함할 수 있다.

[0028] 일부 실시태양에서, 본 발명은 병적 심근비대증의 위험이 있는 대상을 치료하는 방법을 제공한다. 한 실시태양에서, 병적 심근비대증의 위험이 있는 대상은 베타 마이오신 중쇄 유전자에서 돌연변이를 가진다.

[0029] 한 양태에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제의 투여는 투여 후 대상의 심장 세포에서 MYH7B의 발현 또는 활성을 감소시킨다. 다른 양태에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제의 투여는 투여 후 대상의 심장 세포에서 베타 마이오신 중쇄의 발현을 감소시킨다. 또 다른 양태에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제의 투여는 투여 후 대상의 심장 세포에서 miR-499, miR-208a 및/또는 miR-208b의 발현을 현저하게 변화시키지 않는다.

[0030] 일부 실시태양에서, 본 발명은 MYH7B의 억제제를 대상에게 투여하는 단계를 포함하여, 필요한 대상의 병적 심근비대증, 심근경색증 또는 심부전증을 치료 또는 예방하는 방법을 제공한다. 다양한 실시태양에서, MYH7B의 억제제는 안티센스 올리고뉴클레오타이드, 앵타머, 리보자임, 소형 간섭 RNA 또는 소형 헤어핀 RNA로부터 선택되는 핵산 억제제이다.

[0031] 한 실시태양에서, MYH7B의 핵산 억제제는 약 10 내지 약 30개 뉴클레오타이드의 이중 가닥 영역을 포함하는 소형 간섭 RNA 또는 소형 헤어핀 RNA이며, 상기 이중 가닥 영역은 (i) 인간 *Myh7b* 유전자의 서열과 적어도 70% 동일한 서열을 갖는 제 1 RNA 가닥 및 (ii) 제 1 RNA 가닥에 부분적으로, 실질적으로 또는 완전히 상보적인 제 2 RNA 가닥을 포함한다. 한 양태에서, siRNA 또는 shRNA의 제 1 RNA 가닥은 5'-GAGGCCAAGATCAA-3'(SEQ ID NO: 4)의 서열과 적어도 70% 동일한 서열을 가진다. 다른 양태에서, siRNA 또는 shRNA의 제 1 RNA 가닥은 5'-TGGAGGAGCTGCAG-3'(SEQ ID NO: 5)의 서열과 적어도 70% 동일한 서열을 가진다.

발명의 효과

[0032] 본 발명의 내용 중에 포함되어 있다.

도면의 간단한 설명

[0033] 도 1a는 MYH7B에 대한 안티센스 올리고뉴클레오타이드에 의한 형질감염 48시간 후 인간 iPS 심근세포에서 MYH7B mRNA의 수준을 나타낸다. 도 1b는 MYH7B에 대한 안티센스 올리고뉴클레오타이드에 의한 형질감염 48시간 후 인간 iPS 심근 세포에서 MYH7(β-MHC) mRNA의 수준을 나타낸다.

도 2a는 MYH7B에 대한 안티센스 올리고뉴클레오타이드에 의한 형질감염 48시간 후 인간 iPS 심근세포에서 miR-208a의 수준을 나타낸다. 도 2b는 MYH7B에 대한 안티센스 올리고뉴클레오타이드에 의한 형질감염 48시간 후 인간 iPS 심근세포에서 miR-499의 수준을 나타낸다. 도 2c는 MYH7B에 대한 안티센스 올리고뉴클레오타이드에 의한 형질

감염 48시간 후 인간 iPS 심근 세포에서의 miR-208b의 수준을 나타낸다.

도 3a는 MYH7B에 대한 안티센스 올리고뉴클레오타이드에 의한 형질감염 48시간 후 대조군 및 HCM-R663H 심근세포에서 MYH7(β -MHC) mRNA의 수준을 나타낸다. 도 3b는 MYH7B에 대한 안티센스 올리고뉴클레오타이드에 의한 형질감염 168시간(1 주) 후 대조군 및 HCM-R663H 심근세포에서 MYH7(β -MHC) mRNA의 수준을 나타낸다.

도 4는 치료 후 96시간에서 대조군 및 HCM iPS 심근세포로부터의 MYH7(β -MHC) 또는 GAPDH의 웨스턴 블롯 분석을 나타낸다.

도 5는 MYH7B에 대한 테스트 ASO 화합물에 의한 형질감염 48시간 후 인간 iPS 심근세포에서 MYH7B mRNA의 수준을 나타낸다.

도 6a는 MYH7B를 표적으로 하는 1, 5 또는 10 μ M 테스트 ASO 화합물(SEQ ID NOs. 92, 146, 32, 46 및 30)의 수동 투여 168시간 후 인간 iPS 심근세포에서 MYH7B mRNA의 수준을 나타낸다. 도 6b는 1, 5 또는 10 μ M 테스트 ASO 화합물의 수동 투여 168시간 후 인간 iPS 심근세포에서 MYH7(β -MHC) mRNA의 수준을 나타낸다. 도 6c는 1, 5 또는 10 μ M의 테스트 ASO 화합물의 수동 투여 168시간 후 인간 iPS 심근세포에서 MYH6(α -MHC) mRNA의 수준을 나타낸다.

도 7은 SEQ ID NO: 148의 서열을 갖는 5 μ M 테스트 ASO의 수동 투여 168시간 후 인간 iPS 심근 세포에서 MYH7B, MYH7(β -MHC) 및 MYH6(α -MHC) mRNA의 수준을 나타낸다.

도 8은 3일 연속으로 하루 당 25 mg/kg의 테스트 ASO를 주사하고 마지막 투여 48시간 후 희생시킨 쥐의 좌심실에서 MYH7B mRNA 수준을 나타낸다.

도 9a는 SEQ ID NO: 146 및 148의 서열을 포함하는 ASO 화합물로 치료된 토끼의 좌심실에서 MYH7B mRNA의 수준을 나타낸다. 도 9b는 SEQ ID NOs: 146 및 148의 서열을 포함하는 ASO 화합물로 치료된 토끼의 우심실에서 MYH7B mRNA의 수준을 나타낸다. 도 9c는 SEQ ID NOs: 146 및 148의 서열을 포함하는 ASO 화합물로 치료된 토끼의 좌심실에서의 MYH7(β -MHC) mRNA의 수준을 나타낸다. 도 9d는 SEQ ID NOs: 146 및 148의 서열을 포함하는 ASO 화합물로 치료된 토끼의 우심실에서 MYH7(β -MHC) mRNA의 수준을 나타낸다.

도 10a는 SEQ ID NO: 146의 서열을 포함하는 화합물에 기초한 ENA 변형 ASO의 수동 투여 48시간 후 인간 iPS 심근세포에서 MYH7B mRNA의 수준을 나타낸다. 도 10b는 SEQ ID NO: 146의 서열을 포함하는 화합물의 5-메틸 시티딘 및 2'-O-메틸 변형 ASO의 수동 투여 48시간 후 인간 iPS 심근 세포에서 MYH7B mRNA의 수준을 나타낸다. 도 10c는 SEQ ID NO: 146의 서열을 포함하는 화합물의 주쇄-변형 ASO의 수동 투여 48시간 후 인간 iPS 심근 세포에서 MYH7B mRNA의 수준을 나타낸다.

도 11a-11d는 MYH7B에 대한 테스트 ASO 화합물의 수동 투여 48시간 후 인간 iPS 심근 세포에서 MYH7B mRNA의 수준을 나타낸다.

발명을 실시하기 위한 구체적인 내용

- [0034] 본 발명자들은 지금까지 단지 miR-499의 비 기능성 담체로서 작용하는 것으로 생각된 MYH7B가 심장 세포에서 β -MHC의 발현 조절에 중요한 역할을 한다는 것을 발견하였다. 특히, 본 발명자들은 MYH7B의 억제가 심장 세포에서 β -MHC mRNA 및 단백질의 발현을 하향 조절한다는 것을 발견하였다. 따라서, 본 발명은 심장 질환, 특히 심장 근육 질환에 대한 치료적 개입을 위한 새로운 표적으로서 MYH7B를 제공한다.
- [0035] 본 발명은 MYH7B의 발현 또는 활성을 억제하는 제제를 제공한다. 다양한 실시태양에서, MYH7B를 억제하는 제제는 MYH7B 유전자, mRNA 또는 pre-mRNA를 표적으로 하는 핵산 억제제 또는 MYH7B 단백질을 표적으로 하는 항체 또는 그의 결합 단편이다. 특정 실시태양에서, MYH7B의 핵산 억제제는 안티센스 올리고뉴클레오타이드, 앵타머, 리보자임, 소형 간섭 RNA 또는 소형 헤어핀 RNA로부터 선택된다.
- [0036] 한 실시태양에서, MYH7B의 억제제는 안티센스 올리고뉴클레오타이드("ASO")이다. 본 발명의 내용에서, 용어 "안티센스 올리고뉴클레오타이드" 또는 "ASO"는 광범위하게 사용되며, 유전자로부터 단백질로의 정보 전달을 억제하는 리보뉴클레오타이드, 디옥시리보뉴클레오타이드, 변형 리보뉴클레오타이드, 변형 테옥시리보뉴클레오타이드 또는 이들의 조합을 포함하는 올리고머를 포함한다. 본 발명에서 사용된 용어 "안티센스 올리고뉴클레오타이드" 또는 "ASO"는 또한 전사 정지; 캡핑(capping), 스플라이싱(splicing), 핵에서 세포질로의 수송을 포함하는 다양한 단계에서의 pre-mRNA로부터의 mRNA 합성의 파괴; 잭머 ASO 또는 다른 RNase H-유도 ASO에 의해 매개되는 것과 같은 mRNA의 분해; ASO가 표적 mRNA에 결합하여 번역에 사용할 수 없게 하는 번역 정지; 또는 표적 mRNA에 혼성화함으로써

표적 mRNA의 입체적 차단을 촉진시키는 리보뉴클레오타이드, 데옥시리보뉴클레오타이드, 변형 리보뉴클레오타이드, 변형 데옥시리보뉴클레오타이드 또는 이들의 조합을 포함하는 올리고머를 포함한다. 본 발명에서 사용된 용어 "안티센스 올리고뉴클레오타이드"는 또한 링커를 통해 펩타이드 또는 당에 접합되거나 연결된 천연 및/또는 변형 뉴클레오타이드를 함유하는 올리고머를 포함하는 안티센스 올리고뉴클레오타이드 컨주게이트를 포함한다. 안티센스 올리고뉴클레오타이드 컨주게이트를 제조하는데 사용될 수 있는 펩타이드 또는 당 모이어티 및 링커는 아래에서보다 상세히 설명된다.

[0037] 다양한 실시태양에서, MYH7B의 활성을 억제하는데 유용한 안티센스 올리고뉴클레오타이드는 약 5 내지 약 25개 뉴클레오타이드 길이, 약 6 내지 약 22개 뉴클레오타이드 길이, 약 10 내지 약 30개 뉴클레오타이드 길이, 약 12 내지 약 20개 뉴클레오타이드 길이, 또는 약 20 내지 약 25개 뉴클레오타이드 길이이다. 특정 실시태양에서, MYH7B를 표적으로 하는 안티센스 올리고뉴클레오타이드는 약 8 내지 약 18개 뉴클레오타이드 길이이며, 다른 실시태양에서 약 12 내지 약 18개 뉴클레오타이드 길이, 또 다른 실시태양에서 약 12개 내지 약 16개 뉴클레오타이드 길이이다. 일부 실시태양에서, MYH7B를 표적으로 하는 안티센스 올리고뉴클레오타이드는 약 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 또는 25개 뉴클레오타이드 길이이다.

[0038] 일부 실시태양에서, MYH7B에 대한 안티센스 올리고뉴클레오타이드 억제제는 약 50 내지 약 150개 뉴클레오타이드 길이, 약 60 내지 약 140개 뉴클레오타이드 길이, 약 70 내지 약 130개 뉴클레오타이드 길이, 약 80 내지 120개 뉴클레오타이드 길이, 약 90 내지 약 110개 뉴클레오타이드, 또는 약 100 내지 약 120개 뉴클레오타이드 길이이다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 약 100 내지 약 120개 뉴클레오타이드 길이이다. 특정 실시태양에서, MYH7B를 표적으로 하는 안티센스 올리고뉴클레오타이드 억제제는 약 80, 90, 100, 110 또는 120개 뉴클레오타이드 길이이다.

[0039] MYH7B를 표적으로 하는 안티센스 올리고뉴클레오타이드는 생리학적 조건하에서 MYH7B에 혼성화하고 대상의 세포에서 MYH7B의 발현 또는 활성을 억제하기 위해 MYH7B 서열(DNA, mRNA 또는 pre-mRNA)에 적어도 부분적으로 또는 실질적으로 상보적인 서열을 포함한다. 예를 들어, 일부 실시태양에서, 안티센스 올리고뉴클레오타이드는 MYH7B 유전자, mRNA 또는 pre-mRNA 서열에 적어도 부분적으로 상보적인, 예를 들어, MYH7B 유전자, mRNA 또는 pre-mRNA 서열에 적어도 약 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% 또는 99% 상보적인 서열을 포함한다. 일부 실시태양에서, 안티센스 올리고뉴클레오타이드는 MYH7B 유전자, mRNA 또는 pre-mRNA 서열에 실질적으로 상보적일 수 있는데, 즉 표적 뉴클레오타이드 서열과 적어도 약 90%, 95%, 96%, 97%, 98% 또는 99% 상보적일 수 있다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드는 MYH7B 유전자, mRNA 또는 pre-mRNA 서열과 100% 또는 완전히 상보적인 서열을 포함한다. 특정 실시태양에서, MYH7B를 표적으로 하는 안티센스 올리고뉴클레오타이드는 인간 MYH7B 유전자의 서열에 적어도 부분적으로, 실질적으로 또는 완전히 상보적인 서열을 포함한다. 일부 실시태양에서, MYH7B를 표적으로 하는 안티센스 올리고뉴클레오타이드는 인간 MYH7B 유전자(진뱅크 수납번호 NM_020884.4; SEQ ID NO:6)의 암호화 서열에 적어도 부분적으로, 실질적으로 또는 완전히 상보적인 서열을 포함한다.

[0040] 인간 MYH7B 암호화 서열(SEQ ID NO:6):

[0041] 1 taggcttaaa gagcaggcgg ggcagagacc catacctttg gcgctgagtc gaggccagga
[0042] 61 aggggcggtc gatgagaggg cgcccttagc attaggagtgc cgtcggtgc accctggcgg
[0043] 121 atggaccttg ggaaggaggg gaggggacca tcggggtcgg cggggagcta cgggttcccc
[0044] 181 tcttccaaga cgggtgccaa gtgatgagta tcaggtgtct atggtgagat ttggggagga
[0045] 241 tccagacct taacagccct ttggttgaaa cctctcaca tgtggcagag atggggtttt
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[0047] 361 cccaaagtgc tggaattaca gacatgagt gcaataaaag gggtagcaga gcttcctgcc
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[0051] 601 ggaagaagcg agtctgggtg cctgatgaac aggacgccta cgtggaggcc gaggtcaagt
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[0053] 721 gtgaagccga gctgcagccc atgaaccgc ctcgcttcga cttactggag gacatggcca

[0054] 781 tgatgacgca cctgaacgag gcctctgtgc tgcacaacct gcgccagcgc tatgcccgct

[0055] 841 ggatgatcta tacctactca ggccctcttct gtgtcaccat caaccctac aaatggctcc

[0056] 901 cagtctatac ggccctccgta gtggctgctt acaagggaaa gcgccgctca gattccccgc

[0057] 961 cccatatata tgcggtggcg gacaacgcct acaacgacat gctgcgcaac cgagacaacc

[0058] 1021 agtccatgct gatcacccga gaggcggggg ccggttaagac ggtaaacacc aagcgggtca

[0059] 1081 ttcagtactt tgccatcgtc gctgccctgg gagacgggcc gggcaagaag gcccatttc

[0060] 1141 tggcaacaaa gacggggggc acccttgagg atcaaatcat cgaggccaac cctgccatgg

[0061] 1201 aggcctttgg caacgccaa accctgagga atgataactc ctcccgttt ggcaagtcca

[0062] 1261 tccgattca ctttggctcc tctgggaagc tggcatccgc ggatattgac agctatctcc

[0063] 1321 tggagaagtc gcgggtgac ttcagtgc ctggtgagcg cagctacat gtctactacc

[0064] 1381 agatcctctc agggaggaag ccagagctgc aggacatgct gcttctgtct atgaaccct

[0065] 1441 atgactacca ctctgcagc caggcgctca tcaccgtgga caaatgaat gatggggagg

[0066] 1501 agctcatcgc caccgacct gccatggaca tcctaggctt cagcgtggat gagaaatgtg

[0067] 1561 cctgctataa gatcgtgggc gccctcctgc actttggcaa catgaagttc aagcagaagc

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[0070] 1741 acgagtacgt gaccaagggc cagagtgtgg agcagggtgt gtttgctgtg ggggctctgg

[0071] 1801 ccaagggcac ctatgaccgg ctgttcaggt ggctgggtgc tcggatcaac cagaccctgg

[0072] 1861 acacaaagct gccccgcag ttcttcacg gggttctgga catcgtggg tttagatct

[0073] 1921 ttgagttcaa cagcttcgaa cagctgtgca tcaacttcac caatgagaaa ttgcagcagt

[0074] 1981 tcttcaacca gcacatgtt gtgctggagc aggaggagta caagcgggag ggcatcgact

[0075] 2041 gggcttctcat cgacttcggc cttgacctgc agccttgcat cgacctcgc gagaagccac

[0076] 2101 tgggcatcct gtccatcctg gaggaggaat gcatgttccc caaggcctca gacgccagct

[0077] 2161 tccgggcca gctctacgac aaccacgcgg ggaagtcacc caatttcag cagcctcggc

[0078] 2221 ctgacaagaa gcgaagtac caggccact tcgaggtgg cactacgca ggcgtggtgc

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[0083] 2521 ccacacagcc ccacttcgtc cgctgcattg tcccaacga gaacaaaacc ccaggggtca

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[0093] 3121 ctgcggccga ggccaagcgc caggaactgg aggagacgca cgtcagcatc acccaggaga

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[0095] 3241 gctgccactt gctgatcaag tccaaggtgc agctggaggg gaaggtgaag gagctgagt

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[0121] 4801 agtcagagga tgtaacctg gagctggagc gggcgacctc agcagctgct gcgctggaca

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[0132] 5461 ggctgatgca ggcacagctc aaggaggagc aggcaggcg ggacgaggag cagcggttg

[0133] 5521 cagctgagct ccacgagcag gcgcaggctc tggagcgccg ggctctgctg ctggctgcgg

[0134] 5581 agctggagga gctgcgggct gccctggagc agggcgagcg cagccggcga ctggcagagc

[0135] 5641 aggagctttt ggaggccacc gagcgctca acctttctgca ttgcagaac acaggcctcc

[0136] 5701 taaaccagaa gaagaagctg gaggcggact tggcccagct gagcggggag gtggaggagg

[0137] 5761 ctgcacagga gaggcgggag gctgaggaga aggccaaaaa ggccatcact gatgcggcca

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[0146] 6301 cccgggacgc cctgggcccc aagcacaagg agtgacggcc tgacccctg ggctctaaag

[0147] 6361 aggaatgtct gctgttgca atctggctga gggcacctgc ccgatctg ccctctctgc

[0148] 6421 atgccccct gctgccttca gccttccctg ggccctgaat aaacaccaca gccagtttcc

[0149] 6481 ttctcattct tttctttggg gttcaggagg aaaaacacag tcctaggac aaaagccagg

[0150] 6541 tccacagcag tcatttttaa aataaagtta tttaatagtc tccaaaaaaa aaaaaaaaaa

[0151] 6601 aa

[0152] 안티센스 올리고뉴클레오타이드 서열이 자연 발생 뉴클레오타이드 대신에 변형 뉴클레오타이드를 포함하는 경우에도, 안티센스 올리고뉴클레오타이드 억제제의 서열은 MYH7B 유전자, mRNA 또는 pre-mRNA 서열에 상보적인 것으로 생각 되는 것이 이해된다. 예를 들어, MYH7B 유전자, mRNA 또는 pre-mRNA 서열이 특정 위치에서 구아노 신 뉴클레오 티드를 포함하는 경우, 안티센스 올리고뉴클레오타이드 억제제는 상응하는 위치에 잠금 시티딘 뉴클레오타이드 또는 2'-플루오로-시티딘과 같은 변형 시티 딘 뉴클레오타이드를 포함할 수 있다.

[0153] 한 실시태양에서, 본 발명은 MYH7B의 안티센스 올리고뉴클레오타이드 억제제를 제공하며, 상기 안티센스 올리고뉴 클레오타이드는 8 내지 18개 뉴클레오타이드의 길이를 가지며 안티센스 올리고뉴클레오타이드의 서열은 SEQ ID NO: 6 에 실질적으로 상보적이다.

[0154] 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 8 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오 티드 4300-4335로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다.

[0155] 특정 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12-18개 뉴클레오타이드의 길이를 가지며, 뉴클레오타

드 4300-4317, 4300-4316, 4301-4316, 4301-4315, 4302-4317, 4302-4316, 또는 4302-4315로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12-14개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 4302-4315로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 14개 뉴클레오타이드의 길이를 가지며 안티센스 올리고뉴클레오타이드의 서열은 뉴클레오타이드 4302-4315로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적이다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lTslTslGsdAsdTsdCsdTsdTsdGsdGsdCsICsITsIC-3'(SEQ IN NO: 146)의 서열을 포함하며, 여기서 l = 잠금 핵산 변형, d = 테옥시뉴클레오타이드 및 s = 포스포로티오에이트 결합이다.

[0156] 특정 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12-18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 4316-4333, 4316-4332, 4317-4332, 4317-4331, 4318-4333, 4318-4332 또는 4318-4331로부터의 SEQ IN NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12-14개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 4318-4331로부터의 SEQ IN NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 일부 실시태양에서, 안티센스 올리고뉴클레오타이드는 14개 뉴클레오타이드의 길이를 가지며 안티센스 올리고뉴클레오타이드의 서열은 뉴클레오타이드 4318-4331로부터의 SEQ IN NO: 6의 서열에 실질적으로 상보적이다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lCsITsICsdCsdAsdTsdCsdCsdTsICsICsIA-3'(SEQ IN NO: 148)의 서열을 포함한다.

[0157] 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 8 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 1762-1783으로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12-18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 1762-1779, 1763-1779, 1764-1781, 1765-1780 또는 1766-1779로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12-14개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 1764-1777 또는 1766-1779로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lAsICsICsdTsdGsdCsdTsdCsdCsdAsdTsdCsICsIT-3'(SEQ ID NO: 62)의 서열을 포함한다. 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lCsICsICsdCsdTsdGsdCsdTsdCsdCsICsIA-3'(SEQ ID NO: 64)의 서열을 포함한다.

[0158] 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 8 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 511-538으로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12-18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 511-532, 512-530, 513-526, 514-528, 514-527, 515-530, 515-529 또는 515-528로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12-14개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 513-526, 514-527 또는 515-528로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12-18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 521-538, 522-538, 522-537, 523-538, 523-537 또는 523-536으로부터의 SEQ IN NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12-14개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 522-537 또는 523-536으로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lTsICsICsdCsdTsdCsdAsdTsdCsdAsdTsdCsdCsICsIT-3'(SEQ ID NO: 184)의 서열을 포함하며, 여기서 md = 5-메틸 시토신이다. 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lTslTslCsdAsdTsdCsdAsdTsdCsdAsdTsdCsdTsICsICsIA-3'(SEQ ID NO: 186)의 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lGsITsITsdCsdAsdTsdCsdAsdTsdCsdAsdTslTsICsIC-3'(SEQ ID NO: 188)의 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lCsITsICsdCsdCsdCsdAsdTsdGsdTsdTsICsIAIC-3'(SEQ ID NO: 204)의 서열을 포함한다.

[0159] 특정 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 8 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 1640-1701로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 1643-1660, 1648-1665, 1653-1670 또는 1694-1711의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12 내지 14개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 1645-1658, 1650-1663, 1655-1668 또는 1696-1709의 SEQ ID NO: 6의 서열과 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-

lAslCs1TsmcCsdTsmcCsdAsdGsdTsdGsmcCs1Cs1As1T-3'(SEQ ID NO: 230)의 서열을 포함한다. 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lTs1Cs1AsdGsmcCsdAsmdCsdTsmcCsdTsmcCs1As1Gs1T-3'(SEQ ID NO: 238)의 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lCs1Cs1TsdTsdGsdTsmcCsdAsdGsmcCsdAs1Cs1Ts1C-3'(SEQ ID NO: 244)의 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lTs1Ts1TsdGsdAsdGsdGsdGsdGsdGsdTs1Cs1Cs1C-3'(SEQ ID NO: 272)의 서열을 포함한다.

[0160] 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 4280-4300으로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 4280-4297, 4282-4295, 4283-4300 또는 4283-4296으로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lCs1Ts1GsdAsdTsmcCsmcCsdTsmcCsdAsdTs1As1Gs1G-3'(SEQ ID NO: 304)의 서열을 포함한다. 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lGs1Cs1TsdGsdAsdTsmcCsmcCsdTsmcCsdAs1Ts1As1G-3'(SEQ ID NO: 306)의 서열을 포함한다.

[0161] 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 688-705, 689-706, 690-707 또는 690-703으로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lTs1Gs1GsdTsmcCsdTsdTsdTsdGsdGsdTs1Cs1Ts1C-3'(SEQ ID NO: 344)의 서열을 포함한다. 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 890-907, 892-909 또는 892-905로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lGs1As1CsdTsdGsdGsdGsdGsdGsdGsdCsmcCs1As1Ts1T-3'(SEQ ID NO: 350)의 서열을 포함한다.

[0162] 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 1217-1234, 1218-1235, 1219-1236, 또는 1219-1232로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lAs1Ts1TsmcCsmcCsdTsmcCsdAsdGsdGsdGs1Ts1Cs1T-3'(SEQ ID NO: 362)의 서열을 포함한다. 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 1224-1241, 1225-1242, 1226-1243 또는 1226-1239로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lAs1Gs1TsdTsdAsdTsmcCsdAsdTsdTsmcCs1Cs1Ts1C-3'(SEQ ID NO: 370)의 서열을 포함한다.

[0163] 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 1415-1441로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 1415-1432, 1416-1433, 1417-1434 또는 1417-1430으로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lAs1Gs1AsmdCsdAsdGsdAsdGsdGsmcCsdAs1Gs1Cs1A-3'(SEQ ID NO: 396)의 서열을 포함한다. 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 1422-1439, 1423-1440, 1424-1441 또는 1424-1437로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 5'-lGs1Gs1TsdTsmcCsdAsdTsdAsdGsdAsmdCs1As1Gs1A-3'(SEQ ID NO: 410)의 서열을 포함한다.

[0164] 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 8 내지 18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 2348-2371로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12-18개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 2348-2365, 2349-2364, 2348-2361, 2349-2366, 2350-2365, 2351-2366, 2352-2367, 2352-2365, 2353-2370, 2354-2369 또는 2354-2368로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12-14개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 2349-2362 또는 2350-2363으로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 12-14개 뉴클레오타이드의 길이를 가지며 뉴클레오타이드 2350-2363 또는 2351-2364로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클

레오티드 억제제는 12-14개 뉴클레오티드의 길이를 가지며 뉴클레오티드 2352-2365로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 12-14개 뉴클레오티드의 길이를 가지며 뉴클레오티드 2353-2366 또는 2354-2367로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 5'-lAslTs1TsmcCsdTsdGsdTsdGsdAsmdCsdTs1Ts1Cs1T-3'(SEQ ID NO: 474)의 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 5'-lTs1AslTsdTsmcCsdTsdGsdTsdGsdAsmdCs1Ts1Ts1C-3'(SEQ ID NO: 476)의 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 5'-lCs1Ts1AsdTsdTsmcCsdTsdGsdTsdGsdAslCs1Ts1T-3'(SEQ ID NO: 478)의 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 5'-lGs1Cs1CsdTsdAsdTsdTsmcCsdTsdGsdTs1Gs1A1C-3'(SEQ ID NO: 482)의 서열을 포함한다.

[0165] 일부 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 12 내지 18개 뉴클레오티드의 길이를 가지며 뉴클레오티드 2442-2462로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 특정 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 12 내지 14개 뉴클레오티드의 길이를 가지며 뉴클레오티드 2444-2457 또는 2445-2458로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 5'-lAslTs1GsmcCsdTsdGsmcCsmcCsdTsdTsmcCs1Ts1Ts1A-3'(SEQ ID NO: 508)의 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 5'-lGs1AslTsdGsmcCsdTsdGsmcCsmcCsdTsdTs1Cs1Ts1T-3'(SEQ ID NO: 510)의 서열을 포함한다.

[0166] 일부 다른 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 12 내지 18개 뉴클레오티드의 길이를 가지며 뉴클레오티드 4408-4432로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 다양한 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 12 내지 18개 뉴클레오티드의 길이를 가지며 뉴클레오티드 4408-4425, 4409-4426, 4410-4425, 4411-4428, 4411-4427, 4412-4429, 4412-4428 또는 4413-4430으로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 한 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 12 내지 16개 뉴클레오티드의 길이를 가지며 뉴클레오티드 4409-4424 또는 4410-4425로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 다른 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 12 내지 16개 뉴클레오티드의 길이를 가지며 뉴클레오티드 4411-4426 또는 4412-4427로부터의 SEQ ID NO: 6의 서열에 실질적으로 상보적인 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 5'-lAslTs1CsdAsdGsdAsmdCsdAsmdCsdTsmcCs1Cs1Ts1T-3'(SEQ ID NO: 570)의 서열을 포함한다. 다른 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 5'-lTs1Gs1AsdTsmcCsdAsdGsdAsmdCsdAsmdCs1Ts1Cs1C-3'(SEQ ID NO: 572)의 서열을 포함한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 5'-lCs1Ts1GsdAsdTsmcCsdAsdGsdAsmdCsdAslCs1Ts1C-3'(SEQ ID NO: 574)의 서열을 포함한다.

[0167] 일부 실시태양에서, MYH7B를 표적으로 하는 안티센스 올리고뉴클레오티드는 SEQ ID NO: 6의 뉴클레오티드 1182 내지 1213으로부터의 인간 MYH7B 유전자의 서열에 적어도 부분적으로 상보적이거나 완전히 상보적인 서열을 포함한다. 다른 실시태양에서, MYH7B를 표적으로 하는 안티센스 올리고뉴클레오티드는 SEQ ID NO: 6의 뉴클레오티드 1365 내지 1393으로부터 인간 MYH7B 유전자의 서열에 적어도 부분적으로 상보적이거나 완전히 상보적인 서열을 포함한다.

[0168] 한 실시태양에서, MYH7B를 표적으로 하는 안티센스 올리고뉴클레오티드는 5'-GTGAATGCGGATGAA-3'(SEQ ID NO: 1)의 서열을 가진다. 다른 실시태양에서, MYH7B의 안티센스 올리고뉴클레오티드는 5'-GAAGTGGTAGTCATA-3'(SEQ ID NO: 2)의 서열을 가진다. 또 다른 실시태양에서, MYH7B의 안티센스 올리고뉴클레오티드는 5'-lGs1Ts1GsdAsdAsdTsdGsdCsdGsdGsdAsdTs1Gs1A-3'(SEQ ID NO: 3)의 서열을 가진다.

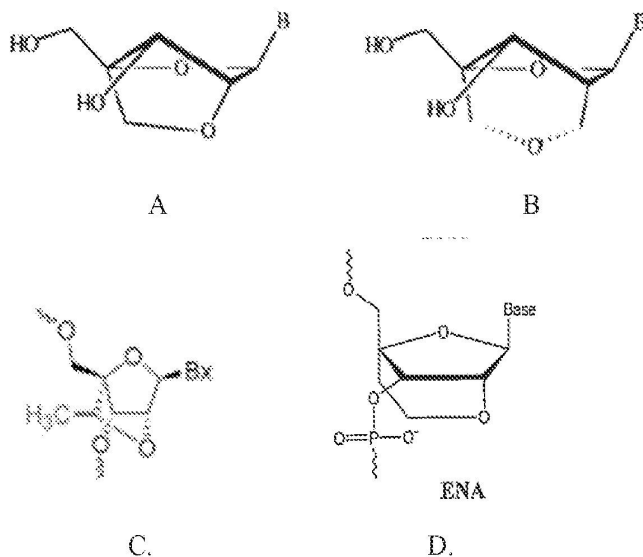
[0169] 본 발명에서 사용된 용어 "약"은 +/- 10% 및 더욱 바람직하게는 +/- 5%의 변화를 포함하는데, 이는 이런 변화가 본 발명을 실시하는데 적절하기 때문이다.

[0170] 한 실시태양에서, MYH7B의 안티센스 올리고뉴클레오티드 억제제는 적어도 하나의 포스포로티오에이트, 모르폴리노 또는 포스포노카복실레이트 인터뉴클레오티드 결합과 같은 적어도 하나의 주쇄 변형을 함유한다(예를 들어, 본 발명에 전문이 참조로 포함된 미국 특허 제 6,693,187호 및 제 7,067,641호 참조). 일부 실시태양에서, MYH7B의 안티센스 올리고뉴클레오티드 억제제는 2개 이상의 포스포로티오에이트 결합을 함유한다. 특정 실시태양에서, MYH7B의 안티센스 올리고뉴클레오티드 억제제는 완전히 포스포로티오에이트 연결된다. 선택적으로, 안

티센스 올리고뉴클레오타이드는 당-포스페이트 주쇄보다는 펩타이드-기초 주쇄를 함유하는 펩타이드 핵산 (PNA)을 포함할 수 있다.

[0171]

한 실시태양에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제는 잠금 뉴클레오타이드 또는 다른 당, 염기 및/또는 주쇄 변형을 함유하는 뉴클레오타이드와 같은 적어도 하나의 변형 뉴클레오타이드를 함유한다. 용어 "잠금 핵산", "잠금 핵산 유닛", "잠금 핵산 잔기", "LNA 유닛", "가교 핵산", "가교 뉴클레오타이드", "BNA"는 본 발명에서 상호 교환하여 사용할 수 있으며 바이사이클릭 뉴클레오시드/뉴클레오타이드 유사체를 의미할 수 있다. 구체적으로, "가교 핵산" 또는 "가교 뉴클레오타이드"는 당 잔기의 2', 4'-위치 또는 3', 4'-위치에 가교를 함유하는 뉴클레오타이드를 의미한다. 예를 들어, 적절한 안티센스 올리고뉴클레오타이드 억제제는 가교 뉴클레오타이드를 함유하는 올리고뉴클레오타이드 및 이의 상보적인 표적 가닥 사이에 형성된 복합체에 향상된 열 안정성을 부여하는 하나 이상의 "입체적으로 제한된" 또는 2', 4'-가교 뉴클레오타이드 변형으로 이루어질 수 있다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 통상적으로 "잠금 뉴클레오타이드" 또는 "LNA"로 공지된 2'-O, 4'-C-메틸렌 가교 리보뉴클레오시드(구조 A)를 함유한다. 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 적어도 하나의 2', 4'-C-가교 2'-데옥시리보뉴클레오시드(CDNA, 구조 B)를 함유한다. 예를 들어, 미국 특허 제 6,403,566호 및 Wang et al. (1999) Bioorganic and Medicinal Chemistry Letters, Vol. 9:1147-1150을 참조하기 바라며, 이들 둘 모두는 전문이 참조로 본 발명에 포함된다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 구조 C로 나타낸 구조를 갖는 적어도 하나의 변형 뉴클레오시드를 함유한다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 적어도 하나의 2'-O, 4'-C-에틸렌 가교 리보뉴클레오시드(구조 D)를 함유한다. 2'-O, 4'-C-에틸렌 가교를 함유하는 핵산 또는 올리고뉴클레오타이드 또는 뉴클레오타이드는 또한 에틸렌 가교 핵산(ENA)으로 알려져있다. 또 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 "아미노-2'-C-가교 바이사이클릭 뉴클레오타이드" 또는 "아미노-CBBN"으로도 알려진 적어도 하나의 2'-CH₂-NH-CH₂-4'-가교 뉴클레오타이드를 함유한다. MYH7B를 표적으로 하는 안티센스 올리고뉴클레오타이드 억제제는 상기 가교 뉴클레오타이드 또는 다른 변형 뉴클레오타이드 및 변형되지 않은 리보뉴클레오타이드 또는 데옥시 리보뉴클레오타이드의 조합을 함유할 수 있다.



[0172]

[0173]

본 발명의 내용에서 DNA 또는 RNA 뉴클레오타이드를 이의 상응하는 가교 또는 잠금 뉴클레오타이드로 치환하는 것을 언급할 때, "상응하는 가교 뉴클레오타이드" 또는 "상응하는 잠금 뉴클레오타이드"라는 용어는 DNA/RNA 뉴클레오타이드가 대체된 DNA/RNA 뉴클레오타이드와 동일한 자연 발생적 질소 함유 염기 또는 화학적으로 변형된 동일한 질소 함유 염기를 함유하는 가교 또는 잠금 뉴클레오타이드에 의해 대체되었다는 것을 의미한다. 예를 들어, 질소 함유 염기 C를 함유하는 DNA 뉴클레오타이드의 상응하는 가교 또는 잠금 뉴클레오타이드는 동일한 질소 함유 염기 C 또는 5-메틸시토신과 같이 화학적으로 변형된 동일한 질소 함유 염기 C를 함유할 수 있다.

[0174]

용어 "비-가교 뉴클레오타이드" 또는 "비-잠금 뉴클레오타이드"는 가교 또는 잠금 뉴클레오타이드와 상이한 뉴클레오타이드를 의미하는데, 즉 용어 "비-가교 뉴클레오타이드" 또는 "비-잠금 뉴클레오타이드"는 DNA 뉴클레오타이드, RNA 뉴클레오타이드뿐만 아니라 변형이 가교 또는 잠금 변형이 아닌 것을 제외하고 염기 및/또는 당이 변형된 변형 뉴클레오타이드를 포함한다.

- [0175] 한 실시태양에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제는 적어도 하나의 잠금 뉴클레오타이드를 함유한다. 일부 실시태양에서, MYH7B의 안티센스 올리고뉴클레오타이드 억제제는 1개 내지 6개의 잠금 뉴클레오타이드를 함유한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제의 3' 말단으로부터의 적어도 처음 3개 뉴클레오타이드는 잠금 뉴클레오타이드이다. 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제의 5' 말단으로부터의 적어도 처음 3개 뉴클레오타이드는 잠금 뉴클레오타이드이다.
- [0176] 특정 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 1 내지 6개의 천연 또는 변형 리보뉴클레오타이드를 함유한다. 한 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제의 3' 말단으로부터의 적어도 처음 3개 뉴클레오타이드는 천연 또는 변형 리보뉴클레오타이드이다. 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제의 5' 말단으로부터의 적어도 처음 3개 뉴클레오타이드는 천연 또는 변형 리보뉴클레오타이드이다. 일부 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제의 3' 말단으로부터의 처음 3개 뉴클레오타이드는 잠금 리보뉴클레오타이드이다. 다른 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제의 5' 말단으로부터의 처음 3개 뉴클레오타이드는 잠금 리보뉴클레오타이드이다.
- [0177] 본 발명의 올리고뉴클레오타이드 억제제는 염기 변형 또는 치환을 갖는 변형 뉴클레오타이드를 포함할 수 있다. 천연 또는 비 변형 염기는 퓨린 염기인 아데닌(A) 및 구아닌(G) 및 피리미딘 염기인 시토신(C), 티민(T) 및 우라실(U)이다. 해테로 사이클릭 염기 모이어티로도 불리는 변형 염기는 5-메틸시토신(5-me-C), 5-하이드 록시메틸 시토신, 잔틴, 하이포잔틴, 2-아미노아데닌, 6-메틸 및 아데닌과 구아닌의 다른 알킬 유도체, 2-프로필 및 아데닌 및 구아닌의 다른 알킬 유도체, 2-티오우라실, 2-티오티민 및 2-티오시토신, 5-할로우라실 및 시토신, 5-프로판일 우라실 및 시토신 및 피리미딘 염기의 다른 알킬일 유도체, 6-아조 우라실, 시토신 및 티민, 5-우라실(pseudouracil), 4-티오우라실, 8-할로, 8-아미노, 8-티올, 8-티오알킬, 8-하이드록실 및 다른 8-치환된 아데닌 및 구아닌, 5-할로(5-브로모, 5-트라이플루오로메틸 및 다른 5-치환 우라실 및 시토신 포함), 7-메틸구아닌 및 7-메틸아데닌, 2-F-아데닌, 2-아미노-아데닌, 8-아자구아닌 및 8-아자아데닌, 7-테아자구아닌 및 7-테아자아데닌 및 3-테아자구아닌 및 3-테아자아데닌과 같은 다른 합성 및 천연 핵염기를 포함한다. 한 실시태양에서, 본 발명의 안티센스 올리고뉴클레오타이드 억제제는 1 내지 5개의 5-메틸시티딘 또는 5-메틸데옥시시티딘 뉴클레오타이드를 포함한다. 특정 실시태양에서, 안티센스 올리고뉴클레오타이드 억제제는 염기 변형 (예를 들어, 5- 메틸시티딘)과 함께 하나 이상의 가교 핵산 변형(예를 들어, LNA, ENA 등)을 포함한다.
- [0178] 본 발명의 올리고뉴클레오타이드 억제제는 변형 당 모이어티를 갖는 뉴클레오타이드를 포함할 수 있다. 대표적인 변형 당은 카보사이클릭 또는 비 사이클릭 당, 2', 3' 또는 4' 위치 중 하나 이상에 치환기를 갖는 당 및 당의 하나 이상의 수소 원자 대신 치환기를 갖는 당을 포함한다. 특정 실시태양에서, 당은 2' 위치에 치환기를 가짐으로써 변형된다. 추가의 실시태양에서, 당은 3' 위치에 치환기를 가짐으로써 변형된다. 다른 실시태양에서, 당은 4' 위치에 치환기를 가짐으로써 변형된다. 당이 이런 위치 중 하나 이상에서 변형을 가질 수 있거나 또는 올리고뉴클레오타이드 억제제가 한 위치에서 당 변형을 갖는 하나 이상의 뉴클레오타이드 및 상이한 위치에서 당 변형을 갖는 하나 이상의 뉴클레오타이드를 가질 수 있다는 것도 또한 고려된다.
- [0179] 본 발명의 올리고뉴클레오타이드 억제제에서 고려되는 당 변형은 OH; F; O-, S- 또는 N-알킬; O-, S- 또는 N-알켄일; O-, S- 또는 N-알카인일; 또는 O-알킬 -O- 알킬로부터 선택된 치환기를 포함하나 이에 제한되지 않으며, 알킬, 알켄일 및 알카인일은 C₁ 내지 C₁₀ 알킬 또는 C₂ 내지 C₁₀ 알켄일 및 알카인일로 치환되거나 비치 환될 수 있다. 한 실시태양에서, 변형은 2'-메톡시에톡시(2'-O-CH₂CH₂OCH₃, 2'-O- (2-메톡시에틸) 또는 2'-MOE로도 알려짐), 즉 알콕시알콕시기를 포함한다. 다른 변형은 2'-다이메틸아미노옥시에톡시, 즉 2'-DMAOE 및 2'-다이메틸아미노에톡시에톡시(이 기술 분야에서 2'-O-다이메틸-아미노-에톡시-에틸 또는 2'-DMAEOE로 공지됨)로도 공지된 O(CH₂)₂ON(CH₃)₂기, 즉 2'-O-CH₂-O-CH₂-N(CH₃)₂를 포함한다.
- [0180] 추가의 당 치환기는 알릴(-CH₂-CH=CH₂), -O-알릴, 메톡시(-O-CH₃), 아미노프로폭시(-OCH₂CH₂CH₂NH₂) 및 플루오로(F)를 포함한다. 2' 위치(2'-) 상의 당 치환기는 아라비노(위) 또는 리보(아래) 위치에 있을 수 있다. 하나의 2'-아라비노 변형은 2'-F이다. 다른 유사한 변형이 당 모이어티 상의 다른 위치, 특히 3' 말단 뉴클레오타이드 또는 2'-5' 연결 올리고뉴클레오타이드에서 당의 3' 위치 및 5' 말단 뉴클레오타이드의 5' 위치에서 이루어질 수 있다. 특정 실시태양에서, 당 변형은 2'-O-알킬 (예를 들어, 2'-O-메틸, 2'-O-메톡시에틸), 2'-할로(예를 들어, 2'-플루오로, 2'-클로로, 2'-브로모) 및 4' 티오 변형이다.
- [0181] 전문이 참조로 본 발명에 포함된 미국 특허 제 6,838,283호에 기술된 바와 같이, 안정성을 향상시키고 효능을 향상시키는 올리고뉴클레오타이드 억제제의 다른 변형은 당해 기술분야에 공지되어 있으며 본 방법에서의 사용에

적합하다. 예를 들어, 생체 내 전달 및 안정성을 촉진하기 위해, 올리고뉴클레오티드 억제제는 이의 3' 말단에서 콜레스테롤 모이어티, 비타민, 지방산, 탄수화물 또는 글리코시드, 펩타이드 또는 다른 소분자 리간드와 같은 스테로이드와 연결될 수 있다. 일부 실시태양에서, 안티센스 올리고뉴클레오티드 억제제는 표적 세포에 의한 억제제의 흡수를 증진시키기 위해 펩타이드에 부착된다. 예를 들어, 일부 실시태양에서, 안티센스 올리고뉴클레오티드 억제제의 3' 말단은 서열 "WLSEAGPVVTVRALRGTSW"를 갖는 펩타이드에 부착된다. 이 펩타이드는 본 발명에 참조로 포함된 McGuire et al("In vitro selection of a peptide with high selectivity for cardiomyocytes in vivo", J. Mol. Bio, 2004, vol. 342, 171-182)에 기술된다. 한 실시태양에서, 펩타이드는 "thiolC3"과 같은 링커를 통해 안티센스 올리고에 부착되어, 티오에터 결합이 링커의 SH기와 펩타이드 상의 올리고 및 말레이미드 사이에 형성된다. 일부 실시태양에서, 안티센스 올리고뉴클레오티드 억제제의 5' 말단은 직접 또는 링커를 통해 당 모이어티에 부착될 수 있다. 링커는 C6-다이설파이드 링커 또는 포스포다이에스테르 dTdT 링커와 같은 1-3 포스포다이에스테르 링커일 수 있다.

[0182] 다양한 실시태양에서, MYH7B의 안티센스 올리고뉴클레오티드는 표 1 내지 5로부터 선택된 서열을 가진다.

표 1

[0183]

SEQ ID NO: 6의 표적 서열의 시작 뉴클레오티드	서열(5'-3')	변형을 가진 서열(5'-3')
733	GTTTCATGGGCTGCA (SEQ ID NO: 7)	IGsITsITsdCsdAsdTsdGsdGsdGsdCsdTsIGsICsIA (SEQ ID NO: 8)
734	GGTTCATGGGCTGC (SEQ ID NO: 9)	IGsIGsITsdTsdCsdAsdTsdGsdGsdGsdCsITsIGsIC (SEQ ID NO: 10)
735	GGGTTCATGGGCTG (SEQ ID NO: 11)	IGsIGsIGsdTsdTsdCsdAsdTsdGsdGsdGsICsITsIG (SEQ ID NO: 12)
867	ATGGTGACACAGAA (SEQ ID NO: 13)	IAsITsIGsdGsdTsdGsdAsdCsdAsdCsdAsIGsIA (SEQ ID NO: 14)
1020	ATCAGCATGGACTG (SEQ ID NO: 15)	IAsITsICsdAsdGsdCsdAsdTsdGsdGsdAsICsITsIG (SEQ ID NO: 16)
1061	GCTTGGTGTAAACC (SEQ ID NO: 17)	IGsICsITsdTsdGsdGsdTsdGsdTsdTsdAsIAcIC (SEQ ID NO: 18)
1188	TCCATGGCAGGGTT (SEQ ID NO: 19)	ITsICsICsdAsdTsdGsdGsdCsdAsdGsdGsIGsITsIT (SEQ ID NO: 20)
1189	CTCCATGGCAGGGT (SEQ ID NO: 21)	ICsITsICsdCsdAsdTsdGsdGsdCsdAsdGsIGsIGsIT (SEQ ID NO: 22)
1190	CCTCCATGGCAGGG (SEQ ID NO: 23)	ICsICsITsdCsdCsdAsdTsdGsdGsdCsdAsIGsIGsIG (SEQ ID NO: 24)
1191	GCCTCCATGGCAGG (SEQ ID NO: 25)	IGsICsICsdTsdCsdCsdAsdTsdGsdGsdCsIAcIGsIG (SEQ ID NO: 26)
1305	AGATAGCTGTCAAT (SEQ ID NO: 27)	IAsIGsIAsdTsdAsdGsdCsdTsdGsdTsdCsIAcIAcIT (SEQ ID NO: 28)
1306	GAGATAGCTGTCAA (SEQ ID NO: 29)	IGsIAcIGsdAsdTsdAsdGsdCsdTsdGsdTsICsIAcIA (SEQ ID NO: 30)
1307	GGAGATAGCTGTCA (SEQ ID NO: 31)	IGsIGsIAsdGsdAsdTsdAsdGsdCsdTsdGsITsICsIA (SEQ ID NO: 32)
1308	AGGAGATAGCTGTC (SEQ ID NO: 33)	IAcIGsIGsdAsdGsdAsdTsdAsdGsdCsdTsIGsITsIC (SEQ ID NO: 34)
1309	CAGGAGATAGCTGT (SEQ ID NO: 35)	ICsIAcIGsdGsdAsdGsdAsdTsdAsdGsdCsITsIGsIT (SEQ ID NO: 36)
1310	CCAGGAGATAGCTG (SEQ ID NO: 37)	ICsICsIAsdGsdGsdAsdGsdAsdTsdAsdGsICsITsIG (SEQ ID NO: 38)
1311	TCCAGGAGATAGCT (SEQ ID NO: 39)	ITsICsICsdAsdGsdGsdAsdGsdAsdTsdAsIGsICsIT (SEQ ID NO: 40)
1443	CAGAAGTGGTAGTC (SEQ ID NO: 41)	ICsIAcIGsdAsdAsdGsdTsdGsdGsdTsdAsIGsITsIC (SEQ ID NO: 42)

1445	TGCAGAAGTGGTAG (SEQ ID NO: 43)	lTs1Gs1CsdAsdGsdAsdAsdGsdTsdGsdGs1Ts1As1G (SEQ ID NO: 44)
1446	CTGCAGAAGTGGTA (SEQ ID NO: 45)	lCs1Ts1GsdCsdAsdGsdAsdAsdGsdTsdGs1Gs1Ts1A (SEQ ID NO: 46)
1647	GCACTCTCAGTGCC (SEQ ID NO: 47)	lGs1Cs1AsdCsdTsdCsdTsdCsdAsdGsdTs1Gs1Cs1C (SEQ ID NO: 48)
1662	TAGGCAGCCTTGTC (SEQ ID NO: 49)	lTs1As1GsdGsdCsdAsdGsdCsdCsdTsdTs1Gs1Ts1C (SEQ ID NO: 50)
1755	AACTCTGGCCCTT (SEQ ID NO: 51)	lAs1Cs1AsdCsdTsdCsdTsdGsdGsdCsdCs1Cs1Ts1T (SEQ ID NO: 52)
1758	TCCACACTCTGGCC (SEQ ID NO: 53)	lTs1Cs1CsdAsdCsdAsdCsdTsdCsdTsdGs1Gs1Cs1C (SEQ ID NO: 54)
1759	CTCCAACTCTGGC (SEQ ID NO: 55)	lCs1Ts1CsdCsdAsdCsdAsdCsdTsdCsdTs1Gs1Gs1C (SEQ ID NO: 56)
1760	GCTCCAACTCTGG (SEQ ID NO: 57)	lGs1Cs1TsdCsdCsdAsdCsdAsdCsdTsdCs1Ts1Gs1G (SEQ ID NO: 58)
1761	TGCTCCAACTCTG (SEQ ID NO: 59)	lTs1Gs1CsdTsdCsdCsdAsdCsdAsdCsdTs1Cs1Ts1G (SEQ ID NO: 60)
1764	ACCTGCTCCAACT (SEQ ID NO: 61)	lAs1Cs1CsdTsdGsdCsdTsdCsdCsdAsdCs1As1Cs1T (SEQ ID NO: 62)
1766	CCAACTGCTCCACA (SEQ ID NO: 63)	lCs1Cs1AsdCsdCsdTsdGsdCsdTsdCsdCs1As1Cs1A (SEQ ID NO: 64)
1767	ACCACCTGCTCCAC (SEQ ID NO: 65)	lAs1Cs1CsdAsdCsdCsdTsdGsdCsdTsdCs1Cs1As1C (SEQ ID NO: 66)
1912	CTCAAAGATCTCAA (SEQ ID NO: 67)	lCs1Ts1CsdAsdAsdAsdGsdAsdTsdCsdTs1Cs1As1A (SEQ ID NO: 68)
1913	ACTCAAAGATCTCA (SEQ ID NO: 69)	lAs1Cs1TsdCsdAsdAsdAsdGsdAsdTsdCs1Ts1Cs1A (SEQ ID NO: 70)
1914	AACTCAAAGATCTC (SEQ ID NO: 71)	lAs1As1CsdTsdCsdAsdAsdAsdGsdAsdTs1Cs1Ts1C (SEQ ID NO: 72)
2037	ATGAAGACCCAGTC (SEQ ID NO: 73)	lAs1Ts1GsdAsdAsdGsdAsdCsdCsdCsdAs1Gs1Ts1C (SEQ ID NO: 74)
2038	GATGAAGACCCAGT (SEQ ID NO: 75)	lGs1As1TsdGsdAsdAsdGsdAsdCsdCsdCs1As1Gs1T (SEQ ID NO: 76)
2253	TAGTGGACCACTC (SEQ ID NO: 77)	lTs1As1GsdTsdGsdGsdAsdCsdCsdAsdCs1Cs1Ts1C (SEQ ID NO: 78)
2481	AGGTTCTCCTGTG (SEQ ID NO: 79)	lAs1Gs1GsdTsdTsdCsdTsdCsdCsdTsdTs1Gs1Ts1G (SEQ ID NO: 80)
2482	GAGGTTCTCCTGT (SEQ ID NO: 81)	lGs1As1GsdGsdTsdTsdCsdTsdCsdCsdTs1Ts1Gs1T (SEQ ID NO: 82)
2483	TGAGGTTCTCCTTG (SEQ ID NO: 83)	lTs1Gs1AsdGsdGsdTsdTsdCsdTsdCsdCs1Ts1Ts1G (SEQ ID NO: 84)
2484	TTGAGGTTCTCCTT (SEQ ID NO: 85)	lTs1Ts1GsdAsdGsdGsdTsdTsdCsdTsdCs1Cs1Ts1T (SEQ ID NO: 86)
2485	GTTGAGGTTCTCCT (SEQ ID NO: 87)	lGs1Ts1TsdGsdAsdGsdGsdTsdTsdCsdTs1Cs1Cs1T (SEQ ID NO: 88)
2488	CTTGTTGAGGTTCT (SEQ ID NO: 89)	lCs1Ts1TsdGsdTsdTsdGsdAsdGsdGsdTs1Ts1Cs1T (SEQ ID NO: 90)
2518	GGGCTGTGTGGCCC (SEQ ID NO: 91)	lGs1Gs1GsdCsdTsdGsdTsdGsdTsdGsdGs1Cs1Cs1C (SEQ ID NO: 92)
2738	CCTTCCTGCTGTCC (SEQ ID NO: 93)	lCs1Cs1TsdTsdCsdCsdTsdGsdCsdTsdGs1Ts1Cs1C (SEQ ID NO: 94)
2740	GGCCTTCCTGCTGT (SEQ ID NO: 95)	lGs1Gs1CsdCsdTsdTsdCsdCsdTsdGsdCs1Ts1Gs1T (SEQ ID NO: 96)
2961	TGGATGGTGAACAG (SEQ ID NO: 97)	lTs1Gs1GsdAsdTsdGsdGsdTsdGsdAsdAs1Cs1As1G (SEQ ID NO: 98)

2962	CTGGATGGTGAACA (SEQ ID NO: 99)	ICsITsIGsdGsdAsdTsdGsdGsdTsdGsdAslAslCsIA (SEQ ID NO: 100)
2964	CACTGGATGGTGAA (SEQ ID NO: 101)	ICsIAslCsdTsdGsdGsdAsdTsdGsdGsdTsIGslAslA (SEQ ID NO: 102)
2966	TCCACTGGATGGTG (SEQ ID NO: 103)	ITsICsICsdAsdCsdTsdGsdGsdAsdTsdGsIGslTsIG (SEQ ID NO: 104)
2967	TTCCACTGGATGGT (SEQ ID NO: 105)	ITsITsICsdCsdAsdCsdTsdGsdGsdAsdTslGsIGslT (SEQ ID NO: 106)
3186	TGCAGGGCCAGGTC (SEQ ID NO: 107)	ITsIGslCsdAsdGsdGsdGsdCsdCsdAsdGslGsITsIC (SEQ ID NO: 108)
3187	CTGCAGGGCCAGGT (SEQ ID NO: 109)	ICsITsIGsdCsdAsdGsdGsdGsdCsdCsdAslGsIGslT (SEQ ID NO: 110)
3255	ACCTTGGACTTGAT (SEQ ID NO: 111)	lAslCsICsdTsdTsdGsdGsdAsdCsdTsdTsIGslAslT (SEQ ID NO: 112)
3257	GCACCTTGGACTTG (SEQ ID NO: 113)	IGslCsIAsdCsdCsdTsdTsdGsdGsdAsdCsITsITsIG (SEQ ID NO: 114)
3258	TGCACCTTGGACTT (SEQ ID NO: 115)	ITsIGslCsdAsdCsdCsdTsdTsdGsdGsdAslCsITsIT (SEQ ID NO: 116)
3259	CTGCACCTTGGACT (SEQ ID NO: 117)	ICsITsIGsdCsdAsdCsdCsdTsdTsdGsdGsIAslCsIT (SEQ ID NO: 118)
3260	GCTGCACCTTGGAC (SEQ ID NO: 119)	IGslCsITsdGsdCsdAsdCsdCsdTsdTsdGsIGslAslC (SEQ ID NO: 120)
3261	AGCTGCACCTTGA (SEQ ID NO: 121)	lAslGsICsdTsdGsdCsdAsdCsdCsdTsdTsIGslGsIA (SEQ ID NO: 122)
4253	CTGCACTGGCCTTG (SEQ ID NO: 123)	ICsITsIGsdCsdAsdCsdTsdGsdGsdCsdCsITsITsIG (SEQ ID NO: 124)
4254	TCTGCACCTGGCCTT (SEQ ID NO: 125)	ITsICsITsdGsdCsdAsdCsdTsdGsdGsdCsICsITsIT (SEQ ID NO: 126)
4255	CTCTGCACCTGGCCT (SEQ ID NO: 127)	ICsITsICsdTsdGsdCsdAsdCsdTsdGsdGsICsICsIT (SEQ ID NO: 128)
4256	TCTCTGCACCTGGCC (SEQ ID NO: 129)	ITsICsITsdCsdTsdGsdCsdAsdCsdTsdGsIGslCsIC (SEQ ID NO: 130)
4257	TTCTCTGCACCTGGC (SEQ ID NO: 131)	ITsITsICsdTsdCsdTsdGsdCsdAsdCsdTsIGslGsIC (SEQ ID NO: 132)
4258	CTTCTCTGCACCTGG (SEQ ID NO: 133)	ICsITsITsdCsdTsdCsdTsdGsdCsdAsdCsITsIGslG (SEQ ID NO: 134)
4259	GCTTCTCTGCACCTG (SEQ ID NO: 135)	IGslCsITsdTsdCsdTsdCsdTsdGsdCsdAslCsITsIG (SEQ ID NO: 136)
4260	AGCTTCTCTGCACT (SEQ ID NO: 137)	lAslGsICsdTsdTsdCsdTsdCsdTsdGsdCsIAslCsIT (SEQ ID NO: 138)
4261	CAGTTCTCTGCAC (SEQ ID NO: 139)	ICsIAslGsdCsdTsdTsdCsdTsdCsdTsdGsICsIAslC (SEQ ID NO: 140)
4262	ACAGCTTCTCTGCA (SEQ ID NO: 141)	lAslCsIAsdGsdCsdTsdTsdCsdTsdCsdTsIGslCsIA (SEQ ID NO: 142)
4263	CACAGCTTCTCTGC (SEQ ID NO: 143)	ICsIAslCsdAsdGsdCsdTsdTsdCsdTsdCsITsIGslC (SEQ ID NO: 144)
4302	TTGATCTTGGCCTC (SEQ ID NO: 145)	ITsITsIGsdAsdTsdCsdTsdTsdGsdGsdCsICsITsIC (SEQ ID NO: 146)
4318	CTGCAGCTCCTCCA (SEQ ID NO: 147)	ICsITsIGsdCsdAsdGsdCsdTsdCsdCsdTsICsICsIA (SEQ ID NO: 148)
4717	TGCCTCCTGCAGCC (SEQ ID NO: 149)	ITsIGslCsdCsdTsdCsdCsdTsdGsdCsdAslGsICsIC (SEQ ID NO: 150)
4773	AGCTTGGCCTTCTC (SEQ ID NO: 151)	lAslGsICsdTsdTsdGsdGsdCsdCsdTsdTsICsITsIC (SEQ ID NO: 152)
4904	TCTCCTCCTCCTGC (SEQ ID NO: 153)	ITsICsITsdCsdCsdTsdCsdCsdTsdCsdCsITsIGslC (SEQ ID NO: 154)

6099	AGGTTCTTCCTGTC (SEQ ID NO: 155)	lAslGslGsdTsdTsdCsdTsdTsdCsdCsdTslGslTslC (SEQ ID NO: 156)
6101	CCAGGTTCTTCCTG (SEQ ID NO: 157)	lCslCslAsdGsdGsdTsdTsdCsdTsdTsdCslCslTslG (SEQ ID NO: 158)
6102	GCCAGGTTCTTCCT (SEQ ID NO: 159)	lGslCslCsdAsdGsdGsdTsdTsdCsdTsdTslCslCslT (SEQ ID NO: 160)
6103	AGCCAGGTTCTTCC (SEQ ID NO: 161)	lAslGslCsdCsdAsdGsdGsdTsdTsdCsdTslTslCslC (SEQ ID NO: 162)
6104	GAGCCAGGTTCTTC (SEQ ID NO: 163)	lGslAslGsdCsdCsdAsdGsdGsdTsdTsdCslTslTslC (SEQ ID NO: 164)
6142	GACCTTGCTCTGCA (SEQ ID NO: 165)	lGslAslCsdCsdTsdTsdGsdCsdTsdCsdTslGslCslA (SEQ ID NO: 166)
6143	TGACCTTGCTCTGC (SEQ ID NO: 167)	lTslGslAsdCsdCsdTsdTsdGsdCsdTsdCslTslGslC (SEQ ID NO: 168)
6144	TTGACCTTGCTCTG (SEQ ID NO: 169)	lTslTslGsdAsdCsdCsdTsdTsdGsdCsdTslCslTslG (SEQ ID NO: 170)
6146	TCTTGACCTTGCTC (SEQ ID NO: 171)	lTslCslTsdTsdGsdAsdCsdCsdTsdTsdGslCslTslC (SEQ ID NO: 172)
6149	AGCTCTTGACCTTG (SEQ ID NO: 173)	lAslGslCsdTsdCsdTsdTsdGsdAsdCsdCslTslTslG (SEQ ID NO: 174)
6151	GTAGCTCTTGACCT (SEQ ID NO: 175)	lGslTslAsdGsdCsdTsdCsdTsdTsdGsdAslCslCslT (SEQ ID NO: 176)
6220	GTGCTGGGCTTGTC (SEQ ID NO: 177)	lGslTslGsdCsdTsdGsdGsdGsdCsdCsdTslTslGslC (SEQ ID NO: 178)
6273	TTGTGGCCTGGGT (SEQ ID NO: 179)	lTslTslGsdTsdTsdGsdGsdCsdCsdTsdGslGslGslT (SEQ ID NO: 180)
6277	CAGCTTGTTGGCCT (SEQ ID NO: 181)	lCslAslGsdCsdTsdTsdGsdTsdTsdGsdGslCslCslT (SEQ ID NO: 182)
	GTGAATGCGGATGAA (SEQ ID NO: 1)	lGslTslGsdAsdAsdTsdGsdCsdGsdGsdAsdTsdGslAslA (SEQ ID NO: 3)

[0184] l = 잠금 뉴클레오티드; d = 데옥시뉴클레오티드; s = 포스포로티오에이트 결합.

표 2

[0185]

SEQ ID NO: 6의 표적 서 열의 시작 뉴 클레오티드	서열(5'-3')	변형을 가진 서열(5'-3')
513	TCACTCACATCCAT (SEQ ID NO: 183)	lTslCslAsmdCsdTsmcCsdAsmdCsdAsdTsmcCslCslAslT (SEQ ID NO: 184)
514	TTCACTCACATCCA (SEQ ID NO: 185)	lTslTslCsdAsmdCsdTsmcCsdAsmdCsdAsdTslCslCslA (SEQ ID NO: 186)
515	GTCACTCACATCC (SEQ ID NO: 187)	lGslTslTsmcCsdAsmdCsdTsmcCsdAsmdCsdAslTslCslC (SEQ ID NO: 188)
516	AGTTCACTCACATC (SEQ ID NO: 189)	lAslGslTsdTsmcCsdAsmdCsdTsmcCsdAsmdCslAslTslC (SEQ ID NO: 190)
517	AAGTTCACTCACAT (SEQ ID NO: 191)	lAslAslGsdTsdTsmcCsdAsmdCsdTsmcCsdAslCslAslT (SEQ ID NO: 192)
518	CAAGTTCACTCACA (SEQ ID NO: 193)	lCslAslAsdGsdTsdTsmcCsdAsmdCsdTsmcCslAslCslA (SEQ ID NO: 194)
519	CCAAGTTCACTCAC (SEQ ID NO: 195)	lCslCslAsdAsdGsdTsdTsmcCsdAsmdCsdTslCslAslC (SEQ ID NO: 196)
520	CCCAAGTTCACTCA (SEQ ID NO: 197)	lCslCslCsdAsdAsdGsdTsdTsmcCsdAsmdCslTslCslA (SEQ ID NO: 198)

521	CCCCAAGTTCACCTC (SEQ ID NO: 199)	ICsICsICsmdCsdAsdAsdGsdTsdTsmcCsdAsICsITsIC (SEQ ID NO: 200)
522	TCCCCAAGTTCACCT (SEQ ID NO: 201)	ITsICsICsmdCsdCsdAsdAsdGsdTsdTsmcCslAsICsIT (SEQ ID NO: 202)
523	CTCCCCAAGTTCAC (SEQ ID NO: 203)	ICsITsICsmdCsdCsdCsdAsdAsdGsdTsdTsICsICsIC (SEQ ID NO: 204)
524	ACTCCCCAAGTTCAC (SEQ ID NO: 205)	ICsITsICsmdCsdCsdCsdCsdAsdAsdGsdTsITsICsICsIA (SEQ ID NO: 206)
525	GACTCCCCAAGTTC (SEQ ID NO: 207)	IGsICsICsdTsmcCsdCsdCsdCsdAsdAsdGslTsITsIC (SEQ ID NO: 208)
526	AGACTCCCCAAGTT (SEQ ID NO: 209)	ICsIGsICsmdCsdTsmcCsdCsdCsdCsdAsdAslGslTsIT (SEQ ID NO: 210)
527	CAGACTCCCCAAGT (SEQ ID NO: 211)	ICsICsICsdAsmdCsdTsmcCsdCsdCsdCsdAslAslGslT (SEQ ID NO: 212)
528	GCAGACTCCCCAAG (SEQ ID NO: 213)	IGsICsICsdAsmdCsdTsmcCsdCsdCsdCslAslAslG (SEQ ID NO: 214)
610	AGGCACCCAGACTC (SEQ ID NO: 215)	ICsICsICsdAsmdCsdCsdCsdCsdAsdGsdAslCslTsIC (SEQ ID NO: 216)
611	CAGGCACCCAGACT (SEQ ID NO: 217)	ICsICsICsdGsdCsdAsmdCsdCsdCsdCsdAsdGslAslCslT (SEQ ID NO: 218)
612	TCAGGCACCCAGAC (SEQ ID NO: 219)	ITsICsICsdGsdGsdCsdAsmdCsdCsdCsdAslGslAslC (SEQ ID NO: 220)
613	ATCAGGCACCCAGA (SEQ ID NO: 221)	ICsITsICsdAsdGsdGsdCsdAsmdCsdCsdCslAslGslIA (SEQ ID NO: 222)
1447	GCTGCAGAAGTGGT (SEQ ID NO: 223)	IGsICsITsdGsdCsdAsdGsdAsdGsdTsIGslGslT (SEQ ID NO: 224)
1518	ATGTCCATGGCATG (SEQ ID NO: 225)	ICsITsIGsdTsmcCsdCsdAsdTsdGsdGsdCslAslTsIG (SEQ ID NO: 226)
1560	ATCTTATAGCAGGC (SEQ ID NO: 227)	ICsITsICsdTsdTsdAsdTsdAsdGsdCsdAslGslGslC (SEQ ID NO: 228)
1645	ACTCTCAGTGCCAT (SEQ ID NO: 229)	ICsICsITsmcCsdTsmcCsdAsdGsdTsdGsdCslCslAslT (SEQ ID NO: 230)
1646	CACTCTCAGTGCCA (SEQ ID NO: 231)	ICsICsICsdTsmcCsdTsmcCsdAsdGsdTsdGslCslCslIA (SEQ ID NO: 232)
1648	AGCACTCTCAGTGC (SEQ ID NO: 233)	ICsIGsICsdAsmdCsdTsmcCsdTsmcCsdAsdGslTsIGslC (SEQ ID NO: 234)
1649	CAGCACTCTCAGTG (SEQ ID NO: 235)	ICsICsICsdAsmdCsdTsmcCsdTsmcCsdAslGslTsIG (SEQ ID NO: 236)
1650	TCAGCACTCTCAGT (SEQ ID NO: 237)	ITsICsICsdGsdCsdAsmdCsdTsmcCsdTsmcCslAslGslT (SEQ ID NO: 238)
1651	GTCAGCACTCTCAG (SEQ ID NO: 239)	IGsITsICsdAsdGsdCsdAsmdCsdTsmcCsdTsICsICsIC (SEQ ID NO: 240)
1652	TGTGAGCACTCTCA (SEQ ID NO: 241)	ITsIGsITsmcCsdAsdGsdCsdAsmdCsdTsmcCslTsICsICsIA (SEQ ID NO: 242)
1655	CCTGTGAGCACTC (SEQ ID NO: 243)	ICsICsITsdTsdGsdTsmcCsdAsdGsdCsdAslCslTsIC (SEQ ID NO: 244)
1682	CACTGTGACCCCC (SEQ ID NO: 245)	ICsICsICsdTsdGsdCsdTsdGsdAsmdCsdCslCslCslC (SEQ ID NO: 246)
1683	CCACTGTGACCCCC (SEQ ID NO: 247)	ICsICsICsdAsmdCsdTsdGsdCsdTsdGsdAsmdCslCslCslC (SEQ ID NO: 248)
1684	CCCACTGTGACCC (SEQ ID NO: 249)	ICsICsICsdAsmdCsdTsdGsdCsdTsdGsdAslCslCslC (SEQ ID NO: 250)
1685	CCCCACTGTGACC (SEQ ID NO: 251)	ICsICsICsdAsmdCsdTsdGsdCsdTsdGslAslCslC (SEQ ID NO: 252)
1686	TCCCCACTGTGAC (SEQ ID NO: 253)	ITsICsICsdCsdAsmdCsdTsdGsdCsdTsIGslAslC (SEQ ID NO: 254)

1687	GTCCCCACTGCTGA (SEQ ID NO: 255)	IGsITsICsmcCsmcCsmcCsdAsmdCsdTsdGsmcCsITsIGsIA (SEQ ID NO: 256)
1688	GGTCCCCACTGCTG (SEQ ID NO: 257)	IGsIGsITsmcCsmcCsmcCsdAsmdCsdTsdGsICsITsIG (SEQ ID NO: 258)
1689	AGGTCCCCACTGCT (SEQ ID NO: 259)	lAsIGsIGsdTsmcCsmcCsmcCsdAsmdCsdTsIGsICsIT (SEQ ID NO: 260)
1690	GAGGTCCCCACTGC (SEQ ID NO: 261)	IGsIGsIGsdGsdTsmcCsmcCsmcCsdAsmdCsITsIGsIC (SEQ ID NO: 262)
1691	GGAGGTCCCCACTG (SEQ ID NO: 263)	IGsIGsIGsdGsdGsdTsmcCsmcCsmcCsdAsICsITsIG (SEQ ID NO: 264)
1692	AGGAGGTCCCCACT (SEQ ID NO: 265)	lAsIGsIGsdAsdGsdGsdTsmcCsmcCsmcCsIGsICsIT (SEQ ID NO: 266)
1693	GAGGAGGTCCCCAC (SEQ ID NO: 267)	IGsIGsIGsdGsdAsdGsdGsdTsmcCsmcCsICsIGsIC (SEQ ID NO: 268)
1694	TGAGGAGGTCCCCA (SEQ ID NO: 269)	ITsIGsIGsdGsdGsdAsdGsdGsdTsmcCsmcCsICsIGsIA (SEQ ID NO: 270)
1696	TTTGAGGAGGTCCC (SEQ ID NO: 271)	ITsITsITsdGsdAsdGsdGsdAsdGsdGsdTsICsICsIC (SEQ ID NO: 272)
1702	AAGGCCTTTGAGGA (SEQ ID NO: 273)	lAsIGsIGsdGsmcCsmcCsdTsdTsdTsdGsdAsIGsIGsIA (SEQ ID NO: 274)
1773	GCAAACACCACCTG (SEQ ID NO: 275)	IGsICsIGsdAsdAsmdCsdAsmdCsmcCsdAsmdCsICsITsIG (SEQ ID NO: 276)
1774	AGCAAACACCACCT (SEQ ID NO: 277)	lAsIGsICsdAsdAsdAsmdCsdAsmdCsmcCsdAsICsICsIT (SEQ ID NO: 278)
1775	CAGCAAACACCACC (SEQ ID NO: 279)	ICsIGsIGsmcCsdAsdAsdAsmdCsdAsmdCsmcCsIGsICsIC (SEQ ID NO: 280)
1776	ACAGCAAACACCAC (SEQ ID NO: 281)	lAsICsIGsdGsmcCsdAsdAsdAsmdCsdAsmdCsICsIGsIC (SEQ ID NO: 282)
1828	AGACACCAGCCACC (SEQ ID NO: 283)	lAsIGsIGsmcCsdAsmdCsmcCsdAsdGsmcCsmcCsIGsICsIC (SEQ ID NO: 284)
1829	GAGACACCAGCCAC (SEQ ID NO: 285)	IGsIGsIGsdAsmdCsdAsmdCsmcCsdAsdGsmcCsICsIGsIC (SEQ ID NO: 286)
2218	CTTCTGTGACGGCC (SEQ ID NO: 287)	ICsITsITsmcCsdTsdTsdGsdTsmcCsdAsdGsIGsICsIC (SEQ ID NO: 288)
2238	AAGTGGGCCTGGTA (SEQ ID NO: 289)	lAsIGsIGsdTsdGsdGsdGsmcCsmcCsdTsdGsIGsITsIA (SEQ ID NO: 290)
2254	GTAGTGGACCACT (SEQ ID NO: 291)	IGsITsIGsdGsdTsdGsdGsdAsmdCsmcCsdAsICsICsIT (SEQ ID NO: 292)
2574	GCATCCATGACCCC (SEQ ID NO: 293)	IGsICsIGsdTsmcCsmcCsdAsdTsdGsdAsmdCsICsICsIC (SEQ ID NO: 294)
2575	GGCATCCATGACCC (SEQ ID NO: 295)	IGsIGsICsdAsdTsmcCsmcCsdAsdTsdGsdAsICsICsIC (SEQ ID NO: 296)
2576	AGGCATCCATGACC (SEQ ID NO: 297)	lAsIGsIGsmcCsdAsdTsmcCsmcCsdAsdTsdGsIGsICsIC (SEQ ID NO: 298)
2577	AAGGCATCCATGAC (SEQ ID NO: 299)	lAsIGsIGsdGsmcCsdAsdTsmcCsmcCsdAsTsIGsIGsIC (SEQ ID NO: 300)
4281	TGATCCTCATAGGT (SEQ ID NO: 301)	ITsIGsIGsdTsmcCsmcCsdTsmcCsdAsdTsAsIGsIGsIT (SEQ ID NO: 302)
4282	CTGATCCTCATAGG (SEQ ID NO: 303)	ICsITsIGsdAsdTsmcCsmcCsdTsmcCsdAsTsAsIGsIG (SEQ ID NO: 304)
4283	GCTGATCCTCATAG (SEQ ID NO: 305)	IGsICsITsdGsdAsdTsmcCsmcCsdTsmcCsdAsTsAsIG (SEQ ID NO: 306)
4284	AGCTGATCCTCATA (SEQ ID NO: 307)	lAsIGsICsdTsdGsdAsdTsmcCsmcCsdTsmcCsIGsITsIA (SEQ ID NO: 308)
4305	ACCTTGATCTTGGC (SEQ ID NO: 309)	lAsICsICsdTsdTsdGsdAsdTsmcCsdTsdTsIGsIGsIC (SEQ ID NO: 310)

4656	ATGGCATCTGCTTC (SEQ ID NO: 311)	lAslTslGsdGsmcCsdAsdTsmcCsdTsdGsmcCslTslTslC (SEQ ID NO: 312)
4657	GATGGCATCTGCTT (SEQ ID NO: 313)	lGslAslTsdGsdGsmcCsdAsdTsmcCsdTsdGslCslTslT (SEQ ID NO: 314)
4661	TCTGGATGGCATCT (SEQ ID NO: 315)	lTslCslTsdGsdGsdAsdTsdGsdGsmcCsdAslTslCslT (SEQ ID NO: 316)
6141	ACCTTGCTCTGCAG (SEQ ID NO: 317)	lAslCslCsdTsdTsdGsmcCsdTsmcCsdTsdGslCslAslG (SEQ ID NO: 318)
436	AGGCAGCAGGCACT (SEQ ID NO: 319)	lAslGslGsmcCsdAsdTsmcCsdAsdTsdGsdGsmcCslAslCslT (SEQ ID NO: 320)
437	AAGGCAGCAGGCAC (SEQ ID NO: 321)	lAslAslGsdGsmcCsdAsdTsmcCsdAsdTsdGslCslAslC (SEQ ID NO: 322)
438	CAAGGCAGCAGGCA (SEQ ID NO: 323)	lCslAslAsdTsdGsdGsmcCsdAsdTsmcCsdAsdTsdGslGslCslA (SEQ ID NO: 324)
439	CCAAGGCAGCAGGC (SEQ ID NO: 325)	lCslCslAsdTsdGsdGsmcCsdAsdTsmcCsdAslGslGslC (SEQ ID NO: 326)
482	GGTGAAGGAGGAGG (SEQ ID NO: 327)	lGslGslTsdGsdAsdTsdGsdGsdAsdTsdGslAslGslG (SEQ ID NO: 328)
483	GGGTGAAGGAGGAG (SEQ ID NO: 329)	lGslGslGsdTsdGsdAsdTsdGsdGsdAsdTsdGslGslAslG (SEQ ID NO: 330)
509	TCACATCCATCATG (SEQ ID NO: 331)	lTslCslAsmCsdAsdTsmcCsmcCsdAsdTsmcCslAslTslG (SEQ ID NO: 332)
511	ACTCACATCCATCA (SEQ ID NO: 333)	lAslCslTsmcCsdAsmCsdAsdTsmcCsmcCsdAslTslCslA (SEQ ID NO: 334)
512	CACTCACATCCATC (SEQ ID NO: 335)	lCslAslCsdTsmcCsdAsmCsdAsdTsmcCsmcCslAslTslC (SEQ ID NO: 336)
616	TTCATCAGGCACCC (SEQ ID NO: 337)	lTslTslCsdAsdTsmcCsdAsdTsdGsdGsmcCsdAslCslCslC (SEQ ID NO: 338)
617	GTTTCATCAGGCACC (SEQ ID NO: 339)	lGslTslTsmcCsdAsdTsmcCsdAsdTsdGsdGsmcCslAslCslC (SEQ ID NO: 340)
622	GTCCTGTTTCATCAG (SEQ ID NO: 341)	lGslTslCsmcCsdTsdGsdTsdTsmcCsdAsdTslCslAslG (SEQ ID NO: 342)
690	TGGTCTTTGGTCTC (SEQ ID NO: 343)	lTslGslGsdTsmcCsdTsdTsdTsdGsdGsdTslCslTslC (SEQ ID NO: 344)
804	TTGTGCAGCACAGA (SEQ ID NO: 345)	lTslTslGsdTsdGsmcCsdAsdTsmcCsdAsmCslAslGslA (SEQ ID NO: 346)
805	GTTGTGCAGCACAG (SEQ ID NO: 347)	lGslTslTsdGsdTsdGsmcCsdAsdTsmcCsdAslCslAslG (SEQ ID NO: 348)
892	GACTGGGAGCCATT (SEQ ID NO: 349)	lGslAslCsdTsdGsdGsdGsdAsdTsmcCsmcCslAslTslT (SEQ ID NO: 350)
893	AGACTGGGAGCCAT (SEQ ID NO: 351)	lAslGslAsmCsdTsdGsdGsdGsdAsdTsmcCslCslAslT (SEQ ID NO: 352)
1102	GTCTCCAGGGCAG (SEQ ID NO: 353)	lGslTslCsdTsmcCsmcCsmcCsdAsdTsdGsdGslCslAslG (SEQ ID NO: 354)
1215	CTCAGGGTCTTGGC (SEQ ID NO: 355)	lCslTslCsdAsdTsdGsdGsdTsmcCsdTsdTslGslGslC (SEQ ID NO: 356)
1216	CCTCAGGGTCTTGG (SEQ ID NO: 357)	lCslCslTsmcCsdAsdTsdGsdGsdTsmcCsdTslTslGslG (SEQ ID NO: 358)
1217	TCCTCAGGGTCTTG (SEQ ID NO: 359)	lTslCslCsdTsmcCsdAsdTsdGsdGsdTsmcCslTslTslG (SEQ ID NO: 360)
1219	ATTCCTCAGGGTCT (SEQ ID NO: 361)	lAslTslTsmcCsmcCsdTsmcCsdAsdTsdGsdGslTslCslT (SEQ ID NO: 362)
1220	CATTCCTCAGGGTC (SEQ ID NO: 363)	lCslAslTsdTsmcCsmcCsdTsmcCsdAsdTsdGslGslTslC (SEQ ID NO: 364)
1222	ATCATTCCTCAGGG (SEQ ID NO: 365)	lAslTslCsdAsdTsdTsmcCsmcCsdTsmcCsdAslGslGslG (SEQ ID NO: 366)

1225	GTTATCATTCCTCA (SEQ ID NO: 366)	IGsITsITsdAsdTsmcCsdAsdTsdTsmcCsmcCsITsICsIA (SEQ ID NO: 368)
1226	AGTTATCATTCCTC (SEQ ID NO: 369)	IAsIGsITsdTsdAsdTsmcCsdAsdTsdTsmcCsICsITsIC (SEQ ID NO: 370)
1268	AGGGACCAAGTGA (SEQ ID NO: 371)	IAsIGsIGsdGsdAsmdCsmcCsdAsdAsdAsdGsITsIGsIA (SEQ ID NO: 372)
1273	CCCAGAGGGACCAA (SEQ ID NO: 373)	ICsICsICsdAsdGsdAsdGsdGsdGsdAsmdCsICsIASIA (SEQ ID NO: 374)
1274	TCCCAGAGGGACCA (SEQ ID NO: 375)	ITsICsICsmcCsdAsdGsdAsdGsdGsdGsdAsICsICsIA (SEQ ID NO: 376)
1275	TTCCCAGAGGGACC (SEQ ID NO: 377)	ITsITsICsmcCsmcCsdAsdGsdAsdGsdGsdGsIASICsIC (SEQ ID NO: 378)
1276	CTTCCCAGAGGGAC (SEQ ID NO: 379)	ICsITsITsmcCsmcCsmcCsdAsdGsdAsdGsdGsIGsIASIC (SEQ ID NO: 380)
1277	GCTTCCCAGAGGGA (SEQ ID NO: 381)	IGsICsITsdTsmcCsmcCsmcCsdAsdGsdAsdGsIGsIGsIA (SEQ ID NO: 382)
1301	AGCTGTCAATATCC (SEQ ID NO: 383)	IAsIGsICsdTsdGsdTsmcCsdAsdAsdTsdAsITsICsIC (SEQ ID NO: 384)
1302	TAGCTGTCAATATC (SEQ ID NO: 385)	ITsIASIGsmcCsdTsdGsdTsmcCsdAsdAsdTslAsITsIC (SEQ ID NO: 386)
1303	ATAGCTGTCAATAT (SEQ ID NO: 387)	IASITsIASdGsmcCsdTsdGsdTsmcCsdAsdAsITsIASIT (SEQ ID NO: 388)
1304	GATAGCTGTCAATA (SEQ ID NO: 389)	IGsIASITsdAsdGsmcCsdTsdGsdTsmcCsdAsIASITsIA (SEQ ID NO: 390)
1379	CTGAGAGGATCTGG (SEQ ID NO: 391)	ICsITsIGsdAsdGsdAsdGsdGsdAsdTsmcCsITsIGsIG (SEQ ID NO: 392)
1380	CCTGAGAGGATCTG (SEQ ID NO: 393)	ICsICsITsdGsdAsdGsdAsdGsdGsdAsdTslCsITsIG (SEQ ID NO: 394)
1417	AGACAGAAGCAGCA (SEQ ID NO: 395)	IASIGsIASmdCsdAsdGsdAsdAsdGsmcCsdAsIGsICsIA (SEQ ID NO: 396)
1418	TAGACAGAAGCAGC (SEQ ID NO: 397)	ITsIASIGsdAsmdCsdAsdGsdAsdAsdGsmcCsIASIGsIC (SEQ ID NO: 398)
1419	ATAGACAGAAGCAG (SEQ ID NO: 399)	IASITsIASdGsdAsmdCsdAsdGsdAsdAsdGsICsIASIG (SEQ ID NO: 400)
1420	CATAGACAGAAGCA (SEQ ID NO: 401)	ICsIASITsdAsdGsdAsmdCsdAsdGsdAsdAsIGsICsIA (SEQ ID NO: 402)
1421	TCATAGACAGAAGC (SEQ ID NO: 403)	ITsICsIASdTsdAsdGsdAsmdCsdAsdGsdAsIASIGsIC (SEQ ID NO: 404)
1422	TTCATAGACAGAAG (SEQ ID NO: 405)	ITsITsICsdAsdTsdAsdGsdAsmdCsdAsdGsIASIASIG (SEQ ID NO: 406)
1423	GTTTCATAGACAGAA (SEQ ID NO: 407)	IGsITsITsmcCsdAsdTsdAsdGsdAsmdCsdAsIGsIASIA (SEQ ID NO: 408)
1424	GGTTCATAGACAGA (SEQ ID NO: 409)	IGsIGsITsdTsmcCsdAsdTsdAsdGsdAsmdCsIASIGsIA (SEQ ID NO: 410)
1425	GGGTTCATAGACAG (SEQ ID NO: 411)	IGsIGsIGsdTsdTsmcCsdAsdTsdAsdGsdAsICsIASIG (SEQ ID NO: 412)
1484	CCCCATCATTCATG (SEQ ID NO: 413)	ICsICsICsmcCsdAsdTsmcCsdAsdTsdTsmcCsIASITsIG (SEQ ID NO: 414)
1557	TTATAGCAGGCACA (SEQ ID NO: 415)	ITsITsIASdTsdAsdGsmcCsdAsdGsdGsmcCsIASICsIA (SEQ ID NO: 416)
1558	CTTATAGCAGGCAC (SEQ ID NO: 417)	ICsITsITsdAsdTsdAsdGsmcCsdAsdGsdGsICsIASIC (SEQ ID NO: 418)
1559	TCTTATAGCAGGCA (SEQ ID NO: 419)	ITsICsITsdTsdAsdTsdAsdGsmcCsdAsdGsIGsICsIA (SEQ ID NO: 420)
1561	GATCTTATAGCAGG (SEQ ID NO: 421)	IGsIASITsmcCsdTsdTsdAsdTsdAsdGsmcCsIASIGsIG (SEQ ID NO: 422)

1582	AAAGTGCAGGAGGG (SEQ ID NO: 423)	lAslAslAsdGsdTsdGsmcCsdAsdGsdGsdAslGslGslG (SEQ ID NO: 424)
1587	TTGCCAAAGTCAG (SEQ ID NO: 425)	lTs1Ts1GsmcCsmcCsdAsdAsdAsdGsdTsdGslCs1As1G (SEQ ID NO: 426)
1703	AAAGGCCTTTGAGG (SEQ ID NO: 427)	lAslAslAsdGsdGsmcCsmcCsdTsdTsdTsdGslAs1GslG (SEQ ID NO: 428)
1704	AAAAGGCCTTTGAG (SEQ ID NO: 429)	lAslAslAsdAsdGsdGsmcCsmcCsdTsdTsdTs1GslAs1G (SEQ ID NO: 430)
1705	CAAAAGGCCTTTGA (SEQ ID NO: 431)	lCs1As1AsdAsdAsdGsdGsmcCsmcCsdTsdTs1Ts1GslA (SEQ ID NO: 432)
1706	GCAAAAGGCCTTTG (SEQ ID NO: 433)	lGslCs1AsdAsdAsdAsdGsdGsmcCsmcCsdTs1Ts1Ts1G (SEQ ID NO: 434)
1707	TGCAAAAGGCCTTT (SEQ ID NO: 435)	lTs1GslCs1AsdAsdAsdAsdGsdGsmcCsmcCs1Ts1Ts1T (SEQ ID NO: 436)
1708	GTGCAAAAGGCCTT (SEQ ID NO: 437)	lGslTs1GsmcCsdAsdAsdAsdAsdGsdGsmcCs1Cs1Ts1T (SEQ ID NO: 438)
1709	GGTGCAAAAGGCCT (SEQ ID NO: 439)	lGslGslTs1GsmcCsdAsdAsdAsdAsdGsdGslCs1Cs1T (SEQ ID NO: 440)
1710	GGGTGCAAAAGGCC (SEQ ID NO: 441)	lGslGslGsdTsdGsmcCsdAsdAsdAsdAsdGslGslCs1C (SEQ ID NO: 442)
1804	GTCATAGGTGGCCT (SEQ ID NO: 443)	lGslTs1Cs1AsdAsdAsdGsdGsdTsdGsdGslCs1Cs1T (SEQ ID NO: 444)
1805	GGTCATAGGTGGCC (SEQ ID NO: 445)	lGslGslTsmdCsdAsdTs1AsdGsdGsdTsdGslGslCs1C (SEQ ID NO: 446)
1819	CCACCTGAACAGCC (SEQ ID NO: 447)	lCs1Cs1AsmdCsmcCsdTsdGsdAsdAsmdCsdAslGslCs1C (SEQ ID NO: 448)
1820	GCCACCTGAACAGC (SEQ ID NO: 449)	lGslCs1Cs1AsmdCsmcCsdTsdGsdAsdAsmdCs1As1GslC (SEQ ID NO: 450)
1827	GACACCAGCCACCT (SEQ ID NO: 451)	lGslAs1Cs1AsmdCsmcCsdAsdGsmcCsmcCsdAs1Cs1Cs1T (SEQ ID NO: 452)
1850	TGTCCAGGTCTGG (SEQ ID NO: 453)	lTs1GslTsmdCsmcCsdAsdGsdGsdGsdTsmdCs1Ts1GslG (SEQ ID NO: 454)
1851	GTGTCCAGGTCTG (SEQ ID NO: 455)	lGslTs1GsdTsmdCsmcCsdAsdGsdGsdGsdTs1Cs1Ts1G (SEQ ID NO: 456)
1852	TGTGTCCAGGTCT (SEQ ID NO: 457)	lTs1GslTs1GsdGsdTsmdCsmcCsdAsdGsdGsdGslTs1Cs1T (SEQ ID NO: 458)
2189	TGGGTGACTTCCCC (SEQ ID NO: 459)	lTs1GslGsdGsdTsdGsdAsmdCsdTsdTsmdCs1Cs1Cs1C (SEQ ID NO: 460)
2190	TTGGGTGACTTCCC (SEQ ID NO: 461)	lTs1Ts1GsdGsdGsdTsdGsdAsmdCsdTsdTs1Cs1Cs1C (SEQ ID NO: 462)
2191	ATTGGGTGACTTCC (SEQ ID NO: 463)	lAs1Ts1Ts1GsdGsdGsdTsdGsdAsmdCsdTs1Ts1Cs1C (SEQ ID NO: 464)
2198	GCTGGAATTTGGGT (SEQ ID NO: 465)	lGslCs1Ts1GsdGsdAsdAsdAsdTs1GslGslTs1G (SEQ ID NO: 466)
2274	CTGTAAGGCACCAC (SEQ ID NO: 467)	lCs1Ts1GsdTsdAsdAsdGsdGsmcCsdAsmdCs1Cs1As1C (SEQ ID NO: 468)
2275	GCTGTAAGGCACCA (SEQ ID NO: 469)	lGslCs1Ts1GsdTsdAsdAsdGsdGsmcCsdAs1Cs1Cs1A (SEQ ID NO: 470)
2276	TGCTGTAAGGCACC (SEQ ID NO: 471)	lTs1GslCs1GsdTsdGsdTsdAsdAsdGsdGsmcCs1As1Cs1C (SEQ ID NO: 472)
2350	ATTCTGTGACTTCT (SEQ ID NO: 473)	lAs1Ts1TsmdCsdTsdGsdTsdGsdAsmdCsdTs1Ts1Cs1T (SEQ ID NO: 474)
2351	TATTCTGTGACTTC (SEQ ID NO: 475)	lTs1As1TsmdCsdTsdGsdTsdGsdAsmdCs1Ts1Ts1C (SEQ ID NO: 476)
2352	CTATTCTGTGACTT (SEQ ID NO: 477)	lCs1Ts1AsdTs1TsmdCsdTsdGsdTsdGsdAs1Cs1Ts1T (SEQ ID NO: 478)

2353	CCTATTCTGTGACT (SEQ ID NO: 479)	ICsICsITsdAsdTsdTsmcCsdTsdGsdTsdGsICsICsIT (SEQ ID NO: 480)
2354	GCCTATTCTGTGAC (SEQ ID NO: 481)	IGsICsICsdTsdAsdTsdTsmcCsdTsdGsdTsIGsICsIC (SEQ ID NO: 482)
2355	AGCCTATTCTGTGA (SEQ ID NO: 483)	ICsICsICsmcCsdTsdAsdTsdTsmcCsdTsdGsICsICsIC (SEQ ID NO: 484)
2356	GAGCCTATTCTGTG (SEQ ID NO: 485)	IGsICsICsmcCsdTsdAsdTsdTsmcCsdTsIGsICsIC (SEQ ID NO: 486)
2357	GGAGCCTATTCTGT (SEQ ID NO: 487)	IGsICsICsdTsdAsdTsdTsmcCsdTsdAsdTsdTsmcCsdTsIGsICsIT (SEQ ID NO: 488)
2358	AGGAGCCTATTCTG (SEQ ID NO: 489)	ICsICsICsdTsdAsdTsdTsmcCsdTsdAsdTsdTsICsICsIC (SEQ ID NO: 490)
2359	CAGGAGCCTATTCT (SEQ ID NO: 491)	ICsICsICsdTsdAsdTsdTsmcCsdTsdAsdTsdTsICsICsIT (SEQ ID NO: 492)
2360	CCAGGAGCCTATTC (SEQ ID NO: 493)	ICsICsICsdTsdAsdTsdTsmcCsdTsdAsdTsdTsICsICsIC (SEQ ID NO: 494)
2361	GCCAGGAGCCTATT (SEQ ID NO: 495)	IGsICsICsdTsdAsdTsdTsmcCsdTsdAsdTsdTsICsICsIT (SEQ ID NO: 496)
2375	TCTCATAGAGAGTC (SEQ ID NO: 497)	ITsICsICsmcCsdTsdAsdTsdTsmcCsdTsdAsdTsdTsICsICsIC (SEQ ID NO: 498)
2376	TTCTCATAGAGAGT (SEQ ID NO: 499)	ITsICsICsdTsdAsdTsdTsmcCsdTsdAsdTsdTsICsICsIT (SEQ ID NO: 500)
2396	TGGAGCAGGAGCCC (SEQ ID NO: 501)	ITsICsICsdTsdAsdTsdTsmcCsdTsdAsdTsdTsICsICsIC (SEQ ID NO: 502)
2397	GTGGAGCAGGAGCC (SEQ ID NO: 503)	IGsICsICsdTsdAsdTsdTsmcCsdTsdAsdTsdTsICsICsIC (SEQ ID NO: 504)
2443	TGCTGCCTTCTTAC (SEQ ID NO: 505)	ITsICsICsdTsdTsmcCsdTsdTsmcCsdTsdTsICsICsIC (SEQ ID NO: 506)
2444	ATGCTGCCTTCTTA (SEQ ID NO: 507)	ICsICsICsmcCsdTsdTsmcCsdTsdTsmcCsdTsICsICsIC (SEQ ID NO: 508)
2445	GATGCTGCCTTCTT (SEQ ID NO: 509)	IGsICsICsdTsdTsmcCsdTsdTsmcCsdTsdTsICsICsIT (SEQ ID NO: 510)
2579	AGAAGGCATCCATG (SEQ ID NO: 511)	ICsICsICsdTsdTsmcCsdTsdTsmcCsdTsICsICsIC (SEQ ID NO: 512)
2580	AAGAAGGCATCCAT (SEQ ID NO: 513)	ICsICsICsdTsdTsmcCsdTsdTsmcCsdTsICsICsIT (SEQ ID NO: 514)
2581	CAAGAAGGCATCCA (SEQ ID NO: 515)	ICsICsICsdTsdTsmcCsdTsdTsmcCsdTsICsICsIC (SEQ ID NO: 516)
2584	CACCAAGAAGGCAT (SEQ ID NO: 517)	ICsICsICsmcCsdTsdTsmcCsdTsdTsmcCsdTsICsICsIT (SEQ ID NO: 518)
2585	GCACCAAGAAGGCA (SEQ ID NO: 519)	IGsICsICsmcCsdTsdTsmcCsdTsdTsmcCsdTsICsICsIC (SEQ ID NO: 520)
2587	TAGCACCAAGAAGG (SEQ ID NO: 521)	ITsICsICsmcCsdTsdTsmcCsdTsdTsmcCsdTsICsICsIC (SEQ ID NO: 522)
2588	GTAGCACCAAGAAG (SEQ ID NO: 523)	IGsICsICsdTsdTsmcCsdTsdTsmcCsdTsICsICsIC (SEQ ID NO: 524)
2589	TGTAGCACCAAGAA (SEQ ID NO: 525)	ITsICsICsdTsdTsmcCsdTsdTsmcCsdTsICsICsIC (SEQ ID NO: 526)
2590	GTGTAGCACCAAGA (SEQ ID NO: 527)	IGsICsICsdTsdTsmcCsdTsdTsmcCsdTsICsICsIC (SEQ ID NO: 528)
2591	GGTGTAGCACCAAG (SEQ ID NO: 529)	IGsICsICsdTsdTsmcCsdTsdTsmcCsdTsICsICsIC (SEQ ID NO: 530)
2592	TGGTGTAGCACCAA (SEQ ID NO: 531)	ITsICsICsdTsdTsmcCsdTsdTsmcCsdTsICsICsIC (SEQ ID NO: 532)
2709	GGGATGGCACTGGG (SEQ ID NO: 533)	IGsICsICsdTsdTsmcCsdTsdTsmcCsdTsICsICsIC (SEQ ID NO: 534)

2731	GCTGTCCATGAAGG (SEQ ID NO: 535)	IGsICsITsdGsdTsmcCsmcCsdAsdTsdGsdAslAslGslG (SEQ ID NO: 536)
2830	GCCTAGAAGCCCAG (SEQ ID NO: 537)	IGsICsICsdTsdAsdTsdGsdAsdTsdGsdCsmcCsICsICsIG (SEQ ID NO: 538)
2986	GGCATTGAAGGCAC (SEQ ID NO: 539)	IGsIGsICsdAsdTsdTsdGsdAsdTsdGsdGsICsICsIC (SEQ ID NO: 540)
3248	ACTTGATCAGCAAG (SEQ ID NO: 541)	lAsICsITsdTsdGsdAsdTsmcCsdAsdTsmcCsICsICsIG (SEQ ID NO: 542)
3249	GACTTGATCAGCAA (SEQ ID NO: 543)	IGsICsICsdTsdTsdGsdAsdTsmcCsdAsdTsmcCsICsICsIA (SEQ ID NO: 544)
3625	GCATTCCAGGTCCT (SEQ ID NO: 545)	IGsICsICsdTsdTsmcCsmcCsdAsdTsdGsdGsdTsICsICsIT (SEQ ID NO: 546)
3626	AGCATTCCAGGTCC (SEQ ID NO: 547)	lAsIGsICsdAsdTsdTsmcCsmcCsdAsdTsdGsdGsITsICsIC (SEQ ID NO: 548)
3628	GGAGCATTCCAGGT (SEQ ID NO: 549)	IGsIGsICsdGsmcCsdAsdTsdTsmcCsmcCsICsICsIT (SEQ ID NO: 550)
3629	GGGAGCATTCCAGG (SEQ ID NO: 551)	IGsIGsICsdAsdTsmcCsdAsdTsdTsmcCsmcCsICsICsIG (SEQ ID NO: 552)
3631	CAGGGAGCATTCCA (SEQ ID NO: 553)	ICsICsICsdGsdGsdAsdTsmcCsdAsdTsdTsICsICsIA (SEQ ID NO: 554)
3793	CAGGCTCAGCTGGC (SEQ ID NO: 555)	ICsICsICsdGsmcCsdTsmcCsdAsdTsmcCsdTsICsICsIC (SEQ ID NO: 556)
3794	GCAGGCTCAGCTGG (SEQ ID NO: 557)	IGsICsICsdGsdGsmcCsdTsmcCsdAsdTsmcCsICsICsIG (SEQ ID NO: 558)
4233	GTCAGAGTCTCCAC (SEQ ID NO: 559)	IGsITsICsdAsdTsdGsdAsdTsmcCsdTsmcCsICsICsIC (SEQ ID NO: 560)
4234	GGTCAGAGTCTCCA (SEQ ID NO: 561)	IGsIGsITsmcCsdAsdTsdGsdAsdTsmcCsdTsICsICsIA (SEQ ID NO: 562)
4235	GGGTCAGAGTCTCC (SEQ ID NO: 563)	IGsIGsICsdTsmcCsdAsdTsdGsdAsdTsmcCsITsICsIC (SEQ ID NO: 564)
4286	TTAGCTGATCCTCA (SEQ ID NO: 565)	ITsITsICsdGsmcCsdTsdGsdAsdTsmcCsmcCsITsICsIA (SEQ ID NO: 566)
4287	CTTAGCTGATCCTC (SEQ ID NO: 567)	ICsITsITsdAsdTsmcCsdTsdGsdAsdTsmcCsICsITsIC (SEQ ID NO: 568)
4410	ATCAGACACTCCTT (SEQ ID NO: 569)	lAsITsICsdAsdTsdGsdAsmCsdAsmCsdTsmcCsICsITsIT (SEQ ID NO: 570)
4412	TGATCAGACACTCC (SEQ ID NO: 571)	ITsIGsICsdTsmcCsdAsdTsdGsdAsmCsdAsmCsITsICsIC (SEQ ID NO: 572)
4413	CTGATCAGACACTC (SEQ ID NO: 573)	ICsITsICsdAsdTsmcCsdAsdTsdGsdAsmCsdAsICsITsIC (SEQ ID NO: 574)
4414	ACTGATCAGACACT (SEQ ID NO: 575)	lAsICsITsdGsdAsdTsmcCsdAsdTsdGsdAsmCsICsITsIT (SEQ ID NO: 576)
4415	GACTGATCAGACAC (SEQ ID NO: 577)	IGsICsICsdTsdGsdAsdTsmcCsdAsdTsdGsdAsICsICsIC (SEQ ID NO: 578)
4416	TGACTGATCAGACA (SEQ ID NO: 579)	ITsIGsICsdAsmCsdTsdGsdAsdTsmcCsdAsdTsdGsdAsICsIA (SEQ ID NO: 580)
4417	CTGACTGATCAGAC (SEQ ID NO: 581)	ICsITsICsdAsmCsdTsdGsdAsdTsmcCsdAsICsICsIC (SEQ ID NO: 582)
4418	GCTGACTGATCAGA (SEQ ID NO: 583)	IGsICsITsdGsdAsmCsdTsdGsdAsdTsmcCsICsICsIA (SEQ ID NO: 584)
4419	AGCTGACTGATCAG (SEQ ID NO: 585)	lAsIGsICsdTsdGsdAsmCsdTsdGsdAsdTsdTsICsICsIG (SEQ ID NO: 586)
4422	CTCAGCTGACTGAT (SEQ ID NO: 587)	ICsITsICsdAsdTsmcCsdTsdGsdAsmCsdTsIGsICsIT (SEQ ID NO: 588)
4423	GCTCAGCTGACTGA (SEQ ID NO: 589)	IGsICsITsmcCsdAsdTsmcCsdTsdGsdAsmCsITsIGsIA (SEQ ID NO: 590)

4424	GGCTCAGCTGACTG (SEQ ID NO: 591)	IGsIGsICsdTsmcCsdAsdGsmcCsdTsdGsdAsICsITsIG (SEQ ID NO: 592)
4797	TCCTCTGACTCTGT (SEQ ID NO: 593)	ITsICsICsdTsmcCsdTsdGsdAsmdCsdTsmcCsITsIGsIT (SEQ ID NO: 594)
4798	ATCCTCTGACTCTG (SEQ ID NO: 595)	lAsITsICsmcCsdTsmcCsdTsdGsdAsmdCsdTsICsITsIG (SEQ ID NO: 596)
4799	CATCCTCTGACTCT (SEQ ID NO: 597)	ICsICsITsmcCsmcCsdTsmcCsdTsdGsdAsmdCsITsICsIT (SEQ ID NO: 598)
4800	ACATCCTCTGACTC (SEQ ID NO: 599)	lAsICsICsdTsmcCsmcCsdTsmcCsdTsdGsdAsICsITsIC (SEQ ID NO: 600)
4801	TACATCCTCTGACT (SEQ ID NO: 601)	ITsICsICsdAsdTsmcCsmcCsdTsmcCsdTsdGsICsICsIT (SEQ ID NO: 602)
4802	TTACATCCTCTGAC (SEQ ID NO: 603)	ITsITsICsmcCsdAsdTsmcCsmcCsdTsmcCsdTsIGsICsIC (SEQ ID NO: 604)
4803	GTTACATCCTCTGA (SEQ ID NO: 605)	IGsITsITsdAsmdCsdAsdTsmcCsmcCsdTsmcCsITsIGsIC (SEQ ID NO: 606)
4804	GGTTACATCCTCTG (SEQ ID NO: 607)	IGsIGsITsdTsdAsmdCsdAsdTsmcCsmcCsdTsICsITsIG (SEQ ID NO: 608)
4805	GGGTTACATCCTCT (SEQ ID NO: 609)	IGsIGsIGsdTsdTsdAsmdCsdAsdTsmcCsmcCsITsICsIT (SEQ ID NO: 610)
4806	AGGGTTACATCCTC (SEQ ID NO: 611)	lAsIGsIGsdGsdTsdTsdAsmdCsdAsdTsmcCsICsITsIC (SEQ ID NO: 612)
4807	CAGGGTTACATCCT (SEQ ID NO: 613)	ICsICsIGsdGsdGsdTsdTsdAsmdCsdAsdTsICsICsIT (SEQ ID NO: 614)
4808	CCAGGGTTACATCC (SEQ ID NO: 615)	ICsICsICsdGsdGsdGsdTsdTsdAsmdCsdAsITsICsIC (SEQ ID NO: 616)
4811	GCTCCAGGGTTACA (SEQ ID NO: 617)	IGsICsITsmcCsmcCsdAsdGsdGsdGsdTsdTsICsICsIC (SEQ ID NO: 618)
4812	AGCTCCAGGGTTAC (SEQ ID NO: 619)	lAsIGsICsdTsmcCsmcCsdAsdGsdGsdGsdTsITsICsIC (SEQ ID NO: 620)
4836	GCAGCTGCTGAGGT (SEQ ID NO: 621)	IGsICsICsdGsmcCsdTsdGsmcCsdTsdGsdAsIGsIGsIT (SEQ ID NO: 622)
4837	AGCAGCTGCTGAGG (SEQ ID NO: 623)	lAsIGsICsdAsdGsmcCsdTsdGsmcCsdTsdGsICsIGsIG (SEQ ID NO: 624)
6033	CCCTTAAGGGCCTC (SEQ ID NO: 625)	ICsICsICsdTsdTsdAsdAsdGsdGsdGsmcCsICsITsIC (SEQ ID NO: 626)

[0186] l = 잠금 뉴클레오티드; d = 데옥시뉴클레오티드; mdC = 5-메틸 시토신; m = 2'-O-메틸 뉴클레오티드; s = 포스 포로티오에이트 결합

표 3

[0187]

SEQ ID NO: 6의 표적 서 열의 시작 뉴 클레오티드	서열(5'-3')	변형을 가진 서열(5'-3')
436	AGGCAGAGGCACT (SEQ ID NO: 627)	lAsdGsIGsdCsICsIGsdCsdAsIGsdGsICsdAsICsIT (SEQ ID NO: 628)
513	TCACACATCCAT (SEQ ID NO: 629)	ITsdCsICsdCsITsICsdAsdCsICsdTsICsdCsICsIT (SEQ ID NO: 630)
524	ACTCCCCAAGTTCA (SEQ ID NO: 631)	lAsdCsITsdCsICsICsdCsdAslAsdGsITsdTsICsIC (SEQ ID NO: 632)
610	AGGCACCCAGACTC (SEQ ID NO: 633)	lAsdGsIGsdCsICsICsdCsdCsICsdGsICsdCsITsIC (SEQ ID NO: 634)
735	GGGTTTCATGGGCTG (SEQ ID NO: 635)	IGsdGsIGsdTsITsICsdAsdTsIGsdGsIGsdCsITsIG (SEQ ID NO: 636)

867	ATGGTGACACAGAA (SEQ ID NO: 637)	lAsdTslGsdGsITslGsdAsdCsIAsdCsIAsdGsIAsIA (SEQ ID NO: 638)
1020	ATCAGCATGGACTG (SEQ ID NO: 639)	lAsdTslCsdAslGslCsdAsdTslGsdGsIAsdCsITslG (SEQ ID NO: 640)
1061	GCTTGGTGTTAACC (SEQ ID NO: 641)	lGsdCsITsdTslGslGsdTsdGsITsdTslAsdAslCsIC (SEQ ID NO: 642)
1188	TCCATGGCAGGGTT (SEQ ID NO: 643)	lTsdCsICsdAsITslGsdGsdCsIAsdGsIGsdGsITslT (SEQ ID NO: 644)
1305	AGATAGCTGTCAAT (SEQ ID NO: 645)	lAsdGsIAsdTslAslGsdCsdTslGsdTslCsdAsIAsIT (SEQ ID NO: 646)
1443	CAGAAGTGGTAGTC (SEQ ID NO: 647)	lCsdAslGsdAslAslGsdTsdGsIGsdTslAsdGsITslC (SEQ ID NO: 648)
1447	GCTGCAGAAGTGGT (SEQ ID NO: 649)	lGsdCsITsdGsICslAsdGsdAslAsdGsITsdGsIGslT (SEQ ID NO: 650)
1518	ATGTCCATGGCATG (SEQ ID NO: 651)	lAsdTslGsdTslCsICsdAsdTslGsdGsICsdAsITslG (SEQ ID NO: 652)
1560	ATCTTATAGCAGGC (SEQ ID NO: 653)	lAsdTslCsdTslTslAsdTsdAslGsdCsIAsdGsIGslC (SEQ ID NO: 654)
1647	GCACTCTCAGTGCC (SEQ ID NO: 655)	lGsdCsIAsdCsITslCsdTsdCsIAsdGsITsdGsICslC (SEQ ID NO: 656)
1662	TAGGCAGCCTTGTC (SEQ ID NO: 657)	lTsdAslGsdGsICslAsdGsdCsICsdTslTsdGsITslC (SEQ ID NO: 658)
1694	TGAGGAGGTCCCCA (SEQ ID NO: 659)	lTsdGsIAsdGsIGslAsdGsdGsITsdCsICsdCsICslA (SEQ ID NO: 660)
1755	ACACTCTGGCCCTT (SEQ ID NO: 661)	lAsdCsIAsdCsITslCsdTsdGsIGsdCsICsdCsITslT (SEQ ID NO: 662)
1764	ACCTGCTCCAACT (SEQ ID NO: 663)	lAsdCsICsdTslGslCsdTsdCsICsdAsICsdAslCsIT (SEQ ID NO: 664)
1828	AGACACCAGCCACC (SEQ ID NO: 665)	lAsdGsIAsdCsIAslCsdCsdAslGsdCsICsdAslCsIC (SEQ ID NO: 666)
1912	CTCAAAGATCTCAA (SEQ ID NO: 667)	lCsdTslCsdAslAslAsdGsdAslTsdCsITsdCsIAsIA (SEQ ID NO: 668)
2037	ATGAAGACCCAGTC (SEQ ID NO: 669)	lAsdTslGsdAslAslGsdAsdCsICsdCsIAsdGsITslC (SEQ ID NO: 670)
2218	CTTCTGTGTCAGGCC (SEQ ID NO: 671)	lCsdTslTsdCsITslTsdGsdTslCsdAslGsdGsICslC (SEQ ID NO: 672)
2253	TAGTGGACCACCTC (SEQ ID NO: 673)	lTsdAslGsdTslGslGsdAsdCsICsdAslCsdCsITslC (SEQ ID NO: 674)
2481	AGTTTCTCCTTGTC (SEQ ID NO: 675)	lAsdGsIGsdTslTslCsdTsdCsICsdTslTsdGsITslG (SEQ ID NO: 676)
2518	GGGCTGTGTGGCCC (SEQ ID NO: 677)	lGsdGsIGsdCsITslGsdTsdGsITsdGsIGsdCsICslC (SEQ ID NO: 678)
2738	CCTTCCTGCTGTCC (SEQ ID NO: 679)	lCsdCsITsdTslCsICsdTsdGsICsdTslGsdTslCsIC (SEQ ID NO: 680)
2961	TGGATGGTGAACAG (SEQ ID NO: 681)	lTsdGsIGsdAsITslGsdGsdTslGsdAslAsdCsIAslG (SEQ ID NO: 682)
3186	TGCAGGGCCAGGTC (SEQ ID NO: 683)	lTsdGsICsdAslGslGsdGsdCsICsdAslGsdGsITslC (SEQ ID NO: 684)
3255	ACCTTGACTTGAT (SEQ ID NO: 685)	lAsdCsICsdTslTslGsdGsdAslCsdTslTsdGsIAsIT (SEQ ID NO: 686)
3625	GCATTCCAGGTCCT (SEQ ID NO: 687)	lGsdCsIAsdTslTslCsdCsdAslGsdGsITsdCsICslT (SEQ ID NO: 688)
3794	GCAGGCTCAGCTGG (SEQ ID NO: 689)	lGsdCsIAsdGsIGslCsdTsdCsIAsdGsICsdTslGslG (SEQ ID NO: 690)
4253	CTGCACTGGCCTTG (SEQ ID NO: 691)	lCsdTslGsdCsIAslCsdTsdGsIGsdCsICsdTslTslG (SEQ ID NO: 692)

4259	GCTTCTCTGCACTG (SEQ ID NO: 693)	lGsdCsITsdTsICsITsdCsdTsIGsdCsIAsdCsITsIG (SEQ ID NO: 694)
4302	TTGATCTGGCCTC (SEQ ID NO: 695)	lTsdTsIGsdAsITsICsdTsdTsIGsdGsICsdCsITsIC (SEQ ID NO: 696)
4318	CTGCAGCTCCTCCA (SEQ ID NO: 697)	lCsdTsIGsdCsIAsIGsdCsdTsICsdCsITsdCsICsIA (SEQ ID NO: 698)
4416	TGACTGATCAGACA (SEQ ID NO: 699)	lTsdGsIAsdCsITsIGsdAsdTslCsdAsIGsdAsICsIA (SEQ ID NO: 700)
4656	ATGGCATCTGCTTC (SEQ ID NO: 701)	lAsdTslGsdGsICsIAsdTsdCsITsdGsICsdTsITsIC (SEQ ID NO: 702)
4717	TGCTCTGCAAGCC (SEQ ID NO: 703)	lTsdGsICsdCsITsICsdCsdTsIGsdCsIAsdGsICsIC (SEQ ID NO: 704)
4773	AGCTTGGCCTTCTC (SEQ ID NO: 705)	lAsdGsICsdTsITsIGsdGsdCsICsdTsITsdCsITsIC (SEQ ID NO: 706)
4802	TTACATCCTCTGAC (SEQ ID NO: 707)	lTsdTsIAsdCsIAsITsdCsdCsITsdCsITsdGsIAsIC (SEQ ID NO: 708)
4837	AGCAGCTGCTGAGG (SEQ ID NO: 709)	lAsdGsICsdAsIGsICsdTsdGsICsdTsIGsdAsIGsIG (SEQ ID NO: 710)
4904	TCTCCTCCTCTGC (SEQ ID NO: 711)	lTsdCsITsdCsICsITsdCsdCsITsdCsICsdTsIGsIC (SEQ ID NO: 712)
6033	CCCTTAAGGGCCTC (SEQ ID NO: 713)	lCsdCsICsdTsITsIAsdAsdGsIGsdGsICsdCsITsIC (SEQ ID NO: 714)
6099	AGTTCTTCCTGTC (SEQ ID NO: 715)	lAsdGsIGsdTsITsICsdTsdTsICsdCsITsdGsITsIC (SEQ ID NO: 716)
6142	GACCTTGCTCTGCA (SEQ ID NO: 717)	lGsdAsICsdCsITsITsdGsdCsITsdCsITsdGsICsIA (SEQ ID NO: 718)
6149	AGCTCTTGACCTTG (SEQ ID NO: 719)	lAsdGsICsdTsICsITsdTsdGsIAsdCsICsdTsITsIG (SEQ ID NO: 720)
6220	GTGCTGGGCCTTGC (SEQ ID NO: 721)	lGsdTsIGsdCsITsIGsdGsdGsICsdCsITsdTsIGsIC (SEQ ID NO: 722)
6273	TTGTTGGCCTGGGT (SEQ ID NO: 723)	lTsdTsIGsdTsITsIGsdGsdCsICsdTsIGsdGsIGsIT (SEQ ID NO: 724)
6277	CAGCTTGTGGCCT (SEQ ID NO: 725)	lCsdAsIGsdCsITsITsdGsdTsITsdGsIGsdCsICsIT (SEQ ID NO: 726)

표 4

[0188]

SEQ ID NO: 6의 표적 서열 의 시작 뉴클 레오티드	서열(5'-3')	변형을 가진 서열(5'-3')
4302	+TT+GA+T+CTT+GG+CC+T+C (SEQ ID NO: 727)	lTsdTsIGsdAsITsICsdTsdTsIGsdGsICsdCsITsIC (SEQ ID NO: 728)
4302	+TT+GA+TC+T+T+GGC+CT+C (SEQ ID NO: 729)	lTsdTsIGsdAsITsdCsITsITsIGsdGsdCsICsdTsIC (SEQ ID NO: 730)
4302		lTsITsIGsdAsdTsmcCsdTsdTsdGsdGsmcCsICsITsIC (SEQ ID NO: 731)
4302	TTGATCTTGGCCTC (SEQ ID NO: 732)	eTseTseGsdAsdTsmcCsdTsdTsdGsdGsmcCseCseTseC (SEQ ID NO: 733)
4302	TTGATCTTGGCCTC (SEQ ID NO: 734)	lTsITsmGsdAsdTsmcCsdTsdTsdGsdGsmcCsmCsITsIC (SEQ ID NO: 735)
4302	TTGATCTTGGCCTC (SEQ ID NO: 736)	eTseTsIGsdAsdTsmcCsdTsdTsdGsdGsmcCseCseTseC (SEQ ID NO: 737)
4302	TTGATCTTGGCCTC (SEQ ID NO: 738)	eTsITsIGsdAsdTsmcCsdTsdTsdGsdGsmcCsICsITsIC (SEQ ID NO: 739)

4302	TTGATCTTGGCCTC (SEQ ID NO: 740)	ITseTsIGsdAsdTsmcCsdTsdTsdGsdGsmcCsICsITsIC (SEQ ID NO: 741)
4302	TTGATCTTGGCCTC (SEQ ID NO: 742)	ITsITseGsdAsdTsmcCsdTsdTsdGsdGsmcCsICsITsIC (SEQ ID NO: 743)
4302	TTGATCTTGGCCTC (SEQ ID NO: 744)	ITsITsIGsdAsdTsmcCsdTsdTsdGsdGsmcCseCsITsIC (SEQ ID NO: 745)
4302	TTGATCTTGGCCTC (SEQ ID NO: 746)	ITsITsIGsdAsdTsmcCsdTsdTsdGsdGsmcCsICseTsIC (SEQ ID NO: 747)
4302	TTGATCTTGGCCTC (SEQ ID NO: 748)	ITsITsIGsdAsdTsmcCsdTsdTsdGsdGsmcCsICsITseC (SEQ ID NO: 749)
4302	TTGATCTTGGCCTC (SEQ ID NO: 750)	ITsITsIGdAdTmdCdTdTdGdGmdCICsITsIC (SEQ ID NO: 751)
4302	TTGATCTTGGCCTC (SEQ ID NO: 752)	ITsITsIGsdAdTmdCdTdTdGdGmdCsICsITsIC (SEQ ID NO: 753)
4302	TTGATCTTGGCCTC (SEQ ID NO: 754)	ITsITsIGdAdTmdCsdTdTsTdGdGsmcCICsITsIC (SEQ ID NO: 755)
4302	TTGATCTTGGCCTC (SEQ ID NO: 756)	ITsITIGsdAdTsmcCdTsTdGsdGmdCsICITsIC (SEQ ID NO: 757)
4302	TTGATCTTGGCCTC (SEQ ID NO: 758)	ITsITsIGdAdTsmcCsdTdTsTdGsdGsmcCICsITsIC (SEQ ID NO: 759)
4318	CTGCAGCTCCTCCA (SEQ ID NO: 760)	ICsITsmGsmcCsdAsdGsmcCsdTsmcCsmcCsdTsmCsICsIA (SEQ ID NO: 761)
4318	CTGCAGCTCCTCCA (SEQ ID NO: 762)	ICsITsIGmdCsdAdGsmcCdTsmcCmdCsdTICsICsIA (SEQ ID NO: 763)
4318	CTGCAGCTCCTCCA (SEQ ID NO: 764)	ICsITsIGmdCsdAsdGsmcCdTsmcCsmcCsdTICsICsIA (SEQ ID NO: 765)
4302	TTGATCTTGGCCTC (SEQ ID NO: 766)	ITsmUsIGsdAsdTsmcCsdTsdTsdGsdGsmcCsICsmusIC (SEQ ID NO: 767)
4302	TTGATCTTGGCCTC (SEQ ID NO: 768)	ITsITsfGsdAsdTsmcCsdTsdTsdGsdGsmcCsICsITsIC (SEQ ID NO: 769)
4661		ITsITcmUsdGsdGsdAsdTsdGsdGsmcCsdAsmUsICsIT (SEQ ID NO: 770)
892		IGsITasmCsdTsdGsdGsdGsdAsdGsmcCsmcCsmAsITsIT (SEQ ID NO: 771)
2360		ICsITcmAsdGsdGsdAsdGsmcCsmcCsdTsdAsmUsITsIC (SEQ ID NO: 772)
1417		IAsITgsmAsmdCsdAsdGsdAsdAsdGsmcCsdAsmGsICsIA (SEQ ID NO: 773)
516		IAsITgsmUsdTsmcCsdAsmdCsdTsmcCsdAsmdCsmAsITsIC (SEQ ID NO: 774)
1582		IAsITasmAsdGsdTsdGsmcCsdAsdGsdGsdAsmGsIGsIG (SEQ ID NO: 775)
1217		ITsITcmCsdTsmcCsdAsdGsdGsdGsdTsmcCsmUsITsIG (SEQ ID NO: 776)
515		IGsITsmUsmdCsdAsmdCsdTsmcCsdAsmdCsdAsmUsICsIC (SEQ ID NO: 777)
514		ITsITsmCsdAsmdCsdTsmcCsdAsmdCsdAsdTsmCsICsIA (SEQ ID NO: 778)
1424		IGsITgsmUsdTsmcCsdAsdTsdAsdGsdAsmdCsmAsIGsIA (SEQ ID NO: 779)
1219		IAsITsmUsmdCsmcCsdTsmcCsdAsdGsdGsdGsmUsICsIT (SEQ ID NO: 780)
513		ITsITcmAsmdCsdTsmcCsdAsmdCsdAsdTsmcCsmCsIAIT (SEQ ID NO: 781)
1226		IAsITgsmUsdTsdAsdTsmcCsdAsdTsdTsmcCsmCsITsIC (SEQ ID NO: 782)

1225		lGs1TsmUsdAsdTsmCdAsdTsdTsmCdCsmUs1Cs1A (SEQ ID NO: 783)
1645		lAs1CsmUsmdCsdTsmCdAsdGsdTsdGsmCdCsmCs1As1T (SEQ ID NO: 784)
4302		avTsavTsavGsdAsdTsmCdTsdTsdGsdGsmCdCsavCsavTsavC (SEQ ID NO: 785)
3257		avGsavCsavAsdCsdCsdTsdTsdGsdGsdAsdCsavTsavTsavG (SEQ ID NO: 786)
2483		avTsavGsavAsdGsdGsdTsdTsdCsdTsdCsdCsavTsavTsavG (SEQ ID NO: 787)

[0189] l = 잠금 뉴클레오티드; d = 테옥시뉴클레오티드; mdC = 5-메틸 시토신; f = 2'-플루오로 뉴클레오티드; av = 아미노-CBBN 뉴클레오티드; s = 포스포로티오에이트 결합; e = 에틸렌-가교 뉴클레오티드

표 5

[0190]	변형을 가진 서열(5'-3')
	lGs1TsdGsdAsdAsdTsdGsdCsdGsdGsdAsdTslGs1As1A (SEQ ID NO: 789)
	lGs1As1AsdGsdTsdGsdGsdTsdAsdGsdTsdCsdAs1Ts1A (SEQ ID NO: 790)
	lGsdTs1GsdAsdAs1Ts1GsdCsdGs1GsdAs1TsdGs1As1A (SEQ ID NO: 791)
	lGsdAs1AsdGsdTs1Gs1GsdTsdAs1GsdTs1CsdAs1Ts1A (SEQ ID NO: 792)

[0191] 한 실시태양에서, 본 발명은 MYH7B의 억제제를 포함하는 조성물을 대상에게 투여하는 단계를 포함하여 β -MHC의 발현이 대상의 심장 세포에서 상향 조절되는 심장 질환을 치료 또는 예방하는 방법을 제공한다. 또 다른 실시태양에서, 본 발명은 대상에게 MYH7B의 억제제를 포함하는 조성물을 투여하는 단계를 포함하여 대상의 심장 세포에서 β -MHC/ α -MHC의 비율이 변경되는 심장 질환을 치료 또는 예방하는 방법을 제공한다. 예를 들어, 본 발명은 MYH7B의 억제제를 사용하여 대상의 심장 세포에서 β -MHC의 발현 또는 활성을 억제 및/또는 β -MHC/ α -MHC의 비율을 변경함으로써 병적 심근비대증, 심근경색증, 심부전증 또는 비대성 심근병증으로 이루어진 그룹으로부터 선택된 심장 질환을 치료 또는 예방하기 위한 조성물 및 방법을 제공한다. 대상에 대한 MYH7B의 억제제의 투여는 투여 후 대상의 심장 세포에서 MYH7B의 발현 또는 활성을 하향 조절한다. 한 실시태양에서, MYH7B의 억제제의 투여는 대상의 심장 세포에서 myomiRs, 특히 miR-499의 발현 또는 활성을 현저하게 변화시키지 않는다. 따라서, 일부 실시태양에서, 심근 세포에서 miR-499, miR-208a 및/또는 miR-208b의 발현은 MYH7B 억제제의 투여 후 통계적으로 상이지 않다.

[0192] 다양한 실시태양에서, 본 발명에 의해 제공된 방법은 본 발명에 개시된 하나 이상의 핵산 억제제를 투여하는 단계를 포함한다. 예를 들어, 한 실시태양에서, 본 발명에 의해 제공된 방법은 표 1-5로부터 선택되는 안티센스 올리고뉴클레오티드 억제제를 투여하는 단계를 포함한다. 또 다른 실시태양에서, 본 발명에 따른 방법은 SEQ ID NO: 146의 서열을 포함하는 안티센스 올리고뉴클레오티드 억제제를 투여하는 단계를 포함한다. 또 다른 실시태양에서, 본 발명에 따른 방법은 SEQ ID NO: 148의 서열을 포함하는 안티센스 올리고뉴클레오티드 억제제를 투여하는 단계를 포함한다.

[0193] 한 특정 실시태양에서, 본 발명은 MYH7B의 억제제를 포함하는 조성물을 대상에게 투여하는 단계를 포함하여 필요한 대상에서 비대성 심근병증을 치료 또는 예방하는 방법을 제공하며, MYH7B의 발현 또는 활성이 억제제의 투여 후 대상의 심장 세포에서 감소된다. 한 실시태양에서, 대상에 대한 MYH7B의 억제제의 투여는 투여 후 대상의 심장 세포에서 β -MHC의 발현 또는 활성을 감소시킨다.

[0194] 한 실시태양에서, 본 발명은 병적 심근비대증 또는 비대성 심근병증의 발병 위험이 있는 대상에서 병적 심근비대증 또는 비대성 심근병증을 치료 또는 예방하는 방법을 제공한다. 병적 심근비대증 또는 비대성 심근병증의 발병 위험이 있는 환자는, 예를 들어, 장기의 비 통제된 고혈압, 교정되지 않은 판막 질환, 만성 협심증, 최근의 심근 경색증, 심장 질환에 대한 선천적 소인 또는 병적 비대증을 포함하는 하나 이상의 위험 인자를 나타낼

수 있다. 일부 실시태양에서, 위험이 있는 대상은 병적 심근비대증 또는 비대성 심근병증에 유전적 소인을 갖는 것으로 진단될 수 있다. 예를 들어, 특정 실시태양에서, 위험이 있는 대상은 근절 유전자에 하나 이상의 돌연변이, 예를 들어 β -MHC(MYH7) 유전자의 돌연변이를 보유할 수 있다. 한 실시태양에서, 위험이 있는 대상은 Arg663His, Lys207Asn, Gly256Glu, Arg403Gln, Arg453Cys, Gly584Arg, Arg719Trp, Arg723Gly, Ile736Thr, Gly741Arg, Asp778Gly, Asp778Val, Asp906Gly 및 Leu908Val로부터 선택된 β -MHC(MYH7) 유전자에 하나 이상의 돌연변이를 가진다. 특정 실시태양에서, 위험이 있는 대상은 β -MHC(MYH7) 유전자에 Arg403Gln, Arg453Cys 또는 Arg719Trp 돌연변이를 가진다. 비대성 심근병증과 관련된 근절 유전자에 다른 돌연변이는 당해 기술분야의 당업자에게 공지되어 있으며(예를 들어, 전문이 참조로 본 발명에 포함된, Moore et al, Circulation Research, Vol. 111:375-385, 2012 참조), 병적 심근비대증 또는 비대성 심근병증의 발병 위험이 있는 대상을 확인하는데 사용될 수 있다. 다른 특정 실시태양에서, 위험이 있는 대상은 병적 심근비대증 또는 비대성 심근병증의 가족력을 가질 수 있다.

[0195] 다른 실시태양에서, 본 발명은 대상에게 MYH7B의 억제제를 투여하는 단계를 포함하여 대상의 심장 세포에서 β -MHC의 발현 또는 활성을 억제하기 위한 조성물 및 방법을 제공한다. 또 다른 실시태양에서, 본 발명은 대상에게 MYH7B의 억제제를 투여하는 단계를 포함하는, 대상의 심장 세포에서 β -MHC/ α -MHC의 비율을 조절하기 위한 조성물 및 방법을 제공한다. 대상에 대한 MYH7B의 억제제의 투여는 MYH7B의 억제제에 의한 치료 전에 MYH7B 및 β -MHC의 발현 또는 활성과 비교하여 대상의 심장 세포에서 MYH7B의 발현 또는 활성 및/또는 β -MHC의 발현 또는 활성을 감소시킨다. 한 실시태양에서, 대상에게 대한 MYH7B의 억제제의 투여는 건강한 대상에서 발견된 정상 수준으로 대상의 심장 세포에서 β -MHC/ α -MHC 비율을 완전 또는 부분적으로 회복시킨다.

[0196] 본 발명의 한 양태에 따라, 대상에 대한 MYH7B의 억제제의 투여는 심장 질환의 하나 이상의 증상의 개선을 초래한다. 예를 들어, 한 실시태양에서, MYH7B 억제제의 투여는 대상에서 병적 심근비대증, 심근경색증 또는 심부전증의 하나 이상의 증상의 개선 또는 병적 심근비대증에서 심부전증으로의 전환의 지연을 초래한다. 다른 실시태양에서, MYH7B 억제제의 투여는 대상에서 병적 심근비대증, 심근경색증 또는 심부전증의 개시에 지연을 초래한다. 하나 이상의 개선된 증상은, 예를 들어, 증가된 운동 능력, 증가된 심장 배출 부피, 감소된 좌심실 이완 말기 압력, 감소된 폐 모세혈관 췌기 압력, 증가된 심박출량, 증가된 심지수, 감소된 폐동맥 압력, 감소된 좌심실 수축 및 이완 말기 치수, 감소된 심장 섬유증, 심근의 감소된 콜라겐 침착, 감소된 좌심실 및 우심실 벽 스트레스, 감소된 벽 긴장, 삶의 질 향상, 및 감소된 질병 관련 이환율 또는 사망률이다.

[0197] 일부 실시태양에서, MYH7B의 억제제는 앵타머이다. 본 발명에 사용된 용어 "앵타머"는 고전적인 왓슨-크릭 염기쌍 형성 이외의 상호 작용을 통해 특정 표적 분자에 대한 특이적 결합 친화력을 갖는 핵산 분자를 의미한다. 이 앵타머는 표적 분자에 결합함으로써 표적 분자의 활성 또는 기능을 억제할 수 있다. 일부 실시태양에서, 본 발명의 앵타머는 MYH7B 단백질의 하나 이상의 에피토프에 결합한다. 특정 실시태양에서, 앵타머는 인간 MYH7B 단백질에 결합한다. 항-MYH7B 앵타머는 리보뉴클레오타이드, 데옥시리보뉴클레오타이드, 변형 뉴클레오타이드 또는 이의 혼합물로 구성될 수 있다. 예를 들어, 앵타머는 하나 이상의 2'-O-알킬(예를 들어, 2'-O-메틸) 또는 2'-할로(예를 들어, 2'-플루오로) 변형을 포함할 수 있다. 일부 실시태양에서, 상기 앵타머는 앵타머의 순환 반감기를 향상시키기 위해 중합체, 예를 들어 PEG 폴리머에 접합될 수 있다.

[0198] MYH7B에 대한 앵타머는 미국특허 제 5,270,163호에 기술된 바와 같이, 지수 적 농축에 의한 리간드의 체계적인 진화(SELEX)를 사용하여 확인될 수 있다(Tuerk & Gold, Science 249:505-510, 1990; Ellington & Szostak, Nature 346:818-822, 1990). 핵산의 라이브러리는 표적 MYH7B와 접촉될 수 있으며, 표적에 특이적으로 결합된 핵산은 표적에 특이적으로 결합하지 않는 라이브러리 내의 핵산의 나머지에서 분할된다. 분할된 핵산은 증폭되어 리간드가 풍부한 풀을 생성한다. 다수의 결합, 분할 및 증폭(즉, 선택)의 여러 사이클은 원하는 활성을 갖는 하나 이상의 앵타머의 동정을 초래한다. 미국 특허공보 제 20090264508호에 기술된 레이저 SELEX 또는 deSELEX와 같은 변형된 방법이 또한 사용될 수 있다.

[0199] 특정 실시태양에서, MYH7B의 발현 또는 활성을 억제하는데 유용한 앵타머의 길이는 약 10 내지 약 30개 뉴클레오타이드, 약 15 내지 약 35개 뉴클레오타이드, 약 20 내지 약 35개 뉴클레오타이드, 약 20 내지 약 40개 뉴클레오타이드, 또는 약 25 내지 약 50개 뉴클레오타이드이다.

[0200] 일부 실시태양에서, MYH7B의 억제제는 리보자임이다. 용어 "리보자임"은 촉매 활성을 갖는 RNA 분자를 의미한다. 다양한 활성을 갖는 리보자임이 공지되어 있다. 선택적으로, 표적 특이적 RNA 절단 활성을 갖는 리보자임이 설계될 수 있다. 특정 실시태양에서, MYH7B의 억제제로서 사용될 수 있는 리보자임은 그룹 I 및 그룹 II 인트론 유형 리보자임, 해머헤드 리보자임 및 헤어핀 리보자임과 같은 공지된 또는 인공적으로 생성된 RNase P

리보자임을 포함한다. 일부 실시태양에서, MYH7B의 억제제로서 사용될 수 있는 리보자임은 약 200 내지 약 500 개 뉴클레오타이드 또는 약 300 내지 약 450개 뉴클레오타이드의 길이를 가진다. 다른 실시태양에서, MYH7B의 리보자임 억제제는 약 30 내지 약 100개 뉴클레오타이드, 약 40 내지 약 80개 뉴클레오타이드, 또는 약 40 내지 약 60개 뉴클레오타이드의 길이를 가질 수 있다.

[0201] 한 실시태양에서, MYH7B의 억제제는 소형 간섭 RNA이다. 본 발명에 사용된 용어 "소형 간섭" 또는 "짧은 간섭 RNA" 또는 "siRNA"는 MYH7B 유전자를 표적으로 하고 MYH7B 유전자의 발현을 억제할 수 있는 뉴클레오타이드의 RNA 이중 가닥을 의미한다. 특정 실시태양에서, MYH7B의 억제제로서 사용될 수 있는 siRNA는 약 10 내지 약 30개 뉴클레오타이드, 특히 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 또는 30개 염기쌍을 형성하고 1개 또는 2개의 뉴클레오타이드의 5' 및/또는 3' 오버행(overhang)을 소유하는 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 또는 30개 뉴클레오타이드의 2개의 상보적인 단일 가닥 RAN를 포함한다. siRNA 이중 가닥은 MYH7B 유전자의 서열과 실질적으로 동일한 서열을 갖는 제 1 가닥 및 제 1 가닥에 부분적으로, 실질적으로 또는 완전히 상보적인 서열을 갖는 제 2 가닥을 포함한다. 특정 실시태양에서, MYH7B siRNA의 제 1 가닥은 MYH7B 유전자의 서열과 적어도 70%, 75%, 80%, 85%, 90%, 95%, 96%, 98%, 99% 또는 100% 동일한 서열을 포함하며 제 2 가닥은 제 1 가닥에 실질적으로 또는 완전히 상보적인 서열을 포함한다. 일부 실시태양에서, MYH7B 유전자를 표적으로 하는 siRNA의 제 1 RNA 가닥은 인간 MYH7B 유전자(SEQ ID NO: 6)의 암호화 영역과 적어도 70%, 75%, 80%, 85%, 90%, 95% 또는 100% 동일하다. 특정 실시태양에서, MYH7B 유전자를 표적으로 하는 siRNA의 제 1 RNA 가닥은 5'-GAGGCCAAGATCAA-3'의 서열(SEQ ID NO: 4)과 적어도 70%, 75%, 80%, 85%, 90%, 95% 또는 100% 동일하다. 다른 실시태양에서, MYH7B 유전자를 표적으로 하는 siRNA의 제 1 RNA 가닥은 5'-TGGAGGAGCTGCAG-3'의 서열(SEQ ID NO: 5)과 적어도 70%, 75%, 80%, 85%, 90%, 95% 또는 100% 동일하다. MYH7B의 siRNA 억제제는 심장 세포에 투여되거나 하나 이상의 벡터로부터 심장 세포에서 발현될 수 있다.

[0202] 다른 실시태양에서, MYH7B의 억제제는 소형 헤어핀 RNA(shRNA)이다. 본 발명에 사용된 용어 "shRNA"는 이중 가닥의 일부가 헤어핀 구조(shRNA)의 일부인 RNA 이중 가닥을 의미한다. 이중 부분 이외에, 헤어핀 구조는 이중 구조를 형성하는 두 개의 제 1 및 제 2 가닥 사이에 위치한 루프 부분을 함유할 수 있다. 루프는 길이가 다를 수 있다. 일부 실시태양에서, 루프는 5, 6, 7, 8, 9, 10, 11, 12 또는 13개 뉴클레오타이드 길이이다. 헤어핀 구조는 또한 3' 또는 5' 오버행을 포함할 수 있다. 일부 양태에서, 오버행은 0, 1, 2, 3, 4 또는 5개 뉴클레오타이드 길이의 3' 또는 5' 오버행이다. MYH7B를 표적으로 하는 shRNA의 이중 가닥 부분 또는 이중 가닥 영역은 약 10 내지 약 30개 뉴클레오타이드 길이이고 적어도 MYH7B 유전자의 서열과 적어도 70%, 75%, 80%, 85%, 90%, 95%, 96%, 98%, 99% 또는 100% 동일한 제 1 RNA 가닥 및 제 1 가닥에 실질적으로 또는 완전히 상보적인 제 2 가닥을 포함한다. 특정 실시태양에서, MYH7B 유전자를 표적으로 하는 이중 가닥 부분의 제 1 RNA 가닥은 인간 MYH7B 유전자(SEQ ID NO: 6)의 암호화 영역과 적어도 70%, 75%, 80%, 85%, 90%, 95% 또는 100% 동일하다. 일부 실시태양에서, MYH7B 유전자를 표적으로 하는 이중 가닥 부분의 제 1 RNA 가닥은 5'-GAGGCCAAGATCAA-3'의 서열(SEQ ID NO: 4)과 적어도 70%, 75%, 80%, 85%, 90%, 95% 또는 100% 동일하다. 다른 실시태양에서, MYH7B 유전자를 표적으로 하는 이중 가닥 부분의 제 1 RNA 가닥은 5'-TGGAGGAGCTGCAG-3'의 서열(SEQ ID NO: 5)과 적어도 70%, 75%, 80%, 85%, 90%, 95% 또는 100% 동일하다. MYH7B의 siRNA 억제제는 심장 세포에 투여되거나 한 벡터로부터 심장 세포에서 발현될 수 있다.

[0203] 안티센스 올리고뉴클레오타이드와 유사하게, 본 발명에 기술된 다른 억제 뉴클레오타이드 분자(엡타머, 리보자임, siRNA, shRNA)는 상기 하나 이상의 화학적으로 변형된 뉴클레오타이드를 함유할 수 있다. 예를 들어, MYH7B를 표적으로 하는 억제 뉴클레오타이드 분자는 2'-O-알킬 (예를 들어, 2'-O-메틸, 2'-O-메톡시에틸), 2'-할로(예를 들어, 2'-플루오로, 2'-클로로, 2'-브로모) 및 4' 티오 변형과 같은 바이사이클릭 당 뉴클레오시드(BSN) 변형, 잠금 핵산(LNA) 변형, 당 변형, 하나 이상의 포스포로티오에이트, 모르폴리노 또는 포스포노카복실레이트 결합과 같은 주쇄 변형, 5- 메틸시티딘 또는 2'-4'-가교 바이사이클릭 뉴클레오시드 변형과 같은 염기 변형을 포함한다.

[0204] 또 다른 실시태양에서, MYH7B의 억제제는 MYH7B 단백질의 하나 이상의 에피토프에 특이적으로 결합하는 항체 또는 이의 결합 단편이다. 예를 들어, 특정 실시 태양에서, MYH7B의 억제제는 MYH7B 단백질의 하나 이상의 에피토프에 특이적으로 결합하는 단클론 또는 다클론 항체이다. 단클론 항체는 키메라화 또는 인간화될 수 있다. 다른 실시태양에서, MYH7B의 억제제는 MYH7B-특이적 항체로부터 유래된 단편이며, 이 단편은 MYH7B 단백질의 하나 이상의 에피토프에 특이적으로 결합한다. 이러한 결합 단편은 Fab 단편, scFv 단편, 또는 다이아바디(diabody), 트라이바디(triabody), 미니바디(minibody) 또는 단일 도메인 항체와 같은 조작된 항체 단편을 포함할 수 있다. 항-MYH7B 항체는 IgM, IgG, IgD, IgE 및 IgA 또는 IgG 서브 클래스(예를 들어, IgG₁, IgG₂, IgG_{2a}, IgG_{2b},

IgG₃ 또는 IgG_M)를 포함하는 이들의 서브 클래스와 같은 모든 면역글로불린 클래스를 포함한다. 바람직하게는, 항-MYH7B 항체 또는 이의 결합 단편은 약 1×10^{-7} M 내지 약 1×10^{-12} M, 약 1×10^{-8} M 내지 약 1×10^{-11} M, 또는 약 1×10^{-9} M 내지 약 5×10^{-10} M 범위의 MYH7B에 대한 결합 친화력을 가진다.

[0205] 본 발명에 사용된 용어 "대상" 또는 "환자"는 인간 및 다른 영장류(예를 들어, 침팬지 및 다른 유인원 및 원숭이 종), 농장 동물(예를 들어, 소, 양, 돼지, 염소 및 말), 가정용 포유류 (예를 들어, 개 및 고양이), 실험용 동물(예를 들어, 생쥐, 쥐 및 기니피그 등의 설치류) 및 조류(예를 들어, 닭, 칠면조 및 다른 가금류, 오리, 오리, 거위 등과 같은 가정용, 야생용 및 경기용 조류)를 포함하나 이에 제한되지 않는 임의의 척추동물을 의미한다. 일부 실시태양에서, 대상은 포유동물이다. 다른 실시태양에서, 대상은 인간이고 억제 분자는 인간 MYH7B 유전자, mRNA, 전 mRNA 또는 단백질을 표적으로 한다.

[0206] 본 발명에 기술된 MYH7B의 임의의 핵산 억제제는 세포에 MYH7B 억제제를 암호화하는 발현 벡터를 전달함으로써 표적 세포(예를 들어, 심장 세포 및 골격근 세포)에 전달될 수 있다. "벡터"는 관심 핵산을 세포의 내부로 전달하기 위해 사용될 수 있는 물질의 조성물이다. 선형 폴리뉴클레오타이드, 이온성 또는 친양쪽성 화합물과 연관된 폴리뉴클레오타이드, 플라스미드, 및 바이러스를 포함하지만 여기에 제한되지 않는 여러 벡터가 당업계에 알려져 있다. 따라서 용어 "벡터"는 자율적으로 복제하는 플라스미드 또는 바이러스를 포함한다. 바이러스성 벡터의 예는 아데노바이러스 벡터, 아데노-연관 바이러스 벡터, 레트로바이러스 벡터 등을 포함하나 여기에 제한되지 않는다. 발현 구조체(expression construct)는 살아있는 세포(living cell)에서 복제되거나 또는 합성적으로 만들어질 수 있다. 본 출원의 목적을 위하여, 용어 "발현 구조체", "발현 벡터", 및 "벡터"라는 용어는 일반적이고, 설명적인 의미에서 본 발명의 출원을 설명하기 위해 상호 교환적으로 사용되며 본 발명을 제한하려는 것은 아니다.

[0207] 한 실시태양에서, MYH7B의 억제제를 발현하기 위한 발현 벡터는 5'-TTGATCTTGGCTC-3'의 서열을 포함하는 안티센스 올리고뉴클레오타이드를 암호화하는 폴리뉴클레오타이드에 작동 가능하게 연결된 프로모터를 포함한다. 다른 실시태양에서, MYH7B의 억제제를 발현하기 위한 발현 벡터는 5'-CTGCAGCTCCTCCA-3'의 서열을 포함하는 안티센스 올리고뉴클레오타이드를 암호화하는 폴리뉴클레오타이드에 작동 가능하게 연결된 프로모터를 포함한다. 본 발명에서 사용된 "작동가능하게 연결된(operably linked)" 또는 "전사 조절 하에서(under transcriptional control)"라는 문구는 프로모터가 RNA 중합효소 및 폴리뉴클레오타이드의 발현에 의해 전사의 개시를 제어하기 위해 폴리뉴클레오타이드에 대한 관계에서 정확한 위치와 방향에 있다는 것을 의미한다.

[0208] 본 발명에 사용된 대로, "프로모터"는 세포의 합성 기관(synthetic machinery) 또는 유전자의 특정 전사를 시작하기에 필요한 유도된 합성 기관(induced synthetic machinery)에 의해 인식된 DNA 서열을 의미한다. 적합한 프로모터는 RNA 중합효소I(polI), RNA 중합효소II(polII), RNA 중합효소III(polIII), 및 바이러스성 프로모터(viral promoter)(예를 들어, 인간 세포거대바이러스 즉각 초기 유전자 프로모터(human cytomegalovirus (CMV) immediate early gene promoter), SV40 초기 프로모터(SV40 early promoter), 및 라우스육종바이러스 긴말단 반복(the Rous sarcoma virus long terminal repeat)를 포함하나 이에 제한되는 것은 아니다. 한 실시태양에서, 프로모터는 조직 특이적 프로모터이다. 특히 관심 있는 것은 근육 특이적 프로모터들이며, 더욱 특히 심장 특이적 프로모터들이다. 이들은 미오신 경쇄-2 프로모터(the myosin light chain-2 promoter)(Franz et al. (1994) *Cardioscience*, Vol. 5(4):235-43; Kelly et al (1995) *J. Cell Biol*, Vol. 129(2):383-396), 알파 액틴 프로모터(the alpha actin promoter)(Moss et al (1996) *Biol. Chem.*, Vol. 271(49):31688-31694), 트로포닌 1 프로모터(the troponin 1 promoter)(Bhavsar et al. (1996) *Genomics*, Vol. 35(1): 11-23); Na⁺/Ca²⁺ 교환기 프로모터(the Na⁺/Ca²⁺ exchanger promoter)(Barnes et al (1997) *J. Biol. Chem.*, Vol. 272(17):11510-11517), 디스트로핀 프로모터(the dystrophin promoter)(Kimura et al. (1997) *Dev. Growth Differ.*, Vol. 39(3):257-265), 알파7 인테그린 프로모터(the alpha7 integrin promoter)(Ziobar and Kramer (1996) *J. Bio. Chem.*, Vol. 271(37):22915-22), 뇌 나트륨이뇨 펩타이드 프로모터(the brain natriuretic peptide promoter)(LaPointe et al (1996) *Hypertension*, Vol. 27(3 Pt 2):715-22) 및 알파 B-크리스탈린/소형열충격단백질 프로모터(the alpha B-crystallin/small heat shock protein promoter)(Gopal-Srivastava (1995) *J. Mol. Cell. Biol*, Vol. 15(12):7081-7090), 알파 미오신 중쇄 프로모터(alpha myosin heavy chain promoter)(Yamauchi-Takahara et al (1989) *Proc. Natl. Acad. Sci USA*, Vol. 86(10):3504-3508) 및 ANF 프로모터(ANF promoter)(LaPointe et al. (1988) *J. Biol. Chem.*, Vol. 263(19):9075-9078)를 포함한다.

[0209] 특정 실시태양에서, MYH7B 억제제를 암호화하는 폴리뉴클레오타이드에 작동 가능하게 연결된 프로모터는 유도성 프로모터일 수 있다. 유도성 프로모터는 당업계에 공지되어 있으며 테트라사이클린 프로모터(tetracycline

promoter), 메탈로티오네인 IIA 프로모터(metallothionein IIA promoter), 열충격 프로모터(heat shock promoter), 스테로이드/갑상선호르몬/레티노산 반응 요소(steroid/thyroid hormone/retinoic acid response elements), 아데노바이러스 후기 프로모터(the adenovirus late promoter), 및 유도성 생쥐 유방 종양 바이러스 LTR(the inducible mouse mammary tumor virus LTR)을 포함하나 이에 제한되지 않는다.

[0210] 발현 구조체와 핵산을 세포에 전달하는 방법은 당업계에 공지되어 있고, 예를 들어, 인산칼슘 공동 침전, 전기 천공법, 미세주사, DEAE-덱스트란, 리포펙션, 폴리아민 형질감염 시약을 사용하는 형질감염, 세포 초음파, 고속 미세주진체를 사용하는 유전자 충돌 및 수용체-매개 형질감염을 포함할 수 있다.

[0211] 또한, 본 발명은 MYH7B의 억제제(예, 안티센스 올리고뉴클레오타이드, 앵타머, 리보자임, siRNA, shRNA 또는 항체) 및 약학적으로 허용가능한 담체 또는 부형제를 포함하는 약학적 조성물을 포함한다. 예를 들어, 본 발명은 MYH7B의 안티센스 올리고뉴클레오타이드 및 약학적으로 허용가능한 담체 또는 부형제를 포함하는 조성물을 제공한다.

[0212] 한 실시태양에서, 약학적 조성물은 유효량의 MYH7B 억제제 및 약학적으로 허용가능한 담체를 포함한다. 예를 들어, 약학적 조성물은 MYH7B를 표적으로 하는 유효량의 안티센스 올리고뉴클레오타이드 또는 본 발명에 기재된 MYH7B를 표적으로 하는 유효량의 변형 안티센스 올리고뉴클레오타이드를 포함한다. 한 실시태양에서, 약학적 조성물은 5'-lTslTslGsdAsdTsdCsdTsdTsdGsdGsdCs1Cs1TslC-3'(SEQ ID NO: 146)의 서열을 갖는 안티센스 올리고뉴클레오타이드를 포함한다. 다른 실시태양에서, 약학적 조성물은 5'-lCs1TslGsdCsdAsdTsdCsdTsdCsdCsdTslCs1Cs1A-3'(SEQ ID NO: 148)의 서열을 갖는 안티센스 올리고뉴클레오타이드를 포함한다. 일부 다른 실시태양에서, 약학적 조성물은 표 1-5에 열거된 서열로부터 선택된 서열을 갖는 안티센스 올리고뉴클레오타이드 및 변형 안티센스 올리고뉴클레오타이드를 포함한다.

[0213] "유효량"은 유익한 또는 원하는 임상 결과를 얻기에 충분한 양이다. 본 발명의 MYH7B 억제제의 유효량은 약 1 mg/kg 내지 약 100 mg/kg, 약 2.5 mg/kg 내지 약 50 mg/kg 또는 약 5 mg/kg 내지 약 25 mg/kg 일 수 있다. 유효량으로 생각될 양의 정확한 결정은 크기, 연령, 장애 유형(예를 들어, 비대성 심근병증, 심근경색증, 심부전증 또는 심장비대증) 및 억제제의 성질(예를 들어, 안티센스 올리고뉴클레오타이드, siRNA, shRNA, 발현 구조체, 항체 등)을 포함하여 각 환자에게 개별적인 요인에 기반할 수 있다. 따라서, 투여량은 본 발명 및 당업계의 지식으로부터 당업자에 의해 용이하게 확인될 수 있다.

[0214] 특정 실시태양에서, 본 발명은 MYH7B의 억제제 및 제 2 치료제를 투여하는 것을 포함하여, 병적 심근비대증, 심근경색증, 심부전증 또는 비대성 심근병증을 치료 또는 예방하는 방법을 제공한다. 일부 실시태양에서, 제 2 치료제는 miR-208a, miR-208b, miR-499, miR-15a, miR-15b, miR-16, miR-195 또는 이들의 조합의 안티센스 올리고뉴클레오타이드 억제제와 같은 심장 치료제이다. 이러한 miRNA의 예시적인 억제제는 WO 2012/083005 및 WO 2013/192486에 기재되어 있으며, 이들의 전문이 본 발명에 참고로 포함된다. 제 2 치료제가 포함되는 실시태양에서, 제 2 제제는 동시에 투여되지만 별도의 제형으로 또는 순차적으로 투여될 수 있다. 다른 실시태양에서, 제 2 치료제는 MYH7B 억제제의 투여 이전 또는 이후 상이한 시간에 투여될 수 있다. 사전 투여는, 예를 들어, 제 2 제제 투여 전 약 1 주 내지 최대 30 분 범위 내에서 제 1 제제의 투여를 포함한다. 사후 투여는 또한, 예를 들어, 제 2 제제의 투여 전 약 2 주 내지 최대 30분 범위 내의 제 1 제제의 투여를 포함할 수 있다. 이후 또는 나중 투여는, 예를 들어, 제 1 제제 투여 후 약 1 주 내지 최대 30분 범위 내에서 제 2 제제 투여를 포함한다. 이후 또는 나중의 투여는 또한, 예를 들어, 제 1 제제 투여 후 약 2 주 내지 최대 30분 범위 내에서 제 2 제제의 투여를 포함할 수 있다.

[0215] 본 발명은 MYH7B의 억제제, 제 2 치료제 및 약학적으로 허용가능한 담체 또는 부형제를 포함하는 약학적 조성물을 포함한다. 특정 실시태양에서, 약학적 조성물은 MYH7B의 둘 이상의 억제제를 포함할 수 있다. 일부 실시태양에서, 제 2 치료제는 miR-208a, miR-208b, miR-499, miR-15a, miR-15b, miR-16, miR-195 또는 이들의 조합의 안티센스 올리고뉴클레오타이드 억제제와 같은 심장 치료제이다. 이런 실시태양에서, 제 2 치료제는 MYH7B의 억제제와 동일한 제제 또는 별도의 제제에 포함될 수 있다. 임상 적용이 고려되는 경우, 약학적 조성물은 의도된 용도에 적합한 형태로 제조될 것이다. 일반적으로, 이것은 발열원뿐만 아니라 인간 또는 동물에게 해를 줄 수 있는 다른 불순물이 본질적으로 없는 조성물의 제조를 수반할 것이다.

[0216] 거대분자 복합체, 나노캡슐, 미소구체, 비드, 및 수용유 에멀전, 미셀, 혼합 미셀, 및 리포솜을 포함하는 지질-기반 시스템과 같은 콜로이드 분산 시스템이 MYH7B의 올리고뉴클레오타이드 억제제 또는 MYH7B 뉴클레오타이드 억제제를 발현하는 구조체를 위한 전달 비히클로서 사용될 수 있다. 본 발명의 핵산을 심장 및 골격 근육 조직으로 전달하기에 적합한 상업적으로 구입가능한 지방 에멀전은 Intralipid®, Liposyn®, Liposyn®II, Liposyn®

III, Nutrilipid, 및 다른 유사한 지질 에멀전을 포함한다. 생체내 전달 비히클로 사용하기 위한 바람직한 콜로이드 시스템은 리포솜(즉, 인공 막소포)이다. 이러한 시스템의 제조 및 사용은 당업계에 주지되어 있다. 예시적인 제제는 또한 전문이 본 발명에 참조로서 포함되는 US 5,981,505; US 6,217,900; US 6,383,512; US 5,783,565; US 7,202,227; US 6,379,965; US 6,127,170; US 5,837,533; US 6,747,014; 및 W003/093449에서 개시된다.

[0217] 특정 실시태양에서, 전달에 사용되는 리포솜은 미국 특허 출원 공개공보 제 20110076322호에 상세히 기술된 SMARTICLES®(Marina Biotech, Inc.)와 같은 친양쪽성 리포솜이다. SMARTICLES®의 표면 전하는 완전히 가역적이라서 이를 핵산 전달에 특히 적합하게 만든다. SMARTICLES®는 주사를 통해 전달되고, 안정적으로 유지되며, 핵산을 전달하기 위해 유리 세포 및 교차 세포막을 응집시킬 수 있다.

[0218] 일부 실시태양에서, 적절한 염 및 버퍼는 전달 비히클을 안정하게 하고 표적 세포에 의한 흡수를 허용한다. 본 발명의 수용성 조성물은 약학적으로 허용 가능한 담체(carrier) 또는 수성 매질(aqueous medium)에 용해 또는 분산된 억제제 폴리뉴클레오티드(예를 들어, 리포솜 또는 다른 복합체 또는 발현 벡터)을 포함하는 유효량의 전달 비히클을 포함한다. "약학적으로 허용가능한" 또는 "약리학적으로 허용가능한"이란 문구는 동물 또는 사람에게 투여되었을 때, 해로운, 알레르기, 또는 다른 뜻밖의(untoward) 반응들을 일으키지 않는 분자 독립체(molecular entities) 및 조성물을 나타낸다. 본 발명에 사용된 "약학적으로 허용가능한 담체"는 용매, 버퍼, 용액, 분산매, 코팅, 향균 및 항진균제, 등장성 및 흡수 지연제 및 인간에 투여하기 적합한 약과 같은 약의 제제화에 사용이 허용되는 다른 물질을 포함한다. 임의의 통상적인 매질 또는 제제가 본 발명의 활성 성분과 양립 불가능한 것을 제외하고는, 치료 조성물에서의 이의 사용이 고려된다. 약학적 활성 물질들을 위한 이와 같은 매질 및 제제의 사용은 당업계에 주지되어 있다. 보충적인 활성 성분들 또한 조성물에 혼합될 수 있다.

[0219] 본 발명의 활성 조성물은 전형적인 약학 제제를 포함할 수 있다. 본 발명에 따른 이들 조성물의 투여는 표적 조직이 그 경로를 통해 이용 가능한 한 임의의 통상적인 경로를 통해 이루어질 수 있다. 여기에는 구강, 비강(흡입) 또는 협측이 포함된다. 선택적으로, 투여는 피내, 피하, 근육 내, 복강 내 또는 정맥 내 주사에 의해, 또는 심근 조직으로의 직접 주사에 의한 것일 수 있다. MYH7B 억제제 또는 MYH7B 억제제를 포함하는 발현 구조체를 포함하는 약학적 조성물은 치료제를 심장에 전달하기 위해 관상 순환을 분리하는 카테터 시스템 또는 시스템에 의해 투여될 수도 있다. 심장 및 관상 혈관계에 치료제를 전달하기 위한 다양한 카테터 시스템이 당업계에 공지되어 있다. 본 발명에서 사용하기에 적합한 카테터-기반 전달 방법 또는 관상 격리 방법의 일부 비 제한적인 예들은 전문이 본 발명에 참조로서 포함되는 미국특허 6,416,510; 미국특허 6,716,196; 미국특허 6,953,466, WO 2005/082440, WO 2006/089340, 미국특허공보 2007/0203445, 미국특허공보 2006/0148742, 및 미국특허공보 2007/0060907에 개시된다. 이런 조성물은 일반적으로 본 발명에 기재된 바와 같은 약학적으로 허용가능한 조성물로서 투여될 것이다.

[0220] 본 발명의 다른 실시태양에서, 본 발명에 기재된 MYH7B 억제제를 포함하는 조성물은 스텐트, 벌룬 또는 카테터와 같은 의료 장치용 코팅제로서 제제화될 수 있다. 대상에서 심장 질환을 치료하는 방법에 특히 유용하게, MYH7B의 억제제는 약물 스텐트를 제조하기 위해 금속 스텐트를 코팅하는데 사용될 수 있다. 약물 용출 스텐트는 협착된 또는 병에 걸린 동맥을 개방시켜 유지하고 화합물을 방출하여 세포 증식 및/또는 염증을 예방하기 위한 스캐폴드이다. MYH7B의 억제제는 억제제를 시간이 지남에 따라 방출하기 위해 얇은 중합체에 묻혀있는 금속 스텐트에 도포될 수 있다. 장치-기반 전달 방법 및 코팅 장치의 방법은 약물-용출 스텐트 및 다른 이식가능한 장치와 마찬가지로 당업계에 주지되어 있다. 예를 들어, 미국 특허 제 7,294,329호, 제 7,247,313호, 제 7,236,821호, 제 7,244,522호, 제 7,105,018호, 제 7,087,263호, 제 7,083,642호, 제 7,055,237호, 제 7,041,127호, 제 6,716,242호 및 제 6,589,286호 및 WO 2004/004602에 개시되어있다. 따라서, 본 발명은 MYH7B 억제제로 코팅된 벌룬, 카테터 또는 스텐트와 같은 의료 장치를 포함한다. 일부 실시태양에서, MYH7B 억제제는 약물-용출 스텐트에 혼입하기 위한 제제를 제조하기 위해 다른 치료제(예를 들어, 항 혈관재협착 화합물)와 조합하여 사용될 수 있다.

[0221] 활성 화합물은 또한 비경구 또는 복강 내 투여될 수 있다. 예시로서, 유리 염기 또는 약리학적으로 허용가능한 염으로서 활성 화합물의 용액은 하이드록시프로필셀룰로오스와 같은 계면 활성제와 적절하게 혼합된 물에서 제조될 수 있다. 분산액은 또한 글리세롤, 액체 폴리에틸렌 글리콜 및 이들의 혼합물 및 오일에서 제조될 수 있다. 통상적인 보관 및 사용 조건하에서, 이들 제제는 일반적으로 미생물의 성장을 방지하기 위한 방부제를 함유한다.

[0222] 주사용 또는 카테터 전달에 적합한 약학적 형태는, 예를 들어, 살균 수성 용액 또는 분산액 및 살균 주사 용액

또는 분산액의 즉석 제조를 위한 살균 분말을 포함한다. 일반적으로 이들 제제는 살균되며 쉬운 주입성이 존재할 정도로 유동성이다. 제제는 제조 및 보관 조건하에서 안정해야 하며 세균 및 균류와 같은 미생물의 오염 작용에 대비하여 보관되어야 한다. 적합한 용매 또는 분산매는 예를 들어, 물, 에탄올, 폴리올(예를 들어, 글리세롤, 프로필렌 글리콜, 및 액체 폴리에틸렌 글리콜 등), 이들의 적절한 혼합물, 및 식물 오일을 포함할 수 있다. 적절한 유동성은, 예를 들어, 레시틴과 같은 코팅제의 사용에 의해, 분산액의 경우에 필요한 입자 크기의 유지에 의해, 계면활성제의 사용에 의해 유지될 수 있다. 미생물 작용의 방지는 예를 들어, 파라벤, 클로로부탄올, 페놀, 소르빈산, 티메로살 등의 다양한 항균 및 항진균제에 의해 이루어질 수 있다. 많은 경우에서, 등장성 물질, 예를 들어, 당 또는 염화나트륨을 포함하는 것이 바람직할 것이다. 주사 가능한 조성물의 장기 흡수는 흡수 지연제, 예를 들어, 알루미늄 모노스테아레이트 및 젤라틴을 조성물에 사용하여 이루어질 수 있다.

[0223] 살균 주사 용액은 적절한 양의 컨주게이트를 용매에 원하는 임의의 다른 성분(예를 들어, 상기에서 열거한 것)과 함께 혼합함으로써 제조될 수 있다. 일반적으로, 분산액은 다양한 살균 활성 성분들을 기본적인 분산매와 원하는 다른 성분들, 예를 들어 앞에서 열거한 것들을 포함하는 살균 비히클에 혼합함으로써 제조된다. 살균 주사 용액 제조를 위한 살균 분말의 경우, 바람직한 제조방법은 상기 살균-여과된 용액으로부터 어떠한 추가적으로 요구되는 성분들을 더한 활성 성분(들)의 파우더를 생산하는 진공-건조 및 동결-건조 기술을 포함한다.

[0224] 본 발명의 조성물은 일반적으로 중성 또는 염 형태로 제제화될 수 있다. 약학적으로 허용가능한 염은, 예를 들어, 무기산(예를 들어, 염산 또는 인산) 또는 유기산(예를 들어, 아세트산, 옥살산, 타르타르산, 만델산 등)으로부터 유도된 산 부가 염(단백질의 유리 아미노기로 형성됨)을 포함한다. 단백질의 유리 카복실기로 형성된 염은 무기 염기(예를 들어, 나트륨, 칼륨, 암모늄, 칼슘 또는 수산화 제 2 철) 또는 유기 염기(예를 들어, 아이소프로필아민, 트라이메틸아민, 히스티딘, 프로카인 등)로부터 유도될 수 있다.

[0225] 제제 시에, 용액들은 되도록 투약 제제에 적합한 방식 및 치료에 효과적인 양으로 투여된다. 제제는 주사 가능한 용액, 약물 방출 캡슐 등과 같은 다양한 투약 형태로 쉽게 투여될 수 있다. 수용액에서 비경구적 투여를 위해서, 예를 들어, 용액은 일반적으로 적절하게 완충되고, 액상 희석액은 먼저 예를 들어 충분한 식염수 또는 글루코스로 등장성을 만든다. 이러한 수용액은 예를 들어, 정맥내, 근육내, 피하 및 복막내 투여에 사용될 수 있다. 바람직하게는, 특히 본 명세서의 관점에서, 당업계의 통상적인 기술을 가진자에게 이미 알려진 바대로 살균 수성 매질이 사용된다. 예로써, 단일 복용량은 1ml의 등장성 NaCl 용액에 용해되고 1000ml의 피하주액 유체(hypodermoclysis fluid)에 첨가되거나 또는 예정된 주입 부위에 주사될 수 있다(예를 들어, "Remington's Pharmaceutical Sciences" 15th Edition, 페이지 1035-1038 및 1570-1580 참조). 치료받는 대상의 질환에 따라 용량의 일부 변화는 필연적으로 생기게 된다. 투여에 대해 책임을 지는 사람은 어떤 일이 있어도 개개의 대상을 위한 적합한 용량을 결정할 것이다. 또한, 인간 투여에 있어, 제제는 제제 당국에 의해 요구되는 살균성, 발열원성, 일반 안전 및 순도 기준을 만족해야만 한다.

[0226] 본 발명의 특정 실시태양에서, 본 발명의 약학적 조성물은 투여용 장치와 함께 포장되거나 또는 투여용 장치 내에 저장된다. 주사 제제용 장치는 주사 포트, 자동 주사기, 주사 펌프 및 주사 펜을 포함하나 이에 제한되지 않는다. 에어로졸 또는 분말 제제용 장치는 흡입기, 흡입기, 흡입기 등을 포함하나 이에 제한되지 않는다. 따라서, 본 발명은 본 발명에 기술된 하나 이상의 장애를 치료 또는 예방하기 위한 본 발명의 약학적 조성물을 포함하는 투여 장치를 포함한다.

[0227] 본 발명은 제한적으로 해석되지 않는 다음 추가 실시예에 의해 추가로 설명된다. 당업자는 본 발명의 관점에서 개시된 특정 실시태양에 여러 변화를 가할 수 있고 본 발명의 취지와 범위를 벗어나지 않고 유사하거나 동일한 결과를 여전히 얻을 수 있다는 것을 안다.

[0228] 본 발명 전체에서 언급된 모든 특허 및 비 특허 문헌은 모든 목적을 위해 전문이 참조로 본 발명에 포함된다.

[0229] **실시예:**

[0230] 실시예 1: MYH7B의 억제제는 MYH7을 하향 조절한다

[0231] 인간 iPS 심근세포를 MYH7B를 표적으로 하는 대조군 올리고뉴클레오타이드 또는 안티센스 올리고뉴클레오타이드(Anti-7b #1(5'-GTGAATGCGGATGAA-3'; SEQ ID NO: 1) 및 Anti-7b #2(5'-GAAGTGGTAGTCATA-3'; SEQ ID NO: 2))로 형질감염시켰다. MYH7B 및 MYH7(β -MHC) mRNA의 수준은 실시간 PCR을 사용하여 48시간에 측정하였다. 갑상선 호르몬, T3로 치료된 인간 iPS 심근세포를 양성 대조군으로 사용하였다. T3는 β -MHC의 발현을 하향 조절하는 것으로 알려져 있다. 형질감염된 세포로부터 분리된 RNA의 실시간 PCR 분석은 MYH7B의 저해가 MYH7B뿐만 아니라 MYH7(β -MHC)의 하향 조절을 초래하였다는 것을 나타내었다. 예상한 대로, T3는 MYH7(β -MHC)을 하향 조절하였

으나(도 1b) MYH7B 또는 miR-208a, miR-208b 및 miR-499의 수준에는 영향을 미치지 않았다(도 1a 및 2).

[0232] MYH7B의 억제가 마이오신 전환을 초래한다는 관찰은 부분적으로 마이오신의 인트론성 miRNA (MYH7B, miR-499; MYH7, miR-208b; MYH6, miR-208a)의 조절을 통해 일어날 수 있다. 따라서, 본 발명자는 인간 iPS 심근세포에서 MYH7B의 억제가 myomiRs, miR-208a, miR-208b 또는 miR-499의 발현에 영향을 주는지 여부를 테스트 하였다.

[0233] 구체적으로, MYH7B의 하향 조절이 myomiRs(miR-208a, miR-499 및 miR-208b)의 발현에 어떠한 영향을 미치는지를 측정하기 위해, miR-208a, miR-499 및 miR-208b의 수준을 형질감염 48시간 후에 측정하였다. miR-208a, miR-499 또는 miR-208b의 발현에 현저한 효과는 관찰되지 않았다(도 2). 시간이 지남에 따라, MYH7의 하향 조절 때문에 miR-208b의 하향 조절이 있을 수 있으나, 도 2의 데이터는 MYH7B에 대한 안티센스 올리고뉴클레오티드가 직접적으로 또는 즉시 myomiRs의 발현에 영향을 미치지 않는 것을 나타낸다. 따라서, MYH7B의 억제에 의한 마이오신 전환의 조절은 심장 세포에서 신규한 miRNA 독립적인 매커니즘을 나타낸다.

[0234] miR-208a는 설치류에서 miR-499의 상류에 작용하고 출생 후의 miR-499, MYH7B 및 β -MHC 발현에 필요하다는 것이 밝혀졌다(van Rooji et al., "Control of stress-dependent cardiac growth and gene expression by a microRNA," Science, 2007, 316(5824), 575-579; Montgomery et al., "Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure," Circulation, 2011, 124(14), 1537-1547). miR-499와 MYH7B의 손실이 설치류의 심장에서 β -MHC 하향 조절에 필요하다(van Rooji et al., "A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance," Dev. Cell, 2009, 17, 662-673). 이 연구는 myomiR은 설치류와 같은 α -MHC 우성 종에서 β -MHC 발현의 조절에 중요한 역할을 한다는 것을 나타내지만, 돼지, 토끼 및 인간과 같은 β -MHC 우성 동물에서의 연구는 대체 매커니즘이 β -MHC 우성 동물에서 β -MHC의 발현을 조절하는데 관련될 수 있다는 것을 나타낸다.

[0235] 실시예 2: 비대성 심근병증 환자로부터 유래된 iPS 심장 세포에서 MYH7B의 억제는 MYH7을 하향 조절한다

[0236] 대조군 iPS 심근세포 및 돌연변이 R663H를 갖는 비대성 심근병증(HCM) 환자로부터 유래된 iPS 심근세포를 대조군 올리고뉴클레오티드 또는 MYH7B 안티센스 올리고뉴클레오티드(Anti-7b # 1 및 Anti-7b # 2; SEQ ID NOs: 1 및 2)로 형질감염시켰다. 실시간 PCR을 사용하여 형질 감염 후 48시간 및 1주(168시간)에 MYH7(β -MHC) mRNA의 수준을 측정하였다. 실시간 PCR 분석은 MYH7B의 억제가 HCM 심근세포에 MYH7(β -MHC) 발현의 하향 조절을 초래한다는 것을 나타내었다(도 3a). 또한, MYH7(β -MHC)의 발현의 하향 조절은 치료 후 1 주까지 지속되었다(도 3b).

[0237] 실시예 3: MYH7B의 억제는 β -MHC 단백질을 하향 조절한다

[0238] MYH7B의 억제가 단백질 수준에서 MYH7의 하향 조절을 초래하는지를 측정하기 위해 대조군 및 HCM R663H 인간 iPS 심근세포를 대조 올리고뉴클레오티드, MYH7B ASO #1(SEQ ID NO: 1), MYH7B ASO 및 TINY로 형질감염시켰다. TINY는 miR-208a, miR-208b 및 miR-499의 씨드 영역에 상보적인 8mer 올리고이다. MYH7(β -MHC) 단백질의 수준은 웨스턴 블롯 분석에 의해 평가되었다. 결과는 MYH7B 안티센스 올리고뉴클레오티드를 사용하는 대조군 또는 HCM iPS 심근세포의 치료가 치료 후 96시간에서 MYH7(β -MHC) 단백질을 현저하게 하향 조절하였다는 것을 나타내었다(도 4). 대조군 올리고는 효과를 나타내지 않았다. T3를 양성 대조군으로 사용하였다.

[0239] 실시예 4 : MYH7B에 대한 안티센스 올리고뉴클레오티드(ASOs)의 스크리닝

[0240] 인간 iPS 심근세포를 대조 올리고뉴클레오티드(모조(Mock)) 또는 MYH7B를 표적으로 하는 1.5 nM의 테스트 ASO로 형질감염시켰다. MYH7B mRNA의 수준은 실시간 PCR을 사용하여 48시간에 측정하였다(도 5). 다수의 ASO는 MYH7B 수준의 50% 초과 억제를 나타냈다. 특히, 하기 ASO 화합물을 테스트하였다:

표 6

[0241]

CPD #	SEQ ID NO:
Comp-1	SEQ ID NO: 52
Comp-2	SEQ ID NO: 8
Comp-3	SEQ ID NO: 22
Comp-4	SEQ ID NO: 82
Comp-5	SEQ ID NO: 96
Comp-6	SEQ ID NO: 110
Comp-7	SEQ ID NO: 3
Comp-8	SEQ ID NO: 140
Comp-9	SEQ ID NO: 154

Comp-10	SEQ ID NO: 170
Comp-11	SEQ ID NO: 38
Comp-12	SEQ ID NO: 54
Comp-13	SEQ ID NO: 24
Comp-14	SEQ ID NO: 68
Comp-15	SEQ ID NO: 84
Comp-16	SEQ ID NO: 98
Comp-17	SEQ ID NO: 112
Comp-18	SEQ ID NO: 126
Comp-19	SEQ ID NO: 142
Comp-20	SEQ ID NO: 156
Comp-21	SEQ ID NO: 172
Comp-22	SEQ ID NO: 56
Comp-23	SEQ ID NO: 10
Comp-24	SEQ ID NO: 26
Comp-25	SEQ ID NO: 70
Comp-26	SEQ ID NO: 86
Comp-27	SEQ ID NO: 100
Comp-28	SEQ ID NO: 114
Comp-29	SEQ ID NO: 128
Comp-30	SEQ ID NO: 144
Comp-31	SEQ ID NO: 158
Comp-32	SEQ ID NO: 174
Comp-33	SEQ ID NO: 42
Comp-34	SEQ ID NO: 58
Comp-35	SEQ ID NO: 12
Comp-36	SEQ ID NO: 28
Comp-37	SEQ ID NO: 72
Comp-38	SEQ ID NO: 88
Comp-39	SEQ ID NO: 102
Comp-40	SEQ ID NO: 116
Comp-41	SEQ ID NO: 130
Comp-42	SEQ ID NO: 146
Comp-43	SEQ ID NO: 160
Comp-44	SEQ ID NO: 176
Comp-45	SEQ ID NO: 44
Comp-46	SEQ ID NO: 60
Comp-47	SEQ ID NO: 14
Comp-48	SEQ ID NO: 30
Comp-49	SEQ ID NO: 74
Comp-50	SEQ ID NO: 90
Comp-51	SEQ ID NO: 104
Comp-52	SEQ ID NO: 118
Comp-53	SEQ ID NO: 132
Comp-54	SEQ ID NO: 148
Comp-55	SEQ ID NO: 162
Comp-56	SEQ ID NO: 178
Comp-57	SEQ ID NO: 46
Comp-58	SEQ ID NO: 62
Comp-59	SEQ ID NO: 16
Comp-60	SEQ ID NO: 32
Comp-61	SEQ ID NO: 76
Comp-62	SEQ ID NO: 92
Comp-63	SEQ ID NO: 3
Comp-64	SEQ ID NO: 120
Comp-65	SEQ ID NO: 134
Comp-66	SEQ ID NO: 150
Comp-67	SEQ ID NO: 164

Comp-68	SEQ ID NO: 180
Comp-69	SEQ ID NO: 48
Comp-70	SEQ ID NO: 64
Comp-71	SEQ ID NO: 18
Comp-72	SEQ ID NO: 34
Comp-73	SEQ ID NO: 78
Comp-74	SEQ ID NO: 106
Comp-75	SEQ ID NO: 122
Comp-76	SEQ ID NO: 136
Comp-77	SEQ ID NO: 152
Comp-78	SEQ ID NO: 166
Comp-79	SEQ ID NO: 182
Comp-80	SEQ ID NO: 50
Comp-81	SEQ ID NO: 66
Comp-82	SEQ ID NO: 36
Comp-83	SEQ ID NO: 80
Comp-84	SEQ ID NO: 94
Comp-85	SEQ ID NO: 108
Comp-86	SEQ ID NO: 124
Comp-87	SEQ ID NO: 138
Comp-88	SEQ ID NO: 168

[0242] 실시예 5 : ASO의 수동적 투여시 MYH7B의 억제

[0243] 실시예 4에서 MYH7B 수준의 50% 초과 억제 나타내는 ASO는 수동적 섭취시 이들의 억제 가능성에 대해 추가로 테스트하였다. 구체적으로, 선택된 ASO를 1, 5 또는 10 μ M ASO 농도에서 168시간 동안 인간 iPS 심근세포와 함께 배양하였다. MYH7B, MYH7(β -MHC) 및 MYH6(α -MHC) mRNA의 수준은 실시간 PCR을 사용하여 168시간에 측정하였다. 실시간 PCR 분석은 많은 ASO가 MYH7B mRNA 수준을 하향 조절하였다는 것을 나타내었다(도 6a). 또한, SEQ ID NO: 146의 서열을 갖는 안티센스 올리고뉴클레오타이드는 마이오신의 수준에서 "전환"을 나타내었고, 여기서 MYH7B의 억제 후에 β -MHC가 하향 조절되고 α -MHC가 상향 조절된다(도 6b 및 6c). 일부 다른 ASO는 또한 β -및 α -MHC 수준에서 유사한 경향을 나타내었다(도 6b 및 6c).

[0244] SEQ ID NO: 146의 서열을 갖는 또 다른 ASO 화합물을 또한 수동적 섭취시 억제 활성화에 대해 테스트하였다. 인간 iPS 심근세포를 SEQ ID NO: 146의 화합물 5 μ M과 함께 168시간 동안 배양하고, 실시간 PCR을 사용하여 MYH7B, MYH7(β -MHC) 및 MYH6(α -MHC) mRNA의 수준을 측정하였다. 도 7에 도시된 바와 같이, SEQ ID NO: 146을 갖는 화합물은 또한 마이오신의 수준에서 "전환"을 나타내었고, 여기서 β -MHC는 MYH7B의 억제 후에 β -MHC가 하향 조절되고 α -MHC가 상향 조절된다.

[0245] 실시예 6 : 테스트 ASO의 생체내 활성화

[0246] MYH7B의 생체외 억제를 나타내는 ASO를 이들의 생체내 억제 잠재성에 대해 추가로 테스트하였다. 구체적으로, 선택된 ASO를 3일 연속 하루 당 25mg/kg의 용량으로 쥐에 피하 주사하였고, 최종 투여 48시간 후 쥐를 희생시켰다. 쥐의 좌심실에서 MYH7B mRNA의 수준을 실시간 PCR을 사용하여 측정하였다(도 8). 도 8에 도시된 바와 같이, 수동적 투여(예를 들어, SEQ ID NO: 146)를 통해 강력한 활성을 나타낸 안티센스 올리고뉴클레오타이드 화합물은 생체 내에서 MYH7B의 하향 조절을 나타내지만, 수동적 투여(예를 들어, SEQ ID NO: 32)를 통해 활성을 나타내지 않는 화합물 또한 생체내에서 활성을 나타내지 않았다.

[0247] 쥐에서 강력한 활성을 나타낸 SEQ ID NO: 146 및 148의 서열을 갖는 화합물을 β -MHC 우성 종에서의 평가를 위한 토끼 연구에 대해 선택하였다. 토끼에게 1일, 3일, 5일, 10일 및 17일에 SEQ ID NO: 146 및 148을 갖는 화합물 10, 15, 20 또는 25mg/kg을 피하 주사하고 18일에 희생시켰다. MYH7B에 대한 실시간 PCR 데이터는 두 심실 모두에서 MYH7B의 용량-의존성 감소를 나타낸다(도 9a-9b). 또한, MYH7B(5003)의 가장 강력한 억제를 가진 토끼는 β -MHC(MYH7)에서 가장 강력한 감소를 나타내었다. 도 9c 및 9d 참조.

[0248] 실시예 7 : MYH7B ASO의 활성화에 대한 뉴클레오타이드 변형의 영향

[0249] SEQ ID NO: 146의 ASO 화합물을 다양한 위치에서 변형시켜 그의 활성화에 대한 변형의 효과를 평가하였다. 변형은 LNA 뉴클레오타이드를 상응하는 ENA 뉴클레오타이드 및 다른 염기 및 당 변형으로 대체하는 것을 포함한다. 도 10a

는 LNA에 대한 ENA의 치환이 MYH7B를 표적으로 하는 수동적 활성을 유지하였다는 것을 나타낸다. 마찬가지로, 시티딘의 5'-메틸 시티딘에 의한 치환 및 LNA 염기의 2'-O-메틸 변형 뉴클레오타이드에 의한 치환은 활성을 보유한 다(도 10b). 도 10c는 주쇄 변형이 화합물의 수동적 활성에 크게 영향을 준다는 것을 나타낸다.

[0250] 실시예 8: 추가 ASO의 스크리닝

[0251] 추가의 안티센스 올리고뉴클레오타이드 화합물을 수동적 투여시 이들의 억제 가능성에 대해 스크리닝하였다. 인간 iPS 심근세포를 48시간 동안 테스트 화합물 5 μ M로 수동적으로 치료하였다. MYH7B mRNA의 수준은 실시간 PCR을 사용하여 48시간에 측정하였다. 다수의 ASO는 MYH7B 수준의 강력한 억제를 나타내었지만 다른 화합물은 MYH7B 수준에 영향을 나타내지 않았다. 도 11a 내지도 11d는 4개의 개별적인 연구 결과를 나타낸다. 특히, 다음 ASO 화합물을 테스트하였다.

표 7

[0252]

CPD #	SEQ ID NO:
Comp-1	SEQ ID NO: 184
Comp-2	SEQ ID NO: 186
Comp-3	SEQ ID NO: 188
Comp-4	SEQ ID NO: 190
Comp-5	SEQ ID NO: 192
Comp-6	SEQ ID NO: 194
Comp-7	SEQ ID NO: 196
Comp-8	SEQ ID NO: 198
Comp-9	SEQ ID NO: 200
Comp-10	SEQ ID NO: 202
Comp-11	SEQ ID NO: 204
Comp-12	SEQ ID NO: 206
Comp-13	SEQ ID NO: 208
Comp-14	SEQ ID NO: 210
Comp-15	SEQ ID NO: 212
Comp-16	SEQ ID NO: 214
Comp-17	SEQ ID NO: 216
Comp-18	SEQ ID NO: 218
Comp-19	SEQ ID NO: 220
Comp-20	SEQ ID NO: 222
Comp-21	SEQ ID NO: 224
Comp-22	SEQ ID NO: 226
Comp-23	SEQ ID NO: 228
Comp-24	SEQ ID NO: 230
Comp-25	SEQ ID NO: 232
Comp-26	SEQ ID NO: 234
Comp-27	SEQ ID NO: 236
Comp-28	SEQ ID NO: 238
Comp-29	SEQ ID NO: 240
Comp-30	SEQ ID NO: 242
Comp-31	SEQ ID NO: 244
Comp-32	SEQ ID NO: 246
Comp-33	SEQ ID NO: 248
Comp-34	SEQ ID NO: 250
Comp-35	SEQ ID NO: 252
Comp-36	SEQ ID NO: 254
Comp-37	SEQ ID NO: 256
Comp-38	SEQ ID NO: 258
Comp-39	SEQ ID NO: 260
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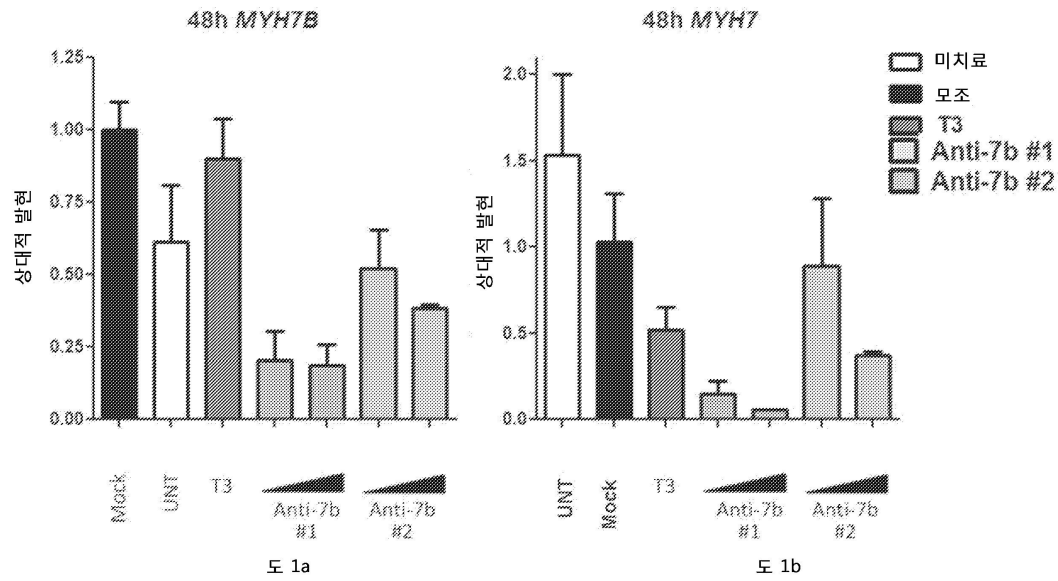
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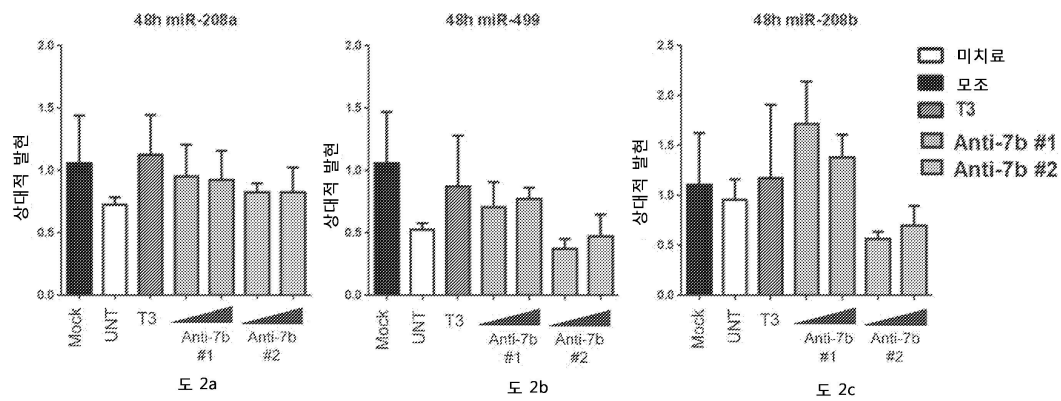
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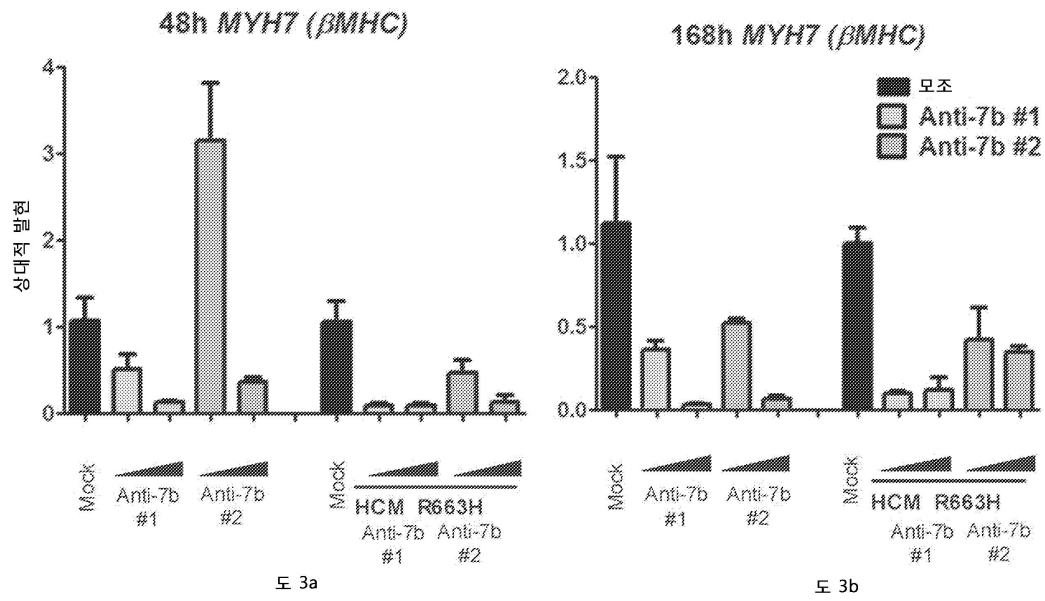
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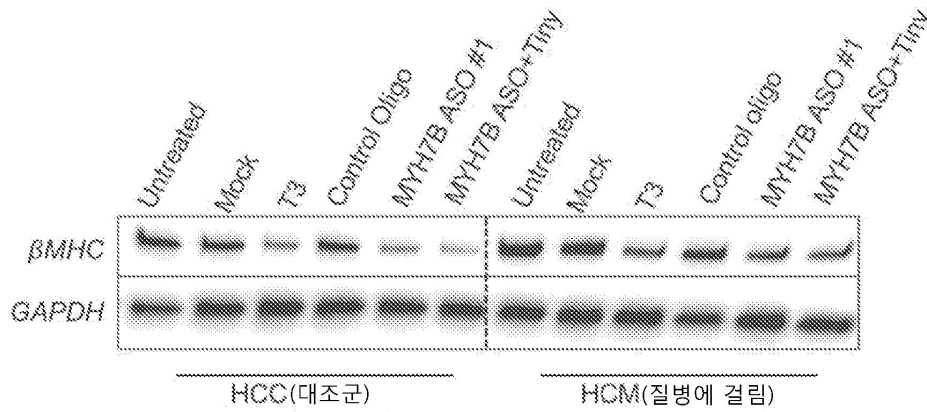
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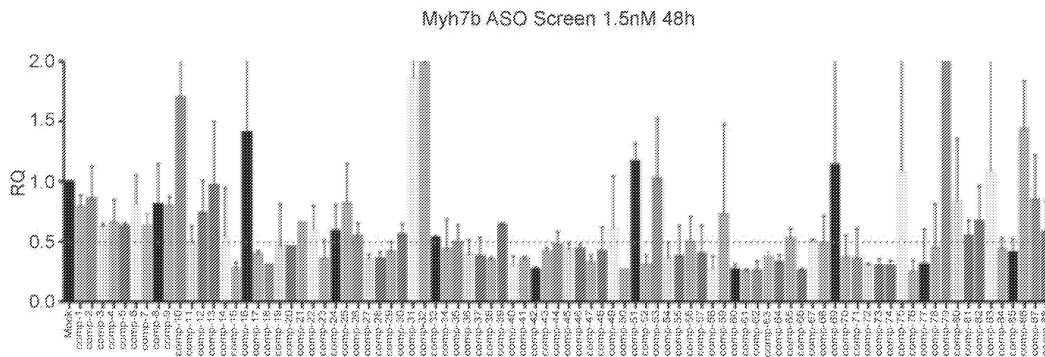
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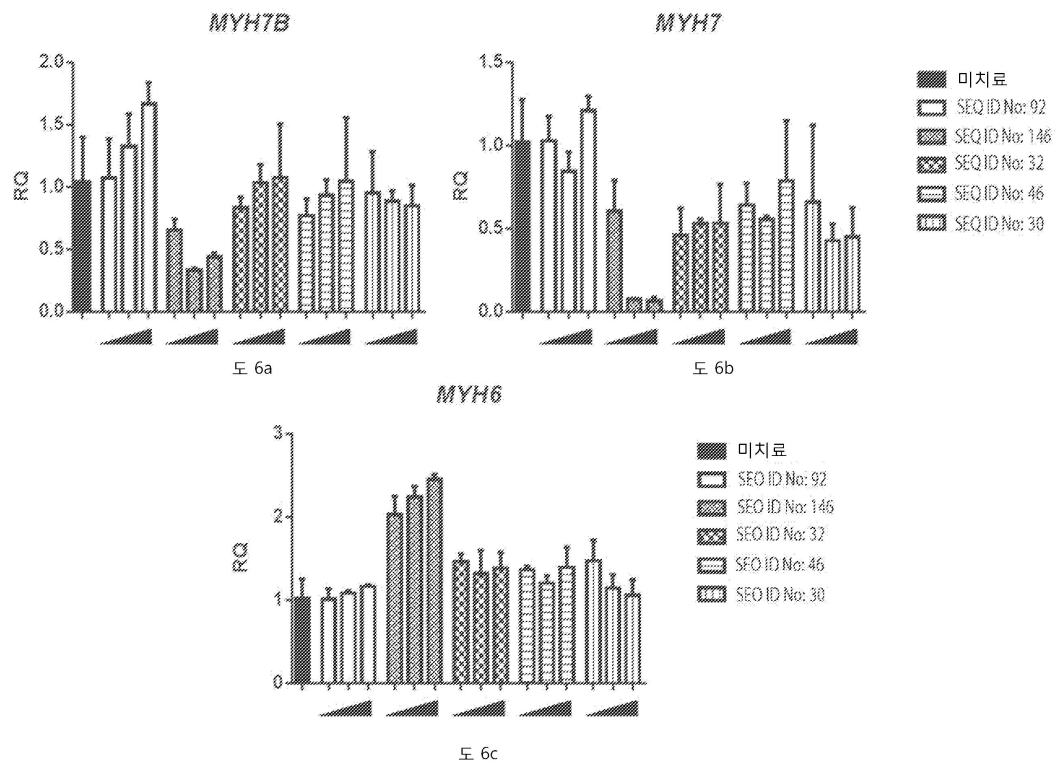
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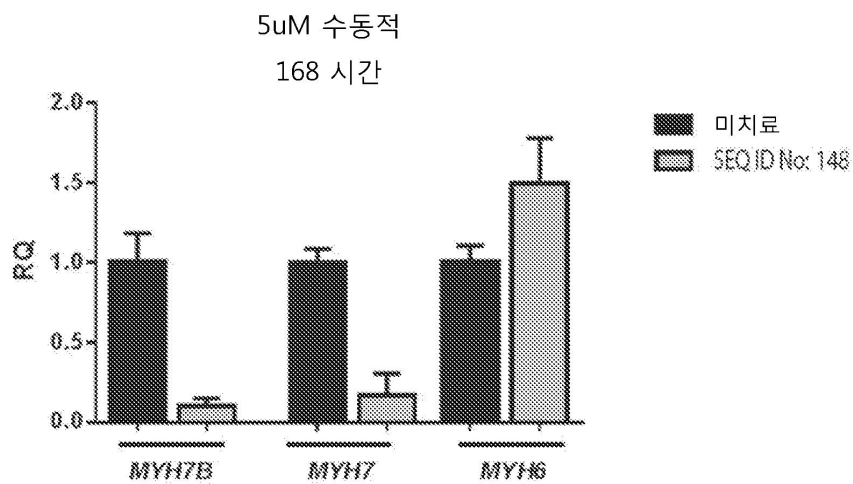
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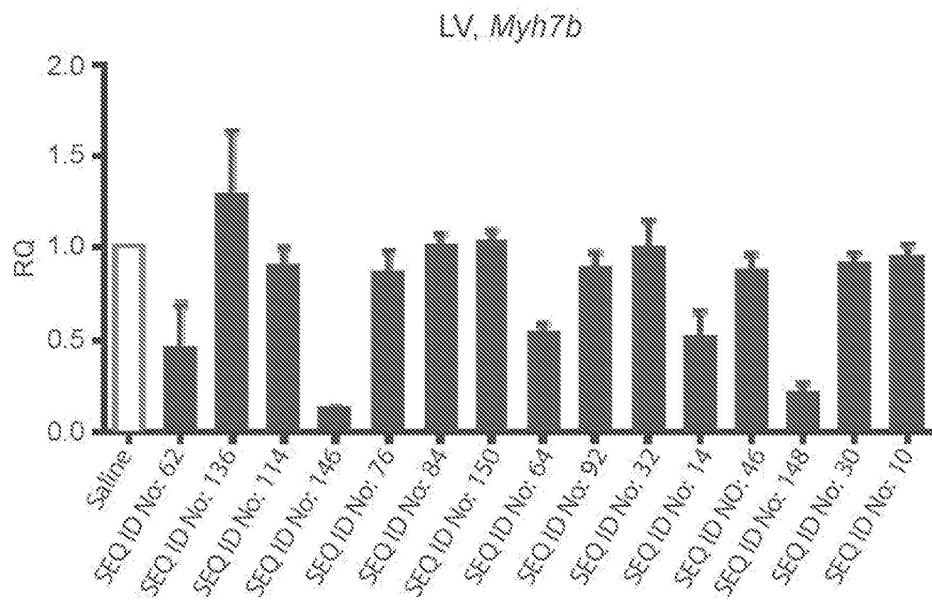
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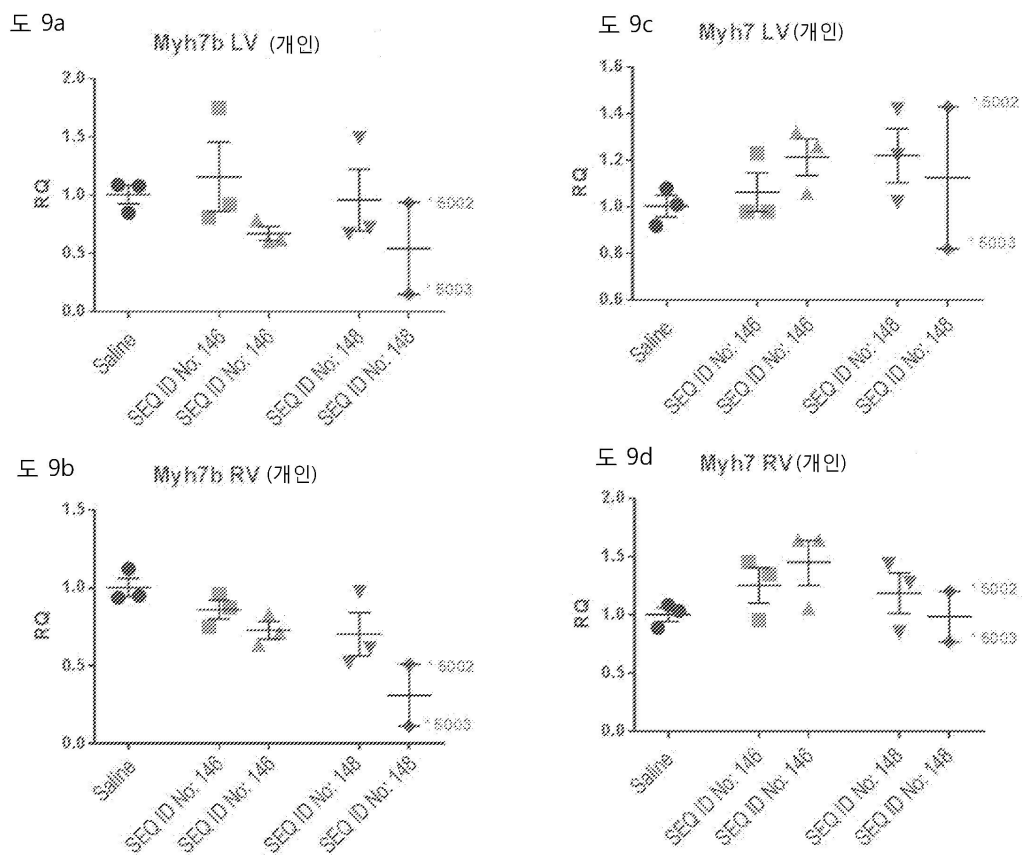
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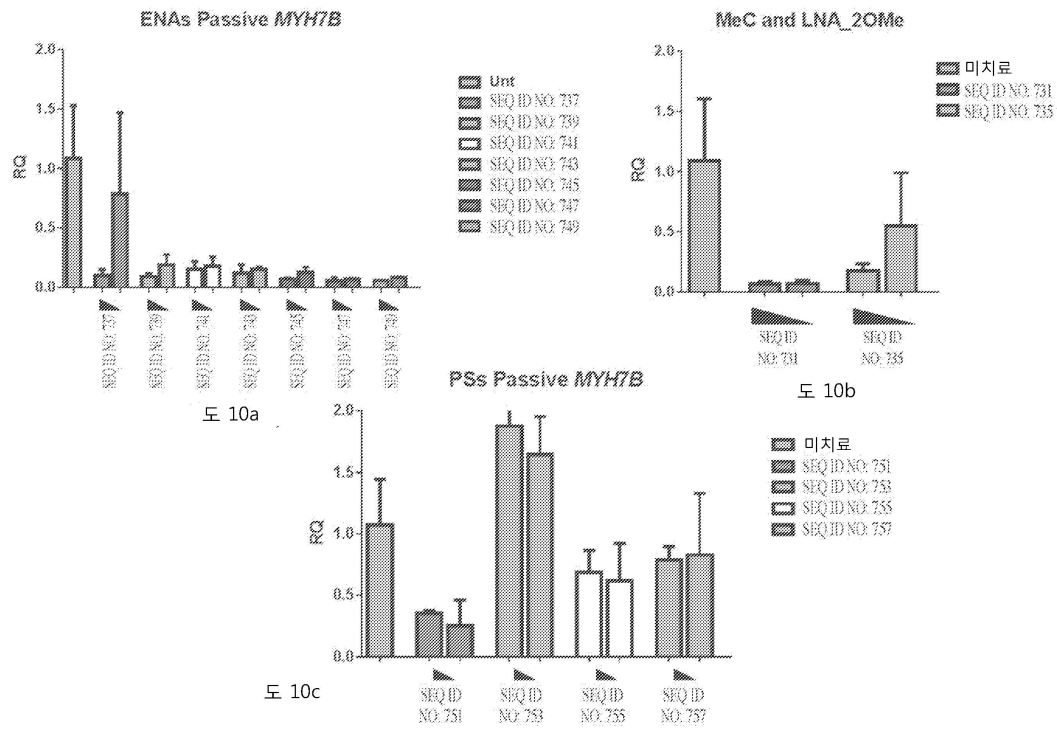
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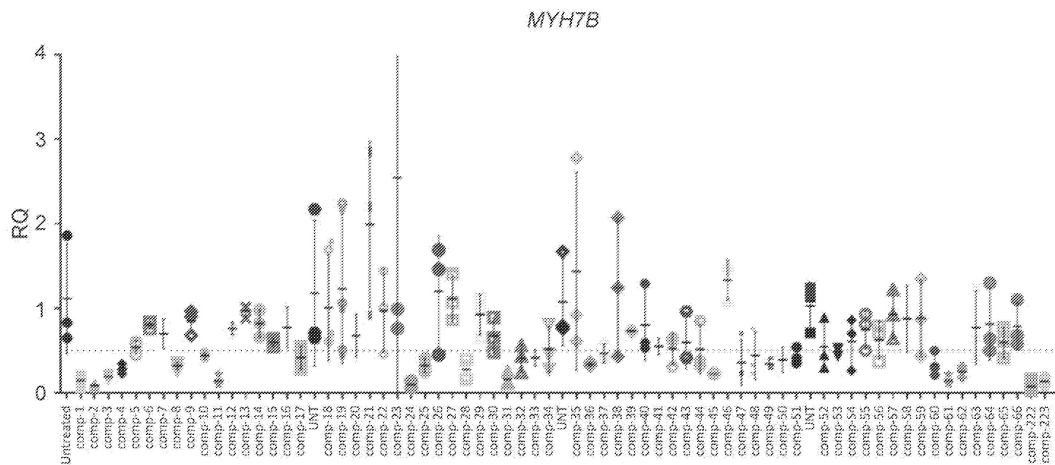
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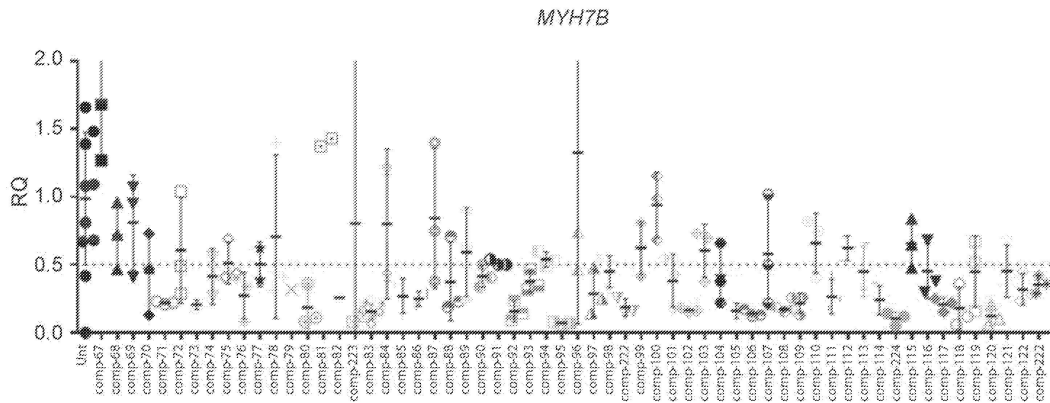
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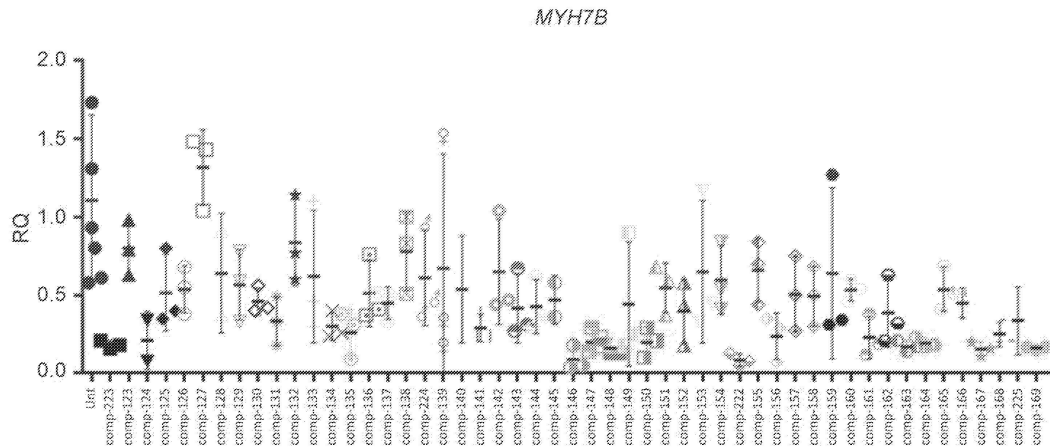
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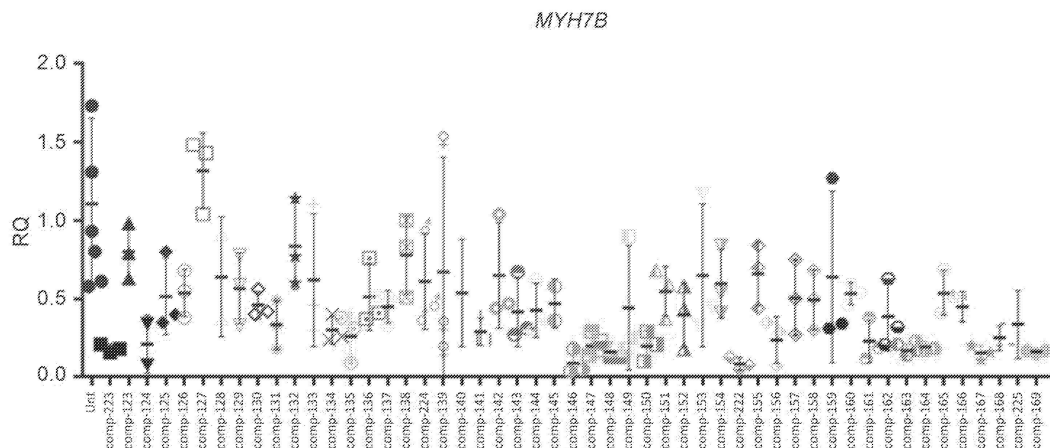
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Dalby, Christina

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<220><221> MOD_RES

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agutcactca catc

<210> 775

<211> 14

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<220><221> MOD_RES

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aaagtgcagg aggg

14

<210> 776

<211> 14

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<210> 777

<211> 14

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gtucactcac aucc 14

<210> 778

<211> 14

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<400> 778

ttcactcaca tcca

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<210> 779

<211> 14

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ggutcataga caga

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atucctcagg guct

14

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tcactcacat ccat

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<210> 782

<211> 14

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1

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10

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