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Inoue et al.

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(45) **Date of Patent: Feb. 9, 2016**

(54) PROTEIN-RESPONSIVE TRANSLATIONAL REGULATORY SYSTEM USING RNA-PROTEIN INTERACTING MOTIF

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(73) Assignee: **Japan Science and Technology Agency**, Saitama (JP)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 633 days.

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§ 371 (c)(1),
(2), (4) Date: **Jul. 21, 2010**

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PCT Pub. Date: **May 28, 2009**

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(30) **Foreign Application Priority Data**

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Jul. 17, 2008 (JP) 2008-186385

(51) **Int. Cl.**
C07H 21/04 (2006.01)
C12P 21/06 (2006.01)
C12N 15/67 (2006.01)

(52) **U.S. Cl.**
CPC ***C12N 15/67*** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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(Continued)

Primary Examiner — Catherine S Hibbert
(74) *Attorney, Agent, or Firm* — Meunier Carlin & Curfman LLC

(57) ABSTRACT

An object of the present invention is to provide a translationally regulatable mRNA which has wider application and can perform specific ON-OFF regulation, an RNA-protein complex specifically bound to the mRNA, and a translational regulatory system. The present invention provides an mRNA having an RNA-protein complex interacting motif-derived nucleotide sequence 5' to the ribosome-binding site or within the 5' region of the open reading frame, and an mRNA having a nucleotide sequence complementary to an RNA-protein complex interacting motif-derived nucleotide sequence 5' to the ribosome-binding site or within the 5' region of the open reading frame.

8 Claims, 21 Drawing Sheets

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FIG.1(A)

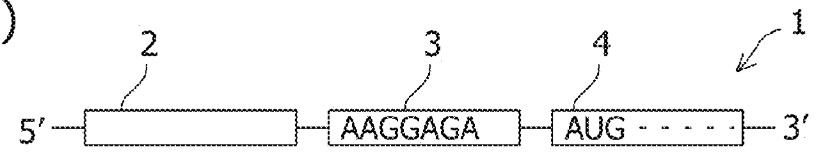


FIG.1(B)

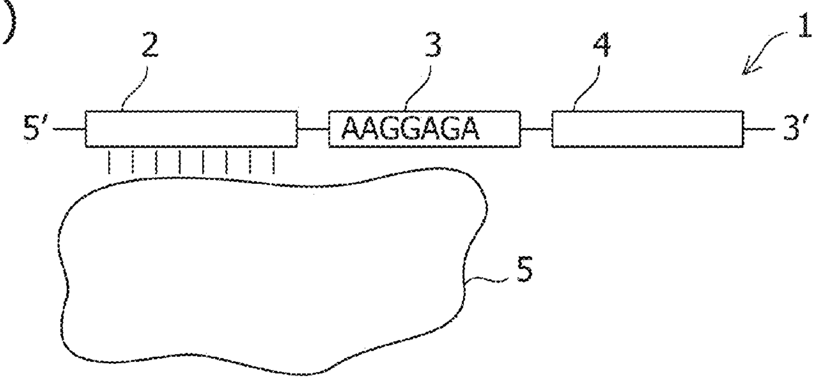


FIG.2

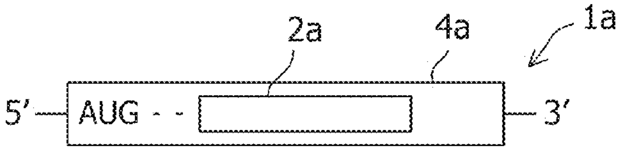


FIG.3(A)

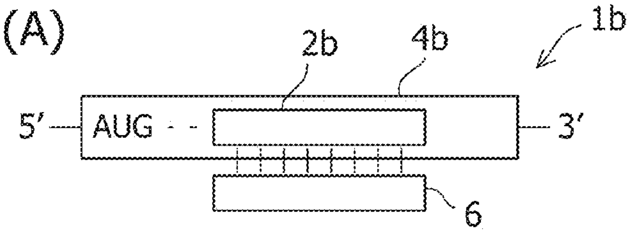


FIG.3(B)

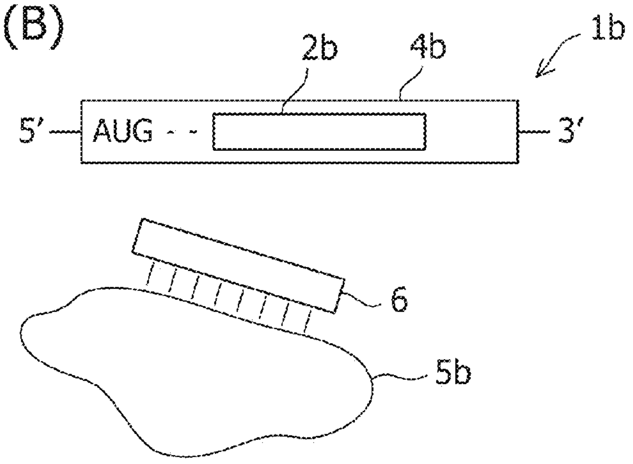


FIG.4

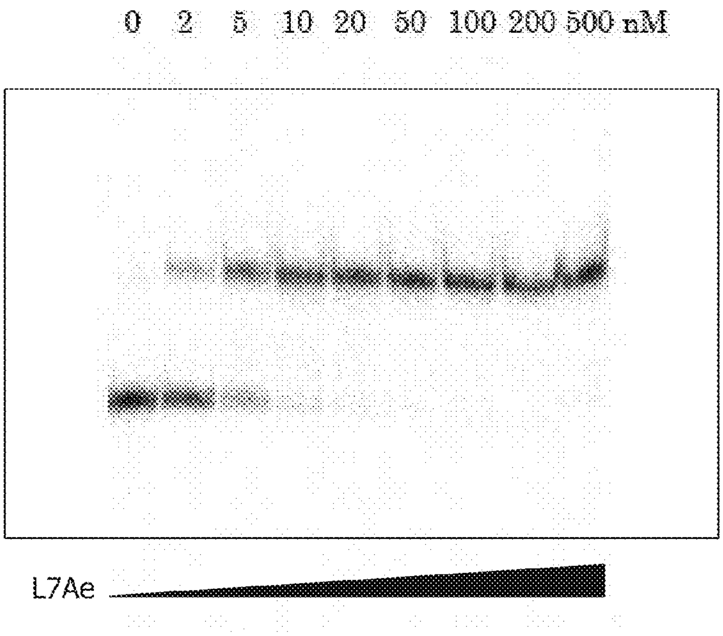

0 2 5 10 20 50 100 200 500 nM
L7Ae

FIG.5

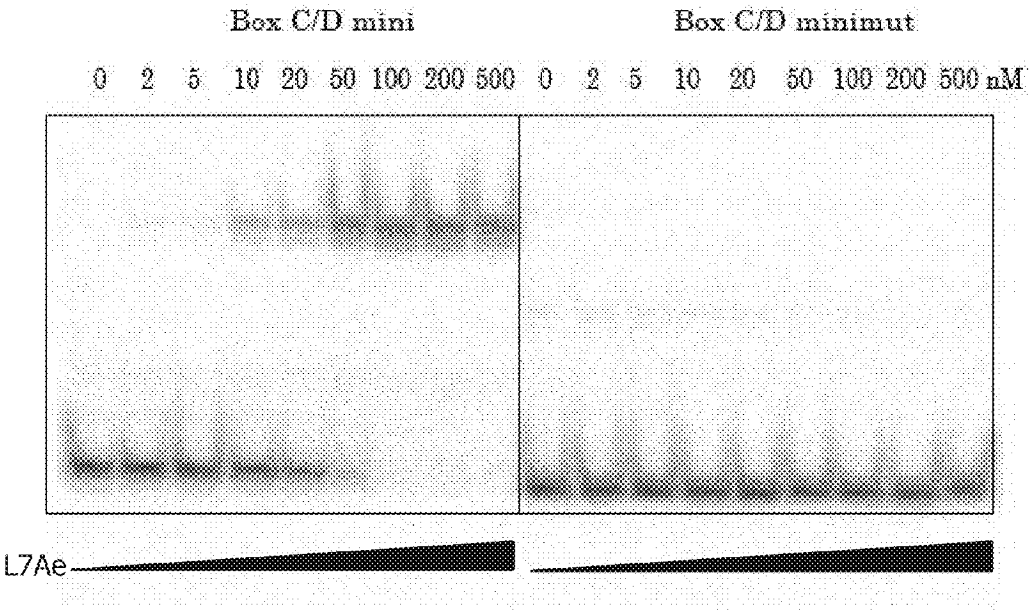

Box C/D mini
Box C/D minimut
0 2 5 10 20 50 100 200 500 0 2 5 10 20 50 100 200 500 nM
L7Ae

FIG.6

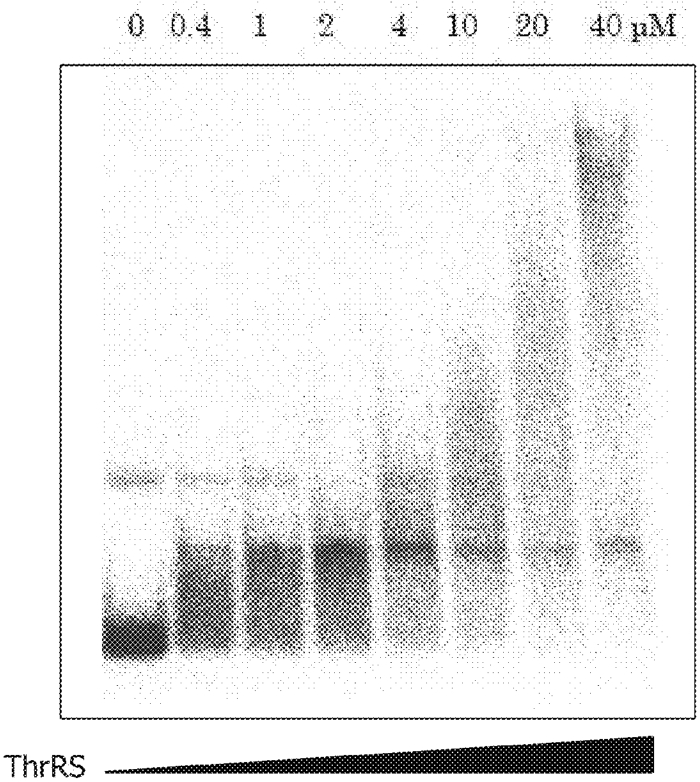


FIG.7

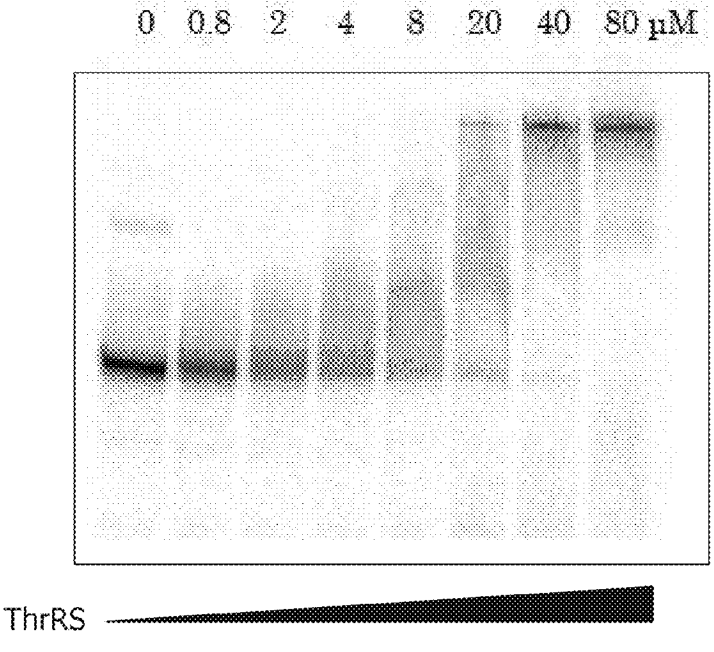


FIG.8A

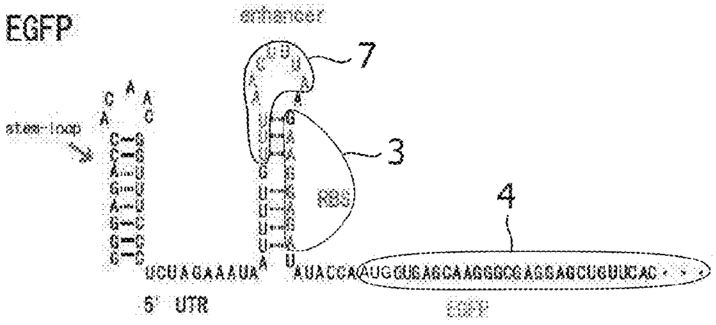


FIG.8B

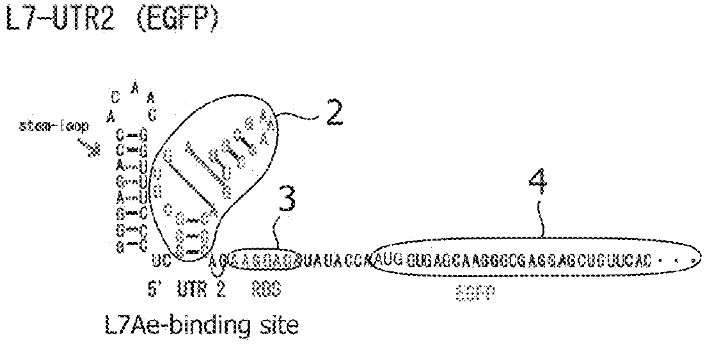


FIG.8C

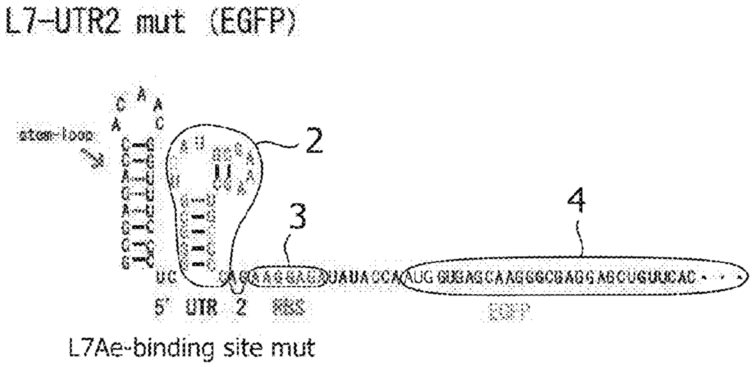


FIG.8D

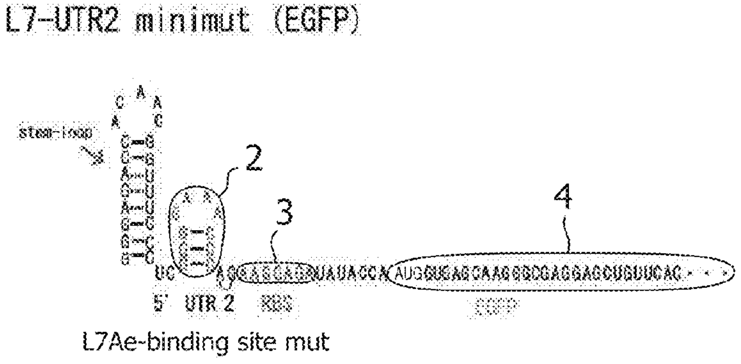


FIG.8E

L7-UTR5 (EGFP)

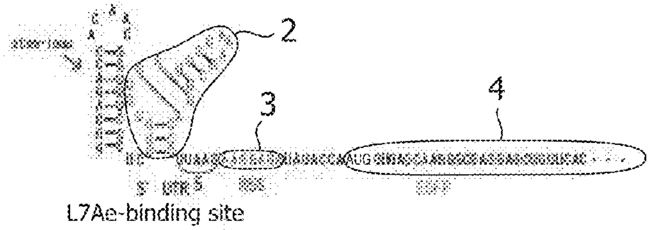


FIG.8F

L7-UTR9 (EGFP)

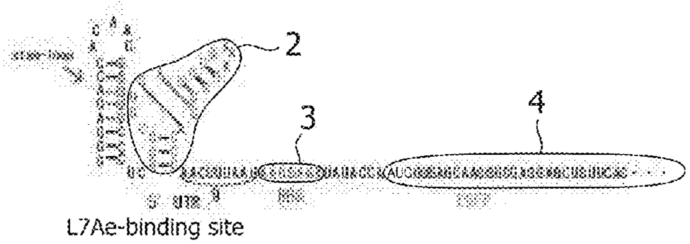


FIG.8G

L7-UTR13 (EGFP)

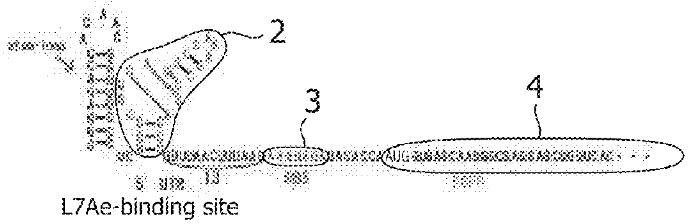


FIG.9

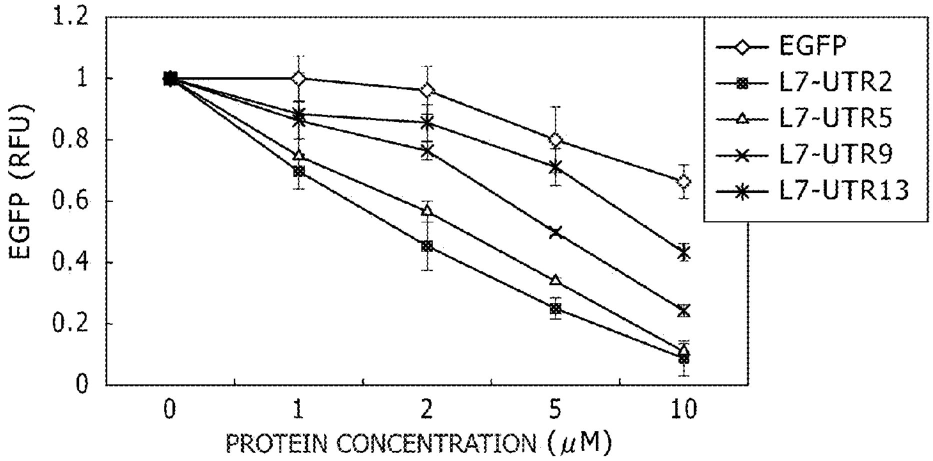


FIG.10

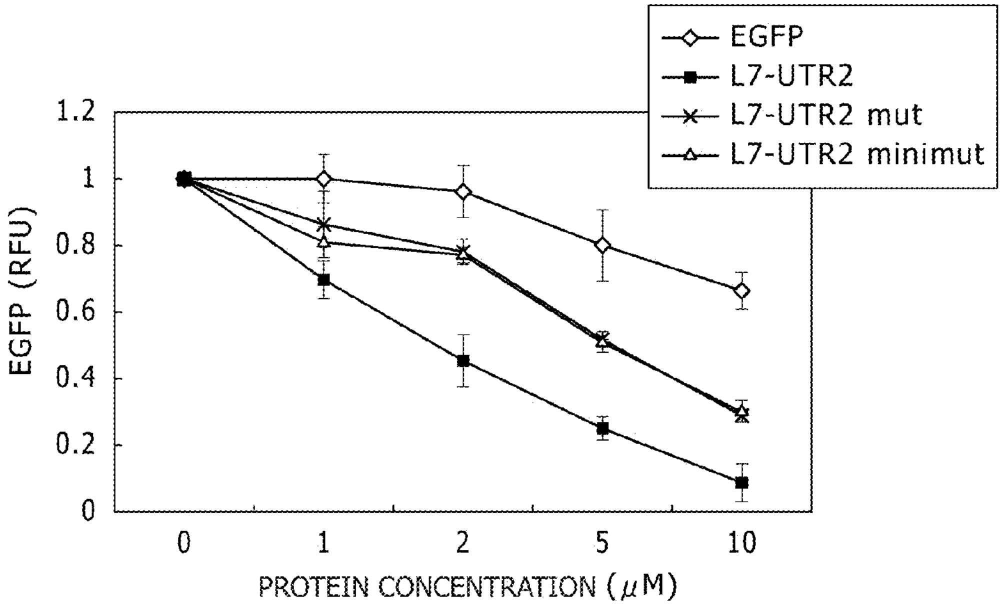
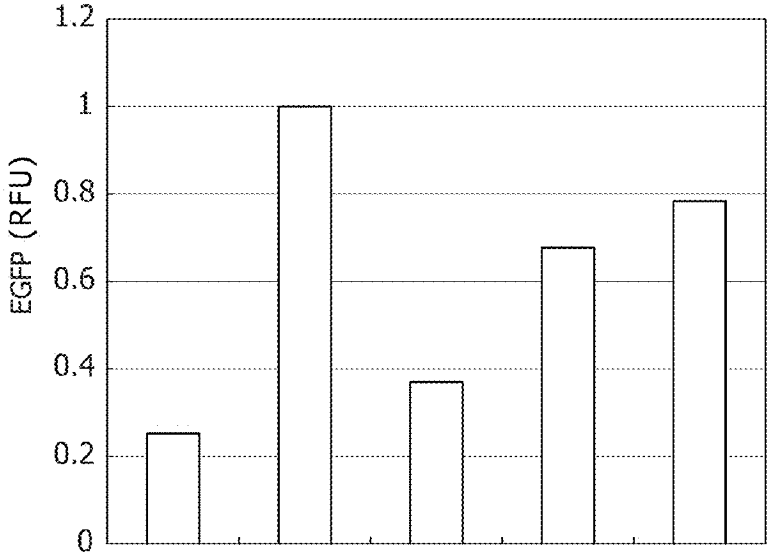


FIG.11



5 μ ML7Ae	+	-	+	+	+
COMPETITOR CONCENTRATION			1	5	10

FIG.12(A)

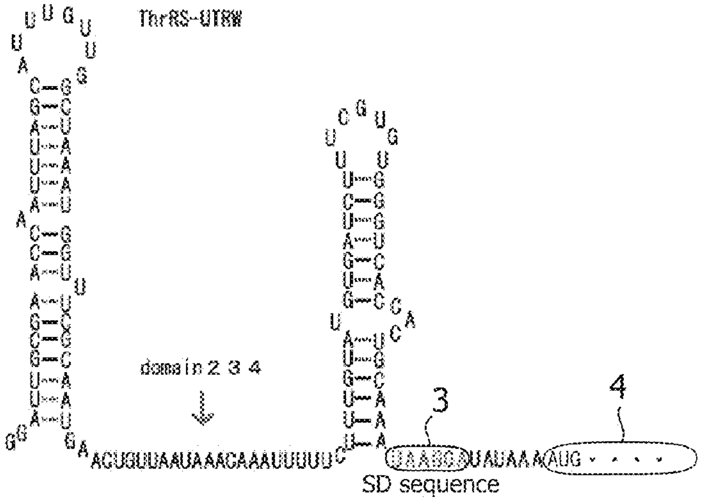


FIG.12(B)

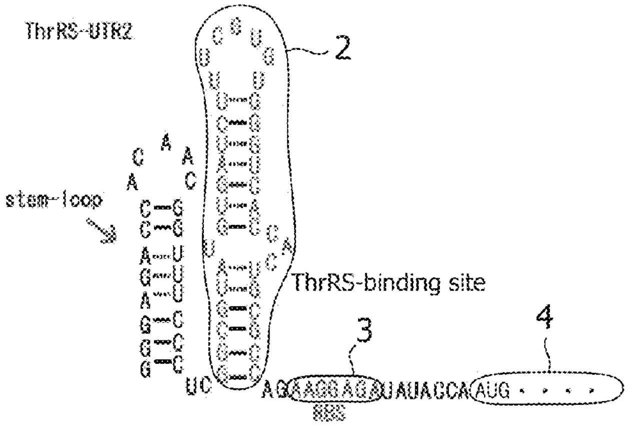


FIG.12(C)

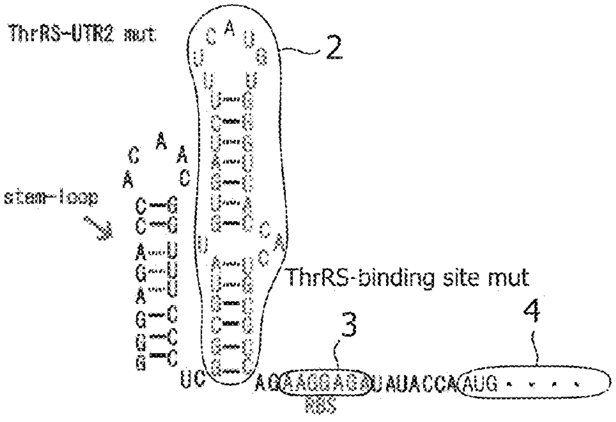


FIG.13

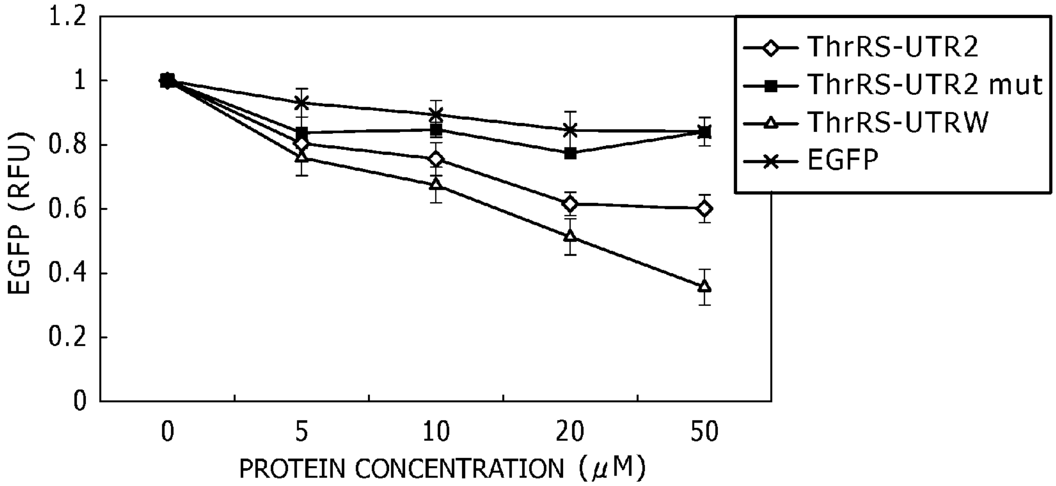


FIG.14(A)

L7-ORF (EGFP)

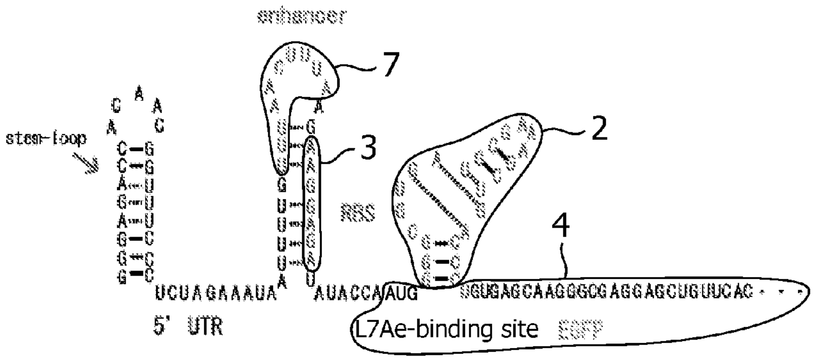


FIG.14(B)

L7-ORF mut (EGFP)

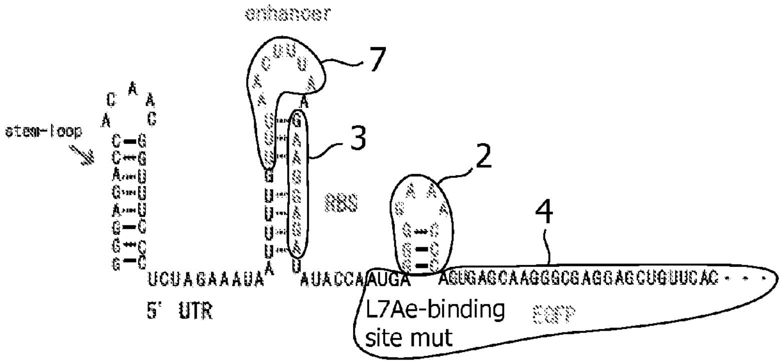


FIG.15

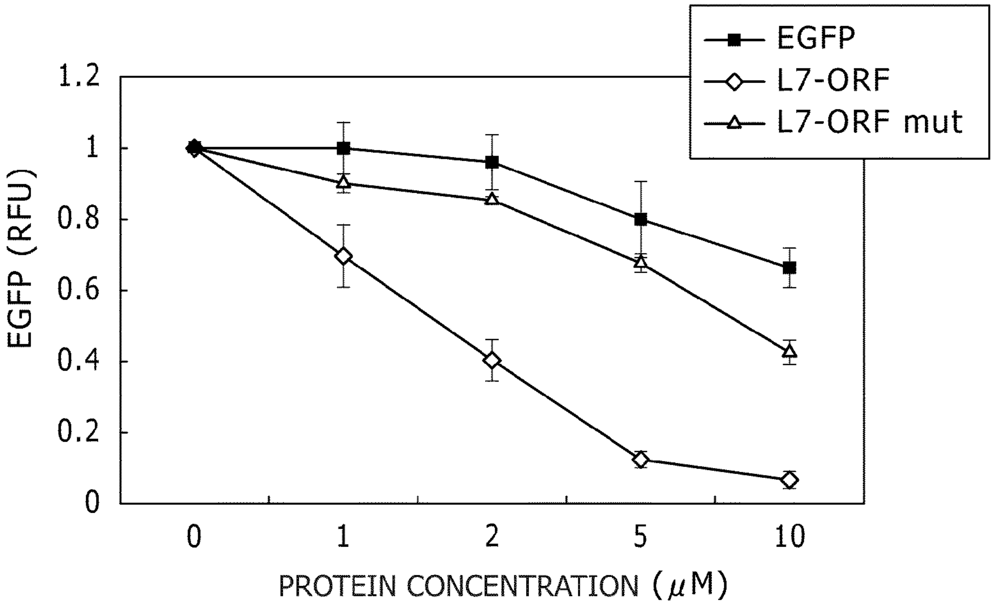

EGFP
L7-ORF
L7-ORF mut
1.2
1
0.8
0.6
0.4
0.2
0
EGFP (RFU)
0
1
2
5
10
PROTEIN CONCENTRATION (μM)

FIG.16

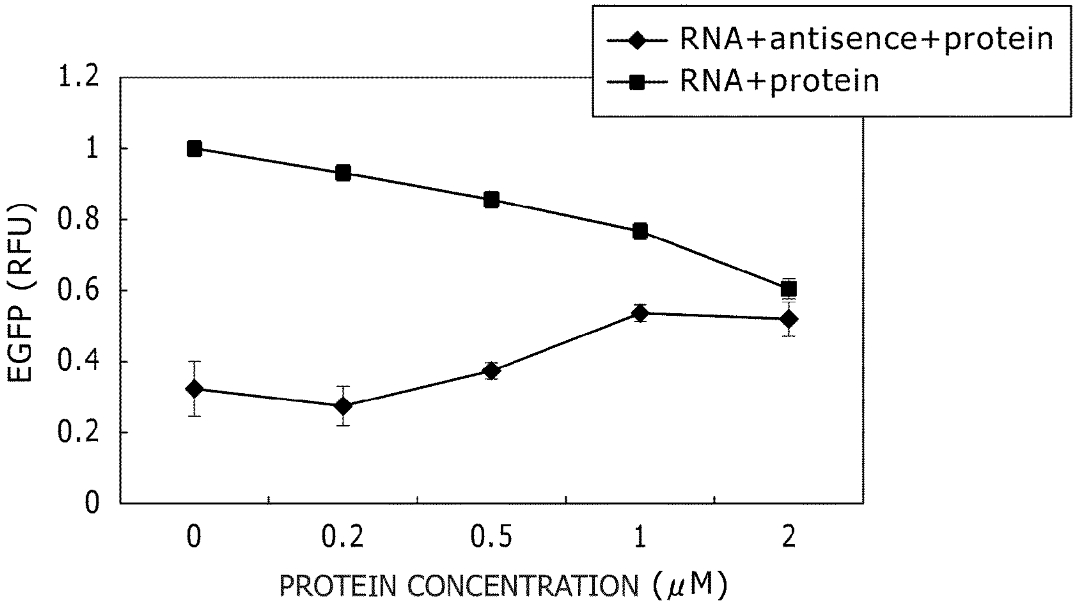

RNA+antisence+protein
RNA+protein
1.2
1
0.8
0.6
0.4
0.2
0
EGFP (RFU)
0
0.2
0.5
1
2
PROTEIN CONCENTRATION (μM)

FIG.17(a)

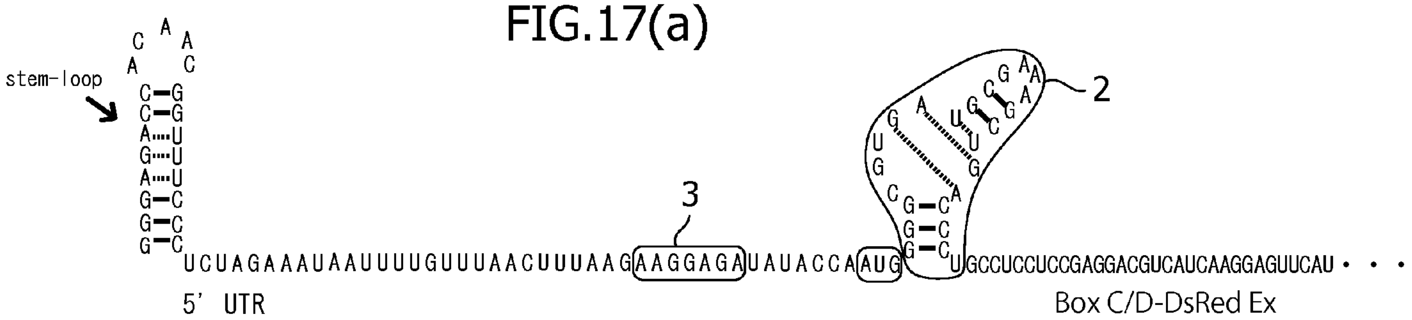


FIG.17(b)

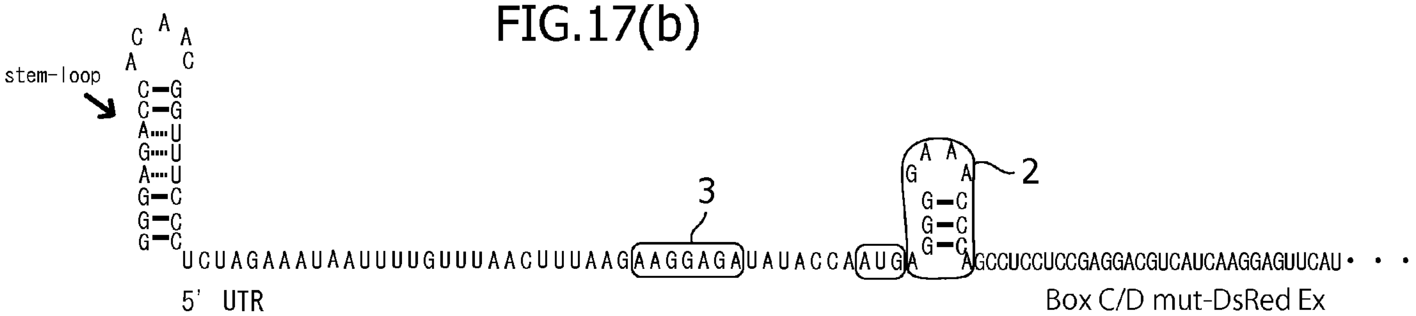


FIG.17(c)

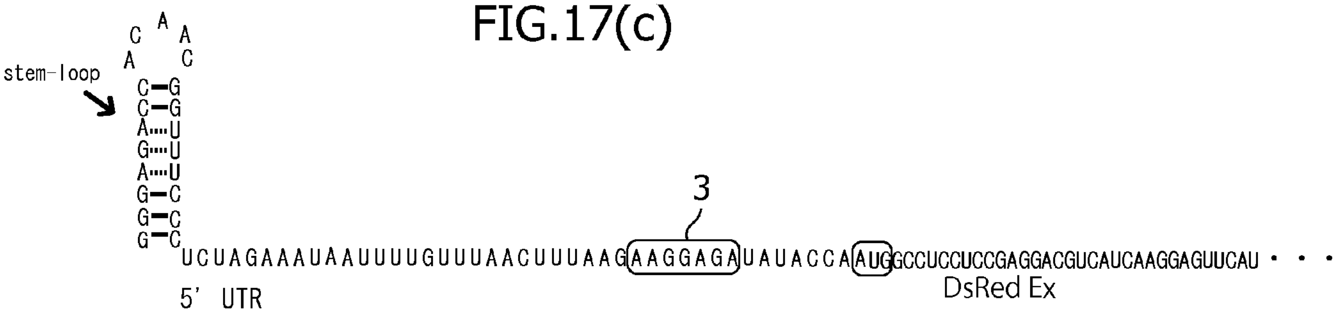


FIG.18

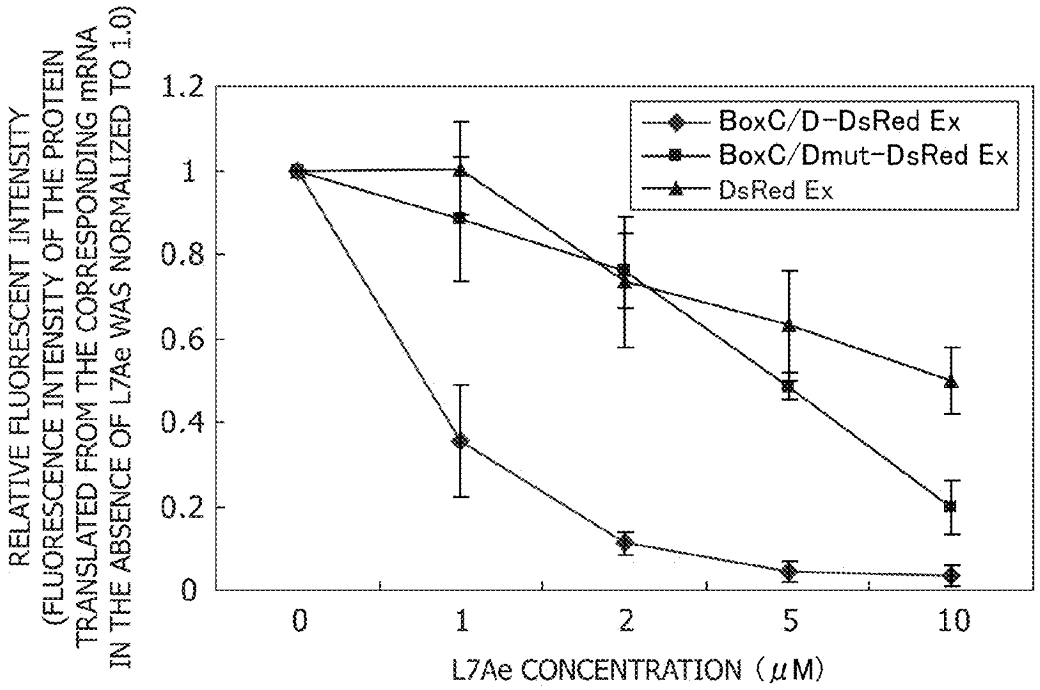

RELATIVE FLUORESCENT INTENSITY
(FLUORESCENCE INTENSITY OF THE PROTEIN
TRANSLATED FROM THE CORRESPONDING mRNA
IN THE ABSENCE OF L7Ae WAS NORMALIZED TO 1.0)
1.2
1
0.8
0.6
0.4
0.2
0
BoxC/D-DsRed Ex
BoxC/Dmut-DsRed Ex
DsRed Ex
0
1
2
5
10
L7Ae CONCENTRATION (μM)

FIG.19

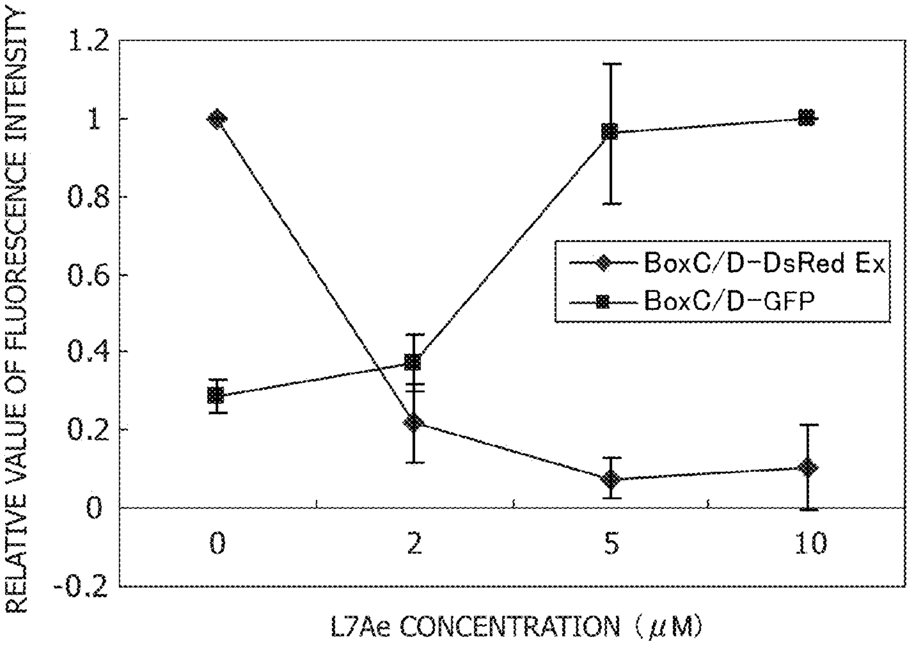

RELATIVE VALUE OF FLUORESCENCE INTENSITY
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
BoxC/D-DsRed Ex
BoxC/D-GFP
0
2
5
10
L7Ae CONCENTRATION (μM)

FIG.20

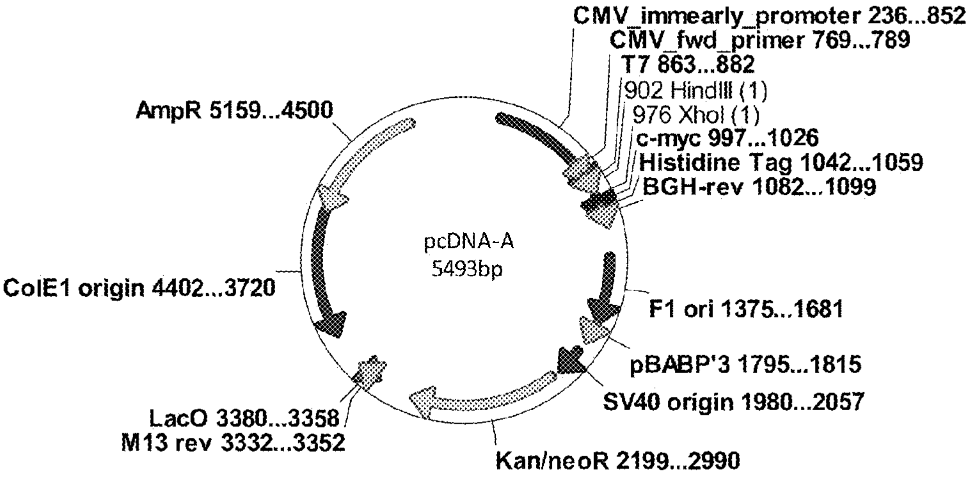

CMV_immearly_promoter 236...852
CMV_fwd_primer 769...789
T7 863...882
902 HindIII (1)
976 XhoI (1)
c-myc 997...1026
Histidine Tag 1042...1059
BGH-rev 1082...1099
AmpR 5159...4500
pcDNA-A
5493bp
ColE1 origin 4402...3720
F1 ori 1375...1681
pBABP'3 1795...1815
SV40 origin 1980...2057
LacO 3380...3358
M13 rev 3332...3352
Kan/neoR 2199...2990

FIG.21

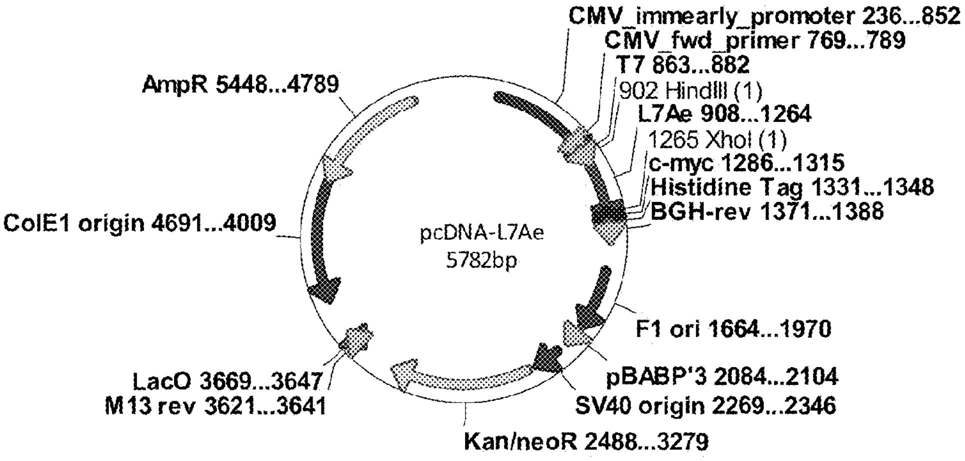

CMV_immearly_promoter 236...852
CMV_fwd_primer 769...789
T7 863...882
902 HindIII (1)
L7Ae 908...1264
1265 XhoI (1)
c-myc 1286...1315
Histidine Tag 1331...1348
BGH-rev 1371...1388
AmpR 5448...4789
ColE1 origin 4691...4009
pcDNA-L7Ae
5782bp
F1 ori 1664...1970
pBABP'3 2084...2104
SV40 origin 2269...2346
LacO 3669...3647
M13 rev 3621...3641
Kan/neoR 2488...3279

FIG.22

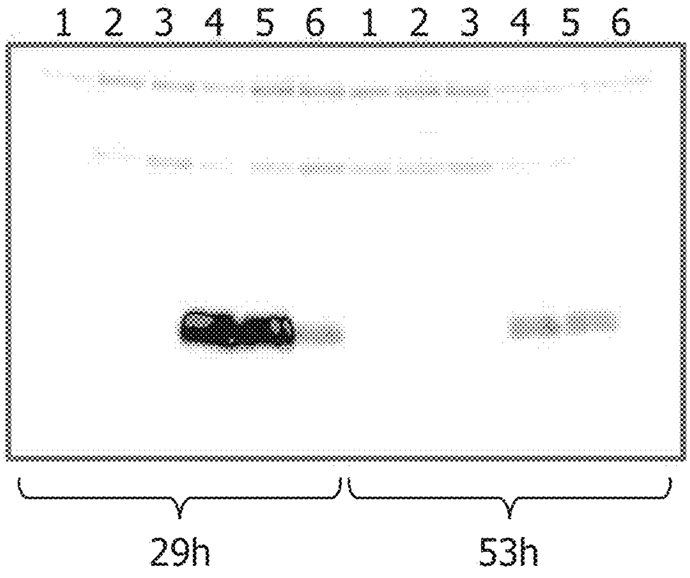

1 2 3 4 5 6 1 2 3 4 5 6
29h
53h

FIG.23

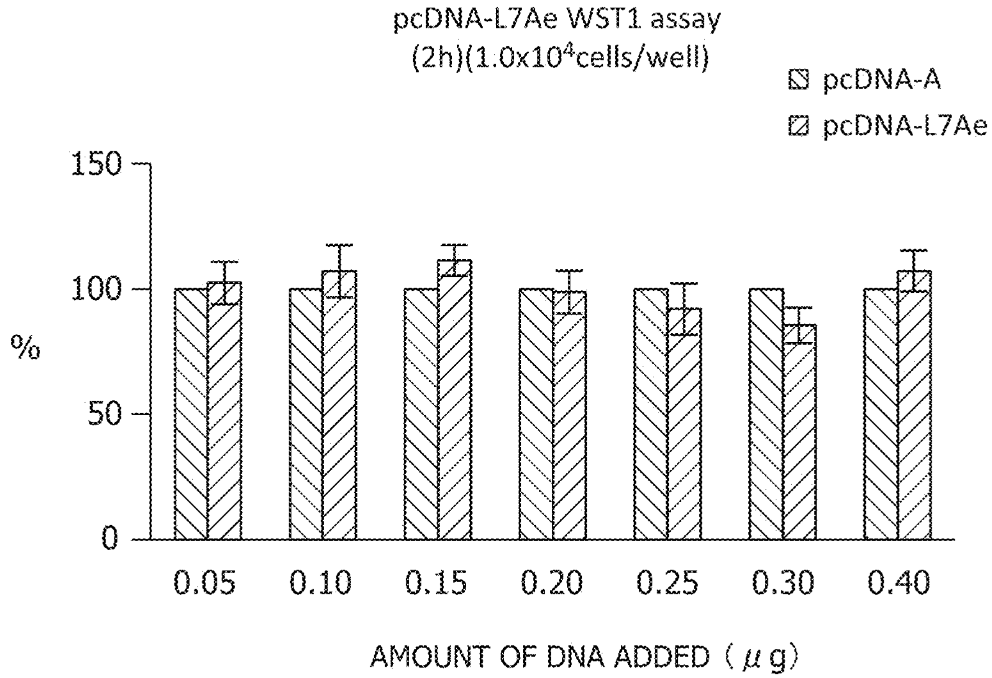

pcDNA-L7Ae WST1 assay
(2h)(1.0x10⁴cells/well)
▧ pcDNA-A
▨ pcDNA-L7Ae
150
100
50
0
%
0.05
0.10
0.15
0.20
0.25
0.30
0.40
AMOUNT OF DNA ADDED（μg）

FIG.24

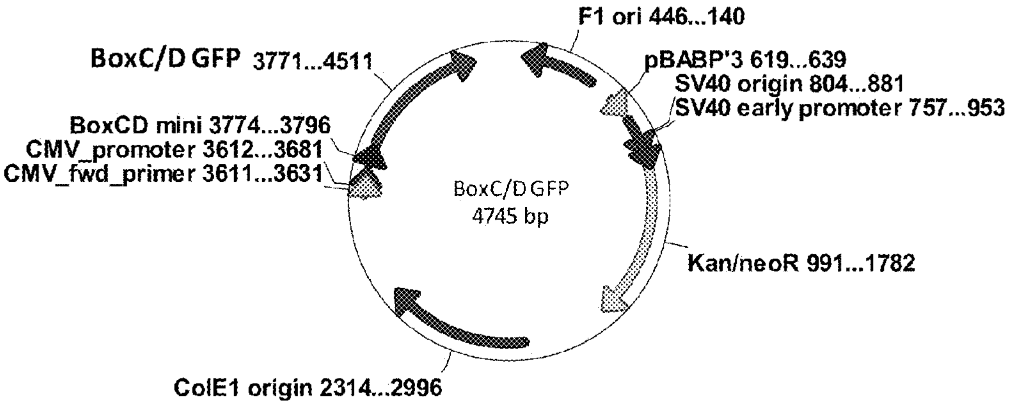


FIG.25

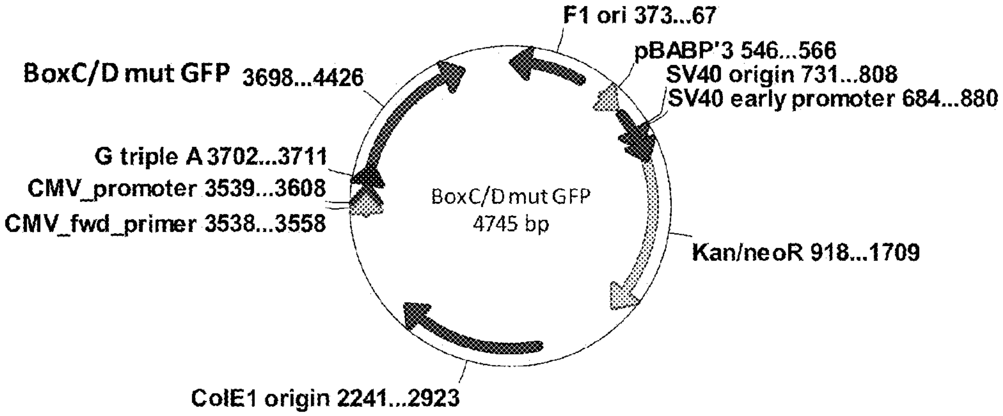


FIG.26

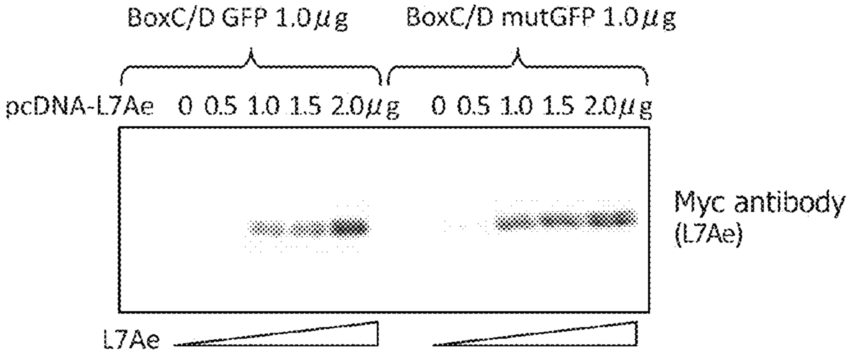


FIG.27

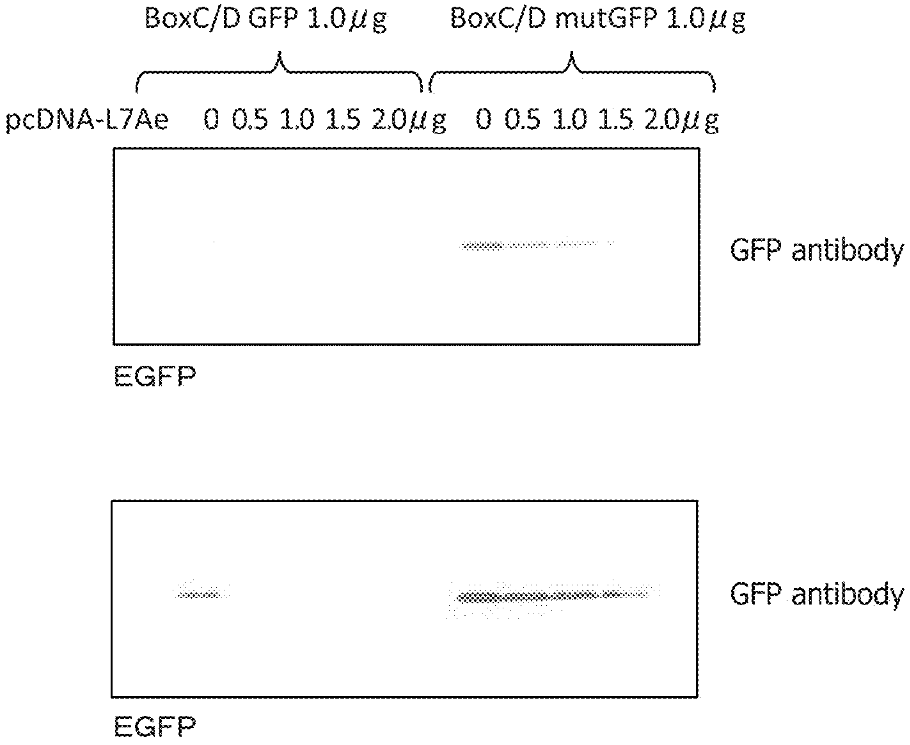

BoxC/D GFP 1.0μg
BoxC/D mutGFP 1.0μg
pcDNA-L7Ae 0 0.5 1.0 1.5 2.0μg 0 0.5 1.0 1.5 2.0μg
GFP antibody
EGFP
GFP antibody
EGFP

FIG.28

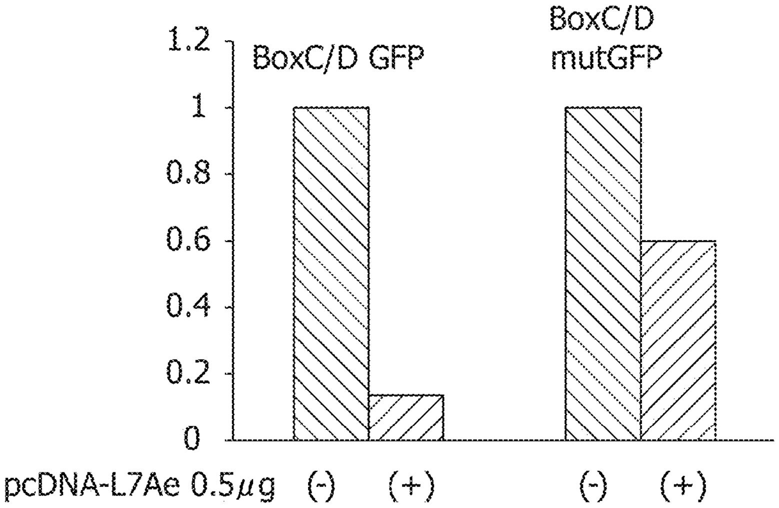

1.2
1
0.8
0.6
0.4
0.2
0
BoxC/D GFP
BoxC/D mutGFP
pcDNA-L7Ae 0.5μg (-) (+) (-) (+)

FIG.29

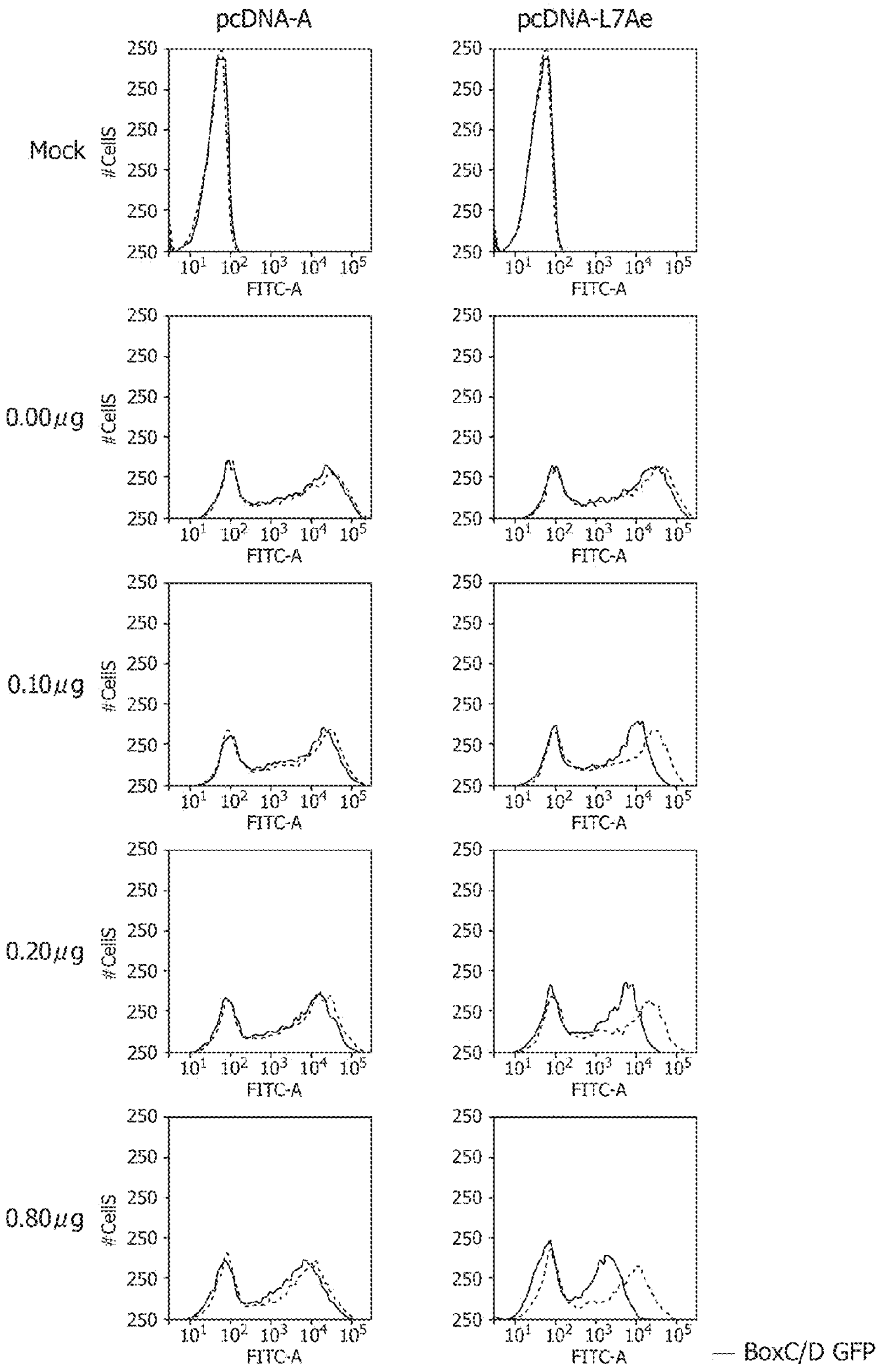

pcDNA-A
pcDNA-L7Ae
Mock
0.00μg
0.10μg
0.20μg
0.80μg
#CellS
250
FITC-A
10^1 10^2 10^3 10^4 10^5
— BoxC/D GFP
··· BoxC/D mut GFP

FIG.30

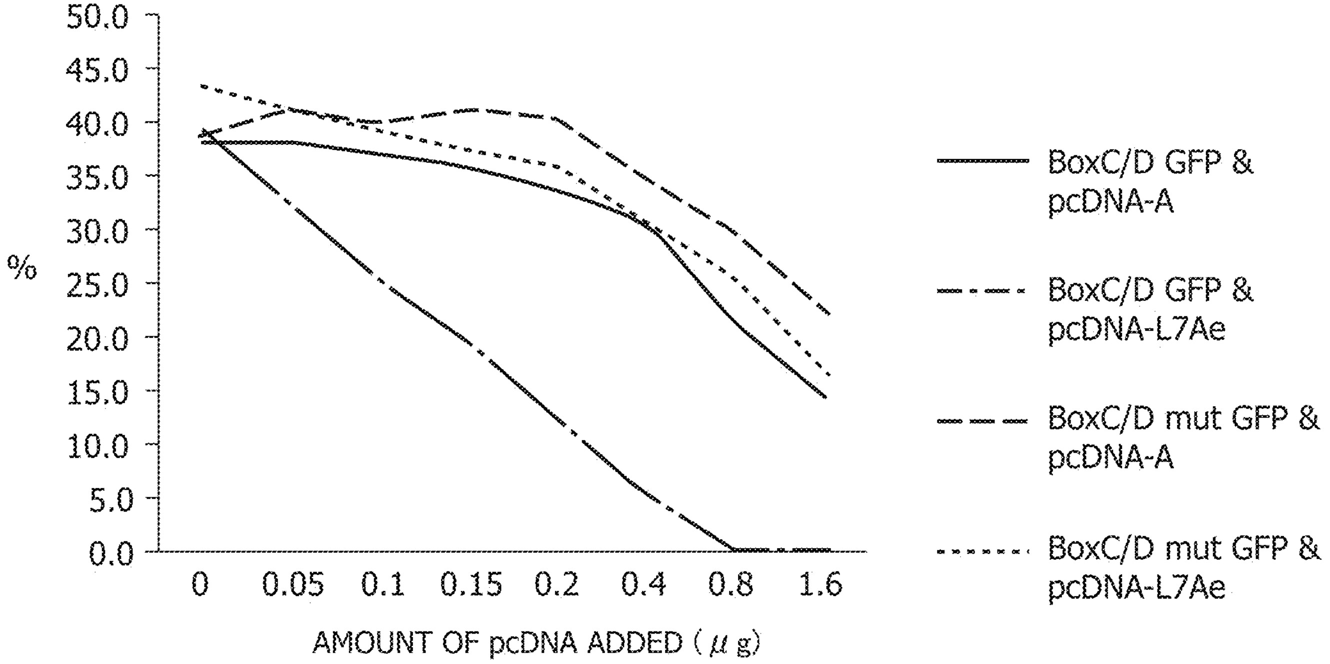

50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
%
0
0.05
0.1
0.15
0.2
0.4
0.8
1.6
AMOUNT OF pcDNA ADDED (μg)
BoxC/D GFP & pcDNA-A
BoxC/D GFP & pcDNA-L7Ae
BoxC/D mut GFP & pcDNA-A
BoxC/D mut GFP & pcDNA-L7Ae

FIG.31

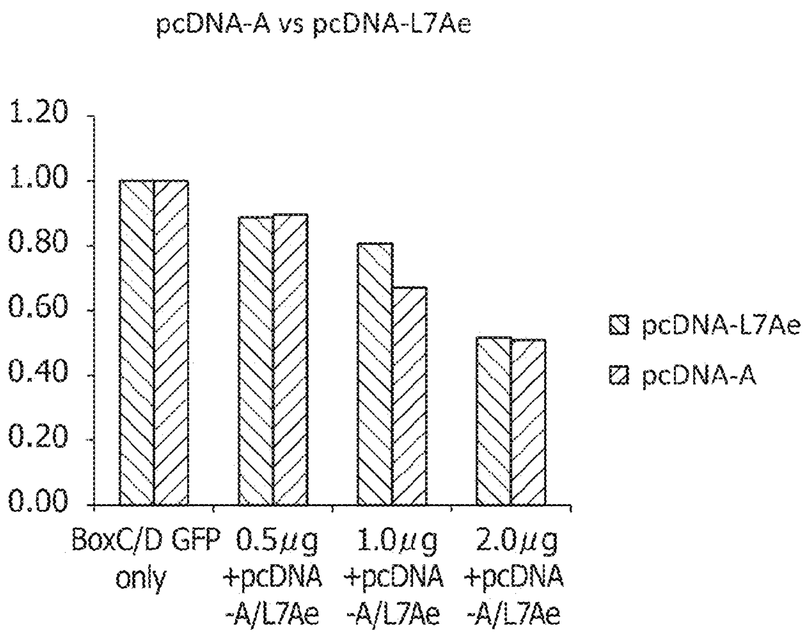

pcDNA-A vs pcDNA-L7Ae
1.20
1.00
0.80
0.60
0.40
0.20
0.00
BoxC/D GFP only
0.5µg +pcDNA -A/L7Ae
1.0µg +pcDNA -A/L7Ae
2.0µg +pcDNA -A/L7Ae
▧ pcDNA-L7Ae
▨ pcDNA-A

FIG.32

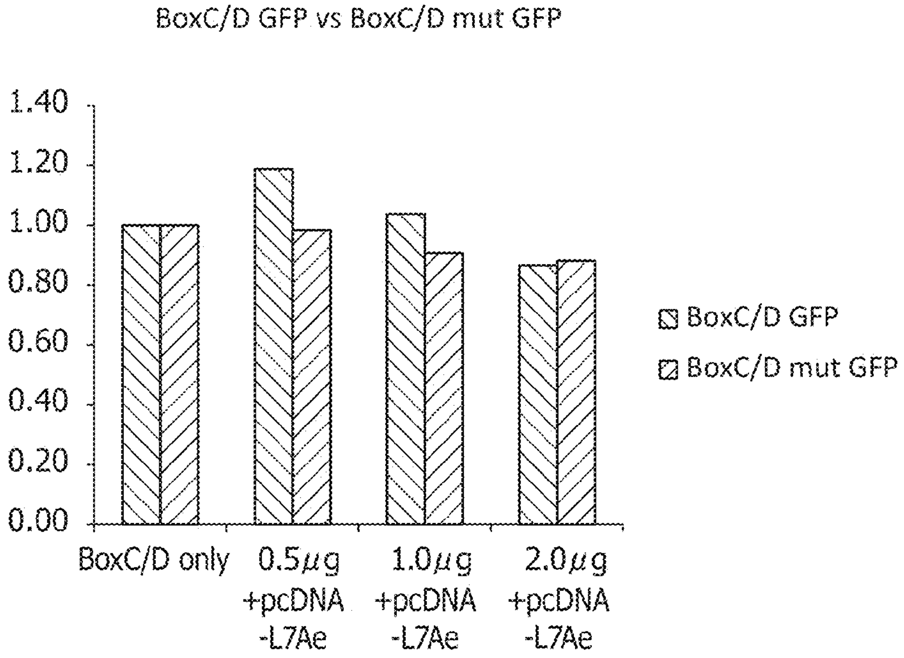

BoxC/D GFP vs BoxC/D mut GFP
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
BoxC/D only
0.5µg +pcDNA -L7Ae
1.0µg +pcDNA -L7Ae
2.0µg +pcDNA -L7Ae
▧ BoxC/D GFP
▨ BoxC/D mut GFP

FIG.33

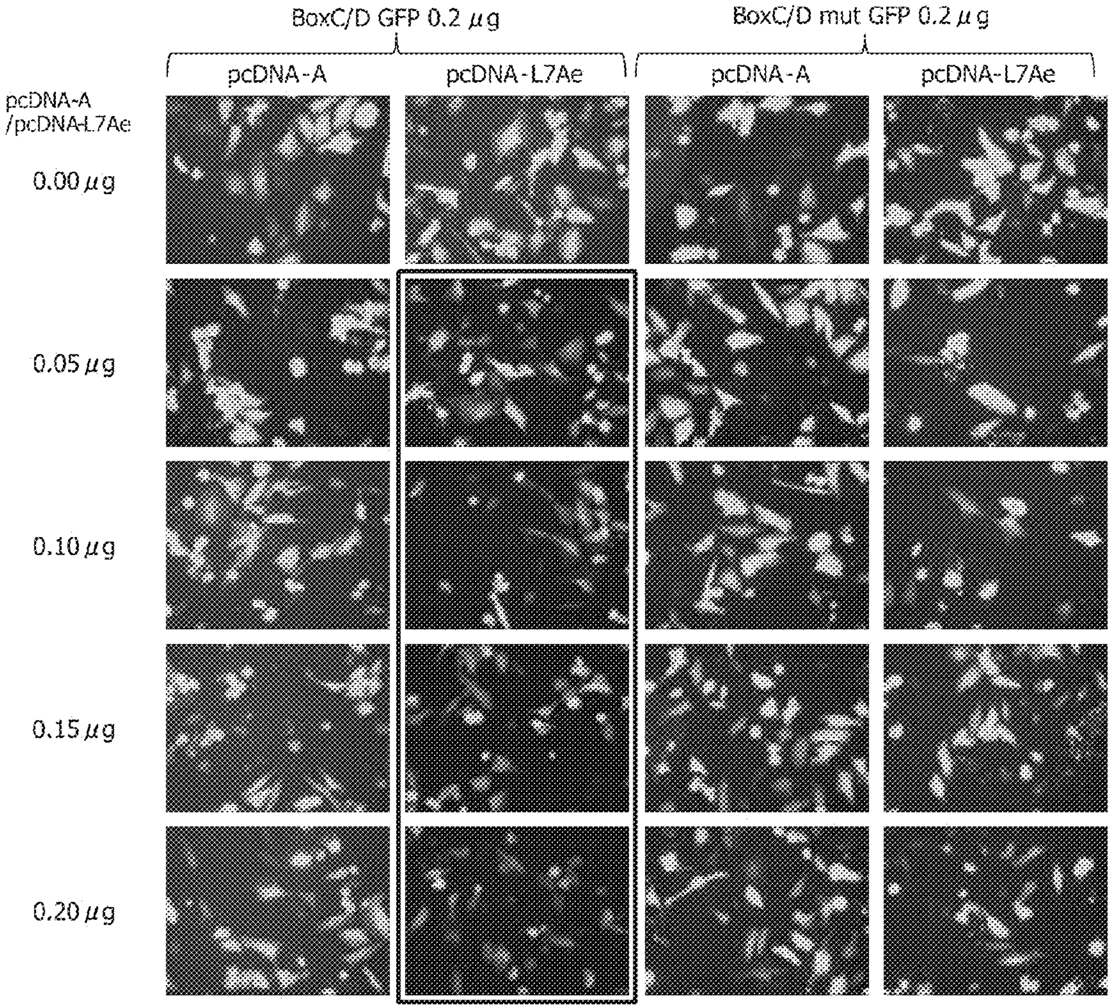

BoxC/D GFP 0.2 μg
BoxC/D mut GFP 0.2 μg
pcDNA-A
pcDNA-L7Ae
pcDNA-A
pcDNA-L7Ae
pcDNA-A
/pcDNA-L7Ae
0.00 μg
0.05 μg
0.10 μg
0.15 μg
0.20 μg

FIG.34

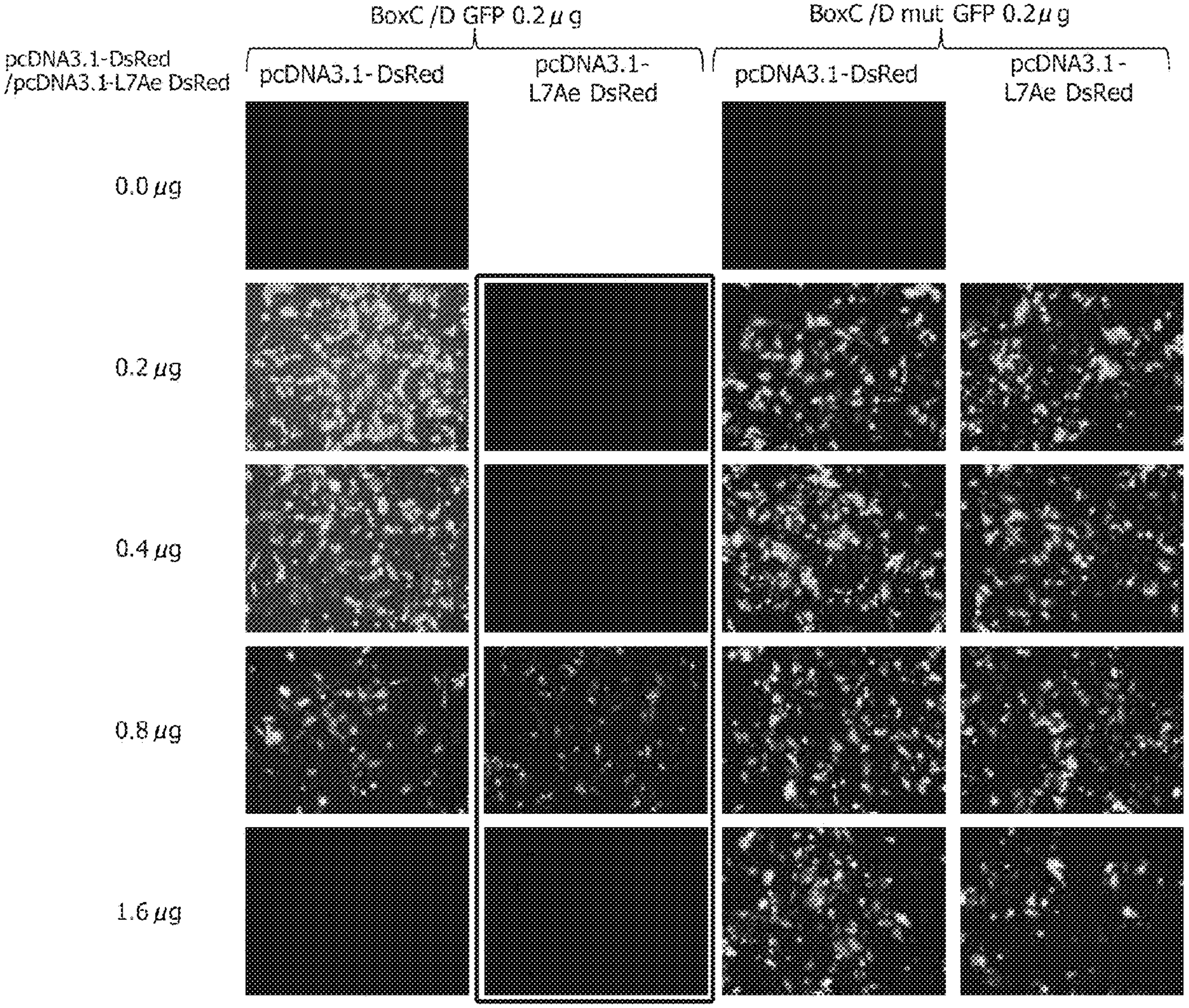
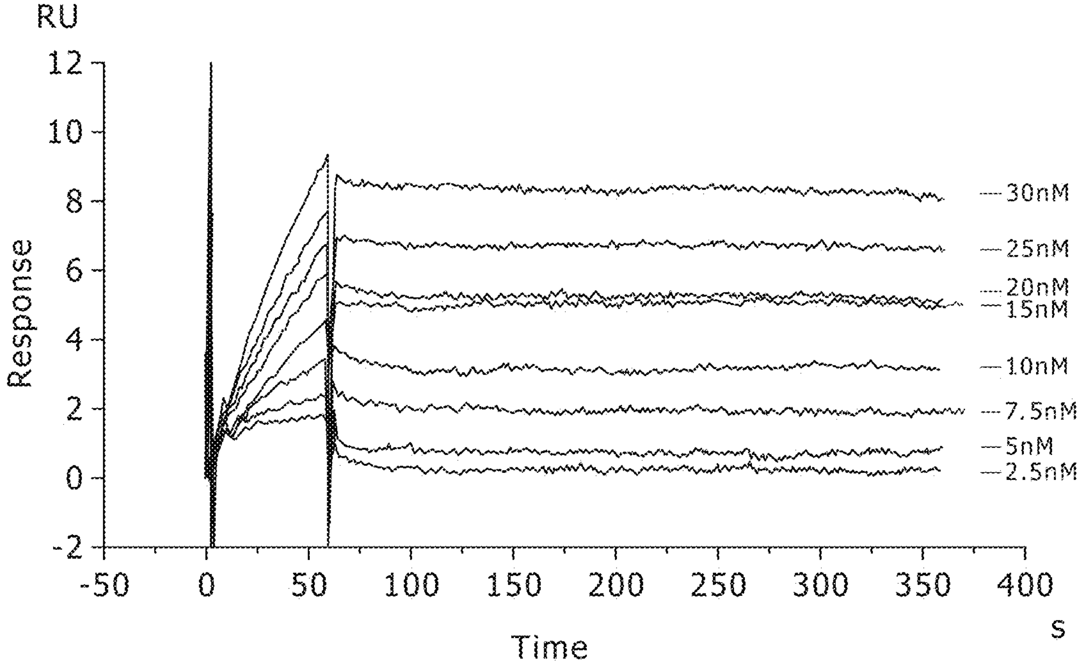

BoxC /D GFP 0.2 μ g
BoxC /D mut GFP 0.2 μ g
pcDNA3.1-DsRed
/pcDNA3.1-L7Ae DsRed
pcDNA3.1- DsRed
pcDNA3.1-
L7Ae DsRed
pcDNA3.1-DsRed
pcDNA3.1 -
L7Ae DsRed
0.0 μg
0.2 μg
0.4 μg
0.8 μg
1.6 μg

FIG.35



K_a (1/Ms)	K_d (1/s)	K_A (1/M)	K_D (M)
1.46E+05	1.02E-04	1.43E+09	7.01E-10

PROTEIN-RESPONSIVE TRANSLATIONAL REGULATORY SYSTEM USING RNA-PROTEIN INTERACTING MOTIF

TECHNICAL FIELD

The present invention relates to a translationally regulatable mRNA, a translational regulatory system, and a translational regulation method using RNA-protein interaction.

BACKGROUND ART

With the progress of post-genomic science, information has accumulated about the structures and functions of biomolecules such as proteins or RNAs. There has been a growing tendency of synthetic biology, which exploits such increasing information to understand the systems of life through "synthesis", in contrast to previous reductive or analytical biology. Particularly, the artificial (re)construction of biomolecules or genetic circuits has received considerable attention in terms of not only life science research but also industrial application. Particularly, there has been a demand for the progress of translational regulatory systems which can recognize a particular protein and regulate arbitrary gene expression.

Heretofore, the conventional technique is known, in which the induction of transcription of DNA is regulated by small molecules or proteins (see Non-Patent Document 1). This technique is a method for modulating the regulation of transcription from DNAs to RNAs. However, this technique had the problem that it cannot be applied directly as a technique of regulating translation from RNAs to proteins. Moreover, there is a naturally occurring system (S15, ThrRS, etc.) in which the protein regulates a translation level upon binding to its own mRNA 5' untranslated region (5'-UTR). However, no artificial translational repression/activation system of a target gene using such an RNP interacting motif has been constructed intracellularly or extracellularly.

Moreover, RNAs called "riboswitches", in which mRNAs induce structural change in response to metabolites, resulting in the regulation of gene expression, have been discovered in recent years in bacteria and have received attention. However, natural riboswitches use substrates limited to small molecules such as vitamins or amino acids and therefore, cannot regulate gene expression in response to biomacromolecules such as RNAs or proteins. Furthermore, natural riboswitches are limited to systems for performing the feedback regulation of their own expressions and therefore, have not been applied so far to the development of artificial systems that regulate arbitrary gene expression. Thus, the development of artificial riboswitches having such functions has been expected.

The conventional technique is known as to translational regulation using RNA aptamers or antisense. There also exists a technique which involves introducing a small molecule theophylline-binding aptamer into an artificial RNA using yeast to prepare an "RNA switch" which performs ON/OFF regulation of gene expression in a manner dependent on the presence of theophylline (Non-Patent Document 2). However, this technique had the problem that it is a system responding to the aptamer for small molecules and therefore, cannot be applied to biomacromolecules such as proteins as substrates.

Non-Patent Document 1: Trends Biochem Sci. 2005; 30 (6): 275-9

Non-Patent Document 2: Nat Biotechnol. 2004 22 (7): 841-7. 2004

DISCLOSURE OF THE INVENTION

Problems to be Solved by the Invention

An object of the present invention is to provide a translationally regulatable mRNA which has wider application and can perform specific ON-OFF regulation, an mRNA-protein complex, and a translational regulatory system and a translational regulation method using the same.

Means for Solving the Problems

The present invention has been achieved for attaining the object. Specifically, according to one embodiment, the present invention provides an mRNA having an RNA-protein complex interacting motif-derived nucleotide sequence 5' to the ribosome-binding site or within the 5' region of the open reading frame.

According to another embodiment, the present invention provides an mRNA having a nucleotide sequence complementary to an RNA-protein complex interacting motif-derived nucleotide sequence 5' to the ribosome-binding site or within the 5' region of the open reading frame.

In any of the mRNAs, the interacting motif is preferably an L7Ae-derived nucleotide sequence.

In any of the mRNAs, the interacting motif is preferably a threonyl-tRNA synthetase (ThrRS)-derived nucleotide sequence.

According to an alternative embodiment, the present invention provides an RNA-protein complex comprising the mRNA and a protein specifically binding to the nucleotide sequence.

According to a further embodiment, the present invention provides a translational regulatory system comprising the mRNA and a protein specifically binding to the nucleotide sequence.

The present invention further provides a method for translational regulation of mRNA, comprising contacting the mRNA with a protein specifically binding to the protein-binding motif. In this context, the term "contacting" refers to mixing in a system in which the mRNA and the protein are movable. For example, such system may be a cell.

According to a further embodiment, the present invention provides a translational regulatory system comprising the mRNA, an RNA which specifically binds to the nucleotide sequence and is complementary to the nucleotide sequence, and a protein specifically binding to the complementary RNA. In other words, this system can be referred to as a translational regulatory system comprising (a) an mRNA having a nucleotide sequence complementary to an RNA-protein complex interacting motif-derived nucleotide sequence 5' to the ribosome-binding site or within the 5' region of the open reading frame, (b) an RNA having the RNA-protein complex interacting motif-derived nucleotide sequence, and (c) a protein specifically binding to the RNA (b).

According to a further embodiment, the present invention provides an artificial information conversion system which converts input information of an arbitrary substrate protein to output information of an arbitrary target protein.

According to a further embodiment, the present invention provides a simultaneous translational regulatory system which regulates the translational repression and activation of different genes using one protein, the system comprising (a) an mRNA having a nucleotide sequence complementary to an RNA-protein complex interacting motif-derived nucleotide sequence 5' to the ribosome-binding site or within the 5' region of the open reading frame, (b) an RNA having the RNA-protein complex interacting motif-derived nucleotide sequence, (c) a protein specifically binding to the RNA (b), and (d) an mRNA having a nucleotide sequence identical to the nucleotide sequence in the RNA (b), 5' to the ribosome-binding site or within the 5' region of the open reading frame, the mRNA encoding a gene different from that encoded by the mRNA (a).

According to a further embodiment, the present invention provides a plasmid vector comprising a nucleic acid sequence encoding any of the mRNAs.

According to a further embodiment, the present invention provides an intracellular translational regulatory system comprising a first plasmid vector comprising a nucleic acid sequence encoding the mRNA, and a second plasmid vector comprising a nucleic acid sequence encoding a protein specifically binding to the RNA-protein complex interacting motif-derived nucleotide sequence in the mRNA produced by the first vector.

The intracellular translational regulatory system is preferably a system for regulating protein translation in a human cancer cell.

According to a further embodiment, the present invention provides a translational regulatory system comprising a fusion protein containing L7Ae as a tag sequence and a first protein. Preferably, the translational regulatory system further comprises an mRNA having a sequence specifically binding to L7Ae and a sequence encoding a second protein.

According to a further embodiment, the present invention provides an intracellular translational regulatory system comprising a plasmid vector containing a nucleic acid sequence encoding an mRNA encoding L7Ae and a first protein. Preferably, the intracellular translational regulatory system further comprises a plasmid vector containing a nucleic acid sequence encoding an mRNA having a sequence specifically binding to L7Ae, the mRNA encoding a second protein.

Advantages of the Invention

The present invention has the advantage that an mRNA of the present invention can regulate the translation reaction of the desired gene. Moreover, the present invention enables intracellular translational regulation and a simultaneous translational regulatory system which regulates the translational repression and activation of different genes using one protein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**(A) is a diagram showing an mRNA according to the first embodiment, and FIG. **1**(B) is a diagram showing the state where a protein is bound to the mRNA according to the first embodiment;

FIG. **2** is a diagram showing an mRNA according to the second embodiment;

FIG. **3**(A) is a diagram showing an mRNA according to the third embodiment, and FIG. **3**(B) is a diagram showing the state where a complementary strand is dissociated from the mRNA according to the third embodiment;

FIG. **4** is a diagram showing EMSA on Box C/D;

FIG. **5** is a diagram showing EMSA on Box C/D mini and Box C/D minimut;

FIG. **6** is a diagram showing EMSA on ThrRS Domain 2;

FIG. **7** is a diagram showing EMSA on ThrRS Domain 234;

FIG. **8**A is a diagram showing the secondary structure of EGFP UTR (SEQ ID NO:98);

FIG. **8**B is a diagram showing the secondary structure of L7-UTR2 (SEQ ID NO:99);

FIG. **8**C is a diagram showing the secondary structure of L7-UTR2 mut of L7Ae (SEQ ID NO:100);

FIG. **8**D is a diagram showing the secondary structure of L7-UTR2 minimut (SEQ ID NO:101);

FIG. **8**E is a diagram showing the secondary structure of L7-UTRS (SEQ ID NO:102);

FIG. **8**F is a diagram showing the secondary structure of L7-UTR9 (SEQ ID NO:103);

FIG. **8**G is a diagram showing the secondary structure of L7-UTR13 (SEQ ID NO:104);

FIG. **9** is a diagram showing the influence of the distance between the ribosome-binding site and the L7Ae-binding site;

FIG. **10** is a diagram showing comparison with translational regulation in mutants;

FIG. **11** is a diagram showing competition assay;

FIG. **12** is a diagram showing the secondary structure of ThrRS-UTRW (SEQ ID NO:105, FIG. **12**A), ThrRS-UTR2 (SEQ ID NO:106, FIG. **12**B) and ThrRS-UTR2 mut (SEQ ID NO:107, FIG. **12**C);

FIG. **13** is a diagram showing that a complex of ThrRS-UTR and ThrRS can inhibit translation;

FIG. **14** is a diagram showing the secondary structure of L7-ORF (EGFP) (SEQ ID NO:108, FIG. **14**A) and L7-ORF mut (EGFP) (SEQ ID NO:109, FIG. **14**B);

FIG. **15** is a diagram showing ORF-based translational regulation;

FIG. **16** is a diagram showing results of translational regulation assay on an ON switch;

FIG. **17**(*a*) is a schematic diagram showing the secondary structure of Box C/D-DsRed Ex (SEQ ID NO:110), FIG. **17**(*b*) is a schematic diagram showing the secondary structure of Box C/D mut-DsRed Ex (SEQ ID NO:111), and FIG. **17**(*c*) is a schematic diagram showing the secondary structure of DsRed Ex (SEQ ID NO:112) (used as a control) having a normal 5'-UTR sequence;

FIG. **18** is a graph showing a Relative fluorescent intensity (fluorescence intensity of the protein translated from the corresponding mRNA in the absence of L7Ae was normalized to 1.0);

FIG. **19** is a graph showing results of simultaneously regulating the translations of two mRNAs by the addition of a protein;

FIG. **20** is a vector diagram showing a pcDNA-A vector;

FIG. **21** is a vector diagram showing an L7Ae expression vector pcDNA-L7Ae, which is a plasmid vector in which the L7Ae gene was inserted downstream of the CMV promoter of a pcDNA3.1 vector (Invitrogen Corp.);

FIG. **22** is a diagram showing L7Ae expression in cultured human cells;

FIG. **23** is a diagram showing that the L7Ae expression in cultured human cells has no cytotoxicity;

FIG. **24** is a vector diagram showing a Box C/D-GFP vector;

FIG. **25** is a vector diagram showing a Box C/D mut GFP vector;

FIG. **26** is a diagram showing L7Ae expression;

FIG. **27** is diagram showing EGFP expression;

FIG. **28** is a diagram showing the quantification of L7Ae-dependent translational repression of EGFP by western blotting;

FIG. **29** is a diagram showing the FACS measurement of L7Ae-dependent translational repression of EGFP;

FIG. **30** is a diagram showing results of quantifying the translational repression of EGFP in an amount of pcDNA or pcDNA-L7Ae added of 0 to 1.6 μg;

FIG. **31** is a graph showing mRNA level comparison among samples (samples derived from Box C/D-GFP and pcDNA-L7Ae and samples derived from Box C/D-GFP and pcDNA-A) with a sample derived from only Box C/D as 1;

FIG. **32** is a graph showing mRNA level comparison among samples (samples derived from Box C/D-GFP and pcDNA-L7Ae and samples derived from Box C/D mut GFP and Box C/D-GFP) with a sample derived from only Box C/D as 1;

FIG. **33** is a fluorescence microscopic image showing the relationship of the amount of an empty vector or an L7Ae-expressing vector added with translational regulation in Box C/D-mut-GFP or Box C/D-GFP;

FIG. **34** is a fluorescence microscopic image showing that L7Ae can be used as a tag sequence for a target protein; and

FIG. **35** is a graph showing measurement of the association rate (Ka), dissociation rate (Kd), and association (KA) and dissociation (KD) constants between the Box C/D RNA and the L7Ae protein, demonstrating that in this RNA-protein complex, a motif having high affinity and a slow dissociation rate is effective for intracellular translational regulation.

DESCRIPTION OF SYMBOLS

1 mRNA
1*a* mRNA
1*b* mRNA
2 RNA-protein complex interacting motif-derived nucleotide sequence
2*a* RNA-protein complex interacting motif-derived nucleotide sequence
2*b* nucleotide sequence complementary to RNA-protein complex interacting motif-derived nucleotide sequence
3 ribosome-binding site
4 open reading frame
4*a* open reading frame
4*b* open reading frame
5 protein
5*b* protein
6 competitor RNA

BEST MODE FOR CARRYING OUT THE INVENTION

Hereinafter, the present invention will be described in detail with reference to the embodiments. However, the description below is not intended to limit the present invention.

With the rapid expansion of molecular biology from the late 20th century to the present, an enormous number of genes have been identified, and the functions of various biomacromolecules, particularly, proteins encoded thereby, have been elucidated. Furthermore, the detailed tertiary structures of DNAs, RNAs, and proteins have been elucidated. They have been demonstrated to function on the atomic level through intermolecular interactions and selective chemical reactions. Accordingly, if these interactions and chemical reactions could be regulated freely, novel disease therapies or methods for solving the energy problem should be developed. Examples of approaches to achieve this include methods which involve: designing and preparing a novel molecule functioning to directly regulate the functions of a targeted molecule through the intermolecular interaction; and regulating cells or tissues using the prepared molecule. RNAs can form diverse tertiary structures. As in proteins, some RNAs have enzymatic functions, and the correlation between the functions and the structures has been revealed in detail through tertiary structure analysis. Moreover, RNAs composed by four basic units are formed based on simple construction principles. Accordingly, RNAs can be used widely in the design and construction of molecules having sophisticated tertiary structures as nano-blocks. On the other hand, proteins, which are composed of basic units as many as 20, have far more diverse and complicated tertiary structures and functions than those of RNAs. Although an enormous number of natural protein structures have been analyzed currently at high resolutions, their molecular designs and constructions are difficult and are thus limited to those having simple structures. As a result, realistically, RNAs or RNPs (RNA-protein complexes) are designed and constructed as nano-scale 3D objects having complicated functions and structures, at this time. Specifically, the combination of an “artificial RNA prepared by molecular design” and a “natural protein having a known structure” is a highly feasible approach for developing functional molecules by molecular design.

The present inventors conceived the idea that ribosome-catalyzed translation reaction is inhibited by binding a particular protein to the 5' side of a ribosome-binding site (RBS) or the 5' region of an open reading frame of an mRNA, and have completed the present invention.

According to the first embodiment, the present invention provides an mRNA having an RNA-protein complex interacting motif-derived protein-binding motif on the 5' side of the ribosome-binding site. FIG. **1**(A) is a diagram schematically showing the mRNA according this embodiment. In FIG. **1**(A), an mRNA **1** comprises an RNA-protein complex interacting motif-derived nucleotide sequence **2**, a ribosome-binding site **3**, and an open reading frame **4**.

[Open Reading Frame]

The mRNA **1** according to this embodiment may be an arbitrary mRNA that has the ribosome-binding site **3** and has translational functions. The sequence of the open reading frame **4** is not limited to a particular sequence. Thus, the sequence of the open reading frame **4** may have a gene that can be expressed into a desired protein, and has a start codon, though it is not limited to a particular sequence. For example, an mRNA having an open reading frame **4** having a gene encoding a fluorescent protein may be used for the purpose of confirming whether the translational functions act. Examples of the fluorescent protein include EGFP, GFP-UV, and DsRed. Their sequences are generally known.

In addition, the sequence of the open reading frame **4** may encode a protein that works as a particular pharmaceutical agent. Specifically, examples of the protein include, but not limited to, Bcl-2 family proteins regulating the apoptosis of cancer cells and antibodies specifically recognizing the surfaces of cancer cells.

[RNA-Protein Complex Interacting Motif-Derived Nucleotide Sequence]

The RNA-protein complex interacting motif-derived nucleotide sequence **2** is a site to which a particular protein specifically binds. The nucleotide sequence **2** may comprise an RNA-protein complex interacting motif-derived nucleotide sequence or a nucleotide sequence mutated from the nucleotide sequence.

In the present invention, the RNA-protein complex interacting motif-derived nucleotide sequence encompasses: a nucleotide sequence known as an RNA sequence in the RNA-protein interacting motif of a known natural RNA-protein complex; and a nucleotide sequence as an RNA sequence in an artificial RNA-protein complex interacting motif obtained by the in vitro evolution method. These RNA-protein complexes are assemblies of proteins and RNAs which are confirmed in vivo in large numbers, and are 3D objects having complicated structures.

The natural RNA-protein complex interacting motif-derived nucleotide sequence is usually composed of approximately 10 to 80 bases and known to specifically bind to a particular amino acid sequence of a particular protein in a noncovalent manner, i.e., through hydrogen bond. Such a natural RNA-protein complex interacting motif-derived nucleotide sequence can be selected from Tables 1 and 2 below. The RNA-protein interacting motif preferably used in this embodiment has such a tertiary structure as to inhibit the translational functions of the mRNA. The tertiary structure capable of inhibition refers to a structure that can compete with ribosome function, owing to its high affinity of RNA-protein interaction. Specifically, it refers to a protein-RNA interacting motif having Kd of approximately 0.1 nM to approximately 1 μM, though the Kd is not limited to this range.

TABLE 1

RNA	Protein	Kd	Reference
5S RNA (*Xenopus laevis* oocyte)	5R1	0.64 ± 0.10 nM	Nat Struct Biol. 1998 July; 5(7): 543-6
5S RNA (*Xenopus laevis* oocyte)	5R2	0.35 ± 0.03 nM	Nat Struct Biol. 1998 July; 5(7): 543-6
dsRNA	B2	1.4 ± 0.13 nM	Nat Struct Mol Biol. 2005 November; 12(11): 952-7
RNA splicing motif with UGCAUGU element	Fox-1	0.49 nM at 150 mM salt	EMBO J. 2006 Jan. 11; 25(1): 163-73.
TGE	GLD-1	9.2 ± 2 nM	J Mol Biol. 2005 Feb. 11; 346(1): 91-104.
sodB mRNA	Hfq	1.8 nM	EMBO J. 2004 Jan. 28; 23(2): 396-405.
RyhB (siRNA)	Hfq	1500 nM	Annu Rev Microbiol. 2004; 58: 303-28
mRNA	HuD	0.7 ± 0.02 nM	Nat Struct Biol. 2001 February; 8(2): 141-5
S domain of 7S RNA	human SRP19		RNA. 2005 July; 11(7): 1043-50. Epub 2005 May 31
Large subunit of SRP RNA	human SRP19	2 nM	Nat Struct Biol. 2001 June; 8(6): 515-20
23S rRNA	L1		Nat Struct Biol. 2003 February; 10(2): 104-8
23S rRNA	L11		Nat Struct Biol. 2000 October; 7(10): 834-7
5S rRNA	L18		Biochem J. 2002 May 1; 363(Pt 3): 553-61
23S rRNA	L20	13 ± 2 nM	J Biol Chem. 2003 Sep. 19; 278(38): 36522-30.
Own mRNA site1	L20	88 ± 23 nM	J Biol Chem. 2003 Sep. 19; 278(38): 36522-30.
Own mRNA site2	L20	63 ± 23 nM	Mol Microbiol. 2005 June; 56(6): 1441-56
23S rRNA	L23		J Biomol NMR. 2003 June; 26(2): 131-7
5S rRNA	L25		EMBO J. 1999 Nov. 15; 18(22): 6508-21
Own mRNA	L30		Nat Struct Biol. 1999 December; 6(12): 1081-3.
mRNA	LicT		EMBO J. 2002 Apr. 15; 21(8): 1987-97
Own mRNA	MS2 coat	39 ± 5 nM	FEBS J. 2006 April; 273(7): 1463-75
Stem-loop RNA motif	Nova-2		Cell. 2000 Feb. 4; 100(3): 323-32
SL2	Nucleocapsid	110 ± 50 nM	J Mol Biol. 2000 Aug. 11; 301(2): 491-511
Pre-rRNA	Nucleolin		EMBO J. 2000 Dec. 15; 19(24): 6870-81
	p19	0.17 ± 0.02 nM	Cell. 2003 Dec. 26; 115(7): 799-811
Box C/D	L7Ae	0.9 ± 0.2 nM	RNA. 2005 August; 11(8): 1192-200.

TABLE 2

RNA	Protein	Kd	Reference
siRNA with the characteristic two-base 3' overhangs	PAZ(PiWi Argonaut and Zwille)		Nat Struct Biol. 2003 December; 10(12): 1026-32.
dsRNA	Rnase III		Cell. 2006 Jan. 27; 124(2): 355-66
HIV-1 RRE (IIB)	RR1-38	3-8 nM	Nat Struct Biol. 1998 July; 5(7): 543-6
Own mRNA	S15	5 nM	EMBO J. 2003 Apr. 15; 22(8): 1898-908
16S rRNA	S15	6 nM	Nat Struct Biol. 2000 April; 7(4): 273-277.
Own mRNA	S15	43 nM	EMBO J. 2003 Apr. 15; 22(8): 1898-908
16S rRNA	S4	6.5 μM in 4° C., 1.7 nM in 42° C.	J Biol Chem. 1979 Mar. 25; 254(6): 1775-7
16S rRNA	S4	18 μM	J Biol Chem. 1979 Mar. 25; 254(6): 1775-7
16S rRNA	S8	26 ± 7 nM	J Mol Biol. 2001 Aug. 10; 311(2): 311-24
mRNA	S8	200 nM	RNA. 2004 June; 10(6): 954-64
mRNA	SacY	1400 nM	EMBO J. 1997 Aug. 15; 16(16): 5019-29
SnRNA	Sm		Cold Spring Harb Symp Quant Biol. 2006; 71: 313-20.
tmRNA	SmpB	21 ± 7 nM	J Biochem (Tokyo). 2005 December; 138(6): 729-39
TD3 of tmRNA	SmpB	650 nM	J Biochem (Tokyo). 2005 December; 138(6): 729-39
U1 snRNA	snRNP U1A	0.032 ± 0.007 nM (salt dependence)	Nat Struct Biol. 2000 October; 7(10): 834-7
S domain of 7S RNA	SRP54	500 nM	RNA. 2005 July; 11(7): 1043-50.
TAR	Tat	200-800 nM	Nucleic Acids Res. 1996 Oct. 15; 24(20): 3974-81
BIV TAR	Tat	1.3 nM or 8 nM or 60 nM (Mg dependence)	Mol Cell. 2000 November; 6(5): 1067-76
tRNA^{Thr}	ThrRS	500 nM	Nat Struct Biol. 2002 May; 9(5): 343-7
thrS mRNA operator	ThrRS	10 nM	Trends Genet. 2003 March; 19(3): 155-61
Single stranded mRNA	TIS11d		Nat Struct Mol Biol. 2004 March; 11(3): 257-64.

TABLE 2-continued

RNA	Protein	Kd	Reference
PSTVd	Virp1	500 nM	Nucleic Acids Res. 2003 Oct. 1; 31(19): 5534-43
RNA hairpin; Smaug recognition element (SRE)	Vts1p	30 nM	Nat Struct Mol Biol. 2006 February; 13(2): 177-8.
λ BoxB	λ N	90 nM	Cell. 1998 Apr. 17; 93(2): 289-99

The artificial RNA-protein complex interacting motif-derived nucleotide sequence is the nucleotide sequence of an RNA in the RNA-protein interacting motif of an artificially designed RNA-protein complex. Such a nucleotide sequence is usually composed of approximately 10 to 80 bases and designed to specifically bind to a particular amino acid sequence of a particular protein in a noncovalent manner, i.e., through hydrogen bond. Examples of such an artificial RNA-protein complex interacting motif-derived nucleotide sequence include, but not limited to, RNA aptamers specifically binding to apoptosis-inducing protein Bcl-2 family, and RNA aptamers specifically recognizing cancer cell surface antigens. Moreover, nucleotide sequences listed in Table 3 below are also known, and these can also be used as the RNA-protein complex interacting motif-derived nucleotide sequence **2** of the present invention.

TABLE 3

RNA	Protein	Kd	Reference
Rev aptamer 5	Rev	190 nM	RNA. 2005 December; 11(12): 1848-57
Aptamer	p50	5.4 ± 2.2 nM	Proc Natl Acad Sci USA. 2003 Aug. 5; 100(16): 9268-73.
BMV Gag aptamer	BMV Gag	20 nM	RNA. 2005 December; 11(12): 1848-57
BMV Gag aptamer	CCMV Gag	260 nM	RNA. 2005 December; 11(12): 1848-57
CCMV Gag aptamer	CCMV Gag	280 nM	RNA. 2005 December; 11(12): 1848-57
CCMV Gag aptamer	BMV Gag	480 nM	RNA. 2005 December; 11(12): 1848-57

The artificial RNA-protein complex can be prepared by using the molecular design and in vitro evolution methods in combination. The in vitro evolution method can produce aptamers or ribozymes by screening functional RNAs from a molecular library having various sequence diversities and repeating the amplification and transcription reactions of the genes (DNAs). Thus, an RNA-protein interacting motif adapted to an RNP having functions and structures of interest based on molecular design in advance can be extracted from natural RNP molecules or can be prepared artificially by the in vitro evolution method.

In this embodiment, for the RNA-protein complex interacting motif-derived nucleotide sequence **2**, the RNA-protein complex serving as an origin of the nucleotide sequence preferably has a dissociation constant Kd of approximately 0.1 nM to approximately 1 μM. This is because affinity sufficient for competing with ribosome-mRNA interaction is necessary.

Specific examples of the RNA-protein complex interacting motif-derived nucleotide sequence **2** include, but not limited to, nucleotide sequences such as a nucleotide sequence 5'-GGGCGUGAUGCGAAAGCUGACCC-3' (SEQ ID NO:9) which can bind to L7Ae (Moore T et al., Structure Vol. 12, pp. 807-818 (2004)) known to participate in RNA modification such as RNA methylation or pseudouridylation, and a nucleotide sequence 5'-GGCGUAUGUGAUCUUUCGUGUGGGUCACCACUGCGCC-3' (SEQ ID NO:19) which can bind to threonyl-tRNA synthetase (Cell (Cambridge, Mass.) v97, pp. 371-381 (1999)), an aminoacylating enzyme, known to have feedback inhibition which inhibits translation upon binding to its own mRNA.

Moreover, a moiety that interacts with a Bcl-xL aptamer protein specifically binding to a cancer cell-specific endogenous protein Bcl-xL may be used as the RNA-protein complex interacting motif-derived nucleotide sequence **2**. Such a Bcl-2 family CED-9-derived nucleotide sequence used as the RNA-protein complex interacting motif-derived nucleotide sequence **2** is R9-2; 5'-GGGUGCUUCGAGCGUAGGAAGAAAGCCGGGGGCUGCAGAUAAUGUAUAGC-3' (SEQ ID NO:113), which is described in detail in Yang C, et al., J Biol Chem. 2006; 281 (14): 9137-44. In addition, a nucleotide sequence derived from an RNA aptamer sequence binding to NF-kappa B can be used as the RNA-protein complex interacting motif-derived nucleotide sequence **2**.

The RNA-protein complex interacting motif-derived nucleotide sequence **2** is incorporated to 5' to the ribosome-binding site **3** in the mRNA **1**. The term "5' to the ribosome-binding site" in the mRNA refers to a position 2 to 10 bases (inclusive) distant from the ribosome-binding site toward the 5' end. In FIG. **1**(A), a nucleotide sequence that may be located between the RNA-protein complex interacting motif-derived nucleotide sequence **2** and the ribosome-binding site **3** is indicated in line. In this embodiment, the nucleotide sequence that may be located between the RNA-protein complex interacting motif-derived nucleotide sequence **2** and the ribosome-binding site **3** is not limited to a particular nucleotide sequence.

Moreover, the mRNA **1** according to this embodiment may have a 5'-terminal sequence forming a stem-loop structure (not shown), which is located 5' to the RNA-protein complex interacting motif-derived nucleotide sequence **2**. This is because the transcriptional efficiency of the mRNA **1** may be enhanced. Examples of the sequence forming a stem-loop structure include usually known structures. Those skilled in the art can introduce an arbitrary stem structure for enhancing transcriptional efficiency into the 5' end using the standard method.

Next, the mechanism of translational regulation according to the first embodiment will be described specifically.

[ON-to-OFF Translational Regulation]

When a protein specifically binding to the RNA-protein complex interacting motif-derived nucleotide sequence **2** is absent in the state shown in FIG. **1**(A), a ribosome, if any, can freely bind to the ribosome-binding site **3** under conditions involving approximately 33 to 41° C. and pH 6.0 to 8.0. Accordingly, the translation of the mRNA is performed as normal. Here, a protein **5** specifically binding to the RNA-protein complex interacting motif-derived nucleotide sequence **2** is added thereto. FIG. **1**(B) shows the relationship of the mRNA **1** and the protein **5** in the presence of the protein. In FIG. **1**(B), the protein **5** is specifically bound to the RNA-protein complex interacting motif-derived nucleotide sequence **2**. Further, the protein **5** blocks the ribosome-binding site **3** through its steric hindrance. Therefore, a ribosome, if any, cannot bind to the ribosome-binding site **3**. Accordingly, the translation reaction of the mRNA **1** fails to function. In this way, the translation reaction of the mRNA **1** can be regulated in an ON-to-OFF manner by adding the particular protein **5** to the protein-free system of the mRNA **1** (state of FIG. **1**(A)).

Moreover, similar ON-to-OFF translational regulation can be achieved not only by adding the particular protein to the system but also by responding to, for example, a protein endogenously expressed in vivo. Specifically, for example, an mRNA **1** that has an aptamer against proteins (e.g., Bcl-xL) specifically expressed in certain cancer cells, as the RNA-protein complex interacting motif-derived nucleotide sequence **2** and has a fluorescent protein-encoding sequence as an open reading sequence may be introduced in cells in vivo. In such a case, fluorescent protein expression is regulated in an ON-to-OFF manner only in cells that have expressed the proteins specifically expressed in certain cancer cells. Therefore, cells that do not emit fluorescence, i.e., cancer-bearing cells, can be detected specifically.

Thus, such an mRNA and a protein can be used as a translational regulatory system. Moreover, in light of the above-mentioned mechanism, a translational regulation method can be provided by contacting the mRNA with the protein. Furthermore, a complex of the mRNA and the protein may be used in such a translational system or translational regulation method. Moreover, the use of them enables construction of an artificial information conversion system which converts input information of an arbitrary substrate protein to output information of an arbitrary target protein.

The mRNA according to the first embodiment of the present invention allows regulation of translation reaction as described above. Moreover, in the applicative aspect of use of the mRNA according to the first embodiment, the RNA-protein complex interacting motif-derived nucleotide sequence **2** is designed to specifically bind to a protein formed due to a particular disease. Further, the open reading frame is designed to incorporate therein a gene encoding a protein that relieves or treats the disease. The resulting mRNA can be used as a drug for the particular disease.

The second embodiment of the present invention provides an mRNA having an RNA-protein complex interacting motif-derived nucleotide sequence within the open reading frame. FIG. **2** is a diagram schematically showing the mRNA according to this embodiment. In FIG. **2**, an mRNA **1***a* according to this embodiment comprises an open reading frame **4***a* and an RNA-protein complex interacting motif-derived nucleotide sequence **2***a* located therewithin.

This embodiment is not only used preferably in the translational regulation of the mRNA free from a ribosome-binding site, specifically, an mRNA derived from an origin other than bacteria (e.g., *E. coli*), but also used in an mRNA containing a ribosome-binding site. In FIG. **2**, the description of the ribosome-binding site is omitted. However, this embodiment is not intended to exclude the presence of the ribosome-binding site.

In this embodiment, the RNA-protein complex interacting motif-derived nucleotide sequence **2***a* is located within the open reading frame **4***a*. The position of the RNA-protein complex interacting motif-derived nucleotide sequence **2***a* may be set to an arbitrary position within the open reading frame **4***a*. The RNA-protein complex interacting motif-derived nucleotide sequence **2***a* can be placed, for example, immediately 3' to the start codon AUG. Moreover, the RNA-protein complex interacting motif-derived nucleotide sequence **2***a* may be placed via approximately 1 to 20 bases 3' to the start codon AUG. Particularly, it may be placed via approximately 1 to 10 bases 3' to the start codon AUG. In this context, when the RNA-protein complex interacting motif-derived nucleotide sequence **2***a* is inserted within the open reading frame **4***a*, the motif-derived nucleotide sequence **2***a* can be supplemented, if necessary, with 1 base or 2 bases such that the base number of the inserted nucleotide sequence is an multiple of 3 to prevent frameshift.

When a protein specifically binding to the RNA-protein complex interacting motif-derived nucleotide sequence **2***a* is absent in the state shown in FIG. **2**, a ribosome, if any, initiates the translation of the mRNA **1***a* under conditions involving approximately 36 to 42° C. and pH 6 to 7.6. However, in the presence of the protein, the protein specifically binds to the RNA-protein complex interacting motif-derived nucleotide sequence **2***a* and sterically blocks the adjacent open reading frame **4***a*. Therefore, the ribosome-catalyzed translation is repressed.

According to the second embodiment, the mRNA translation can be regulated by sterically blocking the open reading frame **4***a*. In this context, the use of the mRNA according to this embodiment can also achieve, as in the first embodiment, a translational regulatory system comprising the mRNA and the protein, a complex of the mRNA and the protein, and a translational regulation method.

According to the third embodiment, the present invention provides an mRNA having a nucleotide sequence complementary to an RNA-protein complex interacting motif-derived nucleotide sequence 5' to the ribosome-binding site or within the 5' region of the open reading frame. FIG. **3**(A) is a diagram schematically showing the mRNA according to this embodiment. In FIG. **3**(A), an mRNA **1***b* according to this embodiment comprises an open reading frame **4***b* and a nucleotide sequence **2***b* complementary to an RNA-protein complex interacting motif-derived nucleotide sequence, located therewithin. In this case as well, the complementary nucleotide sequence **2***b* can be supplemented, if necessary, with 1 base or 2 bases such that the base number of the inserted nucleotide sequence is a multiple of 3.

The mRNA **1***b* according to this embodiment differs from the mRNA of the second embodiment in that the RNA-protein complex interacting motif-derived nucleotide sequence according to the second embodiment is changed to the nucleotide sequence **2***b* complementary to an RNA-protein complex interacting motif-derived nucleotide sequence. In this context, the nucleotide sequence **2***b* complementary to an RNA-protein complex interacting motif-derived nucleotide sequence may comprise not only a completely complementary sequence but also a sequence mutated therefrom.

[OFF-to-ON Translational Regulation]

Next, the OFF-to-ON translational regulation of the mRNA will be described using the mRNA **1***b* according to this embodiment. In the state shown in FIG. **3**(A), the nucleotide sequence **2***b* complementary to an RNA-protein complex interacting motif-derived nucleotide sequence, in the mRNA **1***b*, is bound in advance to a competitor RNA **6** having the RNA-protein complex interacting motif-derived nucleotide sequence of the mRNA **1***b*. When a protein **5***b* shown in FIG. **3**(B) is intracellularly absent, the competitor RNA **6** is bound to the sequence **2***b* in the mRNA **1***b*. This state is the state shown in FIG. **3**(A). This competitor RNA **6** does not have to be completely identical to the RNA-protein complex interacting motif-derived nucleotide sequence and may contain a mutation. In this state, translation does not start even in the presence of a ribosome. This is because the competitor RNA **6** blocks ribosome binding to the mRNA **1***b*.

To this system, a protein **5***b* specifically binding to the competitor RNA **6** having the RNA-protein complex interacting motif-derived nucleotide sequence is added. The added state is shown in FIG. **3**(B). The state shown here in FIG. **3**(B) is brought about by the intracellular expression of the protein **5***b*. Here, the addition of the protein **5***b* can inhibit the specific binding between the competitor RNA **6** having the protein-binding motif-derived sequence and the mRNA **1***b*. The ribosome-catalyzed translation reaction of the open reading frame **4***b* starts upon inhibition of the binding between the RNA **6** and the mRNA **1***b* through the reaction with the particular protein **5***b*. In this way, the translation reaction of the mRNA **1***b* can be regulated in an OFF-to-ON manner by adding the protein **5***b* to the system in which the particular competitor RNA **6** is bound to the mRNA **1***b* (state of FIG. **3**(A)).

In FIG. **3**, the embodiment is shown, in which the nucleotide sequence **2***b* complementary to an RNA-protein complex interacting motif-derived nucleotide sequence is located within the open reading frame **4***b*. However, in a modification of this embodiment, the nucleotide sequence complementary to an RNA-protein complex interacting motif-derived nucleotide sequence may be located 5' to the ribosome-binding site. The aspect may be the same as that of the first embodiment in which the RNA-protein complex interacting motif-derived nucleotide sequence is located 5' to the ribosome-binding site. In this case as well, OFF-to-ON translational regulation can be performed by the same action as in the third embodiment. Moreover, the use of the mRNA according to this embodiment can also achieve a translational regulatory system comprising the mRNA and the protein, a complex of the mRNA and the protein, and a translational regulation method.

According to the fourth embodiment, the present invention provides a modification of the third embodiment and relates to a simultaneous OFF-to-ON/ON-to-OFF translational regulatory system.

The simultaneous translational regulatory system according to the fourth embodiment of the present invention comprises an mRNA **1***b*, a competitor RNA **6**, and a protein **5***b* specifically binding to the competitor RNA **6** shown in FIG. **3**(A) described in the third embodiment and further comprises a second mRNA. The second mRNA has a sequence identical to the competitor RNA **6**, 5' to the ribosome-binding site or within the 5' region of the open reading frame, and encodes a gene different from that encoded by the mRNA **1***b*. Since the second mRNA has a sequence identical to the competitor RNA **6**, and it specifically binds to the protein **5***b*. Specifically, the second mRNA is of type whose translation is inhibited in a manner dependent on the presence of the protein **5***b*. In the description below, the mRNA **1***b* shown in FIG. **3**(A) is referred to as a first mRNA.

[Simultaneous OFF-to-ON/ON-to-OFF Translational Regulation]

In this context, the addition of the protein **5***b* to the system containing the first mRNA **1***b* and the competitor RNA **6** achieves OFF-to-ON translational regulation as described in the third embodiment. Furthermore, when the second mRNA is present in this system in the presence of an excess of the protein **5***b*, this protein **5***b* specifically binds to the second mRNA and hinders its translation. Therefore, the translation of the second mRNA is regulated to achieve ON-to-OFF translational regulation. In this way, the fourth embodiment enables simultaneous OFF-to-ON/ON-to-OFF translational regulation.

For example, the first mRNA **1***b* and the second mRNA may have fluorescent protein genes differing in type as their ORFs. In such a case, OFF-to-ON translational regulation is performed in one of them, while ON-to-OFF translational regulation is performed in the other mRNA. They can be observed easily using a fluorescence microscope or the like by applying EGFP (green) to one of the fluorescent protein genes and DsRed (red) to the other gene. Thus, this system would be useful.

According to the fifth embodiment, the present invention provides an intracellular translational regulatory system comprising a vector containing a nucleic acid encoding any of the RNAs and/or any of the proteins used in the first to fourth embodiments.

Translational regulation can be performed preferably, particularly in cancer cells. Both the repression and promotion of protein expression can be performed according to the procedures of the ON-to-OFF translational regulation and the OFF-to-ON translational regulation, respectively. Moreover, the presence or absence of such regulation can be confirmed based on the expression of a marker protein. In this case, the mRNA and a protein-encoding gene can be introduced into cells using plasmid vectors.

A technique of preparing plasmid vectors expressing the desired RNA or protein is already known by those skilled in the art. These vectors can be prepared by conventional methods. For example, L7Ae-expressing vectors can be constructed by inserting the L7Ae-encoding gene downstream of a CMV promoter within vectors conventionally used in intracellular protein expression for humans. On the other hand, vectors expressing an mRNA in which Box C/D known as a sequence to which L7Ae specifically binds, or its mutant Box C/D mut is inserted within the 5' region of the EGFP open reading frame, can also be prepared by amplifying the corresponding genes by PCR and inserting them within vectors routinely used in intracellular protein expression for humans. Furthermore, when L7Ae is desired to be intracellularly expressed at the intended timing, vectors capable of expressing L7Ae by addition to a tetracycline (Tet) medium may be prepared. Such vectors capable of expressing L7Ae by the addition to a tetracycline medium contain an L7Ae-encoding gene downstream of a Tet operator sequence and comprise, as a component, a vector or cell constitutively expressing a Tet repressor.

The fifth embodiment of the present invention enables intracellular translational regulation. Translational regulation in cells, particularly, cancer cells, is highly possibly applicable therapeutically and can therefore serve as very useful means.

According to the sixth embodiment, the present invention provides a translational regulatory system comprising a fusion protein containing L7Ae as a tag sequence and a first protein.

This fusion protein is specifically a fusion protein comprising L7Ae and a first protein as another arbitrary protein. Hereinafter, such a fusion protein is also referred to as a tag sequence-fused protein. Examples of the first protein as an arbitrary protein include, but not limited to, fluorescent proteins, apoptosis-inducing proteins, apoptosis-repressing proteins, and organellar localized proteins. Theoretically, the desired protein can be used.

The translational regulatory system according to this embodiment further comprises an mRNA having a sequence specifically binding to L7Ae and a sequence encoding a second protein. Specifically, the fusion protein is preferably used together with the mRNA. In the mRNA, the sequence specifically binding to L7Ae is preferably a Box C/D sequence. Alternatively, a sequence mutated from the Box C/D sequence with the Kink-turn motif structure maintained may be used. On the other hand, the second protein encoded by this mRNA is preferably a protein different from the fusion protein. Theoretically, the second protein may be an arbitrary protein and can be determined based on its combination with the first protein constituting the fusion protein. The second protein encoded by the mRNA is preferably a green fluorescent protein for a red fluorescent protein used as the first protein or is preferably an apoptosis-repressing protein for an apoptosis-inducing protein used as the first protein. In addition, some combinations such as some intracellular signaling proteins may be used, in which the translation of the second protein is preferably repressed by the expression of the first protein.

Such a tag sequence-fused protein and an mRNA can be prepared according to the known method as long as genes encoding the desired first and second proteins are known. Moreover, when the protein and the mRNA are used in an intracellular translational regulatory system, plasmid vectors expressing them can be prepared and introduced into cells. These plasmid vectors can be prepared in the same way as in the description of the fifth embodiment by inserting the desired gene thereinto.

Next, the action of the translational regulatory system achieved by such a tag sequence-fused protein and an mRNA will be described. Here, the case will be described, in which the tag sequence-fused protein is a fusion protein of L7Ae and a red fluorescent protein and the mRNA has a Box C/D sequence and encodes a green fluorescent protein, though the present invention is not limited thereto. Plasmid vectors expressing this mRNA are introduced into cells. As a result, the mRNA is translated in the absence of the tag sequence-fused protein to express the green fluorescent protein. To introduce the tag sequence-fused protein into these cells, plasmid vectors having a nucleic acid sequence encoding the tag sequence-fused protein are introduced into the cells. This results in the intracellular expression of the tag sequence-fused protein. Then, the expressed tag sequence-fused protein binds to the mRNA. More specifically, L7Ae constituting the tag sequence-fused protein specifically binds to the Box C/D sequence on the mRNA. Upon this binding, the mRNA translation is repressed to prevent the production of the green fluorescent protein. On the other hand, since the tag sequence-fused protein is continuously produced, the red fluorescent protein constituting the tag-fused protein increases in number. This is observed under a fluorescence microscope such that the green color and the red color become lighter and darker, respectively, with a lapse of time. In this way, the combined use of the tag sequence-fused protein and the mRNA can achieve a translational regulatory system that performs the translation of a target gene in response to the expression of a predetermined gene.

According to the sixth embodiment, a system that represses the translation of a target gene, for example, green fluorescent protein translation, in response to the expression of an arbitrary gene, for example, red fluorescent protein expression, can be constructed intracellularly by adding L7Ae as a tag sequence to the protein. Furthermore, the protein to be fused to the L7Ae tag sequence may be set to, for example, an apoptosis-repressing protein, and the target gene to be regulated may be set to a gene encoding an apoptosis-inducing protein. In such a case, a signaling circuit can be rewired such that it can effectively induce the apoptosis of cells overexpressing apoptosis-repressing proteins, such as cancer cells. Such a translational regulatory system that performs the translation of a target gene in response to the expression of a predetermined gene is a promising tool constituting artificial genetic circuits.

EXAMPLES

A protein-responsive translational regulatory system using a protein-RNA interacting motif (RNP motif) according to the present invention is a technique of using a naturally extracted or artificially prepared RNP motif to regulate translation reaction in an ON-to-OFF or OFF-to-ON manner. Specifically, the ON-to-OFF regulation is established by inserting an RNA-protein complex interacting motif-derived nucleotide sequence into an mRNA. In this regulation, in the presence of a target protein, the protein competes with ribosome binding or entry through its binding to the mRNA to cause translational inhibition. The OFF-to-ON regulation is established by first inserting an antisense sequence of an RNA-protein complex interacting motif-derived nucleotide sequence, 5' region of the open reading frame of an mRNA. Next, an RNA comprising the RNA-protein complex interacting motif-derived nucleotide sequence is added to the reaction solution to form a complementary strand with the antisense strand inserted in the mRNA, resulting in translational inhibition. The addition of a substrate protein thereto inhibits the binding of the RNA comprising the protein-binding motif to the mRNA to activate translation. In Examples below, proteins generally called L7Ae and ThrRS are used. However, proteins that can be used in the reactions are not limited to only L7Ae or ThrRS. Hereinafter, specific examples of experiments or assays will be described.

Example 1

[Preparation of RNA-Protein Complex Interacting Motifs (RNAs and Proteins) Used in Translational Regulation]

[Preparation of L7Ae-Binding RNA Box C/D]

L7Ae-binding RNA Box C/D (SEQ ID NO: 5) was prepared by preparing a DNA template containing a T7 promoter, followed by transcription reaction using T7 RNA polymerase. The details will be shown below. First, 100 μL of reaction solution was prepared for preparing DNA. The reaction solution contained a mixture of 1 ng of Box C/D template (5'-CTAATACGACTCACTATAGGCCA-GAGTGGGCGTGATGCATGTCTAGGAAACTAGA CAT-GCTGACCCACTCTGGCC-3') (SEQ ID NO: 1), 5 μL each of 10 μM Box C/D Fwd (5'-CTAATACGACTCACTATAG-GCCAG-3') (SEQ ID NO: 2) and Box C/D Rev (5'-GGCCA-GAGTGGGTCAGCAT-3') (SEQ ID NO: 3), 8 μL of 2.5 mM dNTP (TAKARA BIO INC.), 10 μL of Ex taq 10× buffer (TAKARA BIO INC.), and 0.5 μL of Ex taq DNA polymerase (TAKARA BIO INC.). 25 cycles each involving 94° C. for 30 seconds, 53° C. for 30 seconds, and 72° C. for 1 minute were performed for extension (SEQ ID NO: 4) using Gradient Master Cycler (Eppendorf). After the reaction, the extension product was subjected to phenol treatment, diethyl ether treatment, and ethanol precipitation and dissolved in 10 μL of ultrapure water. The solution was used as a template for transcription. Transcription reaction was performed under conditions involving, for ^{32}P radiolabeling, 40 mM Tris-Cl (pH 7.5), 5 mM DTT, 1 mM spermidine, 5 mM $MgCl_2$, 1.25 mM ATP, 1.25 mM CTP, 1.25 mM UTP, 0.25 mM GTP,

[^{32}P-α]GTP (PerkinElmer Inc.), 20 U RNase inhibitor (TOYOBO CO., LTD.), and 35 ng/μL T7 RNA polymerase. In 100 μL of the system, 5 μL of the template was used and reacted at 37° C. for 3 hours to overnight. For non-labeling, transcription reaction was performed using MEGAshortscript (trademark) (Ambion, Inc.). The transcription reaction using MEGAshortscript was performed as follows. 1 μg of template DNA dissolved in ultrapure water, 2 μL of T7 10× Reaction Buffer, 2 μL of T7 ATP Solution (75 mM) (the same recipe for CTP, GTP, and UTP), and 2 μL of T7 Enzyme Mix were mixed and adjusted with ultrapure water to the whole amount of 20 μL. This reaction solution was reacted at 37° C. for 4 hours to overnight. Both the solutions, after the reaction, were supplemented with 1 μL of TURBO DNase (MEGAshortscript (trademark), Ambion, Inc.) and incubated at 37° C. for 15 minutes to decompose the template DNA. Then, each transcript was subjected to phenol treatment and ethanol precipitation for purification. After the precipitation, the resulting product was dissolved in 20 μL of denaturing dye (80% formamide, 0.17% XC, 0.27% BPB) and electrophoresed on a 12% polyacrylamide (29:1) denaturing gel. A gel having the size of interest was excised, and elution was performed overnight at 37° C. by the addition of 500 μL of elution buffer (0.3 M sodium acetate (pH 7.0)). The eluted RNA was subjected again to phenol extraction, diethyl ether extraction, and ethanol precipitation for purification.

[Preparation of Box C/D Mini and Box C/D Minimut]

L7Ae-binding RNA Box C/D mini (SEQ ID NO: 9) and Box C/D minimut (SEQ ID NO: 10) were separately prepared through transcription reaction using Box C/D mini primer (5'-GGGTCAGCTTTCGCATCACGCCCTATAGTGAGTCGTATTAGC-3') (SEQ ID NO: 7) or Box C/D minimut primer (5'-GGGGCAGCTTTCGCATGACGCCCTATAGTGAGTCGTATTAGC-3') (SEQ ID NO: 8) as a template and T7 RNA polymerase. Reaction was performed under conditions involving, for ^{32}P radiolabeling, 0.75 μM T7 primer (5'-GCTAATACGACTCACTATA-3') (SEQ ID NO: 6), 0.75 μM template, 40 mM Tris-Cl (pH 7.5), 5 mM DTT, 1 mM spermidine, 5 mM $MgCl_2$, 1.25 mM ATP, 1.25 mM CTP, 1.25 mM UTP, 0.25 mM GTP, [^{32}P-α]GTP (PerkinElmer Inc.), 20 U RNase inhibitor (TOYOBO CO., LTD.), and 35 ng/μL T7 RNA polymerase. In 100 μL of the system, the template was reacted at 37° C. for 3 hours to overnight. For non-labeling, transcription reaction was performed using MEGAshortscript (trademark) (Ambion, Inc.). The transcription reaction using MEGAshortscript was performed as follows. 0.75 μL of 100 μM T7 primer dissolved in ultrapure water, 0.75 μL of 100 μM Box C/D mini, 2 μL of T7 10× Reaction Buffer, 2 μL of T7 ATP Solution (75 mM) (the same recipe for CTP, GTP, and UTP), and 2 μL of T7 Enzyme Mix were mixed and adjusted with ultrapure water to the whole amount of 20 μL. This reaction solution was reacted at 37° C. for 4 hours to overnight. After the reaction, the resulting product was purified in the same way as above using electrophoresis on a 15% polyacrylamide (29:1) denaturing gel.

[Preparation of ThrRS-Binding RNA Domain 234 and Domain 2]

ThrRS-binding RNA Domain 234 (SEQ ID NO: 15) and Domain 2 (SEQ ID NO: 19) were separately prepared in the same way as in Box C/D by preparing a DNA template containing a T7 promoter, followed by transcription reaction using T7 RNA polymerase. First, 100 μL of reaction solution was prepared for preparing DNA. The reaction solution for Domain 234 contained a mixture of 1 μL of 10 ng/μL ThrRS Domain 234 template (5'-GATTGCGAACCAATTTAGCATTTGTTGGCTAAATGGTTTCGCAATGAACTGTTAAT AAACAAATTTTTCTTTGTATGTGATCTTTCGTGTGGGTCACCA-3') (SEQ ID NO: 11), 5 μL each of 10 μM ThrRS Domain 234 Fwd (5'-CTAATACGACTCACTATAGGATTGCGAACCAATTTAGCATTTGTTGG-3') (SEQ ID NO: 12) and ThrRS Domain 234 Rev (5'-TTTGCAGTGGTGACCCACACGAAAGATCAC-3') (SEQ ID NO: 13), 8 μL of 2.5 mM dNTP (TAKARA BIO INC.), 10 μL of Ex taq 10× buffer (TAKARA BIO INC.), and 0.5 μL of Ex taq DNA polymerase (TAKARA BIO INC.). 25 cycles each involving 94° C. for 30 seconds, 55° C. for 30 seconds, and 72° C. for 1 minute were performed for extension (SEQ ID NO: 14) using Gradient Master Cycler (Eppendorf). The reaction solution for Domain 2 contained a mixture of 5 μL each of 10 μM ThrRS Domain 2 Fwd (5'-CTAATACGACTCACTATAGGCGTATGTGATCTTTCGTGTGGGTCAC-3') (SEQ ID NO: 16) and ThrRS Domain 2 Rev (5'-GGCGCAGTGGTGACCCACACGAAAGATCAC-3') (SEQ ID NO: 17), 8 μL of 2.5 mM dNTP (TAKARA BIO INC.), 10 μL of Ex taq 10× buffer (TAKARA BIO INC.), and 0.5 μL of Ex taq DNA polymerase (TAKARA BIO INC.). 10 cycles each involving 94° C. for 30 seconds, 52° C. for 30 seconds, and 72° C. for 1 minute were performed for extension (SEQ ID NO: 18) using Gradient Master Cycler (Eppendorf). After the reaction, each extension product was subjected to phenol treatment, diethyl ether treatment, and ethanol precipitation and dissolved in 10 μL of ultrapure water. The solution was used as a template for transcription. Transcription reaction and purification were performed in the same way as in Box C/D using a 12% polyacrylamide (29:1) denaturing gel for Domain 234 and a 15% polyacrylamide (29:1) denaturing gel for Domain 2.

[Preparation of L7Ae]

The protein L7Ae used in the RNA-protein complex interacting motif was expressed (SEQ ID NO: 66) using plasmids kindly provided by Dr. Alexander Huttenhofer. The plasmids were prepared by amplifying an insert from *A. fulgidus* using primers L7Ae Fwd (5'-CTGACATATGTACGTGAGATTTGAGGTTC 3') (SEQ ID NO: 64) and L7Ae Rev (5'-CTGACTCGAGTTACTTCTGAAGGCCTTTAATC-3') (SEQ ID NO: 65) and incorporating the insert into a pET-28b+ vector (Novagen) cleaved with restriction enzymes NdeI and XhoI. Expression and purification methods will be shown below.

First, *E. coli* BL21(DE3)pLysS was transformed with the plasmids. The obtained colonies were inoculated to 5 mL of LB medium containing 25 μg/mL kanamycin and 100 μg/mL chloramphenicol and shake-cultured overnight at 37° C. Subsequently, the whole amount of the culture solution was subcultured in 500 mL of LB medium containing 25 μg/mL kanamycin and 100 μg/mL chloramphenicol. The solution was shake-cultured at 37° C. until O.D.$_{600}$ of 0.6 to 0.7 and then shake-cultured overnight at 30° C. after addition of 500 μL of 1 M IPTG (final concentration: 1 mM) for expression induction. The bacterial cells were collected by centrifugation (4° C., 6000 rpm, 20 min) and sonicated by the addition of 5 mL of a sonication buffer (50 mM Na phosphate, 0.3 M NaCl, pH 8.0) to disrupt the bacterial cells. The sonication was performed by repeating 6 times the procedure of cooling on ice, followed by ultrasonic application for 15 seconds. Then, impure proteins were denatured at 80° C. for 15 minutes. The supernatant was collected by centrifugation (4° C., 6000 rpm, 20 min). Histidine-tagged proteins were purified by the batch method using an Ni-NTA column (QIAGEN GmbH). Specifically, the supernatant and 1 mL of Ni-NTA were first mixed and stirred at 4° C. for 1 hour. Then, the mixture was charged into a column and washed twice with 4 mL of wash buffer (50 mM Na phosphate, 0.3 M NaCl, 20 mM imidazole, pH 8.0). Stepwise elution was performed using two runs of 1 mL each of 50 mM, 100 mM, 200 mM, and 300 mM imidazole elution buffers (prepared by adding imidazole to 50 mM Na phosphate, 0.3 M NaCl (pH 8.0)). 17% SDS-PAGE was used for confirmation. Subsequently, proteins were concentrated using Microcon YM-3 (Millipore Corp.), and the concentrate was replaced by a dialysis buffer (20 mM Hepes-KOH, 1.5 mM $MgCl_2$, 150 mM KCl, 5% glycerol (pH 7.5)). Moreover, the protein concentration was determined by the Bradford method using Protein Assay (BIO-RAD LABORATORIES INC.).

[Preparation of ThrRS]

The protein ThrRS used in the RNA-protein complex interacting motif was expressed (SEQ ID NO: 67) using plasmids kindly provided by Dr. Yoshihiro Shimizu. The plasmids were prepared by extracting ThrRS from *E. coli* and incorporating it into pQE-30 vectors (QIAGEN GmbH). Expression and purification methods will be shown below.

First, *E. coli* M15(pREP4) was transformed with the plasmids. The obtained colonies were inoculated to 3 mL of LB medium containing 50 μg/mL ampicillin and shake-cultured overnight at 37° C. Subsequently, the whole amount of the culture solution was subcultured in 50 mL of LB medium containing 50 μg/mL ampicillin. The solution was shake-cultured at 37° C. until O.D.$_{600}$ of 0.4 to 0.6 and then shake-cultured overnight at 37° C. after addition of 25 μL of 1 M IPTG (final concentration: 0.5 mM) for expression induction. The bacterial cells were collected by centrifugation (4° C., 6000 rpm, 20 min) and sonicated by the addition of 5 mL of a sonication buffer (50 mM Na phosphate, 0.3 M NaCl, pH 8.0) to disrupt the bacterial cells. The sonication was performed by repeating 6 times the procedure of cooling on ice, followed by ultrasonic application for 15 seconds. Then, impure proteins were denatured at 80° C. for 15 minutes. The supernatant was collected by centrifugation (4° C., 6000 rpm, 20 min). Histidine-tagged proteins were purified by the same batch method as above using an Ni-NTA column (QIAGEN GmbH). 8% SDS-PAGE was used for confirmation. Subsequently, proteins were concentrated using Microcon YM-30 (Millipore Corp.), and the concentrate was replaced by a dialysis buffer (25 mM Hepes-KOH, 5 mM $MgCl_2$, 50 mM KCl, 1 mM DTT, 5% glycerol (pH 7.5)). Moreover, the protein concentration was determined by the Bradford method using Protein Assay (BIO-RAD LABORATORIES INC.).

Example 2

[Confirmation of RNP Complex Formation by EMSA (Electrophoretic Mobility Shift Assay)]

[EMSA on Box C/D, Box C/D Mini, and Box C/D Minimut]

The reaction of L7Ae with Box C/D, Box C/D mini, or Box C/D minimut was performed at a final concentration of 10 nM, 25 nM, or 25 nM RNA, respectively. The reaction was performed as follows under conditions involving 10 nM or 25 nM RNA, 20 mM Hepes-KOH, 150 mM KCl, 1.5 mM $MgCl_2$, 2 mM DTT, 0.001 U/mL tRNA, 3% glycerol, and 0 to 500 nM protein. First, 1 μL of ^{32}P-labeled RNA was denatured at 80° C. for 5 minutes and then supplemented with 4 μL of 5× binding buffer (100 mM Hepes-KOH (pH 7.5), 750 mM KCl, 7.5 mM $MgCl_2$, 10 mM DTT, 0.005 U/ρL tRNA, 15% glycerol) and ultrapure water. Then, the solution was mixed with the protein to adjust the whole amount to 20 μL. The reaction solution was left on ice for 60 minutes. 2 μL of dye (0.25% BPB, 0.25% XC, 30% glycerol) was added thereto, and the mixture was electrophoresed on a 8% nondenaturing polyacrylamide gel at 4° C. for 3 to 4 hours. Then, the gel was dried for 1 hour using a gel drier and analyzed for its radiation dose intensity using Bio-Imaging Analyzer (BAS2500; FUJIFILM) (FIGS. **4** and **5**).

As a result, both Box C/D and Box C/D mini were confirmed to increase the band in an L7Ae protein concentration-dependent manner. This indicates that Box C/D or Box C/D mini binds to L7Ae. On the contrary, no such increase in band was seen in the mutant Box C/D minimut, demonstrating that it does not bind to L7Ae at these protein concentrations.

[EMSA on Domain 234 and Domain 2]

The reaction of ThrRS with Domain 234 or Domain 2 was performed as follows under conditions involving final concentrations of 20 nM RNA, 25 mM Hepes-KOH, 50 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 5% glycerol, and 0 to 40 μM protein. First, 4 μL of 200 nM ^{32}P-labeled RNA was denatured at 80° C. for 5 minutes and then supplemented with 4 μL of 5× binding buffer (75 mM Hepes-KOH, 250 mM KCl, 25 mM $MgCl_2$, 5 mM DTT, 25% glycerol) and ultrapure water. Then, the solution was mixed with the protein to adjust the whole amount to 20 μL. The reaction solution was left on ice for 60 minutes. 2 μL of dye (0.25% BPB, 0.25% XC, 30% glycerol) was added thereto, and the mixture was electrophoresed on a 12% nondenaturing polyacrylamide gel at 4° C. for 3 to 4 hours. Then, the gel was dried for 1 hour using a gel drier and analyzed for its radiation dose intensity using Bio-Imaging Analyzer (BAS2500; FUJIFILM) (FIGS. **6** and **7**).

As a result, both ThrRS Domain 234 and ThrRS Domain 2 were confirmed to increase the band in a ThrRS protein concentration-dependent manner. This indicates that ThrRS Domain 234 or Domain 2 binds to ThrRS. Particularly, a supershifted band was seen in the ThrRS Domain 2. This suggests that ThrRS bound to Domain 2 was dimerized. As is also evident from the degree of band smear, Domain 234 has stronger binding than only Domain 2.

Example 3

[Preparation of Original EGFP and Protein-Responsive Artificial RNA Switches]

Original EGFP and protein-responsive artificial RNAs were prepared by performing PCR twice or three times using pEGFP (Clontech).

[Preparation of Original EGFP]

pEGFP was used as a template to perform 1st PCR using EGFP 1st Fwd (5'-AAGGAGATATACCAATGGTGAGCAAGGGCGAG-3') (SEQ ID NO: 20) and EGFP Rev (5'-TATTCATTACCCGGCGGCGGTCACGAA-3') (SEQ ID NO: 22) as primers. 50 μL of reaction solution contained a mixture of 1 ng of template, 1.5 μL of 10 μM each DNA primers, 5 μL of 2 mM dNTPs, 5 μL of 10×KOD-PLUS-buffer ver. 2, 2 μL of 25 mM $MgSO_4$, and 1 μL of KOD-PLUS-DNA polymerase. Reaction was performed by initially performing incubation at 94° C. for 2 minutes and then 20 cycles each involving 94° C. for 15 seconds, 50° C. for 30 seconds, and 68° C. for 1 minute.

In the description below, only a template and primers will be shown because PCR was performed under the same conditions as above.

After the reaction, the reaction solution was subjected to phenol treatment and ethanol precipitation and dissolved in a nondenaturing dye (30% glycerin, 0.075% xylene cyanol, 0.075% bromophenol blue, 69.85% ultrapure water). The band of interest was separated and excised using low melting point agarose SEAPLAQUE GTG AGAROSE (FMC Corp.). The excised agarose fragment was supplemented with 200 μL of TE, then incubated at 65° C. for 30 minutes, and then subjected to 3 phenol treatments, diethyl ether treatment, and ethanol precipitation for DNA purification.

Next, the product was used as a template to perform 2nd PCR using Universal primer (5'-GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAAT TTTGTTTAACTTTAAGAAGGAGATATACCA-3') (SEQ ID NO: 21) and EGFP Rev as primers. After the reaction, separation and purification were performed in the same way as above, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 23). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). The transcription reaction using MEGAscript was performed in the same way as in MEGAshortscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 24) obtained through the transcription reaction was purified using RNeasy MinElute (trademark) Cleanup Kit (QIAGEN GmbH). The purification using RNeasy MinElute (trademark) Cleanup Kit was performed as follows.

The transcription reaction solution was adjusted to 100 μL by the addition of 80 μL of ultrapure water, further supplemented with 350 μL of Buffer RLT, and sufficiently mixed. 250 μL of ethanol was added thereto and completely mixed by pipetting. The sample was applied to RNeasy MinElute Spin Column loaded in a 2-mL collection tube and centrifuged at 10,000 rpm for 15 seconds using a high-speed refrigerated microcentrifuge MX-100 (TOMY SEIKO CO., LTD.), and the flow-through fraction was discarded. The spin column was transferred to a new 2-mL collection tube, and 500 μL of Buffer RPE was added onto the spin column using a pipette. The sample was centrifuged at 10,000 rpm for 15 seconds, and the flow-through fraction was discarded. After addition of 500 μL of 80% ethanol to the RNeasy MinElute Spin Column, the sample was centrifuged at 10,000 rpm for 2 minutes, and the flow-through fraction was discarded. The RNeasy MinElute Spin Column was transferred to a new 2-mL collection tube. The sample was centrifuged at 14,000 rpm for 5 minutes with the spin column cap opened, and the flow-through fraction was discarded. The spin column was transferred to a new 1.5-mL collection tube, and 20 μL of ultrapure water was added to the center of the silica gel membrane. The sample was centrifuged at 14,000 rpm for 5 minutes for elution. This eluate was used in concentration measurement using DU640 SPECTROPHOTOMETER.

[Preparation of L7-UTR2]

The EGFP 1st PCR product was used as a template to perform 2nd PCR using L7-UTR2 2^{nd} Fwd (5'-GGAGACCACAACGGTTTCCCTCGGGCGTGATGCGAAAGCTGACCCAGAAGGAGA TATACCAATGGTGAGC-3') (SEQ ID NO: 25) and EGFP Rev as primers. Next, the resulting product was used as a template to perform 3rd PCR using stem-loop primer (5'-GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCC-3') (SEQ ID NO: 26) and EGFP Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 27). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 28) obtained through the transcription reaction was purified using RNeasy MinElute (trademark) Cleanup Kit (QIAGEN GmbH), followed by concentration measurement.

[Preparation of L7-UTR5]

The EGFP 1st PCR product was used as a template to perform 2nd PCR using L7-UTR5 2^{nd} Fwd (5'-GGAGACCACAACGGTTTCCCTCGGGCGTGATGCGAAAGCTGACCCTTAAGAAGG AGATATACCAATGGTGAGC-3') (SEQ ID NO: 29) and EGFP Rev as primers. Next, the resulting product was used as a template to perform 3rd PCR using stem-loop primer and EGFP Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 30). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 31) obtained through the transcription reaction was purified in the same way as above, followed by concentration measurement.

[Preparation of L7-UTR9]

The EGFP 1st PCR product was used as a template to perform 2nd PCR using L7-UTR9 2^{nd} Fwd (5'-GGAGACCACAACGGTTTCCCTCGGGCGTGATGCGAAAGCTGACCCAACTTTAAGA AGGAGATATACCAATGGTGAGC-3') (SEQ ID NO: 32) and EGFP Rev as primers. Next, the resulting product was used as a template to perform 3rd PCR using stem-loop primer and EGFP Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 33). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 34) obtained through the transcription reaction was purified in the same way as above, followed by concentration measurement.

[Preparation of L7-UTR13]

The EGFP 1st PCR product was used as a template to perform 2nd PCR using L7-UTR13 2^{nd} Fwd (5'-GGAGACCACAACGGTTTCCCTCGGGCGTGATGCGAAAGCTGACCCGTTTAACTTT AAGAAGGAGATATACCAATGGTGAGC-3') (SEQ ID NO: 35) and EGFP Rev as primers. Next, the resulting product was used as a template to perform 3rd PCR using stem-loop primer and EGFP Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 36). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 37) obtained through the transcription reaction was purified in the same way as above, followed by concentration measurement.

[Preparation of L7-UTR2 Mut]

The EGFP 1st PCR product was used as a template to perform 2nd PCR using L7-UTR2 mut 2^{nd} Fwd (5'-GGAGACCACAACGGTTTCCCTCGGGCGTCATGCGAAAGCTGCCCCAGAAGGAGA TATACCAATGGTGAGC-3') (SEQ ID NO: 38) and EGFP Rev as primers. Next, the resulting product was used as a template to perform 3rd PCR using stem-loop primer and EGFP Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 39). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 40) obtained through the transcription reaction was purified in the same way as above, followed by concentration measurement.

[Preparation of L7-UTR2 Minimut]

The EGFP 1st PCR product was used as a template to perform 2nd PCR using L7-UTR2 minimut 2^{nd} Fwd (5'-GGAGACCACAACGGTTTCCCTCGGGGAAACCCAGAAGGAGATATACCAATGGTG AGC-3') (SEQ ID NO: 41) and EGFP Rev as primers. Next, the resulting product was used as a template to perform 3rd PCR using stem-loop primer and EGFP Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 42). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 43) obtained through the transcription reaction was purified in the same way as above, followed by concentration measurement.

[Preparation of L7-ORF (Box C/D GFP)]

pEGFP was used as a template to perform 1st PCR using L7-ORF 1st Fwd (5'-AAGGAGATATACCAATGGGGCGTGATGCGAAAGCTGACCCTGTGAGCAAGGGCG AGGAG-3') (SEQ ID NO: 44) and EGFP Rev as primers. Next, the resulting product was used as a template to perform 2nd PCR using Universal primer and EGFP Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 45). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 46) obtained through the transcription reaction was purified in the same way as above, followed by concentration measurement.

[Preparation of L7-ORF Mut (Box C/D Mut GFP)]

pEGFP was used as a template to perform 1st PCR using L7-ORF mut 1st Fwd (5'-AAGGAGATATACCAATGAGGGGAAACCCAGTGAGCAAGGGCGAGGAG-3') (SEQ ID NO: 47) and EGFP Rev as primers. Next, the resulting product was used as a template to perform 2nd PCR using Universal primer and EGFP Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 48). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 49) obtained through the transcription reaction was purified in the same way as above, followed by concentration measurement.

[Preparation of ThrRS-UTRW]

pEGFP was used as a template to perform 1st PCR using ThrRS-UTRW 1st Fwd (5'-GTGATCTTTCGTGTGGGTCACCACTGCAAATAAGGATATAAAATGGTGAGCAAGG GCGAG-3') (SEQ ID NO: 50) and EGFP Rev as primers. Next, the resulting product was used as a template to perform 2nd PCR using ThrRS Domain 234 template and EGFP Rev as primers. Next, the resulting product was used as a template to perform 3rd PCR using ThrRS Domain 234 Fwd and EGFP Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 51). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 52) obtained through the transcription reaction was purified in the same way as above, followed by concentration measurement.

[Preparation of ThrRS-UTR2]

The EGFP 1st PCR product was used as a template to perform 2nd PCR using ThrRS-UTR2 2nd Fwd (5'-GGAGACCACAACGGTTTCCCTCGGCGTATGTGATCTTTCGTGTGGGTCACCACTG CGCCAGAAGGAGATATACCAATGGTG-3') (SEQ ID NO: 53) and EGFP Rev as primers. Next, the resulting product was used as a template to perform 3rd PCR using stem-loop primer and EGFP Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 54). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 55) obtained through the transcription reaction was purified in the same way as above, followed by concentration measurement.

[Preparation of ThrRS-UTR2 Mut]

The EGFP 1st PCR product was used as a template to perform 2nd PCR using ThrRS-UTR2 mut 2nd Fwd (5'-GGAGACCACAACGGTTTCCCTCGGCGTATGTGATCTTTCATGTGGGTCACCACTG CGCCAGAAGGAGATATACCAATGGTG-3') (SEQ ID NO: 56) and EGFP Rev as primers. Next, the resulting product was used as a template to perform 3rd PCR using stem-loop primer and EGFP Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 57). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 58) obtained through the transcription reaction was purified in the same way as above, followed by concentration measurement.

[Preparation of ON Switch]

pEGFP was used as a template to perform 1st PCR using ON switch 1st Fwd (5'-AAGGAGATATACCAATGCAGCTTTCGCATCACGTGAGCAAGGGCGAGGAG-3') (SEQ ID NO: 59) and EGFP Rev as primers. Next, the resulting product was used as a template to perform 2nd PCR using Universal primer and EGFP Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 60). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 61) obtained through the transcription reaction was purified in the same way as above, followed by concentration measurement. This RNA had, in the open reading frame (ORF), an insert of a sequence to be hybridized with antisence shown below.

[Preparation of Antisence]

Antisence was prepared using T7 primer and antisence primer (5'-GGTGGGTCAGCTTTCGCATCACGCCCACCTATAGTGAGTCGTATTAGC-3') (SEQ ID NO: 62), and MEGAshortscript (trademark) (Ambion, Inc.). This antisence contains therein an L7Ae-binding site (Box C/D mini). After the reaction, the reaction product was purified (SEQ ID NO: 63) by electrophoresis on a 15% polyacrylamide (29:1) denaturing gel in the same way as in Box C/D mini.

Example 4

[Translational Regulation Assay on L7-UTR]

Translational regulation assay on L7-UTR was conducted using PURE system (Post Genome Institute Co., Ltd.). All ON-to-OFF translational regulations were assayed as follows. First, 5 μL of Solution A, 1 μL of 3.75 μM RNA, and the protein were mixed and adjusted with ultrapure water to the whole amount of 8 μL. The solution was left at 4° C. for 1 hour. Then, 2 μL of Solution B was added thereto and reacted at 37° C. for 75 minutes. After the reaction, the solution was adjusted with ultrapure water to 200 μL and measured at an excitation wavelength of 485 nm and an absorption wavelength of 535 nm using infinite F200 (TECAN Trading AG). The secondary structure of EGFP UTR used as a control is shown in FIG. **8**A. The secondary structure of L7-UTR2 is shown in FIG. **8**B; the secondary structure of L7-UTR5 is shown in FIG. **8**E; the secondary structure of L7-UTR9 is shown in FIG. **8**F; and the secondary structure of L7-UTR13 is shown in FIG. **8**G. In these diagrams, reference numeral **4** depicts an open reading frame; reference numeral **3** depicts a ribosome-binding site; reference numeral **2** depicts an RNA-protein complex interacting motif-derived nucleotide sequence; and reference numeral **7** depicts an enhancer. All the RNAs had an L7Ae-binding motif (Box C/D) nucleotide sequence inserted in EGFP 5'-UTR and were designed to have a distance of 2 bases, 5 bases, 9 bases, or 13 bases between the RBS and the motif.

As is evident from the assay results, the incorporation of the L7Ae motif inhibits translation in response to increase in protein concentration. As is also evident, translational inhibitory effect decreases depending on the distance between the motif and the RBS (FIG. **9**). The secondary structure of L7-UTR2 mut of L7Ae is shown in FIG. **8**C, and the secondary structure of L7-UTR2 minimut is shown in FIG. **8**D. These had a mutation in the L7Ae-binding site of L7-UTR2. Although slight translational inhibition was also observed in these mutants, this translational inhibitory effect was shown to be smaller than that in L7-UTR2 (FIG. **10**).

Competition assay using L7-UTR2 was conducted using Box C/D as a competitor. Specifically, 5 μL of Solution A, 1 μL of 3.75 μM RNA, 1 μL of 10 to 100 μM competitor, and 1 μL of 50 μM protein were mixed and adjusted with ultrapure water to the whole amount of 8 μL. The solution was left at 4° C. for 1 hour. Then, 2 μL of Solution B was added thereto and reacted at 37° C. for 75 minutes. After the reaction, measurement was performed in the same way as above. As is evident from the results, the efficiency of translation decreased due to the addition of the protein shows recovery by the addition of the competitor Box C/D. This result suggests that this translational inhibition is influenced by L7Ae and the L7Ae-binding site (FIG. **11**). These assay results indicated that ribosome binding to the mRNA can be regulated by the steric hindrance of the protein as designed.

[Translational Regulation Assay on ThrRS-UTR]

The same assay as in L7-UTR was conducted on ThrRS-UTR. Assay conditions were the same as in L7-UTR. The secondary structures of ThrRS-UTRW, ThrRS-UTR2, and ThrRS-UTR2 mut are shown in FIG. **12**. In these diagram, reference numeral **4** depicts an open reading frame; reference numeral **3** depicts a ribosome-binding site; and reference numeral **2** depicts an RNA-protein complex interacting motif-derived nucleotide sequence. As in L7-UTR, each protein-binding RNA was inserted in 5'-UTR: in ThrRS-UTRW (FIG. **12**A), Domain 234 was inserted in 5'-UTR; and in ThrRS-UTR2 (FIG. **12**B), Domain 2 was inserted in 5'-UTR. ThrRS-UTR2 mut (FIG. **12**C) had a mutation in the ThrRS-binding site (Domain 2) of ThrRS-UTR2 and was used as a mutant.

As is evident from the results, translation is inhibited depending on the concentration of the ThrRS protein. ThrRS-UTRW had larger inhibitory effect than that of ThrRS-UTR2, owing to difference in binding affinity. Moreover, as in L7-UTR, smaller translational inhibitory effect was observed in the mutant (FIG. **13**). These results indicated that for ON-to-OFF translational regulation, the input protein can be selected arbitrarily by exchanging the protein-binding motif on the mRNA.

[Translational Regulation Assay on L7-ORF (Box C/D GFP)]

The same assay as above was conducted on L7-ORF (Box C/D GFP). Assay conditions were the same as in L7-UTR. The secondary structures of L7-ORF (Box C/D GFP) and L7-ORF mut (Box C/D mut GFP) are shown in FIG. **14**. In these diagram, reference numeral **4** depicts an open reading frame; reference numeral **3** depicts a ribosome-binding site; reference numeral **2** depicts an RNA-protein complex interacting motif-derived nucleotide sequence; and reference numeral **7** depicts an enhancer. Unlike L7-UTR, each protein-binding RNA motif was inserted in ORF: in L7-ORF (Box C/D GFP) (FIG. **14**A), the L7Ae-binding site (Box C/D) was inserted in ORF immediately after the start codon; and in L7-ORF mut (Box C/D mut GFP) (FIG. **14**B) used as a mutant, Stem-Loop was inserted in this site. As a result, translation is inhibited with increase in the concentration of the L7Ae protein. Moreover, as in L7-UTR and ThrRS-UTR, smaller translational inhibitory effect was shown in the mutant. These results indicated that the L7Ae protein bound to the mRNA open reading frame inhibits ribosome entry (FIG. **15**).

[Translational Regulation Assay on ON Switch]

To assay OFF-to-ON translational regulation, 5 μL of Solution A, 1 μL of 500 nM RNA, 1 μL of 10 μM antisence RNA, and the protein were mixed and adjusted with ultrapure water to the whole amount of 8 μL. The solution was heat-treated at 60° C. for 3 minutes and immediately cooled on ice. After the 15-minute cooling on ice, 2 μL of Solution B was added thereto and reacted at 37° C. for 75 minutes. After the reaction, measurement was performed in the same way as above. As a result, the translation inhibited due to the addition of antisence RNA showed a recovery by the addition of the protein (FIG. **16**). This is probably because L7Ae binding to antisence RNA represses the translational inhibition.

Example 5

Next, to demonstrate that the output gene is arbitrarily changed, Example is shown, in which translational regulation was performed with a red fluorescent protein DsRed-Express (DsRed-Ex) as a target, while the translational regulation/activation of two different genes was simultaneously promoted.

[Preparation of Control DsRed-Ex and Protein-Responsive Artificial RNA Switch]

Control DsRed-Ex and a protein-responsive artificial RNA were prepared by performing twice PCR using pDsRed-Ex vectors (Clontech).

[Preparation of Control DsRed-Ex]

pDsRed Ex was used as a template to perform 1st PCR using DsRed Ex 1st Fwd (5'-AAGGAGATATACCAATGGCCTCCTCCGAGGAC-3') (SEQ ID NO: 68) and DsRed Ex Rev (5'-TATTCATTACTACAGGAACAGGTGGTGGC-3') (SEQ ID NO: 69) as primers. 50 μL of reaction solution contained a mixture of 1 ng of template, 1.5 μL of 10 μM each DNA primers, 5 μL of 2 mM dNTPs, 5 μL of 10×KOD-PLUS-buffer ver. 2, 2 μL of 25 mM $MgSO_4$, and 1 μL of KOD-PLUS-DNA polymerase. Reaction was performed by initially performing incubation at 94° C. for 2 minutes and then 20 cycles each involving 94° C. for 15 seconds, 50° C. for 30 seconds, and 68° C. for 1 minute.

In the description below, only a template and primers will be shown because PCR was performed under the same conditions as above. After the reaction, the reaction solution was subjected to phenol treatment and ethanol precipitation and dissolved in a nondenaturing dye (30% glycerin, 0.075% xylene cyanol, 0.075% bromophenol blue, 69.85% ultrapure water). The band of interest was separated and excised using low melting point agarose SEAPLAQUE GTG AGAROSE (FMC Corp.). The excised agarose fragment was supplemented with 200 μL of TE, then incubated at 65° C. for 30 minutes, and then subjected to 3 phenol treatments, diethyl ether treatment, and ethanol precipitation for DNA purification.

Next, the product was used as a template to perform 2nd PCR using Universal primer (5'-GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAAT TTTGTTTAACTTTAAGAAGGAGATATACCA-3') (SEQ ID NO: 21) and DsRed Ex Rev as primers. After the reaction, separation and purification were performed in the same way as above, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 70). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). The transcription reaction using MEGAscript was performed in the same way as in MEGAshortscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 71) obtained through the transcription reaction was purified using RNeasy MinElute (trademark) Cleanup Kit (QIAGEN GmbH). The purification using RNeasy MinElute (trademark) Cleanup Kit was performed as follows.

The transcription reaction solution was adjusted to 100 μl by the addition of 80 μL of ultrapure water, further supplemented with 350 μL of Buffer RLT, and sufficiently mixed. 250 μL of ethanol was added thereto and completely mixed by pipetting. The sample was applied to RNeasy MinElute Spin Column loaded in a 2-mL collection tube and centrifuged at 10,000 rpm for 15 seconds using a high-speed refrigerated microcentrifuge MX-100 (TOMY SEIKO CO., LTD.), and the flow-through fraction was discarded. The spin column was transferred to a new 2-mL collection tube, and 500 μL of Buffer RPE was added onto the spin column using a pipette. The sample was centrifuged at 10,000 rpm for 15 seconds, and the flow-through fraction was discarded. After addition of 500 μL of 80% ethanol to the RNeasy MinElute Spin Column, the sample was centrifuged at 10,000 rpm for 2 minutes, and the flow-through fraction was discarded. The RNeasy MinElute Spin Column was transferred to a new 2-mL collection tube. The sample was centrifuged at 14,000 rpm for 5 minutes with the spin column cap opened, and the flow-through fraction was discarded. The spin column was transferred to a new 1.5-mL collection tube, and 20 μL of ultrapure water was added to the center of the silica gel membrane. The sample was centrifuged at 14,000 rpm for 5 minutes for elution. This eluate was used in concentration measurement using DU640 SPECTROPHOTOMETER.

[Preparation of Box C/D-DsRed-Ex]

pDsRed Ex was used as a template to perform 1st PCR using Box C/D-DsRed-Ex 1st Fwd (5'-AAGGAGATATACCAATGGGGCGTGATGCGAAAGCTGACCCTGCCTCCTCCGAGG ACGTC-3') (SEQ ID NO: 72) and DsRed Ex Rev as primers. Next, the resulting product was used as a template to perform 2nd PCR using Universal primer and DsRed Ex Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 73). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 74) obtained through the transcription reaction was purified in the same way as above, followed by concentration measurement.

[Preparation of Box C/D Mutant-DsRed-Ex]

pDsRed Ex was used as a template to perform 1st PCR using Box C/D mutant 1st Fwd (5'-AAGGAGATATACCAATGAGGGGAAACCCAGCCTCCTCCGAGGACGTC-3') (SEQ ID NO: 75) and DsRed Ex Rev as primers. Next, the resulting product was used as a template to perform 2nd PCR using Universal primer and DsRed Ex Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 76). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 77) obtained through the transcription reaction was purified in the same way as above, followed by concentration measurement.

[Preparation of ON Switch]

pEGFP was used as a template to perform 1st PCR using ON switch 1st Fwd (5'-AAGGAGATATACCAATGCAGCTTTCGCATCACGTGAGCAAGGGCGAGGAG-3') (SEQ ID NO: 59) and EGFP Rev as primers. Next, the resulting product was used as a template to perform 2nd PCR using Universal primer and EGFP Rev as primers. After the reaction, separation and purification were performed, and the purification product was dissolved in ultrapure water. The solution was used as a template for transcription reaction (SEQ ID NO: 60). Transcription reaction was performed using MEGAscript (trademark) (Ambion, Inc.). RNA (SEQ ID NO: 61) obtained through the transcription reaction was purified in the same way as above, followed by concentration measurement. This RNA had, in the open reading frame (ORF), an insert of a sequence to be hybridized with antisence shown below.

[Preparation of Antisence 25 Mer]

Antisence 25 mer was prepared using T7 primer and antisence 25 mer primer (5'-GGGGTCAGCTTTCGCATCACGCCCCTATAGTGAGTCGTATTAGC-3') (SEQ ID NO: 78), and MEGAshortscript (trademark) (Ambion, Inc.). This antisence contains therein an L7Ae-binding site (Box C/D mini). After the reaction, the reaction product was purified by electrophoresis on a 15% polyacrylamide (29:1) denaturing gel in the same way as in Box C/D mini.

[Translational Regulation Assay on Box C/D-DsRed-Ex]

Translational regulation assay on Box C/D-DsRed-Ex was conducted using PURE system (Post Genome Institute Co., Ltd.). All ON-to-OFF translational regulations were assayed as follows. First, 5 μL of Solution A, 1 μL of 3.75 μM RNA, and the protein were mixed and adjusted with ultrapure water to the whole amount of 8 μL. The solution was left at 4° C. for 1 hour. Then, 2 μL of Solution B was added thereto and reacted at 37° C. for 75 minutes. After the reaction, the solution was adjusted with ultrapure water to 200 μL and measured at an excitation wavelength of 535 nm and an absorption wavelength of 595 nm using infinite F200 (TECAN Trading AG). The secondary structure of Box C/D-DsRed-Ex is shown in FIG. **17**(*a*). Moreover, the secondary structure of DsRed-Ex used as a control is shown in FIG. **17**(*c*). In these diagram, reference numeral **3** depicts a ribosome-binding site, and reference numeral **2** depicts an RNA-protein complex interacting motif-derived nucleotide sequence.

As is evident from the assay results, the incorporation of the L7Ae-binding Box C/D motif within the 5' region of mRNA ORF inhibits translation in response to increase in protein concentration. The secondary structure of Box C/D mut-DsRed-Ex is shown in FIG. **17**(*b*). This had a mutation in the L7Ae-binding site (Box C/D motif) of Box C/D-DsRed-Ex. FIG. **18** shows an added L7Ae concentration-dependent fluorescence intensity ratio to 0 μM L7Ae-derived fluorescence intensity defined as 1. Although slight translational inhibition was observed in the controls DsRed-Ex and Box C/D mut-DsRed-Ex using the high concentrations of L7Ae (5 to 10 μM), this translational inhibitory effect was shown to be significantly smaller than that in Box C/D-DsRed-Ex.

[Simultaneous Translational Regulation Assay on ON Switch and Box C/D-DsRed-Ex]

To assay the EGFP gene-targeting simultaneous translational regulation of ON switch and Box C/D-DsRed-Ex, 5 μL of Solution A, 0.5 μL of 1 μM ON switch RNA (SEQ ID NO: 61), 1 μL of 3.75 μM Box C/D-DsRed-Ex RNA (SEQ ID NO: 74), 0.5 μL of 20 μM antisence 25 mer (SEQ ID NO: 63), and the L7Ae protein (SEQ ID NO: 66) were mixed and adjusted with ultrapure water to the whole amount of 8 μL. The solution was heat-treated at 70° C. for 3 minutes and immediately cooled on ice. After the 15-minute cooling on ice, 2 μL of Solution B was added thereto and reacted at 37° C. for 75 minutes. After the reaction, the solution was adjusted with ultrapure water to 200 μL and measured at an excitation wavelength of 485 nm and an absorption wavelength of 535 nm using infinite F200 (TECAN Trading AG). Further, the solution was measured at an excitation wavelength of 535 nm and an absorption wavelength of 595 nm using infinite F200 (TECAN Trading AG). The results are shown in FIG. **19**. As a result, the translation of ON switch RNA inhibited due to the addition of antisence RNA (this inhibition was confirmed based on EGFP expression) showed a recovery by the addition of the L7Ae protein, whereas the translation of Box C/D-DsRed-Ex was repressed by the addition of the L7Ae protein. This indicates that the addition of the protein of one kind could simultaneously regulate the translations of two different mRNAs in opposite directions (translational repression/activation).

[Preparation of pcDNA-L7Ae by Restriction Enzyme Treatment]

pL7Ae was used as a template to perform PCR using Fwd (5'-CACCAAGCTTATGTACGTGAGATTTGAGGTTCC-3') (SEQ ID NO: 79) and Rev (5'-CCGCTCGAGCTTCTGAAGGCCTTTAATTCTTC-3') (SEQ ID NO: 80) as primers. 50 μL of reaction solution contained a mixture of 5 ng of template, 1.5 μL of 10 μM each DNA primers, 4 μL of 2.5 mM dNTPs, 5 μL of 10×KOD-PLUS-buffer ver. 2, 1.6 μL of 25 mM $MgSO_4$, and 1 μL of KOD-PLUS-DNA polymerase. Reaction was performed by initially performing incubation at 94° C. for 2 minutes and then 25 cycles each involving 94° C. for 15 seconds, 52° C. for 30 seconds, and 68° C. for 1 minute. The reaction product was subjected to phenol treatment, diethyl ether treatment, and ethanol precipitation for DNA purification. This purification product was dissolved in 15 μL of ultrapure water. The solution was used as a template for restriction enzyme treatment. A total of 20 μL of system involving 5 μL of template, 2 μL of buffer, 1 μL of HindIII, 1 μL of XhoI, 2 μL of 10×BSA, and 9 μL of ultrapure water was incubated at 37° C. for 2 h. The band of interest was separated and excised using low melting point agarose SEAPLAQUE GTG AGAROSE (FMC Corp.). The excised agarose fragment was supplemented with 200 μL of TE, then incubated at 65° C. for 30 minutes, and then subjected to 2 phenol treatments, diethyl ether treatment, and ethanol precipitation for DNA purification. The same restriction enzyme treatment as above was also performed on pcDNA vectors (Invitrogen Corp.). A total of 20 μL of system involving 2 ng of template, 2 μL of buffer, 1 μL of HindIII, 1 μL of XhoI, 2 μL of 10×BSA, and 13 μL of ultrapure water was incubated at 37° C. for 2 h. The band of interest was separated and excised using low melting point agarose SEAPLAQUE GTG AGAROSE (FMC Corp.). The excised agarose fragment was supplemented with 200 μL of TE, then incubated at 65° C. for 30 minutes, and then subjected to phenol treatment, diethyl ether treatment, and ethanol precipitation for DNA purification. This purification product was dissolved in 10 μL of ultrapure water and used in BAP treatment. A total of 50 μL of system involving 10 μL of template, 33 μL of ultrapure water, 2 μL of BAP, and 5 μL of buffer was incubated at 37° C. for 2 h. The band of interest was separated and excised using low melting point agarose SEAPLAQUE GTG AGAROSE (FMC Corp.). The excised agarose fragment was supplemented with 200 μL of TE, then incubated at 65° C. for 30 minutes, and then subjected to 2 phenol treatments, diethyl ether treatment, and ethanol precipitation for DNA purification.

A total of 4 μL involving 1 μL of insert, 1 μL of vector, and 2 μL of Ligation High was incubated at 16° C. for 2 h, and JM109 was transformed with the ligation product. pcDNA-L7Ae was purified by miniprep.

[Preparation of Box C/D-GFP by Site-Directed Mutagenesis]

Full-length pEGFP-N1 (Clontech) plasmids were amplified as a template using phosphorylated primers and a high-fidelity PCR enzyme KOD-PLUS- (TOYOBO CO., LTD.). The PCR product was self-ligated using Ligation High (TOYOBO CO., LTD.) to prepare Box C/D-GFP. Fwd Box C/D-EGFP primer (5'-GGGCGTGATGCGAAAGCTGACCCTGTGAGCAAGGGCGAGGAGCTG-3') (SEQ ID NO: 81) and Rev Box C/D-EGFP primer (5'-CATGGTGGCGACCGGTGGATC-3') (SEQ ID NO: 82) were used. 50 μL of reaction solution contained a mixture of 5 ng of template, 1.5 μL of 10 μM each DNA primers, 4 μL of 2.5 mM dNTPs, 5 μL of 10×KOD-PLUS- buffer, and 1 μL of KOD-PLUS-DNA polymerase. Reaction was performed by initially performing incubation at 94° C. for 2 minutes and then 25 cycles each involving 98° C. for 10 seconds and 68° C. for 4 minutes. Next, the template plasmid was digested by the action of a restriction enzyme DpnI specifically decomposing methylated DNA. Further, the PCR product was self-circularized by self-ligation.

[Preparation of Box C/D Mut GFP by Site-Directed Mutagenesis]

Fwd Box C/D mut EGFP primer (5'-AGGGGAAACCCAGTGAGCAAGGGCGAGGAGCTG-3') (SEQ ID NO: 83) was prepared and used in gene amplification with pEGFP-N1 (Clontech) plasmids as a template. The other procedures were performed in the same way as above to prepare Box C/D mut GFP.

Example 6

Western blotting was conducted for confirming L7Ae expression in cultured human cancer cells.

On the day before transfection, cervical cancer-derived HeLa cells were seeded at a concentration of 0.5×10^6 cells/well to a 6-well plate and cultured in a 37° C. CO_2 incubator. Next day, the cells were transfected using Lipofectamine 2000 (trademark) (Invitrogen Corp.). The amount of pcDNA-A (FIG. **20**) (SEQ ID NO: 84) or L7Ae expression vector pcDNA-L7Ae (SEQ ID NO: 85) (FIG. **21**) added was set to 1 μg, 2 μg, and 4 μg. According to this amount, the amount of Lipofectamine 2000 was set to 2.5 μl, 5 μl, and 10 μl. These DNA-lipid complexes were incubated at room temperature for 20 minutes and added dropwise to the cells. In this context, the L7Ae expression vector pcDNA-L7Ae is a plasmid vector in which the L7Ae gene is inserted downstream of the CMV promoter of a pcDNA3.1 vector (Invitrogen Corp.). After 4 hours, medium replacement was performed.

29 hours after the transfection, the wells were washed twice with PBS and then supplemented with 300 μl of RIPA buffer (1×PBS, 1% NP40, 0.5% Sodium deoxycholate, 0.1% SDS), and the cells were dissociated from the wells using a cell scraper. The lysates were disrupted using a syringe equipped with 21G needle. After addition of 10 μl of 10 mg/ml PMSF, the mixture was left standing on ice for 30 minutes, and supernatants were collected by centrifugation (4° C., 15000 g, 20 min) Likewise, 53 hours after the transfection, proteins were collected. The protein concentration

was determined by the Lowry method using DC-Protein Assay (BIO-RAD LABORATORIES INC.).

L7Ae was detected by western blotting. The proteins extracted from the cells were deployed by SDS-PAGE and subjected to western blotting. A primary antibody Anti-c-Myc (Ab-1) (Calbiochem) (1/500) and a secondary antibody Goat Anti-Mouse IgG (H+L)-HRP conjugate (BIO-RAD LABORATORIES INC.) (1/2000) were used. A color was developed using ECL Plus (trademark) (GE Healthcare) and detected using LAS3000 (FUJIFILM). From these results, L7Ae expression caused by pcDNA-L7Ae introduction could be confirmed in the HeLa cells. Protein extraction from cells and L7Ae detection shown below were performed in the same way as above. FIG. **22** is a diagram showing intracellular L7Ae expression. In the diagram, the lane 1 was supplemented with 4 μg of pcDNA-A:10 μl of Lipofectamine; the lane 2 was supplemented with 2 μg of pcDNA-A:5 μl of Lipofectamine; the lane 3 was supplemented with 1 μg of pcDNA-A:2.5 μl of Lipofectamine; the lane 4 was supplemented with 4 μg of pcDNA-L7Ae:10 μl of Lipofectamine; the lane 5 was supplemented with 2 μg of pcDNA-L7Ae:5 μl of Lipofectamine; and the lane 6 was supplemented with 1 μg of pcDNA-L7Ae:2.5 μl of Lipofectamine. This diagram demonstrated that L7Ae is expressed within human cancer cells 29 hours after the transfection. Even 53 hours after the transfection, its expression was confirmed, though the expression level was decreased.

To evaluate the influence of L7Ae expression on cytotoxicity, WST1 assay was conducted. On the day before transfection, HeLa cells were seeded at a concentration of 1.0×10^4 cells/well to a 96-well plate and cultured in a 37° C. CO_2 incubator. Next day, the cells were transfected using Lipofectamine 2000 (trademark) (Invitrogen Corp.). The amount of pcDNA-A or pcDNA-L7Ae added was set to 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, and 0.40 μg, and 0.25 μl of Lipofectamine 2000 was added to each sample. These DNA-lipid complexes were incubated at room temperature for 20 minutes and added dropwise to the cells. After 4 hours, medium replacement was performed. 24 hours after the transfection, the number of live cells was measured by WST1 assay using Cell Proliferation Reagent WST-1 (trademark) (Roche Diagnostics Corp.). It was shown that L7Ae expression has no cytotoxicity within this time. FIG. **23** is a diagram showing that L7Ae expression has no cytotoxicity 24 hours after the transfection.

The L7Ae-dependent repression of Box C/D-GFP protein expression was measured by western blotting.

On the day before transfection, HeLa cells were seeded at a concentration of 0.5×10^6 cells/well to a 6-well plate and cultured in a 37° C. CO_2 incubator. Next day, the cells were transfected using Lipofectamine 2000 (trademark) (Invitrogen Corp.). 0, 0.5, 1.0, 1.5, or 2.0 μg of pcDNA-L7Ae was added to 1.0 μg of Box C/D-GFP (FIG. **24**) (SEQ ID NO: 86) or Box C/D mut GFP (FIG. **25**) (SEQ ID NO: 87), and 5 μl of Lipofectamine 2000 was added to each sample. These DNA-lipid complexes were incubated at room temperature for 20 minutes and added dropwise to the cells. After 4 hours, medium replacement was performed. 24 hours after the transfection, proteins were extracted in the same way as above, and L7Ae (FIG. **26**) and EGFP (FIG. **27**) were detected by western blotting. A primary antibody GFP (B-2) SC9996 (Santa Cruz Biotechnology, Inc.) (1/200) and a secondary antibody Goat Anti-Mouse IgG (H+L)-HRP conjugate (BIO-RAD LABORATORIES INC.) (1/2000) were used for EGFP. The L7Ae expression-dependent repression of EGFP expression specific for Box C/D-GFP could be confirmed. FIG. **26** is a diagram showing L7Ae expression. From this diagram, the coexpression of L7Ae with Box C/D-GFP or Box C/D mut GFP could be confirmed by western blotting to exhibit no difference in L7Ae expression level therebetween. FIG. **27** is a diagram showing L7Ae-dependent translational repression of EGFP. As is evident from this diagram, the expression of pcDNA-L7Ae significantly represses Box C/D-GFP expression. On the other hand, these results demonstrated that expression repressive effect on Box C/D mut GFP is smaller than that on Box C/D-GFP.

Moreover, the bands obtained by western blotting were analyzed using LAS3000 (FUJIFILM) and Multi Gauge Ver 3.0 (FUJIFILM). The value of 1.0 μg of Box C/D-GFP or Box C/D mut GFP supplemented with 0.5 μg of pcDNA-L7Ae (+) was calculated with that free from pcDNA-L7Ae (−) defined as 1. The results of this quantification by western blotting are shown in FIG. **28**.

The L7Ae-dependent repression of protein expression was measured by FACS.

On the day before transfection, HeLa cells were seeded at a concentration of 0.5×10^5 cells/well to a 24-well plate and cultured in a 37° C. CO_2 incubator. Next day, the cells were transfected using Lipofectamine 2000 (trademark) (Invitrogen Corp.). 0, 0.05, 0.10, 0.15, 0.20, 0.40, 0.80, or 1.60 μg of pcDNA-A or pcDNA-L7Ae was added to 0.2 μg of Box C/D-GFP or Box C/D mut GFP, and 1 μl of Lipofectamine 2000 was added to each sample. These DNA-lipid complexes were incubated at room temperature for 20 minutes and added dropwise to the cells. After 4 hours, medium replacement was performed.

24 hours after the transfection, the medium was discarded, and the cells were dissociated from the wells using 200 μl of Trypsin EDTA and supplemented with 200 μl of DMEM/F12. The mixture was transferred to a FACS tube and analyzed using FACS Aria (BD). In this context, FACS is a method which involves irradiating free cells passing through a thin tube with laser beam and analyzing cell fractionation based on the intensity of fluorescence generated from the cells. Here, live cells were gated, and 10000 cells were measured by FITC. The results demonstrated that the repression of EGFP expression occurs in a manner specific for the cells transfected with pcDNA-L7Ae and Box C/D-GFP. More detailed analysis was achieved by comparison with the western blotting results. FIG. **29** is a graph showing the measurement results. In the diagram, Mock represents those transfected with only Lipofectamine 2000 (trademark) (Invitrogen Corp.) without the addition of DNA; and 0, 0.10, 0.20, or 0.80 μg of pcDNA-A (shown in the left columns) or pcDNA-L7Ae (shown in the right columns) was added to Box C/D-GFP (solid line) and Box C/D mut GFP (dotted line) fixed to 0.2 μg. FIG. **30** shows results of quantifying L7Ae expression-dependent repression specific for Box C/D-GFP translation by analysis based on the FACS data of FIG. **29**.

Next, change in mRNA level during the L7Ae-dependent repression of protein expression was measured by real-time PCR.

On the day before transfection, HeLa cells were seeded at a concentration of 0.5×10^6 cells/well to a 6-well plate and cultured in a 37° C. CO_2 incubator. 0, 0.5, 1.0, or 2.0 μg of pcDNA-L7Ae was added to 1.0 μg of Box C/D-GFP or Box C/D mut GFP, and 5 μl of Lipofectamine 2000 was added to each sample. Moreover, 0, 0.5, 1.0, or 2.0 μg of pcDNA-L7Ae or pcDNA-A was added to 1.0 μg of Box C/D-GFP, and 5 μl of Lipofectamine 2000 was added to each sample. These DNA-lipid complexes were incubated at room temperature for 20 minutes and added dropwise to the cells. After 4 hours, medium replacement was performed. 24 hours after the transfection, RNA extraction and DNA removal were performed using RNAqueous 4PCR Kit (trademark) (Ambion, Inc.).

1.5 μg (or 0.5 μg) of the extracted RNA was used as a template to synthesize cDNA using High-Capacity cDNA Reverse Transcription Kits (trademark) (Applied Biosystems Inc.), random primers, and reverse transcriptase. Real-time PCR was performed by the intercalation method using 1/20000 diluted cDNA as a template and LightCycler 480 SYBR Green I Master (trademark) (Roche Diagnostics Corp.). PCR reaction and real-time fluorescence detection were performed using LightCycler 480 (trademark) (Roche Diagnostics Corp.). Reaction conditions involved an initial denaturation step set to 95° C. for 5 minutes and an amplification step set to 95° C. for 10 seconds in denaturation, 60° C. for 10 seconds in annealing, and 72° C. for 3 seconds in extension, and this cycle was performed 45 times. Melting curve analysis was conducted at 95° C. for 5 seconds in denaturation, 65° C. for 15 seconds in annealing, and target temperature set to 98° C., and finally, the reaction solution was cooled at 50° C. for 10 seconds to terminate the measurement. The Ct value was determined by the Second Derivative Maximum method. The target EGFP gene was amplified using 481P Fwd (5'-CAAGGAGGACGGCAACA-3') (SEQ ID NO: 88) and Rev (5'-CCTTGATGCCGTTCTTCTGC-3') (SEQ ID NO: 89). A reference gene GAPDH was amplified using GAPDH Fwd (5'-AGCCACATCGCTCAGACAC-3') (SEQ ID NO: 90) and Rev (5'-GCCCAATACGACCAAATCC-3') (SEQ ID NO: 91). The amplification product was confirmed to be a single target product by melting curve analysis and electrophoresis. The results were evaluated by relative quantification. The amount of EGFP was normalized with GAPDH, and the normalized value was used in comparison among samples with a sample supplemented only with Box C/D-GFP (or Box C/D mut GFP) defined as 1. It was shown that the difference in expression level among the samples is within 2 times. From these results, no change in the mRNA level of L7Ae-specific Box C/D-GFP was confirmed, demonstrating that L7Ae does not regulate the transcription level of Box C/D-GFP mRNA.

FIG. **31** is a graph showing Box C/D-GFP mRNA level comparison among samples (samples derived from Box C/D-GFP and pcDNA-L7Ae and samples derived from Box C/D-GFP and pcDNA-A) with a sample derived from only Box C/D-GFP as 1. 0, 0.5, 1.0, or 2.0 μg of pcDNA-L7Ae or pcDNA-A was added to Box C/D-GFP fixed to 1.0 μg. The left bars represent the results from pcDNA-L7Ae added to Box C/D-GFP, and the right bars represent the results from pcDNA-A added to Box C/D-GFP. The ordinate represents the expression levels of samples with the Box C/D-GFP mRNA level of a sample supplemented with 1.0 μg of Box C/D-GFP as 1. The abscissa represents the amounts of pcDNA-L7Ae and pcDNA-A added. FIG. **32** is a graph showing mRNA level comparison among samples (samples derived from Box C/D-GFP and pcDNA-L7Ae and samples derived from Box C/D mut GFP and pcDNA-L7Ae) with a sample derived from only Box C/D as 1. (FIG. **8**)

0, 0.5, 1.0, or 2.0 μg of pcDNA-L7Ae was added to Box C/D-GFP or Box C/D mut GFP fixed to 1.0 μg. The left bars represent the results from pcDNA-L7Ae added to Box C/D-GFP, and the right bars represent the results from pcDNA-L7Ae added to Box C/D mut GFP. The ordinate represents the expression levels of samples with the Box C/D-GFP or Box C/D mut GFP mRNA level of a sample supplemented with 1.0 μg of Box C/D-GFP or Box C/D mut GFP as 1. The abscissa represents the amount of pcDNA-L7Ae added.

The L7Ae expression-dependent repression of Box C/D-GFP expression was observed using fluorescence microscopic photographs.

On the day before transfection, HeLa cells were seeded at a concentration of 0.5×10^5 cells/well to a 24-well plate and cultured in a 37° C. CO_2 incubator. Next day, the cells were transfected using Lipofectamine 2000 (trademark) (Invitrogen Corp.). 0, 0.05, 0.10, 0.15, or 0.20 μg of pcDNA-A or pcDNA-L7Ae was added to 0.2 μg of Box C/D-GFP or Box C/D mut GFP, and 1 μl of Lipofectamine 2000 was added to each sample. These DNA-lipid complexes were incubated at room temperature for 20 minutes and added dropwise to the cells. After 4 hours, medium replacement was performed. 24 hours after the transfection, photographs were taken under a fluorescence microscope. FIG. **33** is a fluorescence microscopic photograph showing the expression repressive effect of L7Ae on Box C/D-GFP. This drawing revealed that the fluorescence intensity of Box C/D-GFP is significantly reduced in the boxed region. This demonstrated that L7Ae expression specifically represses Box C/D-GFP translation.

Next, an experiment will be described which demonstrated that L7Ae can be used as a tag sequence for a target protein. pcDNA-L7Ae could be replaced by pcDNA3.1-L7Ae DsRed.

On the day before transfection, HeLa cells were seeded at a concentration of 0.5×10^5 cells/well to a 24-well plate and cultured in a 37° C. CO_2 incubator. Next day, the cells were transfected using Lipofectamine 2000 (trademark) (Invitrogen Corp.). 0, 0.2, 0.4, 0.8, or 1.6 μg of pcDNA3.1-DsRed or pcDNA3.1-L7Ae DsRed was added to 0.2 μg of Box C/D-GFP or Box C/D mut GFP, and 1 μl of Lipofectamine 2000 was added to each sample. These DNA-lipid complexes were incubated at room temperature for 20 minutes and added dropwise to the cells. After 4 hours, medium replacement was performed. 24 hours after the transfection, photographs were taken under a fluorescence microscope. FIG. **34** is a fluorescence microscopic photograph. This drawing revealed that the fluorescence intensity of Box C/D-GFP is significantly reduced along with the expression of pcDNA3.1-L7Ae-DsRed in the boxed region. As the red fluorescent proteins are expressed by the cells, the expression of the green fluorescent proteins is repressed. This demonstrated that a system that represses the translation of a target gene, for example, green fluorescent protein translation, in response to the expression of an arbitrary gene, for example, red fluorescent protein expression, can be constructed intracellularly by adding L7Ae as a tag sequence to the protein.

Example 7

To examine the binding property of L7Ae to the RNA complexes used in Examples above, reaction rate constants were calculated using inter-biomolecular interaction analyzer “BIACORE3000”.

[Preparation of L7Ae-Binding RNA Box C/D Mini Bia and Box C/D Mini Mutant Bia]

L7Ae-binding RNAs used in BIACORE were prepared by preparing a DNA template containing, at the 3' end of Box C/D mini or Box C/D mini mutant, a complementary strand of a DNA sequence (5'-CCGGGGATCCTCTAGAGTC-3') (SEQ ID NO: 92) immobilized on the BIACORE sensor chip, and a T7 promoter, followed by transcription reaction using T7 RNA polymerase. A reaction solution contained a mixture of 0.1 μM Box C/D mini bia template (5'-CCGGGGATCCTCTAGAGTCGGGTCAGCTTTCGCATCACGCCCTATAGTGAGTCGT ATTAGC-3') (SEQ ID NO: 93), 5 μL each of 10 μM T7 promoter (5'-GCTAATACGACT-

CACTATAGG-3') (SEQ ID NO: 94) and 10 μM Biacore Rev (5'-CCGGGGATCCTCTAGAGT-3') (SEQ ID NO: 95), 8 μl of 2.5 mM dNTP (TAKARA BIO INC.), 10 μL of Ex Taq 10× buffer (TAKARA BIO INC.), and 0.5 μL of Ex Taq DNA polymerase (TAKARA BIO INC.). 25 cycles each involving 94° C. for 30 seconds, 60° C. for 30 seconds, and 72° C. for 30 seconds were performed for extension using DNA Engine PCT-200 (BIO-RAD LABORATORIES INC.). After the reaction, the extension product was subjected to phenol treatment, diethyl ether treatment, and ethanol precipitation and dissolved in 10 μL of ultrapure water. The solution was used as a template for transcription. For Box C/D mini mutant bia, the same procedures as above were performed using 0.1 μM Box C/D minimut template (5'-CCGGGGATCCTCTAGAGTCGGGGCAGCTTTCGCATGACGCCCTATAGTGAGTCGT ATTAGC-3') (SEQ ID NO: 96) as a template in a reaction solution.

For transcription reaction, 10 μL of template DNA, 70 μL of 10× T7 RNA polymerase buffer (400 mM Tris-HCl (pH 7.5), 50 mM DTT, 10 mM Spermidine, 150 mM $MgCl_2$), 70 μL of 10× rNTPs (12.5 mM rATP, 12.5 mM rCTP, 12.5 mM rUTP, 12.5 mM rGTP), and 14 μL of T7 RNA polymerase were mixed and reacted at 37° C. for 3 hours. The reaction solution was supplemented with 5 μL of TURBO DNase (Ambion, Inc.) and incubated at 37° C. for 1 hour to decompose the template DNA. Then, the transcript was subjected to phenol treatment and ethanol precipitation for purification. After the precipitation, the resulting product was dissolved in 20 μL of denaturing dye (80% formamide, 0.17% XC, 0.27% BPB) and electrophoresed on a 12% polyacrylamide (29:1) denaturing gel. A gel having the size of interest was excised, and elution was performed overnight at 37° C. by the addition of 500 μL of elution buffer (0.3 M sodium acetate (pH 7.0), 0.1% SDS). The eluted RNA was subjected again to phenol extraction, diethyl ether extraction, and ethanol precipitation for purification.

[Immobilization of Ligand (Biotin DNA) onto BIACORE Sensor Chip]

Onto a streptavidin-immobilized sensor chip (SA chip) (GE Healthcare), 80 μL of 1 μM N-terminally biotinylated DNAs (5'-CCGGGGATCCTCTAGAGTC-3') (SEQ ID NO: 97) was added at a flow rate of 10 μL/min and immobilized using Amine Coupling Kit (GE Healthcare).

[Immobilization of Ligand RNA onto SA Chip]

RNAs were adjusted to 1 μM with HBS-EP buffer (10 mM HEPES (pH 7.4), 150 mM NaCl, 3 mM EDTA, 0.005% Surfactant P20) (GE Healthcare), then refolded through reaction at 80° C. for 10 min and at room temperature for 10 min, and then diluted 1/100 with 1 M KCl. 300 μL of the dilution was added to the chip at a flow rate of 10 μL/min to immobilize the RNAs corresponding to 52 RU (resonance unit) through the hybridization to the DNAs immobilized on the SA chip.

[L7Ae Association and Dissociation]

L7Ae was adjusted to 0 nM, 2.5 nM, 5 nM, 7.5 nM, 10 nM, 15 nM, 20 nM, and 25 nM with a running buffer (10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5% glycerol, 125 μg/ml tRNA, 62.5 μg/ml BSA, 1 mM DTT, 0.05% Tween 20). Each 50 μL aliquot was added at a flow rate of 50 μL/min for association with the RNA. Dissociation was performed for 5 minutes at the same flow rate as above. After association and dissociation measurements, the addition of 10 μL of 2 M KCl was repeated several times at a flow rate of 20 μL/min to forcedly dissociate, from the RNA, L7Ae undissociated for the 5 minutes. Three measurements were performed for each concentration.

[Calculation of Reaction Rate Constants]

The sensorgram of the flow cell bound with the Box C/D mini mutant bia RNA was subtracted from that of the flow cell bound with the Box C/D mini bia RNA. Based thereon, reaction rate constants (association rate constant (ka), dissociation rate constant (kd), association constant (KD), and dissociation constant (KA)) were calculated by Global fitting using the 1:1 (Langmuir) binding model of BIAevaluation analysis software. The results are shown in Table 4 and FIG. **35**. This diagram demonstrated that the RNP motif that can be used in intracellular translational regulation has strong binding affinity (KD=up to 1 nM) and has a slow dissociation rate (Kd=up to 1×10^{-4}), i.e., has the feature that the RNA and the protein hardly dissociates from each other once forming an RNP complex.

TABLE 4

ka (1/Ms)	kd (1/s)	KA (1/M)	KD (M)
1.46E+05	1.02E−04	1.43E+09	7.01E−10

In Examples above, two expressions GFP and EGFP are used in gene and RNA nomenclatures and both mean a gene and an RNA, respectively, derived from the EGFP (Enhanced Green Fluorescent Protein) gene.

INDUSTRIAL APPLICABILITY

In in vitro applications, the present invention can function as biosensors or artificial genetic circuits that respond to downstream signal proteins (e.g., fluorescent or luminescent proteins) in response to the expression of an arbitrary protein. Alternatively, by intracellular introduction, the present invention can function as systems that detect cells expressing a particular gene without destroying the cells, or as devices for artificial genetic circuits, which convert the expression of an arbitrary protein in an ON-to-OFF or OFF-to-ON manner in response to the expression of an arbitrary protein. Thus, the present invention can be developed into techniques of regulating the fate of cells.

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cgaggacggc agcgtgcagc tcgccgacca ctaccagcag aacaccccca tcgccgacgg    660

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cuggugaacc gcaucgagcu gaagggcauc gacuucaagg aggacggcaa cauccugggg 480

cacaagcugg aguacaacua caacagccac aacgucuaua ucauggccga caagcagaag 540

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gccgaccacu accagcagaa cacccccauc gccgacggcc ccgugcugcu gcccgacaac 660

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acggcgacgu aaacggccac aaguucagcg uguccggcga gggcgagggc gaugccaccu 180

acggcaagcu gacccugaag uucaucugca ccaccggcaa gcugcccgug cccuggccca 240

cccucgugac cacccugacc uacggcgugc agugcuucag ccgcuacccc gaccacauga 300

agcagcacga cuucuucaag uccgccaugc ccgaaggcua cguccaggag cgcaccaucu 360

ucuucaagga cgacggcaac uacaagaccc gcgccgaggu gaaguucgag ggcgacaccc 420

uggugaaccg caucgagcug aagggcaucg acuucaagga ggacggcaac auccuggggc 480

acaagcugga guacaacuac aacagccaca acgucuauau cauggccgac aagcagaaga 540

acggcaucaa ggugaacuuc aagauccgcc acaacaucga ggacggcagc gugcagcucg 600

ccgaccacua ccagcagaac acccccaucg ccgacggccc cgugcugcug cccgacaacc 660

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gtggtgccca tcctggtcga gctggacggc gacgtaaacg gccacaagtt cagcgtgtcc 180

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gggagaccac aacgguuucc cucgggcgug augcgaaagc ugacccaacu uuaagaagga 60

gauauaccaa uggugagcaa gggcgaggag cuguucaccg ggguggugcc cauccugguc 120

gagcuggacg gcgacguaaa cggccacaag uucagcgugu ccggcgaggg cgagggcgau 180

gccaccuacg gcaagcugac ccugaaguuc aucugcacca ccggcaagcu gcccgugccc 240

uggcccaccc ucgugaccac ccugaccuac ggcgugcagu gcuucagccg cuaccccgac 300

cacaugaagc agcacgacuu cuucaagucc gccaugcccg aaggcuacgu ccaggagcgc 360

accaucuucu ucaaggacga cggcaacuac aagacccgcg ccgaggugaa guucgagggc 420

gacacccugg ugaaccgcau cgagcugaag ggcaucgacu ucaaggagga cggcaacauc 480

cuggggcaca agcuggagua caacuacaac agccacaacg ucuauaucau ggccgacaag 540

cagaagaacg gcaucaaggu gaacuucaag auccgccaca acaucgagga cggcagcgug 600

cagcucgccg accacuacca gcagaacacc cccaucgccg acggccccgu gcugcugccc 660

gacaaccacu accugagcac ccaguccgcc cugagcaaag accccaacga gaagcgcgau 720

cacaugguccugcuggaguu cgugaccgcc gccggguaau gaaua 765

<210> SEQ ID NO 35
<211> LENGTH: 81
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 35

ggagaccaca acggtttccc tcgggcgtga tgcgaaagct gacccgttta actttaagaa 60

ggagatatac caatggtgag c 81

<210> SEQ ID NO 36
<211> LENGTH: 791
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 36

gaaattaata cgactcacta tagggagacc acaacggttt ccctcgggcg tgatgcgaaa 60

gctgacccgt ttaactttaa gaaggagata taccaatggt gagcaagggc gaggagctgt 120

tcaccggggt ggtgcccatc ctggtcgagc tggacggcga cgtaaacggc cacaagttca 180

gcgtgtccgg cgagggcgag ggcgatgcca cctacggcaa gctgaccctg aagttcatct 240

gcaccaccgg caagctgccc gtgccctggc ccaccctcgt gaccaccctg acctacggcg 300

tgcagtgctt cagccgctac cccgaccaca tgaagcagca cgacttcttc aagtccgcca 360

tgcccgaagg ctacgtccag gagcgcacca tcttcttcaa ggacgacggc aactacaaga 420

cccgcgccga ggtgaagttc gagggcgaca ccctggtgaa ccgcatcgag ctgaagggca 480

tcgacttcaa ggaggacggc aacatcctgg ggcacaagct ggagtacaac tacaacagcc 540

acaacgtcta tatcatggcc gacaagcaga agaacggcat caaggtgaac ttcaagatcc 600

gccacaacat cgaggacggc agcgtgcagc tcgccgacca ctaccagcag aacaccccca 660

tcgccgacgg ccccgtgctg ctgcccgaca accactacct gagcacccag tccgccctga 720

gcaaagaccc caacgagaag cgcgatcaca tggtcctgct ggagttcgtg accgccgccg 780

ggtaatgaat a 791

<210> SEQ ID NO 37
<211> LENGTH: 769
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 37

gggagaccac aacgguuucc cucgggcgug augcgaaagc ugacccguuu aacuuuaaga 60

aggagauaua ccaauggUga gcaagggcga ggagcuguuc accgggguggugcccauccu 120

ggucgagcug gacggcgacg uaaacggcca caaguucagc guguccggcg agggcgaggg 180

cgaugccacc uacggcaagc ugacccugaa guucaucugc accaccggca agcugcccgu 240

gcccuggccc acccucguga ccacccugac cuacggcgug cagugcuuca gccgcuaccc 300

cgaccacaug aagcagcacg acuucuucaa guccgccaug cccgaaggcu acguccagga 360

gcgcaccauc uucuucaagg acgacggcaa cuacaagacc cgcgccgagg ugaaguucga 420

gggcgacacc cuggugaacc gcaucgagcu gaagggcauc gacuucaagg aggacggcaa 480

cauccugggg cacaagcugg aguacaacua caacagccac aacgucuaua ucauggccga 540

caagcagaag aacggcauca aggugaacuu caagauccgc cacaacaucg aggacggcag 600

cgugcagcuc gccgaccacu accagcagaa cacccccauc gccgacggcc ccgugcugcu 660

gcccgacaac cacuaccuga gcacccaguc cgcccugagc aaagacccca acgagaagcg 720

cgaucacaug guccugcugg aguucgugac cgccgccggg uaaugaaua 769

<210> SEQ ID NO 38
<211> LENGTH: 70
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:

-continued

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<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 38

ggagaccaca acggtttccc tcgggcgtca tgcgaaagct gccccagaag gagatatacc      60

aatggtgagc                                                              70


<210> SEQ ID NO 39
<211> LENGTH: 780
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 39

gaaattaata cgactcacta tagggagacc acaacggttt ccctcgggcg tcatgcgaaa      60

gctgccccag aaggagatat accaatggtg agcaagggcg aggagctgtt caccggggtg     120

gtgcccatcc tggtcgagct ggacggcgac gtaaacggcc acaagttcag cgtgtccggc     180

gagggcgagg gcgatgccac ctacggcaag ctgaccctga agttcatctg caccaccggc     240

aagctgcccg tgccctggcc caccctcgtg accaccctga cctacggcgt gcagtgcttc     300

agccgctacc ccgaccacat gaagcagcac gacttcttca agtccgccat gcccgaaggc     360

tacgtccagg agcgcaccat cttcttcaag gacgacggca actacaagac ccgcgccgag     420

gtgaagttcg agggcgacac cctggtgaac cgcatcgagc tgaagggcat cgacttcaag     480

gaggacggca acatcctggg gcacaagctg gagtacaact acaacagcca caacgtctat     540

atcatggccg acaagcagaa gaacggcatc aaggtgaact tcaagatccg ccacaacatc     600

gaggacggca gcgtgcagct cgccgaccac taccagcaga acacccccat cgccgacggc     660

cccgtgctgc tgcccgacaa ccactacctg agcacccagt ccgccctgag caaagacccc     720

aacgagaagc gcgatcacat ggtcctgctg gagttcgtga ccgccgccgg gtaatgaata     780


<210> SEQ ID NO 40
<211> LENGTH: 758
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 40

gggagaccac aacgguuucc cucgggcguc augcgaaagc ugccccagaa ggagauauac      60

caauggugag caagggcgag gagcuguuca ccgggguggu gcccauccug gucgagcugg     120

acggcgacgu aaacggccac aaguucagcg uguccggcga gggcgagggc gaugccaccu     180

acggcaagcu gacccugaag uucaucugca ccaccggcaa gcugcccgug cccuggccca     240

cccucgugac cacccugacc uacggcgugc agugcuucag ccgcuacccc gaccacauga     300

agcagcacga cuucuucaag uccgccaugc ccgaaggcua cguccaggag cgcaccaucu     360

ucuucaagga cgacggcaac uacaagaccc gcgccgaggu gaaguucgag ggcgacaccc     420

uggugaaccg caucgagcug aagggcaucg acuucaagga ggacggcaac auccuggggc     480

acaagcugga guacaacuac aacagccaca acgucuauau cauggccgac aagcagaaga     540

acggcaucaa ggugaacuuc aagauccgcc acaacaucga ggacggcagc gugcagcucg     600

ccgaccacua ccagcagaac acccccaucg ccgacggccc cgugcugcug cccgacaacc     660

acuaccugag cacccagucc gcccugagca aagaccccaa cgagaagcgc gaucacaugg     720

uccugcugga guucgugacc gccgccgggu aaugaaua                              758
```

<210> SEQ ID NO 41
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 41

ggagaccaca acggtttccc tcggggaaac ccagaaggag atataccaat ggtgagc 57

<210> SEQ ID NO 42
<211> LENGTH: 767
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 42

gaaattaata cgactcacta tagggagacc acaacggttt ccctcgggga aacccagaag 60

gagatatacc aatggtgagc aagggcgagg agctgttcac cggggtggtg cccatcctgg 120

tcgagctgga cggcgacgta aacggccaca agttcagcgt gtccggcgag ggcgagggcg 180

atgccaccta cggcaagctg accctgaagt tcatctgcac caccggcaag ctgcccgtgc 240

cctggcccac cctcgtgacc accctgacct acggcgtgca gtgcttcagc cgctaccccg 300

accacatgaa gcagcacgac ttcttcaagt ccgccatgcc cgaaggctac gtccaggagc 360

gcaccatctt cttcaaggac gacggcaact acaagacccg cgccgaggtg aagttcgagg 420

gcgacaccct ggtgaaccgc atcgagctga agggcatcga cttcaaggag gacggcaaca 480

tcctggggca caagctggag tacaactaca acagccacaa cgtctatatc atggccgaca 540

agcagaagaa cggcatcaag gtgaacttca agatccgcca caacatcgag gacggcagcg 600

tgcagctcgc cgaccactac cagcagaaca cccccatcgc cgacggcccc gtgctgctgc 660

ccgacaacca ctacctgagc acccagtccg ccctgagcaa agaccccaac gagaagcgcg 720

atcacatggt cctgctggag ttcgtgaccg ccgccgggta atgaata 767

<210> SEQ ID NO 43
<211> LENGTH: 745
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 43

gggagaccac aacgguuucc cucggggaaa cccagaagga gauauaccaa uggugagcaa 60

gggcgaggag cuguucaccg ggguggugcc cauccugguc gagcuggacg gcgacguaaa 120

cggccacaag uucagcgugu ccggcgaggg cgagggcgau gccaccuacg gcaagcugac 180

ccugaaguuc aucugcacca ccggcaagcu gcccgugccc uggcccaccc ucgugaccac 240

ccugaccuac ggcgugcagu gcuucagccg cuaccccgac cacaugaagc agcacgacuu 300

cuucaagucc gccaugcccg aaggcuacgu ccaggagcgc accaucuucu ucaaggacga 360

cggcaacuac aagacccgcg ccgaggugaa guucgagggc gacacccugg ugaaccgcau 420

cgagcugaag ggcaucgacu ucaaggagga cggcaacauc cuggggcaca agcuggagua 480

caacuacaac agccacaacg ucuauaucau ggccgacaag cagaagaacg gcaucaaggu 540

gaacuucaag auccgccaca acaucgagga cggcagcgug cagcucgccg accacuacca 600

-continued

gcagaacacc cccaucgccg acggccccgu gcugcugccc gacaaccacu accugagcac 660

ccaguccgcc cugagcaaag accccaacga gaagcgcgau cacauggucc ugcuggaguu 720

cgugaccgcc gccggguaau gaaua 745

<210> SEQ ID NO 44
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 44

aaggagatat accaatgggg cgtgatgcga aagctgaccc tgtgagcaag ggcgaggag 59

<210> SEQ ID NO 45
<211> LENGTH: 805
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 45

gaaattaata cgactcacta tagggagacc acaacggttt ccctctagaa ataattttgt 60

ttaactttaa gaaggagata taccaatggg gcgtgatgcg aaagctgacc ctgtgagcaa 120

gggcgaggag ctgttcaccg ggtggtgcc catcctggtc gagctggacg gcgacgtaaa 180

cggccacaag ttcagcgtgt ccggcgaggg cgagggcgat gccacctacg gcaagctgac 240

cctgaagttc atctgcacca ccggcaagct gcccgtgccc tggcccaccc tcgtgaccac 300

cctgacctac ggcgtgcagt gcttcagccg ctaccccgac cacatgaagc agcacgactt 360

cttcaagtcc gccatgcccg aaggctacgt ccaggagcgc accatcttct tcaaggacga 420

cggcaactac aagacccgcg ccgaggtgaa gttcgagggc gacaccctgg tgaaccgcat 480

cgagctgaag ggcatcgact tcaaggagga cggcaacatc ctggggcaca agctggagta 540

caactacaac agccacaacg tctatatcat ggccgacaag cagaagaacg gcatcaaggt 600

gaacttcaag atccgccaca acatcgagga cggcagcgtg cagctcgccg accactacca 660

gcagaacacc cccatcgccg acggccccgt gctgctgccc gacaaccact acctgagcac 720

ccagtccgcc ctgagcaaag accccaacga gaagcgcgat cacatggtcc tgctggagtt 780

cgtgaccgcc gccgggtaat gaata 805

<210> SEQ ID NO 46
<211> LENGTH: 783
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 46

gggagaccac aacgguuucc cucuagaaau aauuuuguuu aacuuuaaga aggagauaua 60

ccaauggggc gugaugcgaa agcugacccu gugagcaagg gcgaggagcu guucaccggg 120

guggugccca uccuggucga gcuggacggc gacguaaacg gccacaaguu cagcgugucc 180

ggcgagggcg agggcgaugc caccuacggc aagcugaccc ugaaguucau cugcaccacc 240

ggcaagcugc ccgugcccug gcccacccuc gugaccaccc ugaccuacgg cgugcagugc 300

uucagccgcu accccgacca caugaagcag cacgacuucu ucaaguccgc caugcccgaa 360

ggcuacgucc aggagcgcac caucuucuuc aaggacgacg gcaacuacaa gacccgcgcc 420 gaggugaagu ucgagggcga cacccuggug aaccgcaucg agcugaaggg caucgacuuc 480

aaggaggacg gcaacauccu ggggcacaag cuggaguaca acuacaacag ccacaacguc 540

uauaucaugg ccgacaagca gaagaacggc aucaagguga acuucaagau ccgccacaac 600

aucgaggacg gcagcgugca gcucgccgac cacuaccagc agaacacccc caucgccgac 660

ggccccgugc ugcugcccga caaccacuac cugagcaccc aguccgcccu gagcaaagac 720

cccaacgaga agcgcgauca cauggguccug cuggaguucg ugaccgccgc cggguaauga 780

aua 783

<210> SEQ ID NO 47
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 47

aaggagatat accaatgagg ggaaacccag tgagcaaggg cgaggag 47

<210> SEQ ID NO 48
<211> LENGTH: 793
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 48

gaaattaata cgactcacta tagggagacc acaacggttt ccctctagaa ataattttgt 60

ttaactttaa gaaggagata taccaatgag gggaaaccca gtgagcaagg gcgaggagct 120

gttcaccggg gtggtgccca tcctggtcga gctggacggc gacgtaaacg gccacaagtt 180

cagcgtgtcc ggcgagggcg agggcgatgc cacctacggc aagctgaccc tgaagttcat 240

ctgcaccacc ggcaagctgc ccgtgccctg gcccaccctc gtgaccaccc tgacctacgg 300

cgtgcagtgc ttcagccgct accccgacca catgaagcag cacgacttct tcaagtccgc 360

catgcccgaa ggctacgtcc aggagcgcac catcttcttc aaggacgacg gcaactacaa 420

gacccgcgcc gaggtgaagt tcgagggcga caccctggtg aaccgcatcg agctgaaggg 480

catcgacttc aaggaggacg gcaacatcct ggggcacaag ctggagtaca actacaacag 540

ccacaacgtc tatatcatgg ccgacaagca gaagaacggc atcaaggtga acttcaagat 600

ccgccacaac atcgaggacg gcagcgtgca gctcgccgac cactaccagc agaacacccc 660

catcgccgac ggccccgtgc tgctgcccga caaccactac ctgagcaccc agtccgccct 720

gagcaaagac cccaacgaga agcgcgatca catggtcctg ctggagttcg tgaccgccgc 780

cgggtaatga ata 793

<210> SEQ ID NO 49
<211> LENGTH: 771
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 49

gggagaccac aacgguuucc cucuagaaau aauuuuguuu aacuuuaaga aggagauaua 60

ccaaugaggg gaaacccagu gagcaagggc gaggagcugu ucaccggggu ggugcccauc 120 cuggucgagc uggacggcga cguaaacggc cacaaguuca gcguguccgg cgagggcgag 180

ggcgaugcca ccuacggcaa gcugacccug aaguucaucu gcaccaccgg caagcugccc 240

gugcccuggc ccacccucgu gaccacccug accuacggcg ugcagugcuu cagccgcuac 300

cccgaccaca ugaagcagca cgacuucuuc aaguccgcca ugcccgaagg cuacguccag 360

gagcgcacca ucuucuucaa ggacgacggc aacuacaaga cccgcgccga ggugaaguuc 420

gagggcgaca cccuggugaa ccgcaucgag cugaagggca ucgacuucaa ggaggacggc 480

aacauccugg ggcacaagcu ggaguacaac uacaacagcc acaacgucua uaucauggcc 540

gacaagcaga agaacggcau caaggugaac uucaagaucc gccacaacau cgaggacggc 600

agcgugcagc ucgccgacca cuaccagcag aacaccccca ucgccgacgg ccccgugcug 660

cugcccgaca accacuaccu gagcacccag uccgcccuga gcaaagaccc caacgagaag 720

cgcgaucaca ugguccugcu ggaguucgug accgccgccg gguaaugaau a 771

<210> SEQ ID NO 50
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 50

gtgatctttc gtgtgggtca ccactgcaaa taaggatata aaatggtgag caagggcgag 60

<210> SEQ ID NO 51
<211> LENGTH: 833
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 51

ctaatacgac tcactatagg attgcgaacc aatttagcat ttgttggcta aatggtttcg 60

caatgaactg ttaataaaca aatttttctt tgtatgtgat ctttcgtgtg ggtcaccact 120

gcaaataagg atataaaatg gtgagcaagg gcgaggagct gttcaccggg gtggtgccca 180

tcctggtcga gctggacggc gacgtaaacg gccacaagtt cagcgtgtcc ggcgagggcg 240

agggcgatgc cacctacggc aagctgaccc tgaagttcat ctgcaccacc ggcaagctgc 300

ccgtgccctg gcccaccctc gtgaccaccc tgacctacgg cgtgcagtgc ttcagccgct 360

accccgacca catgaagcag cacgacttct tcaagtccgc catgcccgaa ggctacgtcc 420

aggagcgcac catcttcttc aaggacgacg gcaactacaa gacccgcgcc gaggtgaagt 480

tcgagggcga caccctggtg aaccgcatcg agctgaaggg catcgacttc aaggaggacg 540

gcaacatcct ggggcacaag ctggagtaca actacaacag ccacaacgtc tatatcatgg 600

ccgacaagca gaagaacggc atcaaggtga acttcaagat ccgccacaac atcgaggacg 660

gcagcgtgca gctcgccgac cactaccagc agaacacccc catcgccgac ggccccgtgc 720

tgctgcccga caaccactac ctgagcaccc agtccgccct gagcaaagac cccaacgaga 780

agcgcgatca catggtcctg ctggagttcg tgaccgccgc cgggtaatga ata 833

<210> SEQ ID NO 52
<211> LENGTH: 815
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

```
<400> SEQUENCE: 52

ggauugcgaa ccaauuuagc auuuguuggc uaaaugguuu cgcaaugaac uguuaauaaa     60

caaauuuuuc uuuguaugug aucuuucgug ugggucacca cugcaaauaa ggauauaaaa    120

uggugagcaa gggcgaggag cuguucaccg ggguggugcc cauccugguc gagcuggacg    180

gcgacguaaa cggccacaag uucagcgugu ccggcgaggg cgagggcgau gccaccuacg    240

gcaagcugac ccugaaguuc aucugcacca ccggcaagcu gcccgugccc uggcccaccc    300

ucgugaccac ccugaccuac ggcgugcagu gcuucagccg cuaccccgac cacaugaagc    360

agcacgacuu cuucaagucc gccaugcccg aaggcuacgu ccaggagcgc accaucuucu    420

ucaaggacga cggcaacuac aagacccgcg ccgaggugaa guucgagggc gacacccugg    480

ugaaccgcau cgagcugaag ggcaucgacu ucaaggagga cggcaacauc cuggggcaca    540

agcuggagua caacuacaac agccacaacg ucuauaucau ggccgacaag cagaagaacg    600

gcaucaaggu gaacuucaag auccgccaca acaucgagga cggcagcgug cagcucgccg    660

accacuacca gcagaacacc cccaucgccg acggccccgu gcugcugccc gacaaccacu    720

accugagcac ccaguccgcc cugagcaaag accccaacga gaagcgcgau cacaugaucc    780

ugcuggaguu cgugaccgcc gccggguaau gaaua                              815


<210> SEQ ID NO 53
<211> LENGTH: 81
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 53

ggagaccaca acggtttccc tcggcgtatg tgatctttcg tgtgggtcac cactgcgcca     60

gaaggagata taccaatggt g                                               81


<210> SEQ ID NO 54
<211> LENGTH: 795
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 54

gaaattaata cgactcacta taggggagac cacaacggtt tccctcggcg tatgtgatct     60

ttcgtgtggg tcaccactgc gccagaagga gatataccaa tggtgagcaa gggcgaggag    120

ctgttcaccg gggtggtgcc catcctggtc gagctggacg gcgacgtaaa cggccacaag    180

ttcagcgtgt ccggcgaggg cgagggcgat gccacctacg gcaagctgac cctgaagttc    240

atctgcacca ccggcaagct gcccgtgccc tggcccaccc tcgtgaccac cctgacctac    300

ggcgtgcagt gcttcagccg ctaccccgac cacatgaagc agcacgactt cttcaagtcc    360

gccatgcccg aaggctacgt ccaggagcgc accatcttct tcaaggacga cggcaactac    420

aagacccgcg ccgaggtgaa gttcgagggc gacaccctgg tgaaccgcat cgagctgaag    480

ggcatcgact tcaaggagga cggcaacatc ctggggcaca agctggagta caactacaac    540

agccacaacg tctatatcat ggccgacaag cagaagaacg gcatcaaggt gaacttcaag    600

atccgccaca acatcgagga cggcagcgtg cagctcgccg accactacca gcagaacacc    660

cccatcgccg acggccccgt gctgctgccc gacaaccact acctgagcac ccagtccgcc    720
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ctgagcaaag accccaacga gaagcgcgat cacatggtcc tgctggagtt cgtgaccgcc 780

gccgggtaat gaata 795

<210> SEQ ID NO 55
<211> LENGTH: 772
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 55

gggagaccac aacgguuucc cucggcguau gugaucuuuc guguggguca ccacugcgcc 60

agaaggagau auaccaaugg ugagcaaggg cgaggagcug uucaccgggg uggugcccau 120

ccuggucgag cuggacggcg acguaaacgg ccacaaguuc agcguguccg gcgagggcga 180

gggcgaugcc accuacggca agcugacccu gaaguucauc ugcaccaccg gcaagcugcc 240

cgugcccugg cccacccucg ugaccacccu gaccuacggc gugcagugcu ucagccgcua 300

ccccgaccac augaagcagc acgacuucuu caaguccgcc augcccgaag gcuacgucca 360

ggagcgcacc aucuucuuca aggacgacgg caacuacaag acccgcgccg aggugaaguu 420

cgagggcgac acccugguga accgcaucga gcugaagggc aucgacuuca aggaggacgg 480

caacauccug gggcacaagc uggaguacaa cuacaacagc cacaacgucu auaucauggc 540

cgacaagcag aagaacggca ucaaggugaa cuucaagauc cgccacaaca ucgaggacgg 600

cagcgugcag cucgccgacc acuaccagca gaacaccccc aucgccgacg gccccgugcu 660

gcugcccgac aaccacuacc ugagcaccca guccgcccug agcaaagacc ccaacgagaa 720

gcgcgaucac augguccugc uggaguucgu gaccgccgcc ggguaaugaa ua 772

<210> SEQ ID NO 56
<211> LENGTH: 81
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 56

ggagaccaca acggtttccc tcggcgtatg tgatctttca tgtgggtcac cactgcgcca 60

gaaggagata taccaatggt g 81

<210> SEQ ID NO 57
<211> LENGTH: 795
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 57

gaaattaata cgactcacta taggggagac cacaacggtt tccctcggcg tatgtgatct 60

ttcatgtggg tcaccactgc gccagaagga gatataccaa tggtgagcaa gggcgaggag 120

ctgttcaccg gggtggtgcc catcctggtc gagctggacg gcgacgtaaa cggccacaag 180

ttcagcgtgt ccggcgaggg cgagggcgat gccacctacg gcaagctgac cctgaagttc 240

atctgcacca ccggcaagct gcccgtgccc tggcccaccc tcgtgaccac cctgacctac 300

ggcgtgcagt gcttcagccg ctaccccgac cacatgaagc agcacgactt cttcaagtcc 360

gccatgcccg aaggctacgt ccaggagcgc accatcttct tcaaggacga cggcaactac 420

aagacccgcg ccgaggtgaa gttcgagggc gacaccctgg tgaaccgcat cgagctgaag 480

-continued

ggcatcgact tcaaggagga cggcaacatc ctggggcaca agctggagta caactacaac 540

agccacaacg tctatatcat ggccgacaag cagaagaacg gcatcaaggt gaacttcaag 600

atccgccaca acatcgagga cggcagcgtg cagctcgccg accactacca gcagaacacc 660

cccatcgccg acggccccgt gctgctgccc gacaaccact acctgagcac ccagtccgcc 720

ctgagcaaag accccaacga gaagcgcgat cacatggtcc tgctggagtt cgtgaccgcc 780

gccgggtaat gaata 795

<210> SEQ ID NO 58
<211> LENGTH: 772
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 58

gggagaccac aacgguuucc cucggcguau gugaucuuuc augugggguca ccacugcgcc 60

agaaggagau auaccaaugg ugagcaaggg cgaggagcug uucaccgggg uggugcccau 120

ccuggucgag cuggacggcg acguaaacgg ccacaaguuc agcguguccg gcgagggcga 180

gggcgaugcc accuacggca agcugacccu gaaguucauc ugcaccaccg gcaagcugcc 240

cgugcccugg cccacccucg ugaccacccu gaccuacggc gugcagugcu ucagccgcua 300

ccccgaccac augaagcagc acgacuucuu caaguccgcc augcccgaag gcuacgucca 360

ggagcgcacc aucuucuuca aggacgacgg caacuacaag acccgcgccg aggugaaguu 420

cgagggcgac acccugguga accgcaucga gcugaagggc aucgacuuca aggaggacgg 480

caacauccug gggcacaagc uggaguacaa cuacaacagc cacaacgucu auaucauggc 540

cgacaagcag aagaacggca ucaaggugaa cuucaagauc cgccacaaca ucgaggacgg 600

cagcgugcag cucgccgacc acuaccagca gaacaccccc aucgccgacg gccccgugcu 660

gcugcccgac aaccacuacc ugagcaccca guccgcccug agcaaagacc ccaacgagaa 720

gcgcgaucac augguccugc uggaguucgu gaccgccgcc ggguaaugaa ua 772

<210> SEQ ID NO 59
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 59

aaggagatat accaatgcag ctttcgcatc acgtgagcaa gggcgaggag 50

<210> SEQ ID NO 60
<211> LENGTH: 796
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 60

gaaattaata cgactcacta tagggagacc acaacggttt ccctctagaa ataattttgt 60

ttaactttaa gaaggagata taccaatgca gctttcgcat cacgtgagca agggcgagga 120

gctgttcacc ggggtggtgc ccatcctggt cgagctggac ggcgacgtaa acggccacaa 180

gttcagcgtg tccggcgagg gcgagggcga tgccacctac ggcaagctga ccctgaagtt 240

-continued

catctgcacc accggcaagc tgcccgtgcc ctggcccacc ctcgtgacca ccctgaccta 300

cggcgtgcag tgcttcagcc gctaccccga ccacatgaag cagcacgact tcttcaagtc 360

cgccatgccc gaaggctacg tccaggagcg caccatcttc ttcaaggacg acggcaacta 420

caagacccgc gccgaggtga agttcgaggg cgacaccctg gtgaaccgca tcgagctgaa 480

gggcatcgac ttcaaggagg acggcaacat cctggggcac aagctggagt acaactacaa 540

cagccacaac gtctatatca tggccgacaa gcagaagaac ggcatcaagg tgaacttcaa 600

gatccgccac aacatcgagg acggcagcgt gcagctcgcc gaccactacc agcagaacac 660

ccccatcgcc gacggccccg tgctgctgcc cgacaaccac tacctgagca cccagtccgc 720

cctgagcaaa gaccccaacg agaagcgcga tcacatggtc ctgctggagt tcgtgaccgc 780

cgccgggtaa tgaata 796

<210> SEQ ID NO 61
<211> LENGTH: 773
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 61

gggagaccac aacgguuucc cucgggcgug augcgaaagc ugacccagaa ggagauauac 60

caaugcagcu uucgcaucac gugagcaagg gcgaggagcu guucaccggg guggugccca 120

uccuggucga gcuggacggc gacguaaacg gccacaaguu cagcgugucc ggcgagggcg 180

agggcgaugc caccuacggc aagcugaccc ugaaguucau cugcaccacc ggcaagcugc 240

ccgugcccug gcccacccuc gugaccaccc ugaccuacgg cgugcagugc uucagccgcu 300

accccgacca caugaagcag cacgacuucu ucaaguccgc caugcccgaa ggcuacgucc 360

aggagcgcac caucuucuuc aaggacgacg gcaacuacaa gacccgcgcc gaggugaagu 420

ucgagggcga cacccuggug aaccgcaucg agcugaaggg caucgacuuc aaggaggacg 480

gcaacauccu ggggcacaag cuggaguaca acuacaacag ccacaacguc uauaucaugg 540

ccgacaagca gaagaacggc aucaagguga acuucaagau ccgccacaac aucgaggacg 600

gcagcgugca gcucgccgac cacuaccagc agaacacccc caucgccgac ggccccgugc 660

ugcugcccga caaccacuac cugagcaccc aguccgcccu gagcaaagac cccaacgaga 720

agcgcgauca cauggùccug cuggaguucg ugaccgccgc cggguaauga aua 773

<210> SEQ ID NO 62
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 62

ggtgggtcag ctttcgcatc acgcccacct atagtgagtc gtattagc 48

<210> SEQ ID NO 63
<211> LENGTH: 29
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 63

ggugggcgug augcgaaagc ugacccacc 29

```
<210> SEQ ID NO 64
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 64

ctgacatatg tacgtgagat ttgaggttc                                       29


<210> SEQ ID NO 65
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 65

ctgactcgag ttacttctga aggcctttaa tc                                   32


<210> SEQ ID NO 66
<211> LENGTH: 139
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 66

Met Gly Ser Ser His His His His His His Ser Ser Gly Leu Val Pro
1               5                   10                  15

Arg Gly Ser His Met Tyr Val Arg Phe Glu Val Pro Glu Asp Met Gln
            20                  25                  30

Asn Glu Ala Leu Ser Leu Leu Glu Lys Val Arg Glu Ser Gly Lys Val
        35                  40                  45

Lys Lys Gly Thr Asn Glu Thr Thr Lys Ala Val Glu Arg Gly Leu Ala
    50                  55                  60

Lys Leu Val Tyr Ile Ala Glu Asp Val Asp Pro Pro Glu Ile Val Ala
65                  70                  75                  80

His Leu Pro Leu Leu Cys Glu Glu Lys Asn Val Pro Tyr Ile Tyr Val
                85                  90                  95

Lys Ser Lys Asn Asp Leu Gly Arg Ala Val Gly Ile Glu Val Pro Cys
            100                 105                 110

Ala Ser Ala Ala Ile Ile Asn Glu Gly Glu Leu Arg Lys Glu Leu Gly
        115                 120                 125

Ser Leu Val Glu Lys Ile Lys Gly Leu Gln Lys
    130                 135


<210> SEQ ID NO 67
<211> LENGTH: 654
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 67

Met Arg Gly Ser His His His His His His Gly Ser Met Pro Val Ile
1               5                   10                  15

Thr Leu Pro Asp Gly Ser Gln Arg His Tyr Asp His Ala Val Ser Pro
            20                  25                  30

Met Asp Val Ala Leu Asp Ile Gly Pro Gly Leu Ala Lys Ala Cys Ile
        35                  40                  45
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Ala Gly Arg Val Asn Gly Glu Leu Val Asp Ala Cys Asp Leu Ile Glu
    50                  55                  60

Asn Asp Ala Gln Leu Ser Ile Ile Thr Ala Lys Asp Glu Glu Gly Leu
65                  70                  75                  80

Glu Ile Ile Arg His Ser Cys Ala His Leu Leu Gly His Ala Ile Lys
                85                  90                  95

Gln Leu Trp Pro His Thr Lys Met Ala Ile Gly Pro Val Ile Asp Asn
            100                 105                 110

Gly Phe Tyr Tyr Asp Val Asp Leu Asp Arg Thr Leu Thr Gln Glu Asp
        115                 120                 125

Val Glu Ala Leu Glu Lys Arg Met His Glu Leu Ala Glu Lys Asn Tyr
    130                 135                 140

Asp Val Ile Lys Lys Lys Val Ser Trp His Glu Ala Arg Glu Thr Phe
145                 150                 155                 160

Ala Asn Arg Gly Glu Ser Tyr Lys Val Ser Ile Leu Asp Glu Asn Ile
                165                 170                 175

Ala His Asp Asp Lys Pro Gly Leu Tyr Phe His Glu Glu Tyr Val Asp
            180                 185                 190

Met Cys Arg Gly Pro His Val Pro Asn Met Arg Phe Cys His His Phe
        195                 200                 205

Lys Leu Met Lys Thr Ala Gly Ala Tyr Trp Arg Gly Asp Ser Asn Asn
    210                 215                 220

Lys Met Leu Gln Arg Ile Tyr Gly Thr Ala Trp Ala Asp Lys Lys Ala
225                 230                 235                 240

Leu Asn Ala Tyr Leu Gln Arg Leu Glu Glu Ala Ala Lys Arg Asp His
                245                 250                 255

Arg Lys Ile Gly Lys Gln Leu Asp Leu Tyr His Met Gln Glu Glu Ala
            260                 265                 270

Pro Gly Met Val Phe Trp His Asn Asp Gly Trp Thr Ile Phe Arg Glu
        275                 280                 285

Leu Glu Val Phe Val Arg Ser Lys Leu Lys Glu Tyr Gln Tyr Gln Glu
    290                 295                 300

Val Lys Gly Pro Phe Met Met Asp Arg Val Leu Trp Glu Lys Thr Gly
305                 310                 315                 320

His Trp Asp Asn Tyr Lys Asp Ala Met Phe Thr Thr Ser Ser Glu Asn
                325                 330                 335

Arg Glu Tyr Cys Ile Lys Pro Met Asn Cys Pro Gly His Val Gln Ile
            340                 345                 350

Phe Asn Gln Gly Leu Lys Ser Tyr Arg Asp Leu Pro Leu Arg Met Ala
        355                 360                 365

Glu Phe Gly Ser Cys His Arg Asn Glu Pro Ser Gly Ser Leu His Gly
    370                 375                 380

Leu Met Arg Val Arg Gly Phe Thr Gln Asp Asp Ala His Ile Phe Cys
385                 390                 395                 400

Thr Glu Glu Gln Ile Arg Asp Glu Val Asn Gly Cys Ile Arg Leu Val
                405                 410                 415

Tyr Asp Met Tyr Ser Thr Phe Gly Phe Glu Lys Ile Val Val Lys Leu
            420                 425                 430

Ser Thr Arg Pro Glu Lys Arg Ile Gly Ser Asp Glu Met Trp Asp Arg
        435                 440                 445

Ala Glu Ala Asp Leu Ala Val Ala Leu Glu Glu Asn Asn Ile Pro Phe
    450                 455                 460

Glu Tyr Gln Leu Gly Glu Gly Ala Phe Tyr Gly Pro Lys Ile Glu Phe
```

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465                 470                 475                 480

Thr Leu Tyr Asp Cys Leu Asp Arg Ala Trp Gln Cys Gly Thr Val Gln
                485                 490                 495

Leu Asp Phe Ser Leu Pro Ser Arg Leu Ser Ala Ser Tyr Val Gly Glu
            500                 505                 510

Asp Asn Glu Arg Lys Val Pro Val Met Ile His Arg Ala Ile Leu Gly
        515                 520                 525

Ser Met Glu Arg Phe Ile Gly Ile Leu Thr Glu Glu Phe Ala Gly Phe
    530                 535                 540

Phe Pro Thr Trp Leu Ala Pro Val Gln Val Val Ile Met Asn Ile Thr
545                 550                 555                 560

Asp Ser Gln Ser Glu Tyr Val Asn Glu Leu Thr Gln Lys Leu Ser Asn
                565                 570                 575

Ala Gly Ile Arg Val Lys Ala Asp Leu Arg Asn Glu Lys Ile Gly Phe
            580                 585                 590

Lys Ile Arg Glu His Thr Leu Arg Arg Val Pro Tyr Met Leu Val Cys
        595                 600                 605

Gly Asp Lys Glu Val Glu Ser Gly Lys Val Ala Val Arg Thr Arg Arg
    610                 615                 620

Gly Lys Asp Leu Gly Ser Met Asp Val Asn Glu Val Ile Glu Lys Leu
625                 630                 635                 640

Gln Gln Glu Ile Arg Ser Arg Ser Leu Lys Gln Leu Glu Glu
                645                 650
```

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<210> SEQ ID NO 68
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 68

aaggagatat accaatggcc tcctccgagg ac                                   32


<210> SEQ ID NO 69
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 69

tattcattac tacaggaaca ggtggtggc                                       29


<210> SEQ ID NO 70
<211> LENGTH: 772
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 70

gaaattaata cgactcacta tagggagacc acaacggttt ccctctagaa ataattttgt     60

ttaactttaa gaaggagata taccaatggc ctcctccgag gacgtcatca aggagttcat    120

gcgcttcaag gtgcgcatgg agggctccgt gaacggccac gagttcgaga tcgagggcga    180

gggcgagggc cgcccctacg agggcaccca gaccgccaag ctgaaggtga ccaagggcgg    240

ccccctgccc ttcgcctggg acatcctgtc cccccagttc cagtacggct ccaaggtgta    300
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cgtgaagcac cccgccgaca tccccgacta caagaagctg tccttccccg agggcttcaa 360

gtgggagcgc gtgatgaact tcgaggacgg cggcgtggtg accgtgaccc aggactcctc 420

cctgcaggac ggctccttca tctacaaggt gaagttcatc ggcgtgaact tcccctccga 480

cggccccgta atgcagaaga agactatggg ctgggaggcc tccaccgagc gcctgtaccc 540

ccgcgacggc gtgctgaagg gcgagatcca caaggccctg aagctgaagg acggcggcca 600

ctacctggtg gagttcaagt ccatctacat ggccaagaag cccgtgcagc tgcccggcta 660

ctactacgtg gactccaagc tggacatcac ctcccacaac gaggactaca ccatcgtgga 720

gcagtacgag cgcgccgagg gccgccacca cctgttcctg tagtaatgaa ta 772

<210> SEQ ID NO 71
<211> LENGTH: 750
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 71

gggagaccac aacgguuucc cucuagaaau aauuuuguuu aacuuuaaga aggagauaua 60

ccaauggccu ccuccgagga cgucaucaag gaguucaugc gcuucaaggu gcgcauggag 120

ggcuccguga acggccacga guucgagauc gagggcgagg gcgagggccg ccccuacgag 180

ggcacccaga ccgccaagcu gaaggugacc aagggcggcc cccugcccuu cgccugggac 240

auccuguccc cccaguucca guacggcucc aagguguacg ugaagcaccc cgccgacauc 300

cccgacuaca agaagcuguc cuuccccgag ggcuucaagu gggagcgcgu gaugaacuuc 360

gaggacggcg gcguggugac cgugacccag gacuccuccc ugcaggacgg cuccuucauc 420

uacaagguga aguucaucgg cgugaacuuc cccuccgacg gccccguaau gcagaagaag 480

acuaugggcu gggaggccuc caccgagcgc cuguaccccc gcgacggcgu gcugaagggc 540

gagauccaca aggcccugaa gcugaaggac ggcggccacu accuggugga guucaagucc 600

aucuacaugg ccaagaagcc cgugcagcug cccggcuacu acuacgugga cuccaagcug 660

gacaucaccu cccacaacga ggacuacacc aucguggagc aguacgagcg cgccgagggc 720

cgccaccacc uguuccugua guaaugaaua 750

<210> SEQ ID NO 72
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 72

aaggagatat accaatgggg cgtgatgcga aagctgaccc tgcctcctcc gaggacgtc 59

<210> SEQ ID NO 73
<211> LENGTH: 796
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 73

gaaattaata cgactcacta tagggagacc acaacggttt ccctctagaa ataattttgt 60

ttaactttaa gaaggagata taccaatggg gcgtgatgcg aaagctgacc ctgcctcctc 120

cgaggacgtc atcaaggagt tcatgcgctt caaggtgcgc atggagggct ccgtgaacgg 180

```
ccacgagttc gagatcgagg gcgagggcga gggccgcccc tacgagggca cccagaccgc    240

caagctgaag gtgaccaagg gcggccccct gcccttcgcc tgggacatcc tgtcccccca    300

gttccagtac ggctccaagg tgtacgtgaa gcaccccgcc gacatccccg actacaagaa    360

gctgtccttc cccgagggct tcaagtggga gcgcgtgatg aacttcgagg acggcggcgt    420

ggtgaccgtg acccaggact cctccctgca ggacggctcc ttcatctaca aggtgaagtt    480

catcggcgtg aacttcccct ccgacggccc cgtaatgcag aagaagacta tgggctggga    540

ggcctccacc gagcgcctgt acccccgcga cggcgtgctg aagggcgaga tccacaaggc    600

cctgaagctg aaggacggcg gccactacct ggtggagttc aagtccatct acatggccaa    660

gaagcccgtg cagctgcccg gctactacta cgtggactcc aagctggaca tcacctccca    720

caacgaggac tacaccatcg tggagcagta cgagcgcgcc gagggccgcc accacctgtt    780

cctgtagtaa tgaata                                                    796


<210> SEQ ID NO 74
<211> LENGTH: 774
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 74

gggagaccac aacgguuucc cucuagaaau aauuuuguuu aacuuuaaga aggagauaua     60

ccaaugggc gugaugcgaa agcugacccu gccuccuccg aggacgucau caaggaguuc    120

augcgcuuca aggugcgcau ggagggcucc gugaacggcc acgaguucga gaucgagggc    180

gagggcgagg gccgccccua cgagggcacc cagaccgcca agcugaaggu gaccaagggc    240

ggcccccugc ccuucgccug ggacauccug ucccccagu uccaguacgg cuccaaggug    300

uacgugaagc accccgccga cauccccgac uacaagaagc uguccuuccc cgagggcuuc    360

aagugggagc gcgugaugaa cuucgaggac ggcggcgugg ugaccgugac ccaggacucc    420

ucccugcagg acggcuccuu caucuacaag gugaaguuca ucggcgugaa cuuccccucc    480

gacggccccg uaaugcagaa gaagacuaug ggcugggagg ccuccaccga gcgccuguac    540

ccccgcgacg gcgugcugaa gggcgagauc cacaaggccc ugaagcugaa ggacggcggc    600

cacuaccugg uggaguucaa guccaucuac auggccaaga agcccgugca gcugcccggc    660

uacuacuacg uggacuccaa gcuggacauc accuccccaca acgaggacua caccaucgug    720

gagcaguacg agcgcgccga gggccgccac caccuguucc uguaguaaug aaua          774


<210> SEQ ID NO 75
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 75

aaggagatat accaatgagg ggaaacccag cctcctccga ggacgtc                   47


<210> SEQ ID NO 76
<211> LENGTH: 784
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct
```

<400> SEQUENCE: 76

gaaattaata cgactcacta tagggagacc acaacggttt ccctctagaa ataattttgt 60

ttaactttaa gaaggagata taccaatgag ggaaacccca gcctcctccg aggacgtcat 120

caaggagttc atgcgcttca aggtgcgcat ggagggctcc gtgaacggcc acgagttcga 180

gatcgagggc gagggcgagg gccgccccta cgagggcacc cagaccgcca agctgaaggt 240

gaccaagggc ggcccccctgc ccttcgcctg ggacatcctg tccccccagt tccagtacgg 300

ctccaaggtg tacgtgaagc accccgccga catccccgac tacaagaagc tgtccttccc 360

cgagggcttc aagtgggagc gcgtgatgaa cttcgaggac ggcggcgtgg tgaccgtgac 420

ccaggactcc tccctgcagg acggctcctt catctacaag gtgaagttca tcggcgtgaa 480

cttcccctcc gacggccccg taatgcagaa gaagactatg ggctgggagg cctccaccga 540

gcgcctgtac cccgcgacg gcgtgctgaa gggcgagatc cacaaggccc tgaagctgaa 600

ggacggcggc cactacctgg tggagttcaa gtccatctac atggccaaga agcccgtgca 660

gctgcccggc tactactacg tggactccaa gctggacatc acctcccaca acgaggacta 720

caccatcgtg gagcagtacg agcgcgccga gggccgccac cacctgttcc tgtagtaatg 780

aata 784

<210> SEQ ID NO 77
<211> LENGTH: 762
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 77

gggagaccac aacgguuucc cucuagaaau aauuuuguuu aacuuuaaga aggagauaua 60

ccaaugaggg gaaacccagc cuccuccgag gacgucauca aggaguucau gcgcuucaag 120

gugcgcaugg agggcuccgu gaacggccac gaguucgaga ucgagggcga gggcgagggc 180

cgccccuacg agggcaccca gaccgccaag cugaagguga ccaagggcgg cccccugccc 240

uucgccuggg acauccuguc cccccaguuc caguacggcu ccaaggugua cgugaagcac 300

cccgccgaca uccccgacua caagaagcug uccuuccccg agggcuucaa gugggagcgc 360

gugaugaacu ucgaggacgg cggcguggug accgugaccc aggacuccuc ccugcaggac 420

ggcuccuuca ucuacaaggu gaaguucauc ggcgugaacu uccccuccga cggccccgua 480

augcagaaga agacuauggg cugggaggcc uccaccgagc gccuguaccc ccgcgacggc 540

gugcugaagg gcgagaucca caaggcccug aagcugaagg acggcggcca cuaccuggug 600

gaguucaagu ccaucuacau ggccaagaag cccgugcagc ugcccggcua cuacuacgug 660

gacuccaagc uggacaucac cucccacaac gaggacuaca ccaucgugga gcaguacgag 720

cgcgccgagg gccgccacca ccuguuccug uaguaaugaa ua 762

<210> SEQ ID NO 78
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 78

ggggtcagct ttcgcatcac gcccctatag tgagtcgtat tagc 44

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<210> SEQ ID NO 79
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 79

caccaagctt atgtacgtga gatttgaggt tcc                                 33


<210> SEQ ID NO 80
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 80

ccgctcgagc ttctgaaggc ctttaattct tc                                  32


<210> SEQ ID NO 81
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 81

gggcgtgatg cgaaagctga ccctgtgagc aagggcgagg agctg                    45


<210> SEQ ID NO 82
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 82

catggtggcg accggtggat c                                              21


<210> SEQ ID NO 83
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 83

aggggaaacc cagtgagcaa gggcgaggag ctg                                 33


<210> SEQ ID NO 84
<211> LENGTH: 5493
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 84

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ccgcatagtt aagccagtat ctgctccctg cttgtgtgtt ggaggtcgct gagtagtgcg    120

cgagcaaaat ttaagctaca acaaggcaag gcttgaccga caattgcatg aagaatctgc    180

ttagggttag gcgttttgcg ctgcttcgcg atgtacgggc cagatatacg cgttgacatt    240

gattattgac tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata    300
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tggagttccg cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc    360

cccgcccatt gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc    420

attgacgtca atgggtggag tatttacggt aaactgccca cttggcagta catcaagtgt    480

atcatatgcc aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt    540

atgcccagta catgacctta tgggactttc ctacttggca gtacatctac gtattagtca    600

tcgctattac catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg    660

actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc    720

aaaatcaacg ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggcg    780

gtaggcgtgt acggtgggag gtctatataa gcagagctct ctggctaact agagaaccca    840

ctgcttactg gcttatcgaa attaatacga ctcactatag ggagacccaa gctggctagt    900

taagcttggt accgagctcg gatccactag tccagtgtgg tggaattctg cagatatcca    960

gcacagtggc ggccgctcga gtctagaggg cccttcgaac aaaaactcat ctcagaagag   1020

gatctgaata tgcataccgg tcatcatcac catcaccatt gagtttaaac ccgctgatca   1080

gcctcgactg tgccttctag ttgccagcca tctgttgttt gcccctcccc cgtgccttcc   1140

ttgaccctgg aaggtgccac tcccactgtc ctttcctaat aaaatgagga aattgcatcg   1200

cattgtctga gtaggtgtca ttctattctg gggggtgggg tggggcagga cagcaagggg   1260

gaggattggg aagacaatag caggcatgct ggggatgcgg tgggctctat ggcttctgag   1320

gcggaaagaa ccagctgggg ctctaggggg tatccccacg cgccctgtag cggcgcatta   1380

agcgcggcgg gtgtggtggt tacgcgcagc gtgaccgcta cacttgccag cgccctagcg   1440

cccgctcctt tcgctttctt cccttccttt ctcgccacgt tcgccggctt tccccgtcaa   1500

gctctaaatc gggggctccc tttagggttc cgatttagtg ctttacggca cctcgacccc   1560

aaaaaacttg attagggtga tggttcacgt agtgggccat cgccctgata gacggttttt   1620

cgccctttga cgttggagtc cacgttcttt aatagtggac tcttgttcca aactggaaca   1680

acactcaacc ctatctcggt ctattctttt gatttataag ggattttgcc gatttcggcc   1740

tattggttaa aaaatgagct gatttaacaa aaatttaacg cgaattaatt ctgtggaatg   1800

tgtgtcagtt agggtgtgga aagtccccag gctccccagc aggcagaagt atgcaaagca   1860

tgcatctcaa ttagtcagca accaggtgtg gaaagtcccc aggctcccca gcaggcagaa   1920

gtatgcaaag catgcatctc aattagtcag caaccatagt cccgccccta actccgccca   1980

tcccgcccct aactccgccc agttccgccc attctccgcc ccatggctga ctaatttttt   2040

ttatttatgc agaggccgag gccgcctctg cctctgagct attccagaag tagtgaggag   2100

gcttttttgg aggcctaggc ttttgcaaaa agctcccggg agcttgtata tccattttcg   2160

gatctgatca agagacagga tgaggatcgt ttcgcatgat tgaacaagat ggattgcacg   2220

caggttctcc ggccgcttgg gtggagaggc tattcggcta tgactgggca caacagacaa   2280

tcggctgctc tgatgccgcc gtgttccggc tgtcagcgca ggggcgcccg gttctttttg   2340

tcaagaccga cctgtccggt gccctgaatg aactgcagga cgaggcagcg cggctatcgt   2400

ggctggccac gacgggcgtt ccttgcgcag ctgtgctcga cgttgtcact gaagcgggaa   2460

gggactggct gctattgggc gaagtgccgg ggcaggatct cctgtcatct caccttgctc   2520

ctgccgagaa agtatccatc atggctgatg caatgcggcg gctgcatacg cttgatccgg   2580

ctacctgccc attcgaccac caagcgaaac atcgcatcga gcgagcacgt actcggatgg   2640

aagccggtct tgtcgatcag gatgatctgg acgaagagca tcaggggctc gcgccagccg   2700
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aactgttcgc caggctcaag gcgcgcatgc ccgacggcga ggatctcgtc gtgacccatg   2760

gcgatgcctg cttgccgaat atcatggtgg aaaatggccg cttttctgga ttcatcgact   2820

gtggccggct gggtgtggcg gaccgctatc aggacatagc gttggctacc cgtgatattg   2880

ctgaagagct tggcggcgaa tgggctgacc gcttcctcgt gctttacggt atcgccgctc   2940

ccgattcgca gcgcatcgcc ttctatcgcc ttcttgacga gttcttctga gcgggactct   3000

ggggttcgcg aaatgaccga ccaagcgacg cccaacctgc catcacgaga tttcgattcc   3060

accgccgcct tctatgaaag gttgggcttc ggaatcgttt tccgggacgc cggctggatg   3120

atcctccagc gcggggatct catgctggag ttcttcgccc accccaactt gtttattgca   3180

gcttataatg gttacaaata aagcaatagc atcacaaatt tcacaaataa agcatttttt   3240

tcactgcatt ctagttgtgg tttgtccaaa ctcatcaatg tatcttatca tgtctgtata   3300

ccgtcgacct ctagctagag cttggcgtaa tcatggtcat agctgtttcc tgtgtgaaat   3360

tgttatccgc tcacaattcc acacaacata cgagccggaa gcataaagtg taaagcctgg   3420

ggtgcctaat gagtgagcta actcacatta attgcgttgc gctcactgcc cgctttccag   3480

tcgggaaacc tgtcgtgcca gctgcattaa tgaatcggcc aacgcgcggg gagaggcggt   3540

ttgcgtattg ggcgctcttc cgcttcctcg ctcactgact cgctgcgctc ggtcgttcgg   3600

ctgcggcgag cggtatcagc tcactcaaag gcggtaatac ggttatccac agaatcaggg   3660

gataacgcag gaaagaacat gtgagcaaaa ggccagcaaa aggccaggaa ccgtaaaaag   3720

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ggaagctccc tcgtgcgctc tcctgttccg accctgccgc ttaccggata cctgtccgcc   3900

tttctccctt cgggaagcgt ggcgctttct catagctcac gctgtaggta tctcagttcg   3960

gtgtaggtcg ttcgctccaa gctgggctgt gtgcacgaac cccccgttca gcccgaccgc   4020

tgcgccttat ccggtaacta tcgtcttgag tccaacccgg taagacacga cttatcgcca   4080

ctggcagcag ccactggtaa caggattagc agagcgaggt atgtaggcgg tgctacagag   4140

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ctgctgaagc cagttacctt cggaaaaaga gttggtagct cttgatccgg caaacaaacc   4260

accgctggta gcggtggttt ttttgtttgc aagcagcaga ttacgcgcag aaaaaaagga   4320

tctcaagaag atcctttgat cttttctacg gggtctgacg ctcagtggaa cgaaaactca   4380

cgttaaggga ttttggtcat gagattatca aaaaggatct tcacctagat ccttttaaat   4440

taaaaatgaa gttttaaatc aatctaaagt atatatgagt aaacttggtc tgacagttac   4500

caatgcttaa tcagtgaggc acctatctca gcgatctgtc tatttcgttc atccatagtt   4560

gcctgactcc ccgtcgtgta gataactacg atacgggagg gcttaccatc tggccccagt   4620

gctgcaatga taccgcgaga cccacgctca ccggctccag atttatcagc aataaaccag   4680

ccagccggaa gggccgagcg cagaagtggt cctgcaactt tatccgcctc catccagtct   4740

attaattgtt gccgggaagc tagagtaagt agttcgccag ttaatagttt gcgcaacgtt   4800

gttgccattg ctacaggcat cgtggtgtca cgctcgtcgt ttggtatggc ttcattcagc   4860

tccggttccc aacgatcaag gcgagttaca tgatccccca tgttgtgcaa aaaagcggtt   4920

agctccttcg gtcctccgat cgttgtcaga agtaagttgg ccgcagtgtt atcactcatg   4980

gttatggcag cactgcataa ttctcttact gtcatgccat ccgtaagatg cttttctgtg   5040
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actggtgagt actcaaccaa gtcattctga gaatagtgta tgcggcgacc gagttgctct 5100

tgcccggcgt caatacggga taataccgcg ccacatagca gaactttaaa agtgctcatc 5160

attggaaaac gttcttcggg gcgaaaactc tcaaggatct taccgctgtt gagatccagt 5220

tcgatgtaac ccactcgtgc acccaactga tcttcagcat cttttacttt caccagcgtt 5280

tctgggtgag caaaaacagg aaggcaaaat gccgcaaaaa agggaataag ggcgacacgg 5340

aaatgttgaa tactcatact cttccttttt caatattatt gaagcattta tcagggttat 5400

tgtctcatga gcggatacat atttgaatgt atttagaaaa ataaacaaat aggggttccg 5460

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<210> SEQ ID NO 85
<211> LENGTH: 5782
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 85

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ccgcatagtt aagccagtat ctgctccctg cttgtgtgtt ggaggtcgct gagtagtgcg 120

cgagcaaaat ttaagctaca acaaggcaag gcttgaccga caattgcatg aagaatctgc 180

ttagggttag gcgttttgcg ctgcttcgcg atgtacgggc cagatatacg cgttgacatt 240

gattattgac tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata 300

tggagttccg cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc 360

cccgcccatt gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc 420

attgacgtca atgggtggag tatttacggt aaactgccca cttggcagta catcaagtgt 480

atcatatgcc aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt 540

atgcccagta catgacctta tgggactttc ctacttggca gtacatctac gtattagtca 600

tcgctattac catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg 660

actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc 720

aaaatcaacg ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggcg 780

gtaggcgtgt acggtgggag gtctatataa gcagagctct ctggctaact agagaaccca 840

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caagaacgac cttggaaggg ctgtgggcat tgaggtgcca tgcgcttcgg cagcgataat 1200

caacgaggga gagctgagaa aggagcttgg aagccttgtg gagaagatta aaggccttca 1260

gaagctcgag tctagagggc ccttcgaaca aaaactcatc tcagaagagg atctgaatat 1320

gcataccggt catcatcacc atcaccattg agtttaaacc cgctgatcag cctcgactgt 1380

gccttctagt tgccagccat ctgttgtttg cccctccccc gtgccttcct tgaccctgga 1440

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taggtgtcat tctattctgg ggggtggggt ggggcaggac agcaaggggg aggattggga 1560

agacaatagc aggcatgctg ggatgcggt gggctctatg gcttctgagg cggaaagaac 1620

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cagctggggc tctagggggt atccccacgc gccctgtagc ggcgcattaa gcgcggcggg   1680

tgtggtggtt acgcgcagcg tgaccgctac acttgccagc gccctagcgc ccgctccttt   1740

cgctttcttc ccttcctttc tcgccacgtt cgccggcttt ccccgtcaag ctctaaatcg   1800

ggggctccct ttagggttcc gatttagtgc tttacggcac ctcgacccca aaaaacttga   1860

ttagggtgat ggttcacgta gtgggccatc gccctgatag acggtttttc gccctttgac   1920

gttggagtcc acgttcttta atagtggact cttgttccaa actggaacaa cactcaaccc   1980

tatctcggtc tattcttttg atttataagg gattttgccg atttcggcct attggttaaa   2040

aaatgagctg atttaacaaa aatttaacgc gaattaattc tgtggaatgt gtgtcagtta   2100

gggtgtggaa agtccccagg ctccccagca ggcagaagta tgcaaagcat gcatctcaat   2160

tagtcagcaa ccaggtgtgg aaagtcccca ggctccccag caggcagaag tatgcaaagc   2220

atgcatctca attagtcagc aaccatagtc ccgcccctaa ctccgcccat cccgccccta   2280

actccgccca gttccgccca ttctccgccc catggctgac taattttttt tatttatgca   2340

gaggccgagg ccgcctctgc ctctgagcta ttccagaagt agtgaggagg cttttttgga   2400

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gagacaggat gaggatcgtt tcgcatgatt gaacaagatg gattgcacgc aggttctccg   2520

gccgcttggg tggagaggct attcggctat gactgggcac aacagacaat cggctgctct   2580

gatgccgccg tgttccggct gtcagcgcag gggcgcccgg ttctttttgt caagaccgac   2640

ctgtccggtg ccctgaatga actgcaggac gaggcagcgc ggctatcgtg gctggccacg   2700

acgggcgttc cttgcgcagc tgtgctcgac gttgtcactg aagcgggaag ggactggctg   2760

ctattgggcg aagtgccggg gcaggatctc ctgtcatctc accttgctcc tgccgagaaa   2820

gtatccatca tggctgatgc aatgcggcgg ctgcatacgc ttgatccggc tacctgccca   2880

ttcgaccacc aagcgaaaca tcgcatcgag cgagcacgta ctcggatgga agccggtctt   2940

gtcgatcagg atgatctgga cgaagagcat caggggctcg cgccagccga actgttcgcc   3000

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aaagaacatg tgagcaaaag gccagcaaaa ggccaggaac cgtaaaaagg ccgcgttgct   4020

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gaggtggcga aacccgacag gactataaag ataccaggcg tttccccctg gaagctccct   4140

cgtgcgctct cctgttccga ccctgccgct taccggatac ctgtccgcct ttctcccttc   4200

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tcgctccaag ctgggctgtg tgcacgaacc ccccgttcag cccgaccgct gcgccttatc   4320

cggtaactat cgtcttgagt ccaacccggt aagacacgac ttatcgccac tggcagcagc   4380

cactggtaac aggattagca gagcgaggta tgtaggcggt gctacagagt tcttgaagtg   4440

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agttaccttc ggaaaaagag ttggtagctc ttgatccggc aaacaaacca ccgctggtag   4560

cggtggtttt tttgtttgca agcagcagat tacgcgcaga aaaaaaggat ctcaagaaga   4620

tcctttgatc ttttctacgg ggtctgacgc tcagtggaac gaaaactcac gttaagggat   4680

tttggtcatg agattatcaa aaaggatctt cacctagatc cttttaaatt aaaaatgaag   4740

ttttaaatca atctaaagta tatatgagta aacttggtct gacagttacc aatgcttaat   4800

cagtgaggca cctatctcag cgatctgtct atttcgttca tccatagttg cctgactccc   4860

cgtcgtgtag ataactacga tacgggaggg cttaccatct ggccccagtg ctgcaatgat   4920

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ctcaaccaag tcattctgag aatagtgtat gcggcgaccg agttgctctt gcccggcgtc   5400

aatacgggat aataccgcgc cacatagcag aactttaaaa gtgctcatca ttggaaaacg   5460

ttcttcgggg cgaaaactct caaggatctt accgctgttg agatccagtt cgatgtaacc   5520

cactcgtgca cccaactgat cttcagcatc ttttactttc accagcgttt ctgggtgagc   5580

aaaaacagga aggcaaaatg ccgcaaaaaa gggaataagg gcgacacgga aatgttgaat   5640

actcatactc ttcctttttc aatattattg aagcatttat cagggttatt gtctcatgag   5700

cggatacata tttgaatgta tttagaaaaa taaacaaata ggggttccgc gcacatttcc   5760

ccgaaaagtg ccacctgacg tc                                            5782
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<210> SEQ ID NO 86
<211> LENGTH: 4757
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 86

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cccatcctgg tcgagctgga cggcgacgta aacggccaca agttcagcgt gtccggcgag    120

ggcgagggcg atgccaccta cggcaagctg accctgaagt tcatctgcac caccggcaag    180

ctgcccgtgc cctggcccac cctcgtgacc accctgacct acggcgtgca gtgcttcagc    240
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cgctaccccg accacatgaa gcagcacgac ttcttcaagt ccgccatgcc cgaaggctac    300
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aagttcgagg gcgacaccct ggtgaaccgc atcgagctga agggcatcga cttcaaggag    420
gacggcaaca tcctggggca caagctggag tacaactaca acagccacaa cgtctatatc    480
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tctggattca tcgactgtgg ccggctgggt gtggcggacc gctatcagga catagcgttg   2640

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tacggtatcg ccgctcccga ttcgcagcgc atcgccttct atcgccttct tgacgagttc   2760

ttctgagcgg gactctgggg ttcgaaatga ccgaccaagc gacgcccaac ctgccatcac   2820

gagatttcga ttccaccgcc gccttctatg aaaggttggg cttcggaatc gttttccggg   2880

acgccggctg gatgatcctc cagcgcgggg atctcatgct ggagttcttc gcccacccta   2940

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caataaaaag acagaataaa acgcacggtg ttgggtcgtt tgttcataaa cgcggggttc   3060

ggtcccaggg ctggcactct gtcgataccc caccgagacc ccattggggc caatacgccc   3120

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tgggaggtct atataagcag agctggttta gtgaaccgtc agatccgcta gcgctaccgg   4680

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<210> SEQ ID NO 87
<211> LENGTH: 4745
<212> TYPE: DNA
<213> ORGANISM: Artificial

<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 87

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gccacctacg gcaagctgac cctgaagttc atctgcacca ccggcaagct gcccgtgccc 180

tggcccaccc tcgtgaccac cctgacctac ggcgtgcagt gcttcagccg ctaccccgac 240

cacatgaagc agcacgactt cttcaagtcc gccatgcccg aaggctacgt ccaggagcgc 300

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gacaccctgg tgaaccgcat cgagctgaag ggcatcgact tcaaggagga cggcaacatc 420

ctggggcaca agctggagta caactacaac agccacaacg tctatatcat ggccgacaag 480

cagaagaacg gcatcaaggt gaacttcaag atccgccaca acatcgagga cggcagcgtg 540

cagctcgccg accactacca gcagaacacc cccatcggcg acggccccgt gctgctgccc 600

gacaaccact acctgagcac ccagtccgcc ctgagcaaag accccaacga gaagcgcgat 660

cacatggtcc tgctggagtt cgtgaccgcc gccgggatca ctctcggcat ggacgagctg 720

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ttaaagaacg tggactccaa cgtcaaaggg cgaaaaaccg tctatcaggg cgatggccca 1200

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ccatg 4745

<210> SEQ ID NO 88
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 88

caaggaggac ggcaaca 17

<210> SEQ ID NO 89
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 89

ccttgatgcc gttcttctgc 20

<210> SEQ ID NO 90
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 90

agccacatcg ctcagacac 19

<210> SEQ ID NO 91
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 91

gcccaatacg accaaatcc 19

<210> SEQ ID NO 92
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 92

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<210> SEQ ID NO 93
<211> LENGTH: 61
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 93

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<210> SEQ ID NO 94
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 94

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<210> SEQ ID NO 95
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 95

ccggggatcc tctagagt                                                     18


<210> SEQ ID NO 96
<211> LENGTH: 61
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 96

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c                                                                       61


<210> SEQ ID NO 97
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 97

ccggggatcc tctagagtc                                                    19


<210> SEQ ID NO 98
<211> LENGTH: 92
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 98

gggagaccac aacgguuucc cucuagaaau aauuuuguuu aacuuuaaga aggagauaua      60

ccaaugguga gcaagggcga ggagcuguuc ac                                     92


<210> SEQ ID NO 99
<211> LENGTH: 91
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 99

gggagaccac aacgguuucc cucgggcgug augcgaaagc ugacccagaa ggagauauac      60

caauggugag caagggcgag gagcuguuca c                                      91
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<210> SEQ ID NO 100
<211> LENGTH: 91
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 100

gggagaccac aacgguuucc cucgggcguc augcgaaagc ugccccagaa ggagauauac 60

caauggugag caagggcgag gagcuguuca c 91

<210> SEQ ID NO 101
<211> LENGTH: 78
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 101

gggagaccac aacgguuucc cucggggaaa cccagaagga gauauaccaa uggugagcaa 60

gggcgaggag cuguucac 78

<210> SEQ ID NO 102
<211> LENGTH: 94
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 102

gggagaccac aacgguuucc cucgggcgug augcgaaagc ugacccuuaa gaaggagaua 60

uaccaauggu gagcaagggc gaggagcugu ucac 94

<210> SEQ ID NO 103
<211> LENGTH: 98
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 103

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gauauaccaa uggugagcaa gggcgaggag cuguucac 98

<210> SEQ ID NO 104
<211> LENGTH: 102
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 104

gggagaccac aacgguuucc cucgggcgug augcgaaagc ugacccguuu aacuuuaaga 60

aggagauaua ccaauggugа gcaagggcga ggagcuguuc ac 102

<210> SEQ ID NO 105
<211> LENGTH: 122
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

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<400> SEQUENCE: 105

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caaauuuuuc uuuguaugug aucuuucgug ugggucacca cugcaaauaa ggauauaaaa    120

ug                                                                   122


<210> SEQ ID NO 106
<211> LENGTH: 79
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 106

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agaaggagau auaccaaug                                                  79


<210> SEQ ID NO 107
<211> LENGTH: 79
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 107

gggagaccac aacgguuucc cucggcguau gugaucuuuc augugggucа ccacugcgcc     60

agaaggagau auaccaaug                                                  79


<210> SEQ ID NO 108
<211> LENGTH: 116
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 108

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ccaaugggc gugaugcgaa agcugacccu gugagcaagg gcgaggagcu guucac          116


<210> SEQ ID NO 109
<211> LENGTH: 104
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 109

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ccaaugaggg gaaacccagu gagcaagggc gaggagcugu ucac                     104


<210> SEQ ID NO 110
<211> LENGTH: 122
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 110

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The invention claimed is:

1. An isolated non-naturally occurring mRNA encoding a protein comprising an RNA-protein complex interacting motif nucleotide sequence incorporated 5' to a ribosome-binding site in a position 2 to 10 bases distant from the ribosome-binding site or within the 5' region of an open reading frame, wherein the interacting motif comprises nucleic acid sequence SEQ ID NO:9, wherein an RNA-protein complex of the interacting motif and L7Ae protein has a dissociation constant Kd of approximately 0.1 nM to approximately 1 μM between the motif and L7Ae and the interacting motif interacts with L7Ae protein.

2. An RNA-protein complex comprising an mRNA according to claim **1** and a protein specifically binding to the nucleotide sequence.

3. A translational regulatory kit comprising an mRNA according to claim **1** and a protein specifically binding to the nucleotide sequence.

4. A method for translational regulation of mRNA, comprising contacting the mRNA according to claim **1** with a protein specifically binding to the RNA-protein complex interacting motif nucleotide sequence.

5. An artificial information conversion method which converts input information of an arbitrary substrate protein to output information of an arbitrary target protein using an mRNA according to claim **1**, comprising steps of

preparing the mRNA of claim **1** having an open reading frame encoding the arbitrary target protein; and

contacting the mRNA with the substrate protein that specifically binds to the RNA-protein complex interacting motif nucleotide sequence.

6. A plasmid vector comprising a nucleic acid sequence encoding an mRNA according to claim **1**.

7. An intracellular translational regulatory kit comprising

a first plasmid vector comprising a nucleic acid sequence encoding an mRNA according to claim **1**, and

a second plasmid vector comprising a nucleic acid sequence encoding a protein specifically binding to the RNA-protein complex interacting motif nucleotide sequence.

8. The kit according to claim **7**, for regulating protein translation in a human cancer cell.

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