



US 20150176004A1

(19) **United States**

(12) **Patent Application Publication**  
**Yomo et al.**

(10) **Pub. No.: US 2015/0176004 A1**

(43) **Pub. Date: Jun. 25, 2015**

(54) **IN VITRO MEMBRANE PROTEIN  
MOLECULAR EVOLUTIONARY  
ENGINEERING TECHNIQUE**

**Publication Classification**

(71) Applicant: **Japan Science and Technology Agency,**  
Saitama (JP)

(51) **Int. Cl.**  
*C12N 15/11* (2006.01)  
*C12Q 1/68* (2006.01)

(72) Inventors: **Tetsuya Yomo,** Osaka (JP); **Tomoaki  
Matsuura,** Osaka (JP); **Haruka Soga,**  
Osaka (JP); **Hajime Watanabe,** Osaka  
(JP); **Satoshi Fujii,** Osaka (JP)

(52) **U.S. Cl.**  
CPC ..... *C12N 15/11* (2013.01); *C12Q 1/686*  
(2013.01)

(21) Appl. No.: **14/411,892**

(57) **ABSTRACT**

(22) PCT Filed: **Jun. 17, 2013**

The objective of the present invention is to improve the efficiency of screening/selection of a membrane protein in molecular evolutionary engineering (for example, an enzyme evolutionary method).

(86) PCT No.: **PCT/JP2013/003767**

The above-described objective is achieved by providing a unilamellar liposome comprising:

§ 371 (c)(1),

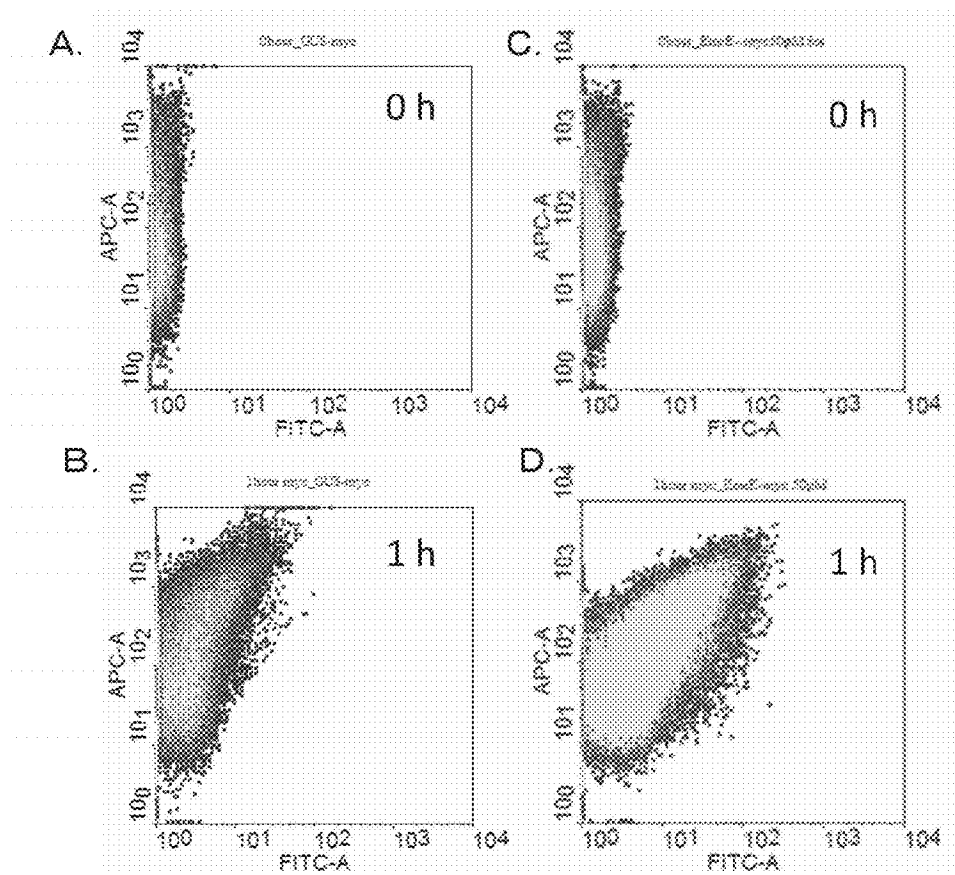
(2) Date: **Dec. 29, 2014**

- (a) a DNA comprising a promoter sequence, a translational initiation sequence, and a sequence encoding a membrane protein;
- (b) an RNA polymerase;
- (c) a ribonucleotide; and
- (d) a cell-free protein synthesis system. In one aspect of the present invention, the membrane protein is a transporter, and the unilamellar liposome further comprises
- (e) a factor that binds to a ligand transported by the membrane protein.

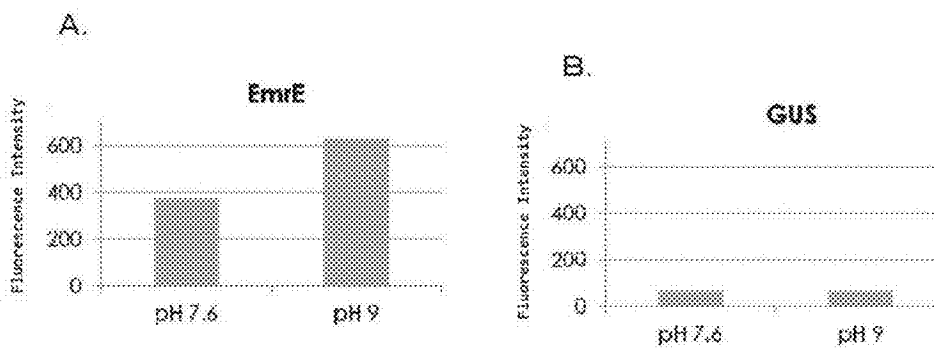
(30) **Foreign Application Priority Data**

Jun. 28, 2012 (JP) ..... 2012-145795

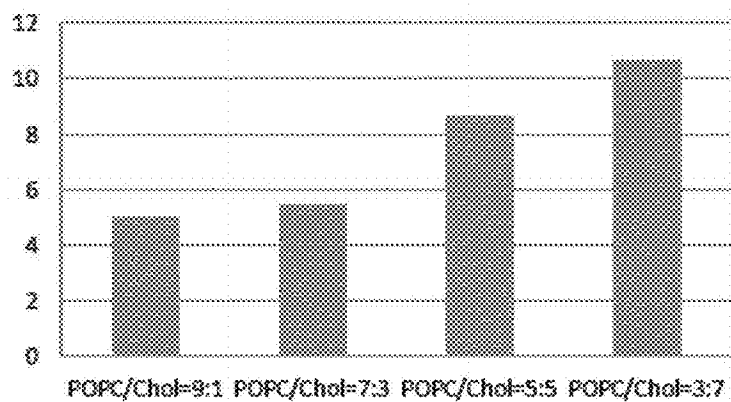
[Fig. 1]



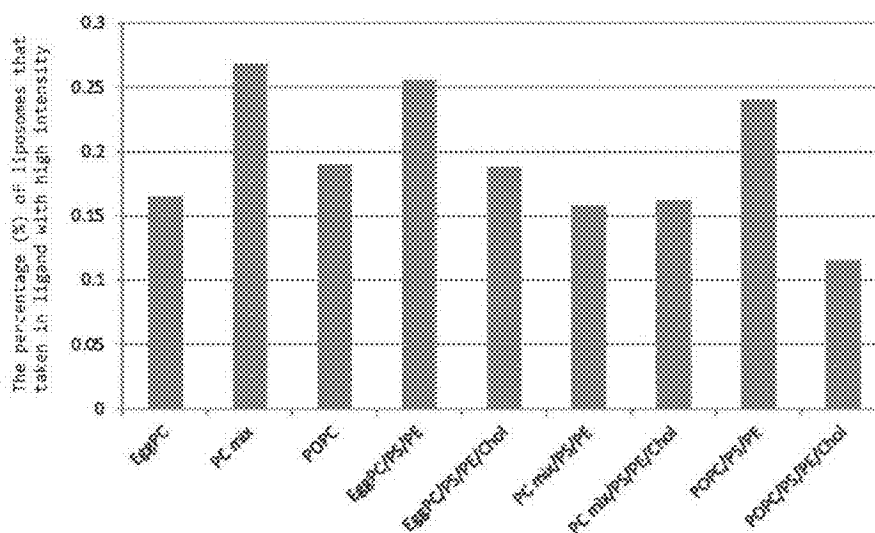
[Fig. 2]



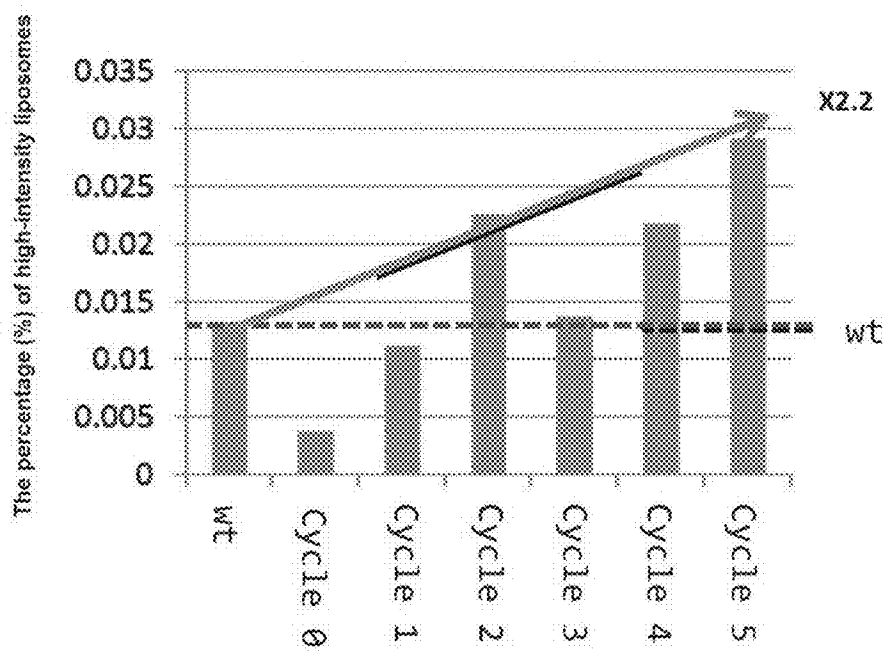
[Fig. 3]



[Fig. 4]



[Fig. 5]



**IN VITRO MEMBRANE PROTEIN  
MOLECULAR EVOLUTIONARY  
ENGINEERING TECHNIQUE**

**TECHNICAL FIELD**

**[0001]** The present invention relates to the field of novel unilamellar liposomes for utilization in in-vitro molecular evolutionary engineering of membrane proteins. The present invention further relates to novel molecular evolutionary engineering, particularly enzyme evolutionary engineering, targeting membrane proteins that uses the unilamellar liposomes.

**BACKGROUND ART**

**[0002]** As a method of improving an enzyme by evolutionary engineering, a method using liposomes in which a gene library and a cell-free protein synthesis system are enclosed, and a cell sorter has been utilized. In this method, a gene library in which random mutation is introduced into an enzyme gene and a cell-free protein synthesis system are enclosed in liposomes for internal expression of an enzyme. Further, a liposome that contains an enzyme having a higher function is selected by the cell sorter to enable selection of a gene encoding an enzyme having a higher function. By repeating this selection, a gene encoding an enzyme can be evolved (Non Patent Literature 1). This conventional method is solely targeted to soluble proteins.

**[0003]** It is well known that membrane proteins play an important role in functions of cells. Thus, novel molecular evolutionary engineering, particularly enzyme evolutionary engineering, targeting membrane proteins has been required.

**CITATION LIST**

**Non Patent Literature**

**[0004]** [NPL 1] Sunami, T., Sato, K., Matsuura, T., Tsukada, K., Urabe, I., and Yomo, T. (2006) Analytical biochemistry 357, 128-136

**SUMMARY OF INVENTION**

**Technical Problem**

**[0005]** The objective of the present invention is to provide a novel molecular evolutionary engineering technique, particularly an enzyme evolutionary engineering technique, targeting membrane proteins.

**Solution to Problem**

**[0006]** The above-described objective has been achieved by providing the following.

(Item 1)

**[0007]** A unilamellar liposome comprising:

**[0008]** (a) a DNA comprising a promoter sequence, a translational initiation sequence, and a sequence encoding a membrane protein;

**[0009]** (b) an RNA polymerase;

**[0010]** (c) a ribonucleotide; and

**[0011]** (d) a cell-free protein synthesis system.

(Item 2)

**[0012]** The unilamellar liposome of item 1, wherein the membrane protein is a transporter, and the unilamellar liposome further comprises

**[0013]** (e) a factor that binds to a ligand transported by the membrane protein.

(Item 3)

**[0014]** The unilamellar liposome of item 1 or 2, wherein the unilamellar liposome is treated with a nuclease.

(Item 4)

**[0015]** The unilamellar liposome of item 3, wherein the nuclease is selected from the group consisting of a ribonuclease and a deoxyribonuclease.

(Item 5)

**[0016]** The unilamellar liposome of item 4, wherein the nuclease is a ribonuclease.

(Item 6)

**[0017]** A library comprising a plurality of unilamellar liposomes, wherein the unilamellar liposome comprises:

**[0018]** (a) a DNA comprising a promoter sequence, a translational initiation sequence, and a sequence encoding a membrane protein;

**[0019]** (b) an RNA polymerase;

**[0020]** (c) a ribonucleotide; and

**[0021]** (d) a cell-free protein synthesis system.

(Item 7)

**[0022]** The library of item 6, wherein the membrane protein is a transporter, and the unilamellar liposome further comprises

**[0023]** (e) a factor that binds to a ligand transported by the membrane protein.

(Item 8)

**[0024]** The library of item 6 or 7, wherein the unilamellar liposome is treated with a nuclease.

(Item 9)

**[0025]** The library of item 8, wherein the nuclease is selected from the group consisting of a ribonuclease and a deoxyribonuclease.

(Item 10)

**[0026]** The library of item 9, wherein the nuclease is a ribonuclease.

(Item 11)

**[0027]** A unilamellar liposome comprising:

**[0028]** (a) an RNA comprising a translational initiation sequence, and a sequence encoding a membrane protein; and

**[0029]** (d) a cell-free protein synthesis system.

(Item 12)

**[0030]** The unilamellar liposome of item 11, wherein the membrane protein is a transporter, and the unilamellar liposome further comprises

**[0031]** (e) a factor that binds to a ligand transported by the membrane protein.

(Item 13)

**[0032]** The unilamellar liposome of item 11 or 12, wherein the unilamellar liposome is treated with a nuclease.

(Item 14)

**[0033]** The unilamellar liposome of item 13, wherein the nuclease is selected from the group consisting of a ribonuclease and a deoxyribonuclease.

(Item 15)

**[0034]** The unilamellar liposome of item 14, wherein the nuclease is a ribonuclease.

(Item 16)

**[0035]** A library comprising a plurality of unilamellar liposomes, wherein the unilamellar liposome comprises:

**[0036]** (a) an RNA comprising a translational initiation sequence, and a sequence encoding a membrane protein; and

**[0037]** (d) a cell-free protein synthesis system.

(Item 17)

**[0038]** The library of item 16, wherein the membrane protein is a transporter, and the unilamellar liposome further comprises

**[0039]** (e) a factor that binds to a ligand transported by the membrane protein.

(Item 18)

**[0040]** The library of item 16 or 17, wherein the unilamellar liposome is treated with a nuclease.

(Item 19)

**[0041]** The library of item 18, wherein the nuclease is selected from the group consisting of a ribonuclease and a deoxyribonuclease.

(Item 20)

**[0042]** The library of item 19, wherein the nuclease is a ribonuclease.

(Item 21)

**[0043]** A method of producing a unilamellar liposome treated with a nuclease, comprising:

**[0044]** (1) preparing a unilamellar liposome enclosing:

**[0045]** (a) a DNA comprising a promoter sequence, a translational initiation sequence, and a sequence encoding a membrane protein;

**[0046]** (b) an RNA polymerase;

**[0047]** (c) a ribonucleotide; and

**[0048]** (d) a cell-free protein synthesis system; and

**[0049]** (2) treating the unilamellar liposome prepared in (1) with a nuclease.

(Item 22)

**[0050]** A method of producing a unilamellar liposome treated with a nuclease, comprising:

**[0051]** (1) preparing a unilamellar liposome enclosing:

**[0052]** (a) a DNA comprising a promoter sequence, a translational initiation sequence, and a sequence encoding a membrane protein that is a transporter;

**[0053]** (b) an RNA polymerase;

**[0054]** (c) a ribonucleotide;

**[0055]** (d) a cell-free protein synthesis system; and

**[0056]** (e) a factor that binds to a ligand transported by the membrane protein; and

**[0057]** (2) treating the unilamellar liposome prepared in (1) with a nuclease.

(Item 23)

**[0058]** The method of item 21 or 22, wherein the nuclease is selected from the group consisting of a ribonuclease and a deoxyribonuclease.

(Item 24)

**[0059]** The method of item 23, wherein the nuclease is a ribonuclease.

(Item 25)

**[0060]** A method of producing a unilamellar liposome treated with a nuclease, comprising:

**[0061]** (1) preparing a unilamellar liposome enclosing:

**[0062]** (a) an RNA comprising a translational initiation sequence, and a sequence encoding a membrane protein; and

**[0063]** (d) a cell-free protein synthesis system; and

**[0064]** (2) treating the unilamellar liposome prepared in (1) with a nuclease.

(Item 26)

**[0065]** A method of producing a unilamellar liposome treated with a nuclease, comprising:

**[0066]** (1) preparing a unilamellar liposome enclosing:

**[0067]** (a) an RNA comprising a translational initiation sequence, and a sequence encoding a membrane protein that is a transporter;

**[0068]** (d) a cell-free protein synthesis system; and

**[0069]** (e) a factor that binds to a ligand transported by the membrane protein; and

**[0070]** (2) treating the unilamellar liposome prepared in (1) with a nuclease.

(Item 27)

**[0071]** The method of item 25 or 26, wherein the nuclease is selected from the group consisting of a ribonuclease and a deoxyribonuclease.

(Item 28)

**[0072]** The method of item 27, wherein the nuclease is a ribonuclease.

(Item 29)

**[0073]** A screening method using a library of unilamellar liposomes, comprising:

**[0074]** (i) providing a library of any of items 6 to 10;

**[0075]** (ii) selecting a unilamellar liposome having a desired feature from the library;

**[0076]** (iii) amplifying a DNA included in the unilamellar liposome; and

**[0077]** (iv) isolating the amplified DNA.

(Item 30)

**[0078]** A screening method using a library of unilamellar liposomes, comprising:

**[0079]** (i) providing a library of any of items 16 to 20;

**[0080]** (ii) selecting a unilamellar liposome having a desired feature from the library;

**[0081]** (iii) generating a DNA by operating a reverse transcriptase on an RNA included in the unilamellar liposome;

**[0082]** (iv) amplifying the generated DNA; and

**[0083]** (v) isolating the amplified DNA.

#### Advantageous Effects of Invention

**[0084]** The present invention enables an in-vitro molecular evolutionary engineering technique targeting membrane proteins that utilizes liposomes. The present invention further enables large-scale screening/selection of a gene encoding a membrane protein having a desired function.

**[0085]** If a membrane protein is a transporter, a factor that binds to a ligand transported by the membrane protein would be enclosed within a liposome to capture the transported ligand within the liposome, thereby enhancing the sensitivity of screening/selection.

**[0086]** Further, by using unilamellar liposomes that are processed by a nuclease according to the present invention, screening efficiency will be enhanced. While not wishing to be bound by theory, the following reason can be mentioned as a reason that the present invention exerts a remarkable effect. Conventionally-used liposomes are multilamellar liposomes that are prepared by a freeze-drying method, and since those liposomes internally have a multiple structure, the volume of a reaction vessel is not possible to be controlled. The volume of liposomes affects the internal enzymatic kinetics. Thus, in order to efficiently improve an enzyme, the use of unilamellar liposomes which do not have a multiple structure is preferable. However, in methods so far, when unilamellar liposomes that are prepared by a centrifugal sedimentation method are used as reaction vessels, selection and collection of a gene encoding an enzyme having a high function were not possible even by selecting liposomes that were more reactive than others by a cell sorter. In contrast, in the present invention, treatment of unilamellar liposomes with a nuclease enables further highly-efficient screening compared to unilamellar liposomes that are not treated with an enzyme and multilamellar liposomes used in conventional methods, thereby allowing selection and collection of a gene encoding a highly-functional enzyme.

**[0087]** In addition, by optimizing the composition/ratio of a lipid forming a liposome and the magnesium concentration when preparing the liposome according to the disclosure of the present invention, the sensitivity of screening/selection will be further enhanced.

#### BRIEF DESCRIPTION OF DRAWINGS

**[0088]** FIG. 1 is the result of using a DNA comprising an EmrE-myc-his sequence (SEQ ID NO: 1) or a DNA comprising a GUS sequence (SEQ ID NO: 3), wherein a labeling anti-Myc tag antibody is added to liposomes before and after the expression of proteins, and an analysis is performed by a cell sorter. The vertical axis shows the internal volume of liposomes and the horizontal axis shows the fluorescence intensity of Alexa 488. A and B show the results of using the GUS sequence, and C and D show the results of using the EmrE-myc-his sequence. A and C are results of liposomes

before the expression of proteins by incubation at 37° C., and B and D are results of liposomes that expressed proteins by an hour incubation at 37° C.

**[0089]** FIG. 2 is the result of measuring the transport activity of EtBr with different pH, in liposomes comprising a DNA comprising an EmrE-myc-his sequence (SEQ ID NO: 1; FIG. 2A) or a DNA comprising a GUS sequence (SEQ ID NO: 3; FIG. 2B), wherein proteins are expressed.

**[0090]** FIG. 3 is the result showing the percentage of expression of a membrane protein having a function when various lipid compositions are used. When hemolysin exerts the activity, Halo Tag Alexa Fluor 488 ligand is taken in with high intensity, and thus the vertical axis shows the percentage (%) of liposomes that taken in ligands with high intensity. That is, the vertical axis shows the percentage of exertion of membrane protein activity in liposomes. The results of using the mixture of POPC:Chol=9:1; the mixture of POPC:Chol=7:3; the mixture of POPC:Chol=5:5; and the mixture of POPC:Chol=3:7 are shown in order from the left. Further, POPC is an abbreviation of 1-palmitoyl-2-oleoylphosphatidylcholine, and Chol is an abbreviation of cholesterol. [FIG. 4] The vertical axis of FIG. 4 shows the percentage (%) of liposomes that taken in Halo Tag Alexa Fluor 488 ligand with high intensity among all the liposomes when various lipids are used. That is, FIG. 4 is a graph showing the relative activity of channels. The lipids that are used are as follows: EggPC is an abbreviation of phosphatidylcholine purified from a hen's egg; POPC is an abbreviation of 1-palmitoyl-2-oleoylphosphatidylcholine; PS is an abbreviation of 1-palmitoyl-2-oleoylphosphoserine; PE is an abbreviation of 1-palmitoyl-2-oleoylphosphoethanolamine; and Chol is an abbreviation of cholesterol. PC mix is an abbreviation of the mixture of 1-palmitoyl-2-oleoylphosphatidylcholine:1-palmitoyl-2-linoleoylphosphatidylcholine:1-stearoyl-2-oleoylphosphatidylcholine:1-stearoyl-2-linoleoylphosphatidylcholine=129:67:48:24 (mass ratio); EggPC/PS/PE is an abbreviation of the mixture of each of them at the ratio of 3:1:1 (mass ratio) in order; EggPC/PS/PE/Chol is an abbreviation of the mixture of each of them at the ratio of 2:1:1:1 (mass ratio) in order; PCmix/PS/PE is an abbreviation of the mixture of each of them at the ratio of 3:1:1 (mass ratio) in order; PCmix/PS/PE/Chol is an abbreviation of the mixture of each of them at the ratio of 2:1:1:1 (mass ratio) in order; POPC/PS/PE is an abbreviation of the mixture of each of them at the ratio of 3:1:1 (mass ratio) in order; and POPC/POPE/POPS/Chol is an abbreviation of the mixture of each of them at the ratio of 2:1:1:1 (mass ratio) in order.

**[0091]** FIG. 5 is a graph showing the result of an evolutionary experiment. The vertical axis shows the percentage of high intensity liposomes (the percentage of red dots). By repeating the cycle, the percentage of a group having high activity increased.

#### DESCRIPTION OF EMBODIMENTS

**[0092]** Hereinafter, the present invention will be described. It should be understood that unless particularly stated otherwise, the terms used in the present specification have the meanings that are conventionally used in the art.

**[0093]** Hereinafter, the definitions of the terms that are used particularly in the present specification will be listed.

#### DEFINITION

**[0094]** The term "micro-compartment" as used herein refers to a closed minute space composed of a lipid layer and

an internal aqueous layer. Examples of the “micro-compartment” include liposomes, but are not limited thereto.

**[0095]** The term “liposome” as used herein generally means a closed vesicle composed of a lipid layer gathered in a membrane state and an internal aqueous layer. Other than phospholipid which is representatively used, cholesterol, glycolipid and the like can be incorporated. In the present invention, a liposome preferably contains cholesterol as the component. In the present invention, in order to have a modifying group, a liposome may have a constitutional unit having a functional group that allows ester bond (for example, glycolipid, ganglioside and phosphatidylglycerol) or a constitutional unit having a functional group that allows peptide bond (for example, phosphatidylethanolamine). The liposome that is used in the present invention is a “unilamellar liposome” consisting of a single membrane consisting of a lipid bilayer. As the preparation method of the unilamellar liposome, various well-known methods can be utilized.

**[0096]** The term “promoter sequence” as used herein refers to a region on a DNA that determines an initiation site of transcription of a gene and that directly regulates the frequency thereof, which is a base sequence to which an RNA polymerase bound and starts transcription. Although a putative promoter region varies in each structural gene, a putative promoter region is generally located in the upstream of a structural gene. However, the location is not limited thereto, and a putative promoter region also may be located in the downstream of a structural gene. The promoter may be inducible, structural, site-specific or stage-specific. The promoter may be any promoter as long as the promoter is able to be expressed in a host cell such as a mammalian cell, a colon *bacillus* and yeast. Representative promoter sequences include a T7 promoter sequence, a T5 promoter sequence, a Sp6 promoter sequence and a T3 promoter sequence, but are not limited thereto.

**[0097]** The “RNA polymerase” as used herein may be any RNA polymerase as long as it adapts to a promoter sequence to be used, that is, performs transcription from the promoter to be used. Preferably, the promoter sequence and the RNA polymerase are derived from the same or close species. For example, when a promoter sequence derived from a prokaryote is used, an RNA polymerase to be used is also preferably derived from a prokaryote. Alternatively, when a promoter sequence derived from a bacteriophage is used, an RNA polymerase to be used is also preferably derived from the same or similar bacteriophage.

**[0098]** The term “translational initiation sequence” as used herein means any sequence that is able to provide a functional ribosome entry site. In the system of bacteria, this region is also referred to as Shine-Dalgarno sequence.

**[0099]** The term “cell-free protein synthesis system” as used herein is a component derived from a cell that has lost autonomous replication ability by treating the cell, and is a component that is able to synthesize a protein. As the cell-free protein synthesis system, for example, PURESYSYSTEM (registered trademark) (BioComber Co., Ltd.; Bunkyo-ku, Tokyo) that is commercially available can be utilized. Alternatively, the cell-free protein synthesis system is possible to be prepared by performing purification and/or recombinant expression of a component that is required for the cell-free protein synthesis system.

**[0100]** The term “operably linked” as used herein refers to a state in which the expression (operation) of a desired sequence is disposed under the control of a certain transcrip-

tional/translational regulatory sequence (for example, a promoter and an enhancer) or a translational regulatory sequence. In order to allow for a promoter to be operably linked to a gene, the promoter is generally disposed in just upstream of the gene. However, the promoter is not necessarily adjacently disposed.

**[0101]** The term “membrane protein” as used herein refers to a protein that is attached to a lipid bilayer. The membrane protein may be a protein that contains a transmembrane region or may be a protein that does not contain a transmembrane region.

(Membrane Protein)

**[0102]** The present invention is applicable to various membrane proteins. Representative membrane proteins include, for example, transporters and receptors, but are not limited thereto. The sequence encoding the membrane protein of the present invention may comprise a leader sequence for inserting a protein into a membrane, as necessary.

(Transporter)

**[0103]** The membrane protein of the present invention may be or may not be a transporter. Examples of the transporter of the present invention include proteins related to substance transportation in cells (for example, EmrE protein) and proteins that allow permeation of a substance that does not permeate a lipid bilayer (for example, hemolysin), but are not limited thereto.

(Production of Unilamellar Liposome)

**[0104]** The unilamellar liposome used in the present invention is possible to be prepared by using the centrifugal sedimentation method described in the Examples. However, the preparation method is not limited thereto. For example, other than the centrifugal sedimentation method, a swelling hydration method (P. Mueller and T. F. Chien, *Biophys. J.*, 1983, 44, 375-381) and an electro-formation method (Miglina I. Angelove and Dimiter S. Dimitrov, *Faraday Discuss. Chem. Soc.*, 1986, 81, 303-311) can be utilized.

**[0105]** The swelling hydration method is a method that representatively encompasses the following steps: (1) a step of dissolving a lipid in a solvent for natural drying within a flask to form a lipid membrane on a surface of the flask; and (2) a step of adding an aqueous solution to enlarge the lipid membrane. By this second step, a liposome in which the lipid membrane taken in the aqueous solution floats up.

**[0106]** The electro-formation method is a method that representatively encompasses the following steps: (1) a step of applying a lipid solution on a conductive electrode for drying to form a lipid film; (2) a step of placing a conductive electrode also in the opposite side intervened by an insulating spacer and filling an aqueous solution therebetween; and (3) a step of applying an electric field between the two electrodes to remove the lipid film from the electrodes and prepare a giant thin film liposome.

(Component/Composition of Lipid Used in Production of Unilamellar Liposome)

**[0107]** The component/composition of a lipid used in the production of unilamellar liposomes preferably include, although not particularly limited, phospholipid and cholesterol. Examples of the lipid include L-alpha-phosphatidylcholine, cholesterol, L-alpha-dilauroylphosphatidylcholine,



L-alpha-dilauroylphosphatidylethanolamine, L-alpha-dilauroylphosphatidylglycerolsodium, L-alpha-monomyristoylphosphatidylcholine, L-alpha-dimyristoylphosphatidylcholine, L-alpha-dimyristoylphosphatidylethanolamine, L-alpha-dimyristoylphosphatidylglycerol ammonium, L-alpha-dimyristoylphosphatidylglycerol sodium, L-alpha-dimyristoylphosphatidic acid sodium, L-alpha-dioleoylphosphatidylcholine, L-alpha-dioleoylphosphatidylethanolamine, L-alpha-dioleoylphosphatidylserine sodium, L-alpha-monopalmitoylphosphatidylcholine, L-alpha-dipalmitoylphosphatidylcholine, L-alpha-dipalmitoylphosphatidylethanolamine, L-alpha-dipalmitoylphosphatidylglycerol ammonium, L-alpha-dipalmitoylphosphatidylglycerol sodium, L-alpha-dipalmitoylphosphatidic acid sodium, L-alpha-stearoylphosphatidylcholine, L-alpha-distearoylphosphatidylcholine, L-alpha-distearoylphosphatidylethanolamine, L-alpha-distearoylphosphatidylglycerol sodium, L-alpha-distearoylphosphatidylglycerol ammonium, L-alpha-distearoylphosphatidic acid sodium, L-alpha-dierucoylphosphatidylcholine, 1-palmitoyl-2-oleoylphosphatidylcholine, beta-oleyl-gamma-palmitoyl-L-alpha-phosphatidylethanolamine, beta-oleyl-gamma-palmitoyl-L-alpha-phosphatidylglycerol sodium, sphingomyelin and stearylamine, but are not limited thereto.

**[0108]** The proportion of the cholesterol is preferably 10% or more, more preferably 30% or more, even more preferably 50% or more, and most preferably 700 or more.

(Magnesium Concentration Appropriate for Production of Unilamellar Liposome)

**[0109]** The concentration of magnesium is preferably 15 mM to 50 mM, more preferably 18.88 mM to 42.48 mM, even more preferably 28.32 mM to 37.76 mM, and most preferably 33.04 mM.

(Nuclease)

**[0110]** Examples of the nuclease used in the present invention include a ribonuclease and a deoxyribonuclease, but are not limited thereto. The source of supply of the nuclease to be used is not particularly limited. When DNase is used as the nuclease, the enzyme activity to be used is 1 U to 20 U, more preferably 5 U to 15 U and most preferably about 12.5 U per 100  $\mu$ L of a liposome solution. When RNase is used as the nuclease, enzyme activity to be used is 1  $\mu$ g to 20  $\mu$ g, more preferably 5  $\mu$ g to 15  $\mu$ g, and most preferably about 10  $\mu$ g per 100  $\mu$ L of a liposome solution. Those skilled in the art are able to readily determine the amount of an enzyme to be used.

(DNA or RNA to be Used)

**[0111]** For example, if genetic information to be included in a liposome is a DNA, a coding sequence of a protein, a translational regulatory sequence operably linked to the coding sequence, and a transcriptional/translational regulatory sequence operably linked to the coding sequence will be included in the DNA.

**[0112]** Examples of the translational regulatory sequence include a translational initiation sequence, but are not limited thereto. A translation termination codon may be included as necessary. The translational regulatory sequence to be linked preferably adapts to a cell-free protein synthesis system to be

used. For example, if a cell-free protein synthesis system that is derived from *E. coli* is to be utilized, a translational regulatory sequence to be linked is preferably a translational initiation sequence of *E. coli*. A translational regulatory sequence and a cell-free protein synthesis system to be used are not necessarily required to be derived from the same species. A translational regulatory sequence and a cell-free protein synthesis system to be used can be derived from any species as long as they are adaptable, that is, the cell-free protein synthesis system is able to initiate translation from the translational regulatory sequence.

**[0113]** Examples of the transcriptional/translational regulatory sequence include a promoter sequence, but are not limited thereto. An enhancer sequence, a suppressor sequence, an operator sequence, and a transcription termination site may be included as necessary. A transcriptional/translational regulatory sequence to be linked preferably adapts to an RNA polymerase to be used. For example, if an RNA polymerase derived from *E. coli* is to be utilized, a transcriptional/translational regulatory sequence to be linked is preferably a transcriptional/translational regulatory sequence of *E. coli*. A transcriptional/translational regulatory sequence and an RNA polymerase to be used are not necessarily required to be derived from the same species. The transcriptional/translational regulatory sequence and the RNA polymerase to be used can be derived from any species as long as they are adaptable, that is, the RNA polymerase is able to initiate (or control) transcription from the transcriptional/translational regulatory sequence.

**[0114]** For example, if genetic information to be included in a liposome is an RNA, a coding sequence of a protein, and a translational regulatory sequence operably linked to the coding sequence will be included in the RNA. Examples of the translational regulatory sequence include a translational initiation sequence, but are not limited thereto. A translation termination codon may be included as necessary. A translational regulatory sequence to be linked preferably adapts to a cell-free protein synthesis system to be used. For example, if a cell-free protein synthesis system derived from *E. coli* is to be utilized, a translational regulatory sequence to be linked is preferably a translational initiation sequence of *E. coli*. A translational regulatory sequence and a cell-free protein synthesis system to be used are not necessarily required to be derived from the same species. A translational regulatory sequence and a cell-free protein synthesis system to be used can be derived from any species as long as they are adaptable, that is, the cell-free protein synthesis system is able to initiate translation from the translational regulatory sequence.

(Application of Liposome of the Present Invention to Molecular Evolutionary Engineering)

**[0115]** The liposomes of the present invention can be utilized for molecular evolutionary engineering.

**[0116]** For example, unilamellar liposomes treated by a nuclease are incubated under the condition that the internal DNA or RNA generates protein products, and (1) by using the presence of proteins expressed on the surface of the liposomes as an indicator, or (2) by measuring the activity of the generated membrane proteins and using this activity as an indicator, selection (screening) of unilamellar liposomes including high-functional genetic information is performed. Activity to be utilized is representatively activity of a protein that is encoded by a DNA or an RNA within the unilamellar liposomes. For example, if a DNA or an RNA within the

unilamellar liposomes encodes a transporter, activity to be utilized is representatively the transport activity thereof. If the transport activity of a transporter is used as an indicator, for example, substances that are transported into the liposomes by the transporter are labeled (for example, fluorescent labeling), and liposomes in which the labeled substances are accumulated are selected by using a cell sorter (FACS: fluorescence-activated cell sorter). For example, a factor that binds to a ligand transported by the transporter can be enclosed within the liposomes to capture the transported ligand within the liposomes, thereby enhancing the sensitivity of screening/selection.

**[0117]** Alternatively, the enzyme activity possessed by a membrane protein may be used as an indicator.

**[0118]** In order to detect phosphorylation of a protein or bonding with other proteins as an indicator of the activity of a membrane protein, for example, the following methods are used: a step of labeling an edge of a target protein with fluorescent dye that causes FRET; and when conformation is changed by phosphorylation or bonding with other proteins and the degree of FRET is changed, a step of selection by using the fluorescence change as an indicator. Alternatively, by disposing a GFP gene in the downstream of a T3RNA polymerase promoter for example, and using a T3RNA polymerase RNA at the same time, a T3RNA polymerase having higher RNA synthetic activity is possible to be obtained.

**[0119]** In addition, by introducing mutation into sequences (sequences related to the control of gene expression such as a promoter sequence, an enhancer sequence, a ribosome-binding sequence, and a translation initiation site) other than a coding sequence of a protein, and selecting the sequence to which mutation is introduced, a sequence can be evolved to have high activity (for example, high promoter activity, enhancer activity and translation activity).

**[0120]** The unilamellar liposome obtained as a result of screening is used to isolate genetic information included therein as a DNA or an RNA. If the genetic information is a DNA, the isolation can be performed by using a primer that specifically amplifies the DNA, thereby amplifying the genetic information by PCR. Alternatively, if the DNA includes a sequence that is required for autonomous replication within a host cell, the DNA can be introduced into an appropriate host cell, and the isolation can be performed after the amplification.

**[0121]** If genetic information is an RNA, (1) the RNA may be converted into a DNA using a reverse transcriptase, and then the DNA may be amplified by PCR using a thermostable DNA polymerase enzyme, or (2) genetic information of the RNA may be amplified in a single step using a thermostable reverse transcriptase. If the RNA includes a sequence that is required for autonomous replication within a host cell, the RNA can be introduced into an appropriate host cell, and the isolation can be performed after the amplification.

**[0122]** Genetic information is not necessarily required to be isolated (purified) after a first round of screening. For example, instead of obtaining a monoclonal DNA or RNA by the first round of screening, a second round of screening may be performed by obtaining a group of DNAs or RNAs and using the group as a starting material. A group of DNAs or RNAs obtained by the second round of screening or the subsequent rounds of screening may be used as a starting material of the next round.

**[0123]** Alternatively, mutagenesis may be performed on a clone (purified clone) obtained after the screening to prepare

a group comprising a plurality of different clones, and the group may be used as a starting material of the screening of the next round.

## EXAMPLES

**[0124]** Hereinafter, the present invention will be described in detail by Examples and the like. However, the present invention is not limited thereto.

### Example 1

#### Preparation of Unilamellar Liposome

**[0125]** Unilamellar liposomes were prepared by the centrifugal sedimentation method described below.

**[0126]** 10 mg of lipid (phosphatidylcholine:cholesterol=9:1) was dissolved into 100  $\mu$ l of chloroform for mixture with 2 ml of liquid paraffin.

**[0127]** Incubation was performed for 30 minutes at 80° C.

**[0128]** An extraliposomal solution (333 mM glucose, and a solution in which a group of translated proteins and tRNA are removed from a cell-free protein synthesis system) and an intraliposomal solution (330 mM sucrose, 1  $\mu$ M Transferrin Alexa 647, a cell-free protein synthesis system, 40 U/ $\mu$ l RNase inhibitor (Promega), 0.4  $\mu$ M ribosome S1 subunit and 50 pM DNA) were prepared. A DNA comprising an EmrE-myc-his sequence (SEQ ID NO: 1; a sequence comprising a myc tag and a his tag in the C-terminus of an EmrE gene) or a DNA comprising a GUS sequence (SEQ ID NO: 3; negative control comprising a myc sequence and a GUS sequence) was used. This condition is a condition that a single molecule of DNA is enclosed in each liposome. The composition of the cell-free protein synthesis system that was used is as follows: amino acids 0.3 mM each (alanine, glycine, leucine, isoleucine, valine, serine, threonine, proline, tryptophan, phenylalanine, glutamine, glutamic acid, asparagine, aspartic acid, lysine, arginine, histidine, methionine, cysteine, tyrosine); 3.6  $\mu$ g/ $\mu$ l tRNA; 2 mM ATP; 2 mM GTP; 1 mM CTP; 1 mM UTP; 14 mM magnesium acetate; 50 mM Hepes-KOH (pH7.8); 100 mM potassium glutamate; 2 mM spermidine; 20 mM creatine phosphate; 2 mM dithiothreitol; 10 ng/ $\mu$ l 10-formyl-5,6,7,8-tetrahydrofolic acid; a group of translated proteins (2500 nM IF1, 411 nM IF2, 728 nM IF3, 247 nM RF1, 484 nM RF2, 168 nM RF3, 485 nM RRF, 727 nM AlaRS, 99 nM ArgRS, 420 nM AsnRS, 121 nM AspRS, 100 nM CysRS, 101 nM GlnRS, 232 nM GluRS, 86 nM GlyRS, 85 nM HisRS, 365 nM IleRS, 99 nM LeuRS, 115 nM LysRS, 109 nM MetRS, 134 nM PheRS, 166 nM ProRS, 99 nM SerRS, 84 nM ThrRS, 102 nM TrpRS, 101 nM TyrRS, 100 nM ValRS, 588 nM MTF, 926 nM MK, 465 nM CK, 1307 nM NDK, 621 nM Ppiase2, 1290 nM EF-G, 2315 nM EF-Tu, 3300 nM EF-Ts, 529 nM Tig, 22 nM HrpA, 1440 nM TrxC).

**[0129]** 20  $\mu$ l of intraliposomal solution was put into 400  $\mu$ l of liquid paraffin in which a lipid is dissolved, and the solution was placed on ice for 1 minute.

**[0130]** Emulsion was prepared by stirring for 40 seconds at the maximum strength of a vortex mixer, and the emulsion was placed on ice for 10 minutes.

**[0131]** 150  $\mu$ l of extraliposomal solution was put into a new tube and the prepared emulsion was laminated thereon, and they were placed on ice for 10 minutes.

**[0132]** Centrifugation was performed for 30 minutes at 14 kxg, 4° C.

**[0133]** A hole was made at the bottom of the tube, and 80  $\mu$ l of liposome suspension accumulated at the bottom was collected.

**[0134]** 2  $\mu$ l of 5 U/ $\mu$ l DNase or 4 mg/ml RNase was added to the liposome suspension.

**[0135]** The liposome suspension was incubated for 3 hours at 37° C., and protein synthesis was performed.

**[0136]** An antibody (anti-Myc tag antibody (mouse IgG1) labeled with Alexa Fluor 488) was diluted with a PBS+1% BSA solution and added to the liposome suspension such that the final concentration becomes 5  $\mu$ g/ml (1  $\mu$ l of 50 g/ml antibody was added to 9  $\mu$ l of liposome solution).

**[0137]** After standing for 30 minutes at room temperature, the antibody was observed by microscopy (Ex: 470-490 Em: 510-550).

**[0138]** As a result, Alexa 488 fluorescence that is caused by an antibody bound to a polypeptide consisting of a sequence comprising a myc tag and a his tag in the C-terminus of an EmrE gene was confirmed as being localized in a liposome membrane. That is, by the above-described method, it was confirmed that a membrane protein was in-vitro synthesized within the liposome, and the membrane protein was incorporated into the liposome membrane.

**[0139]** Next, a DNA comprising an EmrE-myc-his sequence (SEQ ID NO: 1; a sequence comprising a myc tag and a his tag in the C-terminus of an EmrE gene) or a DNA comprising a GUS sequence (SEQ ID NO: 3; negative control comprising a myc sequence and a GUS sequence) was used, and an antibody (anti-Myc tag antibody (mouse IgG1) labeled with Alexa Fluor 488, final concentration 5  $\mu$ g/ml) diluted with a PBS+1% BSA solution was added to liposomes before and after the expression of proteins (1  $\mu$ l of 50 g/ml antibody was added to 9  $\mu$ l of liposome solution) followed by 30 minutes of standing at room temperature for analysis by a cell sorter. The results are shown in FIG. 1. The vertical axis shows the internal volume of liposomes and the horizontal axis shows the fluorescence intensity of Alexa 488. A and B show the results of using the GUS sequence, and C and D show the results of using the EmrE-myc-his sequence. A and C are results of liposomes before the expression of proteins by incubation at 37° C., and B and D are results of liposomes that expressed proteins by an hour incubation at 37° C. As is apparent from FIG. 1, liposomes are prepared under the condition that a single molecule of DNA is enclosed in each liposome, and it was confirmed that a membrane protein was expressed and the membrane protein was able to be detected by an antibody.

#### Example 2

##### Confirmation of Function of Membrane Protein Expressed in Unilamellar Liposome

**[0140]** 5 nM of a DNA comprising an EmrE-myc-his sequence (SEQ ID NO: 1; a sequence comprising a myc tag and a his tag in the C-terminus of an EmrE gene) or a DNA comprising a GUS sequence (SEQ ID NO: 3; negative control comprising a myc sequence and a GUS sequence) and a PURE system were enclosed within liposomes. The liposomes were incubated for 2 hours at 37° C. to express EmrE-myc-his and GUS-myc. After the preparation of the liposomes, external solution 1 was replaced with external solution 2 containing EtBr 5  $\mu$ g/ml. Fluorescence was measured every minute, and the intake of EtBr was observed.

Subsequently, the same sample was observed with a fluorescence microscope (Ex: 520-550 Em: 580-).

**[0141]** The composition of external solution 1 (that is, the external solution at the time of synthesis of liposomes) is as follows: HEPES-KOH (pH7.6) 100 mM; K-Glu 200 mM; spermidine 4 mM; magnesium acetate 25 mM; CP 40 mM; DTT 2 mM; FD 20  $\mu$ g/ml; 20 types of amino acids 0.4 mM each; ATP 8 mM; GTP 8 mM; UTP 4 mM; CTP 4 mM.

**[0142]** The composition of external solution 2 (that is, the external solution for making a proton gradient) is as follows: Tris-HCl (pH9.0 or 7.6) 100 mM; K-Glu 200 mM; spermidine 4 mM, magnesium acetate 25 mM; CP 40 mM; DTT 2 mM; FD 20  $\mu$ g/ml; 20 types of amino acids 0.4 mM each; ATP 8 mM; GTP 8 mM; UTP 4 mM; CTP 4 mM.

**[0143]** The results are shown in FIG. 2. FIG. 2A shows the result of using the DNA comprising the EmrE-myc-his sequence (SEQ ID NO: 1), and FIG. 2B shows the result of using the DNA containing the GUS sequence (SEQ ID NO: 3). In the liposomes that expressed a membrane protein from the EmrE-myc-his sequence, pH-dependent fluorescence intensity was observed. This result verifies that the membrane protein expressed in the liposomes exerted transport ability.

#### Example 3

##### Examination on Mg Concentration

**[0144]** DNA 5 nM comprising a hemolysin sequence, a halo tag protein and a PURE system were enclosed within liposomes. At this time, liposomes were prepared under 9 conditions of Mg concentration of an intraliposomal solution and an extraliposomal solution, which are 18.88, 23.6, 28.32, 33.04, 37.76, 42.28, 47.2, 51.92, 56.64 mM. After the preparation of liposomes, incubation was performed for 16 hours at 37° C. to express hemolysin. 1  $\mu$ M of Halo Tag Alexa Fluor 488 ligand was added to the extraliposomal solution to measure the function of expressed alpha hemolysin, and after 3 hours, the amount of fluorescence of Halo Tag Alexa Fluor 488 ligand accumulated within the liposomes was measured. As a result, Halo Tag Alexa Fluor 488 ligand was accumulated the most in liposomes that were prepared by the Mg concentration value of 33.04 mM. Accordingly, it was ascertained that the condition for the detection of activity of hemolysin is preferably 18.88 mM-23.6 mM, more preferably 23.6 mM-28.32 mM, and most preferably 28.32-42.48 mM.

#### Example 4

##### Examination on Lipid Component/Composition-1

**[0145]** Instead of the EmrE-myc-his sequence used in Example 1, a sequence encoding hemolysin (SEQ ID NO: 5) was used to express a transporter. Further, a halo tag protein (SEQ ID NO: 7) was used as a factor to which Halo Tag Alexa Fluor 488 ligand, which is the ligand transported by hemolysin, bound. Hemolysin is a membrane protein that creates a pore in a membrane, and hemolysin allows permeation of substances smaller than 3 kDa. Thus, when hemolysin is expressed, a pore is generated in liposomes, and as a result, permeation of Halo Tag Alexa Fluor 488 ligand, which is unable to permeate lipid membranes, is allowed. Halo Tag Alexa Fluor 488 ligand that permeated through the pore binds to the halo tag protein, and as a result, Halo Tag Alexa Fluor 488 ligand that moved into the liposomes accumulate within the liposomes.

**[0146]** As a lipid forming liposomes, a mixture of POPC:Chol=9:1, a mixture of POPC:Chol=7:3, a mixture of POPC:Chol=5:5, and a mixture of POPC:Chol=3:7 were used. Further, POPC is an abbreviation of 1-palmitoyl-2-oleoylphosphatidylcholine, and Chol is an abbreviation of cholesterol. As a result, as shown in FIG. 3, the percentage of exertion of membrane protein activity in liposomes comprising a DNA raised as the ratio of cholesterol increased.

#### Example 5

##### Examination on Lipid Component/Composition-2

**[0147]** Next, liposomes were synthesized using various lipids by the same technique as Example 4, and the activity of the expressed membrane protein was compared. The results are shown in FIG. 4.

**[0148]** The vertical axis of FIG. 4 shows the percentage (%) of liposomes that taken in Halo Tag Alexa Fluor 488 ligand with high intensity among all the liposomes when various lipids were used. The lipids that were used are as follows: EggPC is an abbreviation of phosphatidylcholine purified from a hen's egg; POPC is an abbreviation of 1-palmitoyl-2-oleoylphosphatidylcholine; PS is an abbreviation of 1-palmitoyl-2-oleoylphosphoserine; PE is an abbreviation of 1-palmitoyl-2-oleoylphosphoethanolamine; and Chol is an abbreviation of cholesterol. PC mix is an abbreviation of the mixture of 1-palmitoyl-2-oleoylphosphatidylcholine:1-palmitoyl-2-linoleoylphosphatidylcholine:1-stearoyl-2-oleoylphosphatidylcholine=129:67:48:24 (mass ratio); EggPC/PS/PE is an abbreviation of the mixture of each of them at the ratio of 3:1:1 (mass ratio) in order; EggPC/PS/PE/Chol is an abbreviation of the mixture of each of them at the ratio of 2:1:1:1 (mass ratio) in order; PCmix/PS/PE is an abbreviation of the mixture of each of them at the ratio of 3:1:1 (mass ratio) in order; PCmix/PS/PE/Chol is an abbreviation of the mixture of each of them at the ratio of 2:1:1:1 (mass ratio) in order; POPC/PS/PE is an abbreviation of the mixture of each of them at the ratio of 3:1:1 (mass ratio) in order; and POPC/POPE/POPS/Chol is an abbreviation of the mixture of each of them at the ratio of 2:1:1:1 (mass ratio) in order.

**[0149]** These results ascertained that change in types of phosphatidylcholine and mixture of a plurality of types, and mixture of 1-palmitoyl-2-oleoylphosphoserine and 1-palmitoyl-2-oleoylphosphoethanolamine do not significantly affect the exertion of activity of hemolysin.

#### Example 6

##### Concentration of Desired Nucleic Acid

**[0150]** An experiment was performed by using wild type hemolysin (SEQ ID NO: 5) and lethal mutation type hemolysin (SEQ ID NO: 8) and by using the same technique as Example 4. The proportion of wild type to lethal mutation type was set to 1:12, and tenfold or more of lethal mutation type were used. Culturing was performed for 160 minutes at 37° C. to express a membrane protein, and then liposomes that showed transport activity were selected by a cell sorter to determine the percentage of wild type genes and mutated genes included in the liposomes. The result was wild type: mutant type=8:1. This result verifies that hundredfold concentration was performed by the screening/selection of the present invention.

**[0151]** For example, by selecting a liposome showing a desired property and performing mutation induction (for example, random mutation) on the included DNA (or RNA), selection by a cell sorter can be performed by using the group to which mutation is induced as a starting material. By repeating this procedure, concentration of mutated genes having a desired property is possible.

#### Example 7

##### Evolutionary Experiment

**[0152]** An evolutionary experiment was performed by using the following procedures.

**[0153]** 1) Liposomes are created by a centrifugal sedimentation method.

**[0154]** POPC:Chol=1:1 (wt/wt) was used as the lipid composition. As the composition of the internal solution, the same composition as the cell-free protein synthesis system described in Example 1 (except that the magnesium acetate concentration was changed to 33.04 mM) was used. Further, 100 nM T7 RNA polymerase, 200 mM sucrose, 5 mM  $\beta$ -glucuronidase conjugated halo peptide, 1 mM transferrin conjugated alexa fluor 647, 5 pM DNA (ORF of hemolysin was disposed under the control of a T7 promoter) were used. As the composition of the external solution, a solution containing only a small molecule having the same composition as the cell-free protein synthesis system described in Example 1 (except that the magnesium acetate concentration was changed to 33.04 mM), and 200 mM glucose was used.

**[0155]** 2) The external solution was replaced to remove the intraliposomal solution that was mixed into the external solution. Centrifugation was performed for 5 minutes at 6000 G, and after the supernatant was thrown away, the precipitation was resuspend with 300 ml of new extraliposomal solution.

**[0156]** 3) A hemolysin protein was synthesized within the liposomes and the hemolysin protein was presented in the lipid membrane. Incubation was performed for 16 hours at 37° C.

**[0157]** 4) DNase was added to degrade the DNA remained in the extraliposomal solution. 4  $\mu$ l of DNase (TAKARA recombinant Dnase1) was added to the liposome solution.

**[0158]** 5) A fluorescent substrate was added to the external environment. 900 ml of new external solution was added to the liposome solution such that the final volume becomes 1.2 ml. The final concentration was set to 2 nM, and Halo Tag Alexa Fluor 488 ligand was added to the external solution. The fluorescence intensity of liposomes was successively measured with a flow cytometer.

**[0159]** 6) The intake of the fluorescent substrate was suspended by competitive inhibitory substrate that is non-fluorescent and that is permeable to lipid bilayer. When appropriate fluorescence intensity was obtained, final concentration 200 nM halo tag biotin ligand was added to the external solution.

**[0160]** 7) Concentration of the liposome solution. Centrifugation was performed for 5 minutes at 6000 G, and after the supernatant was thrown away, the precipitation was resuspended with 300 ml of new external solution.

**[0161]** 8) 10,000 high-intensity liposomes were sorted from the highest intensity value with a cell sorter (BD, FACS Aria 2).

**[0162]** 9) Genetic information was amplified. The sorted liposome solution was purified by using a simplified DNA purification column (QIAGEN MinElute PCR Purification

Kit). Subsequently, PCR was performed for 40 cycles (TOYOBO KOD FX Neo was used for the DNA polymerase). PCR was purified by using the DNA purification column again. Subsequently, a gel band was purified by using agarose electrophoresis (life technologies, E-Gel CloneWell SYBR Safe Gel was used). After performing purification by using the DNA purification column again, PCR was performed again for 20 cycles. The PCR product was purified by DNA purification column again for reuse as the DNA stock of the next cycle.

**[0163]** The results are shown in FIG. 5. FIG. 5 is a graph showing the percentage of a group of high-intensity liposomes in which the fluorescence intensity is 260 or over. The upper limit of fluorescence values in which Halo Tag Alexa Fluor 488 ligand adheres to negative-control liposomes not having hemolysin activity is 260. Thus, samples that showed a value over this fluorescence value are samples that showed specific Halo Tag Alexa Fluor 488 ligand intake by hemolysin.

**[0164]** It was shown that the percentage of genes having higher activity increased by repeating the cycle of screening/selection. Further, mutation may be introduced after the isolation of the DNA.

## INDUSTRIAL APPLICABILITY

**[0165]** By the use of unilamellar liposomes treated with a nuclease, further highly-efficient screening is enabled, and a gene encoding a membrane protein having a desired function can be selected and obtained.

[Sequence Listing Free Text]

**[0166]** SEQ ID NO: 1: the nucleotide sequence of EmrE-myc-his

SEQ ID NO: 2: the amino acid sequence of EmrE-myc-his

SEQ ID NO: 3: the nucleotide sequence of GUS derived from *Escherichia coli*

SEQ ID NO: 4: the amino acid sequence of GUS derived from *Escherichia coli*

SEQ ID NO: 5: the nucleotide sequence encoding hemolysin derived from *Staphylococcus aureus*

SEQ ID NO: 6: the amino acid sequence of hemolysin derived from *Staphylococcus aureus*

SEQ ID NO: 7: the amino acid sequence of the halo tag protein

SEQ ID NO: 8: the nucleotide sequence encoding the lethal mutation type hemolysin derived from *Staphylococcus aureus*

SEQ ID NO: 9: the amino acid sequence of the lethal mutation type hemolysin derived from *Staphylococcus aureus*

## SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 9

<210> SEQ ID NO 1

<211> LENGTH: 414

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: EmrE-myc-his

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(414)

<400> SEQUENCE: 1

```

atg aac cct tat att tat ctt ggt ggt gca ata ctt gca gag gtc att      48
Met Asn Pro Tyr Ile Tyr Leu Gly Gly Ala Ile Leu Ala Glu Val Ile
1           5           10           15

ggt aca acc tta atg aag ttt tca gaa ggt ttt aca cgg tta tgg cca      96
Gly Thr Thr Leu Met Lys Phe Ser Glu Gly Phe Thr Arg Leu Trp Pro
                20           25           30

tct gtt ggt aca att att tgt tat tgt gca tca ttc tgg tta tta gct     144
Ser Val Gly Thr Ile Ile Cys Tyr Cys Ala Ser Phe Trp Leu Leu Ala
                35           40           45

cag acg ctg gct tat att cct aca ggg att gct tat gct atc tgg tca     192
Gln Thr Leu Ala Tyr Ile Pro Thr Gly Ile Ala Tyr Ala Ile Trp Ser
                50           55           60

gga gtc ggt att gtc ctg att agc tta ctg tca tgg gga ttt ttc ggc     240
Gly Val Gly Ile Val Leu Ile Ser Leu Leu Ser Trp Gly Phe Phe Gly
65           70           75           80

caa cgg ctg gac ctg cca gcc att ata ggc atg atg ttg att tgt gcc     288
Gln Arg Leu Asp Leu Pro Ala Ile Ile Gly Met Met Leu Ile Cys Ala
                85           90           95

ggt gtg ttg att att aat tta ttg tca cga agc aca cca cat gaa ttt     336
Gly Val Leu Ile Ile Asn Leu Leu Ser Arg Ser Thr Pro His Glu Phe
                100          105          110

gag gca tat gtt gag caa aaa tta ata agt gaa gaa gat ttg aat agc     384

```

-continued

---

```

Glu Ala Tyr Val Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ser
    115                                120                                125

gct gta gac cat cac cat cac cat cac taa
Ala Val Asp His His His His His His
    130                                135

```

414

```

<210> SEQ ID NO 2
<211> LENGTH: 137
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

```

&lt;400&gt; SEQUENCE: 2

```

Met Asn Pro Tyr Ile Tyr Leu Gly Gly Ala Ile Leu Ala Glu Val Ile
 1      5      10      15

Gly Thr Thr Leu Met Lys Phe Ser Glu Gly Phe Thr Arg Leu Trp Pro
 20      25      30

Ser Val Gly Thr Ile Ile Cys Tyr Cys Ala Ser Phe Trp Leu Leu Ala
 35      40      45

Gln Thr Leu Ala Tyr Ile Pro Thr Gly Ile Ala Tyr Ala Ile Trp Ser
 50      55      60

Gly Val Gly Ile Val Leu Ile Ser Leu Leu Ser Trp Gly Phe Phe Gly
 65      70      75      80

Gln Arg Leu Asp Leu Pro Ala Ile Ile Gly Met Met Leu Ile Cys Ala
 85      90      95

Gly Val Leu Ile Ile Asn Leu Leu Ser Arg Ser Thr Pro His Glu Phe
 100     105     110

Glu Ala Tyr Val Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ser
 115                                120                                125

Ala Val Asp His His His His His His
 130                                135

```

```

<210> SEQ ID NO 3
<211> LENGTH: 2406
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(2406)

```

&lt;400&gt; SEQUENCE: 3

```

tta cgt cct gta gaa acc cca acc cgt gaa atc aaa aaa ctc gac ggc
Leu Arg Pro Val Glu Thr Pro Thr Arg Glu Ile Lys Lys Leu Asp Gly
 1      5      10      15

ctg tgg gca ttc agt ctg gat cgc gaa aac tgt gga att gat cag cgt
Leu Trp Ala Phe Ser Leu Asp Arg Glu Asn Cys Gly Ile Asp Gln Arg
 20      25      30

tgg tgg gaa agc gcg tta caa gaa agc cgg gca att gct gtg cca ggc
Trp Trp Glu Ser Ala Leu Gln Glu Ser Arg Ala Ile Ala Val Pro Gly
 35      40      45

agt ttt aac gat cag ttc gcc gat gca gat att cgt aat tat gcg ggc
Ser Phe Asn Asp Gln Phe Ala Asp Ala Asp Ile Arg Asn Tyr Ala Gly
 50      55      60

aac gtc tgg tat cag cgc gaa gtc ttt ata ccg aaa ggt tgg gca ggc
Asn Val Trp Tyr Gln Arg Glu Val Phe Ile Pro Lys Gly Trp Ala Gly
 65      70      75      80

cag cgt atc gtg ctg cgt ttc gat gcg gtc act cat tac ggc aaa gtg

```

48

96

144

192

240

288

-continued

Gln	Arg	Ile	Val	Leu	Arg	Phe	Asp	Ala	Val	Thr	His	Tyr	Gly	Lys	Val	
				85					90					95		
tgg	gtc	aat	aat	cag	gaa	gtg	atg	gag	cat	cag	ggc	ggc	tat	acg	cca	336
Trp	Val	Asn	Asn	Gln	Glu	Val	Met	Glu	His	Gln	Gly	Gly	Tyr	Thr	Pro	
		100						105					110			
ttt	gaa	gcc	gat	gtc	acg	ccg	tat	gtt	att	gcc	ggg	aaa	agt	gta	cgt	384
Phe	Glu	Ala	Asp	Val	Thr	Pro	Tyr	Val	Ile	Ala	Gly	Lys	Ser	Val	Arg	
		115					120						125			
atc	acc	gtt	tgt	gtg	aac	aac	gaa	ctg	aac	tgg	cag	act	atc	ccg	ccg	432
Ile	Thr	Val	Cys	Val	Asn	Asn	Glu	Leu	Asn	Trp	Gln	Thr	Ile	Pro	Pro	
		130				135						140				
gga	atg	gtg	att	acc	gac	gaa	aac	ggc	aag	aaa	aag	cag	tct	tac	ttc	480
Gly	Met	Val	Ile	Thr	Asp	Glu	Asn	Gly	Lys	Lys	Lys	Gln	Ser	Tyr	Phe	
		145			150					155					160	
cat	gat	ttc	ttt	aac	tat	gcc	ggg	atc	cat	cgc	agc	gta	atg	ctc	tac	528
His	Asp	Phe	Phe	Asn	Tyr	Ala	Gly	Ile	His	Arg	Ser	Val	Met	Leu	Tyr	
				165					170					175		
acc	acg	ccg	aac	acc	tgg	gtg	gac	gat	atc	acc	gtg	gtg	acg	cat	gtc	576
Thr	Thr	Pro	Asn	Thr	Trp	Val	Asp	Asp	Ile	Thr	Val	Val	Thr	His	Val	
			180					185					190			
gcg	caa	gac	tgt	aac	cac	gcg	tct	gtt	gac	tgg	cag	gtg	gtg	gcc	aat	624
Ala	Gln	Asp	Cys	Asn	His	Ala	Ser	Val	Asp	Trp	Gln	Val	Val	Ala	Asn	
		195					200						205			
ggt	gat	gtc	agc	gtt	gaa	ctg	cgt	gat	gcg	gat	caa	cag	gtg	gtt	gca	672
Gly	Asp	Val	Ser	Val	Glu	Leu	Arg	Asp	Ala	Asp	Gln	Gln	Val	Val	Ala	
		210				215					220					
act	gga	caa	ggc	act	agc	ggg	act	ttg	caa	gtg	gtg	aat	ccg	cac	ctc	720
Thr	Gly	Gln	Gly	Thr	Ser	Gly	Thr	Leu	Gln	Val	Val	Asn	Pro	His	Leu	
					230					235					240	
tgg	caa	ccg	ggt	gaa	ggt	tat	ctc	tat	gaa	ctg	tgc	gtc	aca	gcc	aaa	768
Trp	Gln	Pro	Gly	Glu	Gly	Tyr	Leu	Tyr	Glu	Leu	Cys	Val	Thr	Ala	Lys	
				245					250					255		
agc	cag	aca	gag	tgt	gat	atc	tac	ccg	ctt	cgc	gtc	ggc	atc	cgg	tca	816
Ser	Gln	Thr	Glu	Cys	Asp	Ile	Tyr	Pro	Leu	Arg	Val	Gly	Ile	Arg	Ser	
			260					265					270			
gtg	gca	gtg	aag	ggc	gaa	cag	ttc	ctg	att	aac	cac	aaa	ccg	ttc	tac	864
Val	Ala	Val	Lys	Gly	Glu	Gln	Phe	Leu	Ile	Asn	His	Lys	Pro	Phe	Tyr	
			275				280						285			
ttt	act	ggc	ttt	ggt	cgt	cat	gaa	gat	gcg	gac	ttg	cgt	ggc	aaa	gga	912
Phe	Thr	Gly	Phe	Gly	Arg	His	Glu	Asp	Ala	Asp	Leu	Arg	Gly	Lys	Gly	
			290			295					300					
ttc	gat	aac	gtg	ctg	atg	gtg	cac	gac	cac	gca	tta	atg	gac	tgg	att	960
Phe	Asp	Asn	Val	Leu	Met	Val	His	Asp	His	Ala	Leu	Met	Asp	Trp	Ile	
					305		310			315					320	
ggg	gcc	aac	tcc	tac	cgt	acc	tcg	cat	tac	cct	tac	gct	gaa	gag	atg	1008
Gly	Ala	Asn	Ser	Tyr	Arg	Thr	Ser	His	Tyr	Pro	Tyr	Ala	Glu	Glu	Met	
				325					330					335		
ctc	gac	tgg	gca	gat	gaa	cat	ggc	atc	gtg	gtg	att	gat	gaa	act	gct	1056
Leu	Asp	Trp	Ala	Asp	Glu	His	Gly	Ile	Val	Val	Ile	Asp	Glu	Thr	Ala	
			340					345					350			
gct	gtc	ggc	ttt	aac	ctc	tct	tta	ggc	att	ggt	ttc	gaa	gcg	ggc	aac	1104
Ala	Val	Gly	Phe	Asn	Leu	Ser	Leu	Gly	Ile	Gly	Phe	Glu	Ala	Gly	Asn	
			355				360						365			
aag	ccg	aaa	gaa	ctg	tac	agc	gaa	gag	gca	gtc	aac	ggg	gaa	act	cag	1152
Lys	Pro	Lys	Glu	Leu	Tyr	Ser	Glu	Glu	Ala	Val	Asn	Gly	Glu	Thr	Gln	
		370				375					380					
caa	gcg	cac	tta	cag	gcg	att	aaa	gag	ctg	ata	gcg	cgt	gac	aaa	aac	1200

-continued

Gln 385	Ala	His	Leu	Gln	Ala	Ile	Lys	Glu	Leu	Ile	Ala	Arg	Asp	Lys	Asn	400
					390					395						
cac	cca	agc	gtg	gtg	atg	tgg	agt	att	gcc	aac	gaa	ccg	gat	acc	cgt	1248
His	Pro	Ser	Val	Val	Met	Trp	Ser	Ile	Ala	Asn	Glu	Pro	Asp	Thr	Arg	
			405						410					415		
ccg	caa	ggt	gca	ccg	gaa	tat	ttc	gcg	cca	ctg	gcg	gaa	gca	acg	cgt	1296
Pro	Gln	Gly	Ala	Arg	Glu	Tyr	Phe	Ala	Pro	Leu	Ala	Glu	Ala	Thr	Arg	
			420					425					430			
aaa	ctc	gac	ccg	acg	cgt	ccg	atc	acc	tgc	gtc	aat	gta	atg	ttc	tgc	1344
Lys	Leu	Asp	Pro	Thr	Arg	Pro	Ile	Thr	Cys	Val	Asn	Val	Met	Phe	Cys	
		435					440				445					
gac	gct	cac	acc	gat	acc	atc	agc	gat	ctc	ttt	gat	gtg	ctg	tgc	ctg	1392
Asp	Ala	His	Thr	Asp	Thr	Ile	Ser	Asp	Leu	Phe	Asp	Val	Leu	Cys	Leu	
	450					455				460						
aac	cgt	tat	tac	gga	tgg	tat	gtc	caa	agc	ggc	gat	ttg	gaa	acg	gca	1440
Asn	Arg	Tyr	Tyr	Gly	Trp	Tyr	Val	Gln	Ser	Gly	Asp	Leu	Glu	Thr	Ala	
465				470					475					480		
gag	aag	gta	ctg	gaa	aaa	gaa	ctt	ctg	gcc	tgg	cag	gag	aaa	ctg	cat	1488
Glu	Lys	Val	Leu	Glu	Lys	Glu	Leu	Leu	Ala	Trp	Gln	Glu	Lys	Leu	His	
			485					490						495		
cag	ccg	att	atc	atc	acc	gaa	tac	ggc	gtg	gat	acg	tta	gcc	ggg	ctg	1536
Gln	Pro	Ile	Ile	Ile	Thr	Glu	Tyr	Gly	Val	Asp	Thr	Leu	Ala	Gly	Leu	
		500					505					510				
cac	tca	atg	tac	acc	gac	atg	tgg	agt	gaa	gag	tat	cag	tgt	gca	tgg	1584
His	Ser	Met	Tyr	Thr	Asp	Met	Trp	Ser	Glu	Glu	Tyr	Gln	Cys	Ala	Trp	
		515				520						525				
ctg	gat	atg	tat	cac	cgc	gtc	ttt	gat	cgc	gtc	agc	gcc	gtc	gtc	ggt	1632
Leu	Asp	Met	Tyr	His	Arg	Val	Phe	Asp	Arg	Val	Ser	Ala	Val	Val	Gly	
	530				535				540							
gaa	cag	gta	tgg	aat	ttc	gcc	gat	ttt	gcg	acc	tcg	caa	ggc	ata	ttg	1680
Glu	Gln	Val	Trp	Asn	Phe	Ala	Asp	Phe	Ala	Thr	Ser	Gln	Gly	Ile	Leu	
545				550					555					560		
cgc	ggt	ggc	ggt	aac	aag	aaa	ggg	atc	ttc	act	cgc	gac	cgc	aaa	ccg	1728
Arg	Val	Gly	Gly	Asn	Lys	Lys	Gly	Ile	Phe	Thr	Arg	Asp	Arg	Lys	Pro	
			565				570							575		
aag	tcg	gcg	gct	ttt	ctg	ctg	caa	aaa	cgc	tgg	act	ggc	atg	aac	ttc	1776
Lys	Ser	Ala	Ala	Phe	Leu	Leu	Gln	Lys	Arg	Trp	Thr	Gly	Met	Asn	Phe	
		580					585					590				
ggt	gaa	aaa	ccg	cag	cag	gga	ggc	aaa	caa	ggc	cta	tgc	ggc	cgc	aag	1824
Gly	Glu	Lys	Pro	Gln	Gln	Gly	Gly	Lys	Gln	Gly	Leu	Cys	Gly	Arg	Lys	
		595				600					605					
ctt	atg	gac	aaa	gat	tgc	gaa	atg	aaa	cgt	acc	acc	ctg	gat	agc	ccg	1872
Leu	Met	Asp	Lys	Asp	Cys	Glu	Met	Lys	Arg	Thr	Thr	Leu	Asp	Ser	Pro	
	610				615						620					
ctg	ggc	aaa	ctg	gaa	ctg	agc	ggc	tgc	gaa	cag	ggc	ctg	cat	gaa	att	1920
Leu	Gly	Lys	Leu	Glu	Leu	Ser	Gly	Cys	Glu	Gln	Gly	Leu	His	Glu	Ile	
625				630					635					640		
aaa	ctg	ctg	ggt	aaa	ggc	acc	agc	gcg	gcc	gat	gcg	ggt	gaa	ggt	ccg	1968
Lys	Leu	Leu	Gly	Lys	Gly	Thr	Ser	Ala	Ala	Asp	Ala	Val	Glu	Val	Pro	
			645						650					655		
gcc	ccg	gcc	gcc	gtg	ctg	ggt	ggt	ccg	gaa	ccg	ctg	atg	cag	gcg	acc	2016
Ala	Pro	Ala	Ala	Val	Leu	Gly	Gly	Pro	Glu	Pro	Leu	Met	Gln	Ala	Thr	
			660					665					670			
gcg	tgg	ctg	aac	gcg	tat	ttt	cat	cag	ccg	gaa	gcg	att	gaa	gaa	ttt	2064
Ala	Trp	Leu	Asn	Ala	Tyr	Phe	His	Gln	Pro	Glu	Ala	Ile	Glu	Glu	Phe	
		675				680						685				
ccg	ggt	ccg	gcg	ctg	cat	cat	ccg	gtg	ttt	cag	cag	gag	agc	ttt	acc	2112



-continued

---

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr	
690	695 700
cgt cag gtg ctg tgg aaa ctg ctg aaa gtg gtt aaa ttt ggc gaa gtg	2160
Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val	
705	710 715 720
att agc tat cag cag ctg gcg gcc ctg gcg ggt aat ccg gcg gcc acc	2208
Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr	
	725 730 735
gcc gcc gtt aaa acc gcg ctg agc ggt aac ccg gtg ccg att ctg att	2256
Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile	
	740 745 750
ccg tgc cat cgt gtg gtt agc tct agc ggt gcg gtt ggc ggt tat gaa	2304
Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu	
	755 760 765
ggg ggt ctg gcg gtg aaa gag tgg ctg ctg gcc cat gaa ggt cat cgt	2352
Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg	
	770 775 780
ctg ggt aaa ccg ggt ctg gga cct gca ggt ata ggg cac cac cac cac	2400
Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly His His His His	
	785 790 795 800
cac cac	2406
His His	

<210> SEQ ID NO 4  
 <211> LENGTH: 802  
 <212> TYPE: PRT  
 <213> ORGANISM: Escherichia coli

<400> SEQUENCE: 4

Leu Arg Pro Val Glu Thr Pro Thr Arg Glu Ile Lys Lys Leu Asp Gly	
1	5 10 15
Leu Trp Ala Phe Ser Leu Asp Arg Glu Asn Cys Gly Ile Asp Gln Arg	
	20 25 30
Trp Trp Glu Ser Ala Leu Gln Glu Ser Arg Ala Ile Ala Val Pro Gly	
	35 40 45
Ser Phe Asn Asp Gln Phe Ala Asp Ala Asp Ile Arg Asn Tyr Ala Gly	
	50 55 60
Asn Val Trp Tyr Gln Arg Glu Val Phe Ile Pro Lys Gly Trp Ala Gly	
	65 70 75 80
Gln Arg Ile Val Leu Arg Phe Asp Ala Val Thr His Tyr Gly Lys Val	
	85 90 95
Trp Val Asn Asn Gln Glu Val Met Glu His Gln Gly Gly Tyr Thr Pro	
	100 105 110
Phe Glu Ala Asp Val Thr Pro Tyr Val Ile Ala Gly Lys Ser Val Arg	
	115 120 125
Ile Thr Val Cys Val Asn Asn Glu Leu Asn Trp Gln Thr Ile Pro Pro	
	130 135 140
Gly Met Val Ile Thr Asp Glu Asn Gly Lys Lys Lys Gln Ser Tyr Phe	
	145 150 155 160
His Asp Phe Phe Asn Tyr Ala Gly Ile His Arg Ser Val Met Leu Tyr	
	165 170 175
Thr Thr Pro Asn Thr Trp Val Asp Asp Ile Thr Val Val Thr His Val	
	180 185 190
Ala Gln Asp Cys Asn His Ala Ser Val Asp Trp Gln Val Val Ala Asn	
	195 200 205

-continued

---

Gly Asp Val Ser Val Glu Leu Arg Asp Ala Asp Gln Gln Val Val Ala  
 210 215 220  
 Thr Gly Gln Gly Thr Ser Gly Thr Leu Gln Val Val Asn Pro His Leu  
 225 230 235 240  
 Trp Gln Pro Gly Glu Gly Tyr Leu Tyr Glu Leu Cys Val Thr Ala Lys  
 245 250 255  
 Ser Gln Thr Glu Cys Asp Ile Tyr Pro Leu Arg Val Gly Ile Arg Ser  
 260 265 270  
 Val Ala Val Lys Gly Glu Gln Phe Leu Ile Asn His Lys Pro Phe Tyr  
 275 280 285  
 Phe Thr Gly Phe Gly Arg His Glu Asp Ala Asp Leu Arg Gly Lys Gly  
 290 295 300  
 Phe Asp Asn Val Leu Met Val His Asp His Ala Leu Met Asp Trp Ile  
 305 310 315 320  
 Gly Ala Asn Ser Tyr Arg Thr Ser His Tyr Pro Tyr Ala Glu Glu Met  
 325 330 335  
 Leu Asp Trp Ala Asp Glu His Gly Ile Val Val Ile Asp Glu Thr Ala  
 340 345 350  
 Ala Val Gly Phe Asn Leu Ser Leu Gly Ile Gly Phe Glu Ala Gly Asn  
 355 360 365  
 Lys Pro Lys Glu Leu Tyr Ser Glu Glu Ala Val Asn Gly Glu Thr Gln  
 370 375 380  
 Gln Ala His Leu Gln Ala Ile Lys Glu Leu Ile Ala Arg Asp Lys Asn  
 385 390 395 400  
 His Pro Ser Val Val Met Trp Ser Ile Ala Asn Glu Pro Asp Thr Arg  
 405 410 415  
 Pro Gln Gly Ala Arg Glu Tyr Phe Ala Pro Leu Ala Glu Ala Thr Arg  
 420 425 430  
 Lys Leu Asp Pro Thr Arg Pro Ile Thr Cys Val Asn Val Met Phe Cys  
 435 440 445  
 Asp Ala His Thr Asp Thr Ile Ser Asp Leu Phe Asp Val Leu Cys Leu  
 450 455 460  
 Asn Arg Tyr Tyr Gly Trp Tyr Val Gln Ser Gly Asp Leu Glu Thr Ala  
 465 470 475 480  
 Glu Lys Val Leu Glu Lys Glu Leu Leu Ala Trp Gln Glu Lys Leu His  
 485 490 495  
 Gln Pro Ile Ile Ile Thr Glu Tyr Gly Val Asp Thr Leu Ala Gly Leu  
 500 505 510  
 His Ser Met Tyr Thr Asp Met Trp Ser Glu Glu Tyr Gln Cys Ala Trp  
 515 520 525  
 Leu Asp Met Tyr His Arg Val Phe Asp Arg Val Ser Ala Val Val Gly  
 530 535 540  
 Glu Gln Val Trp Asn Phe Ala Asp Phe Ala Thr Ser Gln Gly Ile Leu  
 545 550 555 560  
 Arg Val Gly Gly Asn Lys Lys Gly Ile Phe Thr Arg Asp Arg Lys Pro  
 565 570 575  
 Lys Ser Ala Ala Phe Leu Leu Gln Lys Arg Trp Thr Gly Met Asn Phe  
 580 585 590  
 Gly Glu Lys Pro Gln Gln Gly Gly Lys Gln Gly Leu Cys Gly Arg Lys  
 595 600 605

-continued

---

Leu Met Asp Lys Asp Cys Glu Met Lys Arg Thr Thr Leu Asp Ser Pro  
 610 615 620

Leu Gly Lys Leu Glu Leu Ser Gly Cys Glu Gln Gly Leu His Glu Ile  
 625 630 635 640

Lys Leu Leu Gly Lys Gly Thr Ser Ala Ala Asp Ala Val Glu Val Pro  
 645 650 655

Ala Pro Ala Ala Val Leu Gly Gly Pro Glu Pro Leu Met Gln Ala Thr  
 660 665 670

Ala Trp Leu Asn Ala Tyr Phe His Gln Pro Glu Ala Ile Glu Glu Phe  
 675 680 685

Pro Val Pro Ala Leu His His Pro Val Phe Gln Gln Glu Ser Phe Thr  
 690 695 700

Arg Gln Val Leu Trp Lys Leu Leu Lys Val Val Lys Phe Gly Glu Val  
 705 710 715 720

Ile Ser Tyr Gln Gln Leu Ala Ala Leu Ala Gly Asn Pro Ala Ala Thr  
 725 730 735

Ala Ala Val Lys Thr Ala Leu Ser Gly Asn Pro Val Pro Ile Leu Ile  
 740 745 750

Pro Cys His Arg Val Val Ser Ser Ser Gly Ala Val Gly Gly Tyr Glu  
 755 760 765

Gly Gly Leu Ala Val Lys Glu Trp Leu Leu Ala His Glu Gly His Arg  
 770 775 780

Leu Gly Lys Pro Gly Leu Gly Pro Ala Gly Ile Gly His His His His  
 785 790 795 800

His His

<210> SEQ ID NO 5  
 <211> LENGTH: 885  
 <212> TYPE: DNA  
 <213> ORGANISM: Staphylococcus aureus  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(885)

<400> SEQUENCE: 5

atg gca gat tct gat att aat att aaa acc ggt act aca gat att gga	48
Met Ala Asp Ser Asp Ile Asn Ile Lys Thr Gly Thr Thr Asp Ile Gly	
1 5 10 15	
agc aat act aca gta aaa aca ggt gat tta gtc act tat gat aaa gaa	96
Ser Asn Thr Thr Val Lys Thr Gly Asp Leu Val Thr Tyr Asp Lys Glu	
20 25 30	
aat ggc atg cac aaa aaa gta ttt tat agt ttt atc gat gat aaa aat	144
Asn Gly Met His Lys Lys Val Phe Tyr Ser Phe Ile Asp Asp Lys Asn	
35 40 45	
cac aat aaa aaa ctg cta gtt att aga acg aaa ggt acc att gct ggt	192
His Asn Lys Lys Leu Leu Val Ile Arg Thr Lys Gly Thr Ile Ala Gly	
50 55 60	
caa tat aga gtt tat agc gaa gaa ggt gct aac aaa agt ggt tta gcc	240
Gln Tyr Arg Val Tyr Ser Glu Glu Gly Ala Asn Lys Ser Gly Leu Ala	
65 70 75 80	
tgg cct tca gcc ttt aag gta cag ttg caa cta cct gat aat gaa gta	288
Trp Pro Ser Ala Phe Lys Val Gln Leu Gln Leu Pro Asp Asn Glu Val	
85 90 95	
gct caa ata tct gat tac tat cca aga aat tcg att gat aca aaa gag	336
Ala Gln Ile Ser Asp Tyr Tyr Pro Arg Asn Ser Ile Asp Thr Lys Glu	
100 105 110	

-continued

---

tat atg agt act tta act tat gga ttc aac ggt aat gtt act ggt gat	384
Tyr Met Ser Thr Leu Thr Tyr Gly Phe Asn Gly Asn Val Thr Gly Asp	
115 120 125	
gat aca gga aaa att ggc ggc ctt att ggt gca aat gtt tcg att ggt	432
Asp Thr Gly Lys Ile Gly Gly Leu Ile Gly Ala Asn Val Ser Ile Gly	
130 135 140	
cat aca ctg aaa tat gtt caa cct gat ttc aaa aca att tta gag agc	480
His Thr Leu Lys Tyr Val Gln Pro Asp Phe Lys Thr Ile Leu Glu Ser	
145 150 155 160	
cca act gat aaa aaa gta ggc tgg aaa gtg ata ttt aac aat atg gtg	528
Pro Thr Asp Lys Lys Val Gly Trp Lys Val Ile Phe Asn Asn Met Val	
165 170 175	
aat caa aat tgg gga cca tat gat aga gat tct tgg aac ccg gta tat	576
Asn Gln Asn Trp Gly Pro Tyr Asp Arg Asp Ser Trp Asn Pro Val Tyr	
180 185 190	
ggc aat caa ctt ttc atg aaa act aga aat ggt tct atg aaa gca gca	624
Gly Asn Gln Leu Phe Met Lys Thr Arg Asn Gly Ser Met Lys Ala Ala	
195 200 205	
gat aac ttc ctt gat cct aac aaa gca agt tct cta tta tct tca ggg	672
Asp Asn Phe Leu Asp Pro Asn Lys Ala Ser Ser Leu Leu Ser Ser Gly	
210 215 220	
ttt tca cca gac ttc gct aca gtt att act atg gat aga aaa gca tcc	720
Phe Ser Pro Asp Phe Ala Thr Val Ile Thr Met Asp Arg Lys Ala Ser	
225 230 235 240	
aaa caa caa aca aat ata gat gta ata tac gaa cga gtt cgt gat gat	768
Lys Gln Gln Thr Asn Ile Asp Val Ile Tyr Glu Arg Val Arg Asp Asp	
245 250 255	
tac caa ttg cat tgg act tca aca aat tgg aaa ggt acc aat act aaa	816
Tyr Gln Leu His Trp Thr Ser Thr Asn Trp Lys Gly Thr Asn Thr Lys	
260 265 270	
gat aaa tgg aca gat cgt tct tca gaa aga tat aaa atc gat tgg gaa	864
Asp Lys Trp Thr Asp Arg Ser Ser Glu Arg Tyr Lys Ile Asp Trp Glu	
275 280 285	
aaa gaa gaa atg aca aat taa	885
Lys Glu Glu Met Thr Asn	
290	

&lt;210&gt; SEQ ID NO 6

&lt;211&gt; LENGTH: 294

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Staphylococcus aureus

&lt;400&gt; SEQUENCE: 6

Met Ala Asp Ser Asp Ile Asn Ile Lys Thr Gly Thr Thr Asp Ile Gly	
1 5 10 15	
Ser Asn Thr Thr Val Lys Thr Gly Asp Leu Val Thr Tyr Asp Lys Glu	
20 25 30	
Asn Gly Met His Lys Lys Val Phe Tyr Ser Phe Ile Asp Asp Lys Asn	
35 40 45	
His Asn Lys Lys Leu Leu Val Ile Arg Thr Lys Gly Thr Ile Ala Gly	
50 55 60	
Gln Tyr Arg Val Tyr Ser Glu Glu Gly Ala Asn Lys Ser Gly Leu Ala	
65 70 75 80	
Trp Pro Ser Ala Phe Lys Val Gln Leu Gln Leu Pro Asp Asn Glu Val	
85 90 95	
Ala Gln Ile Ser Asp Tyr Tyr Pro Arg Asn Ser Ile Asp Thr Lys Glu	

-continued

---

	100						105						110			
Tyr	Met	Ser	Thr	Leu	Thr	Tyr	Gly	Phe	Asn	Gly	Asn	Val	Thr	Gly	Asp	
	115						120						125			
Asp	Thr	Gly	Lys	Ile	Gly	Gly	Leu	Ile	Gly	Ala	Asn	Val	Ser	Ile	Gly	
	130					135						140				
His	Thr	Leu	Lys	Tyr	Val	Gln	Pro	Asp	Phe	Lys	Thr	Ile	Leu	Glu	Ser	
145					150					155					160	
Pro	Thr	Asp	Lys	Lys	Val	Gly	Trp	Lys	Val	Ile	Phe	Asn	Asn	Met	Val	
			165						170					175		
Asn	Gln	Asn	Trp	Gly	Pro	Tyr	Asp	Arg	Asp	Ser	Trp	Asn	Pro	Val	Tyr	
			180					185						190		
Gly	Asn	Gln	Leu	Phe	Met	Lys	Thr	Arg	Asn	Gly	Ser	Met	Lys	Ala	Ala	
	195						200						205			
Asp	Asn	Phe	Leu	Asp	Pro	Asn	Lys	Ala	Ser	Ser	Leu	Leu	Ser	Ser	Gly	
	210					215					220					
Phe	Ser	Pro	Asp	Phe	Ala	Thr	Val	Ile	Thr	Met	Asp	Arg	Lys	Ala	Ser	
225					230					235					240	
Lys	Gln	Gln	Thr	Asn	Ile	Asp	Val	Ile	Tyr	Glu	Arg	Val	Arg	Asp	Asp	
				245					250					255		
Tyr	Gln	Leu	His	Trp	Thr	Ser	Thr	Asn	Trp	Lys	Gly	Thr	Asn	Thr	Lys	
			260					265						270		
Asp	Lys	Trp	Thr	Asp	Arg	Ser	Ser	Glu	Arg	Tyr	Lys	Ile	Asp	Trp	Glu	
		275					280					285				
Lys	Glu	Glu	Met	Thr	Asn											
	290															

<210> SEQ ID NO 7  
 <211> LENGTH: 901  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Halo-tag protein

<400> SEQUENCE: 7

Leu	Arg	Pro	Val	Glu	Thr	Pro	Thr	Arg	Glu	Ile	Lys	Lys	Leu	Asp	Gly
1			5						10					15	
Leu	Trp	Ala	Phe	Ser	Leu	Asp	Arg	Glu	Asn	Cys	Gly	Ile	Asp	Gln	Arg
		20						25					30		
Trp	Trp	Glu	Ser	Ala	Leu	Gln	Glu	Ser	Arg	Ala	Ile	Ala	Val	Pro	Gly
		35					40					45			
Ser	Phe	Asn	Asp	Gln	Phe	Ala	Asp	Ala	Asp	Ile	Arg	Asn	Tyr	Ala	Gly
	50					55					60				
Asn	Val	Trp	Tyr	Gln	Arg	Glu	Val	Phe	Ile	Pro	Lys	Gly	Trp	Ala	Gly
65					70					75					80
Gln	Arg	Ile	Val	Leu	Arg	Phe	Asp	Ala	Val	Thr	His	Tyr	Gly	Lys	Val
			85						90					95	
Trp	Val	Asn	Asn	Gln	Glu	Val	Met	Glu	His	Gln	Gly	Gly	Tyr	Thr	Pro
		100						105					110		
Phe	Glu	Ala	Asp	Val	Thr	Pro	Tyr	Val	Ile	Ala	Gly	Lys	Ser	Val	Arg
		115					120						125		
Ile	Thr	Val	Cys	Val	Asn	Asn	Glu	Leu	Asn	Trp	Gln	Thr	Ile	Pro	Pro
	130					135					140				
Gly	Met	Val	Ile	Thr	Asp	Glu	Asn	Gly	Lys	Lys	Lys	Gln	Ser	Tyr	Phe

-continued

145	150	155	160
His Asp Phe Phe Asn Tyr Ala Gly Ile His Arg Ser Val Met Leu Tyr	165	170	175
Thr Thr Pro Asn Thr Trp Val Asp Asp Ile Thr Val Val Thr His Val	180	185	190
Ala Gln Asp Cys Asn His Ala Ser Val Asp Trp Gln Val Val Ala Asn	195	200	205
Gly Asp Val Ser Val Glu Leu Arg Asp Ala Asp Gln Gln Val Val Ala	210	215	220
Thr Gly Gln Gly Thr Ser Gly Thr Leu Gln Val Val Asn Pro His Leu	225	230	235
Trp Gln Pro Gly Glu Gly Tyr Leu Tyr Glu Leu Cys Val Thr Ala Lys	245	250	255
Ser Gln Thr Glu Cys Asp Ile Tyr Pro Leu Arg Val Gly Ile Arg Ser	260	265	270
Val Ala Val Lys Gly Glu Gln Phe Leu Ile Asn His Lys Pro Phe Tyr	275	280	285
Phe Thr Gly Phe Gly Arg His Glu Asp Ala Asp Leu Arg Gly Lys Gly	290	295	300
Phe Asp Asn Val Leu Met Val His Asp His Ala Leu Met Asp Trp Ile	305	310	315
Gly Ala Asn Ser Tyr Arg Thr Ser His Tyr Pro Tyr Ala Glu Glu Met	325	330	335
Leu Asp Trp Ala Asp Glu His Gly Ile Val Val Ile Asp Glu Thr Ala	340	345	350
Ala Val Gly Phe Asn Leu Ser Leu Gly Ile Gly Phe Glu Ala Gly Asn	355	360	365
Lys Pro Lys Glu Leu Tyr Ser Glu Glu Ala Val Asn Gly Glu Thr Gln	370	375	380
Gln Ala His Leu Gln Ala Ile Lys Glu Leu Ile Ala Arg Asp Lys Asn	385	390	395
His Pro Ser Val Val Met Trp Ser Ile Ala Asn Glu Pro Asp Thr Arg	405	410	415
Pro Gln Gly Ala Arg Glu Tyr Phe Ala Pro Leu Ala Glu Ala Thr Arg	420	425	430
Lys Leu Asp Pro Thr Arg Pro Ile Thr Cys Val Asn Val Met Phe Cys	435	440	445
Asp Ala His Thr Asp Thr Ile Ser Asp Leu Phe Asp Val Leu Cys Leu	450	455	460
Asn Arg Tyr Tyr Gly Trp Tyr Val Gln Ser Gly Asp Leu Glu Thr Ala	465	470	475
Glu Lys Val Leu Glu Lys Glu Leu Leu Ala Trp Gln Glu Lys Leu His	485	490	495
Gln Pro Ile Ile Ile Thr Glu Tyr Gly Val Asp Thr Leu Ala Gly Leu	500	505	510
His Ser Met Tyr Thr Asp Met Trp Ser Glu Glu Tyr Gln Cys Ala Trp	515	520	525
Leu Asp Met Tyr His Arg Val Phe Asp Arg Val Ser Ala Val Val Gly	530	535	540
Glu Gln Val Trp Asn Phe Ala Asp Phe Ala Thr Ser Gln Gly Ile Leu	545	550	555
			560

-continued

---

Arg Val Gly Gly Asn Lys Lys Gly Ile Phe Thr Arg Asp Arg Lys Pro  
                   565                                  570                                  575  
 Lys Ser Ala Ala Phe Leu Leu Gln Lys Arg Trp Thr Gly Met Asn Phe  
                   580                                  585                                  590  
 Gly Glu Lys Pro Gln Gln Gly Gly Lys Gln Gly Leu Cys Gly Arg Lys  
                   595                                  600                                  605  
 Leu Met Ala Glu Ile Gly Thr Gly Phe Pro Phe Asp Pro His Tyr Val  
                   610                                  615                                  620  
 Glu Val Leu Gly Glu Arg Met His Tyr Val Asp Val Gly Pro Arg Asp  
                   625                                  630                                  635                                  640  
 Gly Thr Pro Val Leu Phe Leu His Gly Asn Pro Thr Ser Ser Tyr Val  
                   645                                  650                                  655  
 Trp Arg Asn Ile Ile Pro His Val Ala Pro Thr His Arg Cys Ile Ala  
                   660                                  665                                  670  
 Pro Asp Leu Ile Gly Met Gly Lys Ser Asp Lys Pro Asp Leu Gly Tyr  
                   675                                  680                                  685  
 Phe Phe Asp Asp His Val Arg Phe Met Asp Ala Phe Ile Glu Ala Leu  
                   690                                  695                                  700  
 Gly Leu Glu Glu Val Val Leu Val Ile His Asp Trp Gly Ser Ala Leu  
                   705                                  710                                  715                                  720  
 Gly Phe His Trp Ala Lys Arg Asn Pro Glu Arg Val Lys Gly Ile Ala  
                   725                                  730                                  735  
 Phe Met Glu Phe Ile Arg Pro Ile Pro Thr Trp Asp Glu Trp Pro Glu  
                   740                                  745                                  750  
 Phe Ala Arg Glu Thr Phe Gln Ala Phe Arg Thr Thr Asp Val Gly Arg  
                   755                                  760                                  765  
 Lys Leu Ile Ile Asp Gln Asn Val Phe Ile Glu Gly Thr Leu Pro Met  
                   770                                  775                                  780  
 Gly Val Val Arg Pro Leu Thr Glu Val Glu Met Asp His Tyr Arg Glu  
                   785                                  790                                  795                                  800  
 Pro Phe Leu Asn Pro Val Asp Arg Glu Pro Leu Trp Arg Phe Pro Asn  
                   805                                  810                                  815  
 Glu Leu Pro Ile Ala Gly Glu Pro Ala Asn Ile Val Ala Leu Val Glu  
                   820                                  825                                  830  
 Glu Tyr Met Asp Trp Leu His Gln Ser Pro Val Pro Lys Leu Leu Phe  
                   835                                  840                                  845  
 Trp Gly Thr Pro Gly Val Leu Ile Pro Pro Ala Glu Ala Ala Arg Leu  
                   850                                  855                                  860  
 Ala Lys Ser Leu Pro Asn Cys Lys Ala Val Asp Ile Gly Pro Gly Leu  
                   865                                  870                                  875                                  880  
 Asn Leu Leu Gln Glu Asp Asn Pro Asp Leu Ile Gly Ser Glu Ile Ala  
                   885                                  890                                  895  
 Arg Trp Leu Ser Thr  
                   900

<210> SEQ ID NO 8  
 <211> LENGTH: 771  
 <212> TYPE: DNA  
 <213> ORGANISM: Staphylococcus aureus  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(771)

-continued

&lt;400&gt; SEQUENCE: 8

```

atg ttt tat agt ttt atc gat gat aaa aat cac aat aaa aaa ctg cta      48
Met Phe Tyr Ser Phe Ile Asp Asp Lys Asn His Asn Lys Lys Leu Leu
1           5           10           15

ggt att aga acg aaa ggt acc att gct ggt caa tat aga gtt tat agc      96
Val Ile Arg Thr Lys Gly Thr Ile Ala Gly Gln Tyr Arg Val Tyr Ser
20           25           30

gaa gaa ggt gct aac aaa agt ggt tta gcc tgg cct tca gcc ttt aag     144
Glu Glu Gly Ala Asn Lys Ser Gly Leu Ala Trp Pro Ser Ala Phe Lys
35           40           45

gta cag ttg caa cta cct gat aat gaa gta gct caa ata tct gat tac     192
Val Gln Leu Gln Leu Pro Asp Asn Glu Val Ala Gln Ile Ser Asp Tyr
50           55           60

tat cca aga aat tcg att gat aca aaa gag tat atg agt act tta act     240
Tyr Pro Arg Asn Ser Ile Asp Thr Lys Glu Tyr Met Ser Thr Leu Thr
65           70           75           80

tat gga ttc aac ggt aat gtt act ggt gat gat aca gga aaa att ggc     288
Tyr Gly Phe Asn Gly Asn Val Thr Gly Asp Asp Thr Gly Lys Ile Gly
85           90           95

ggc ctt att ggt gca aat gtt tcg att ggt cat aca ctg aaa tat gtt     336
Gly Leu Ile Gly Ala Asn Val Ser Ile Gly His Thr Leu Lys Tyr Val
100          105          110

caa cct gat ttc aaa aca att tta gag agc cca act gat aaa aaa gta     384
Gln Pro Asp Phe Lys Thr Ile Leu Glu Ser Pro Thr Asp Lys Lys Val
115          120          125

ggc tgg aaa gtg ata ttt aac aat atg gtg aat caa aat tgg gga cca     432
Gly Trp Lys Val Ile Phe Asn Asn Met Val Asn Gln Asn Trp Gly Pro
130          135          140

tat gat aga gat tct tgg aac ccg gta tat ggc aat caa ctt ttc atg     480
Tyr Asp Arg Asp Ser Trp Asn Pro Val Tyr Gly Asn Gln Leu Phe Met
145          150          155          160

aaa act aga aat ggt tct atg aaa gca gca gat aac ttc ctt gat cct     528
Lys Thr Arg Asn Gly Ser Met Lys Ala Ala Asp Asn Phe Leu Asp Pro
165          170          175

aac aaa gca agt tct cta tta tct tca ggg ttt tca cca gac ttc gct     576
Asn Lys Ala Ser Ser Leu Leu Ser Ser Gly Phe Ser Pro Asp Phe Ala
180          185          190

aca gtt att act atg gat aga aaa gca tcc aaa caa caa aca aat ata     624
Thr Val Ile Thr Met Asp Arg Lys Ala Ser Lys Gln Gln Thr Asn Ile
195          200          205

gat gta ata tac gaa cga gtt cgt gat gat tac caa ttg cat tgg act     672
Asp Val Ile Tyr Glu Arg Val Arg Asp Asp Tyr Gln Leu His Trp Thr
210          215          220

tca aca aat tgg aaa ggt acc aat act aaa gat aaa tgg aca gat cgt     720
Ser Thr Asn Trp Lys Gly Thr Asn Thr Lys Asp Lys Trp Thr Asp Arg
225          230          235          240

tct tca gaa aga tat aaa atc gat tgg gaa aaa gaa gaa atg aca aat     768
Ser Ser Glu Arg Tyr Lys Ile Asp Trp Glu Lys Glu Glu Met Thr Asn
245          250          255

taa                                                                 771

```

&lt;210&gt; SEQ ID NO 9

&lt;211&gt; LENGTH: 256

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Staphylococcus aureus

&lt;400&gt; SEQUENCE: 9



-continued

---

Met	Phe	Tyr	Ser	Phe	Ile	Asp	Asp	Lys	Asn	His	Asn	Lys	Lys	Leu	Leu
1			5					10						15	
Val	Ile	Arg	Thr	Lys	Gly	Thr	Ile	Ala	Gly	Gln	Tyr	Arg	Val	Tyr	Ser
		20						25					30		
Glu	Glu	Gly	Ala	Asn	Lys	Ser	Gly	Leu	Ala	Trp	Pro	Ser	Ala	Phe	Lys
		35					40					45			
Val	Gln	Leu	Gln	Leu	Pro	Asp	Asn	Glu	Val	Ala	Gln	Ile	Ser	Asp	Tyr
	50					55					60				
Tyr	Pro	Arg	Asn	Ser	Ile	Asp	Thr	Lys	Glu	Tyr	Met	Ser	Thr	Leu	Thr
65					70					75					80
Tyr	Gly	Phe	Asn	Gly	Asn	Val	Thr	Gly	Asp	Asp	Thr	Gly	Lys	Ile	Gly
			85						90					95	
Gly	Leu	Ile	Gly	Ala	Asn	Val	Ser	Ile	Gly	His	Thr	Leu	Lys	Tyr	Val
			100					105					110		
Gln	Pro	Asp	Phe	Lys	Thr	Ile	Leu	Glu	Ser	Pro	Thr	Asp	Lys	Lys	Val
		115					120					125			
Gly	Trp	Lys	Val	Ile	Phe	Asn	Asn	Met	Val	Asn	Gln	Asn	Trp	Gly	Pro
	130					135					140				
Tyr	Asp	Arg	Asp	Ser	Trp	Asn	Pro	Val	Tyr	Gly	Asn	Gln	Leu	Phe	Met
145					150					155					160
Lys	Thr	Arg	Asn	Gly	Ser	Met	Lys	Ala	Ala	Asp	Asn	Phe	Leu	Asp	Pro
			165						170					175	
Asn	Lys	Ala	Ser	Ser	Leu	Leu	Ser	Ser	Gly	Phe	Ser	Pro	Asp	Phe	Ala
		180						185					190		
Thr	Val	Ile	Thr	Met	Asp	Arg	Lys	Ala	Ser	Lys	Gln	Gln	Thr	Asn	Ile
		195					200						205		
Asp	Val	Ile	Tyr	Glu	Arg	Val	Arg	Asp	Asp	Tyr	Gln	Leu	His	Trp	Thr
	210					215					220				
Ser	Thr	Asn	Trp	Lys	Gly	Thr	Asn	Thr	Lys	Asp	Lys	Trp	Thr	Asp	Arg
225					230					235					240
Ser	Ser	Glu	Arg	Tyr	Lys	Ile	Asp	Trp	Glu	Lys	Glu	Glu	Met	Thr	Asn
				245					250						255

---

1. A unilamellar liposome comprising:
  - (a) a DNA comprising a promoter sequence, a translational initiation sequence, and a sequence encoding a membrane protein;
  - (b) an RNA polymerase;
  - (c) a ribonucleotide; and
  - (d) a cell-free protein synthesis system,
 wherein the unilamellar liposome is treated with a nuclease, and the nuclease is selected from the group consisting of a ribonuclease and a deoxyribonuclease.
2. The unilamellar liposome of claim 1, wherein the membrane protein is a transporter, and the unilamellar liposome further comprises
  - (e) a factor that binds to a ligand transported by the membrane protein.
3. The unilamellar liposome of claim 1, wherein the nuclease is a ribonuclease.
4. A library comprising a plurality of unilamellar liposomes of claim 1.
5. The library of claim 4, wherein the membrane protein is a transporter, and the unilamellar liposome further comprises
  - (e) a factor that binds to a ligand transported by the membrane protein.
6. The library of claim 4, wherein the nuclease is a ribonuclease.
7. A unilamellar liposome comprising:
  - (a) an RNA comprising a translational initiation sequence, and a sequence encoding a membrane protein; and
  - (b) a cell-free protein synthesis system,
 wherein the unilamellar liposome is treated with a nuclease, and the nuclease is a ribonuclease.
8. The unilamellar liposome of claim 7, wherein the membrane protein is a transporter, and the unilamellar liposome further comprises
  - (c) a factor that binds to a ligand transported by the membrane protein.
9. A library comprising a plurality of unilamellar liposomes of claim 7.
10. The library of claim 9, wherein the membrane protein is a transporter, and the unilamellar liposome further comprises
  - (c) a factor that binds to a ligand transported by the membrane protein.

**11.** A method of producing the unilamellar liposome of claim **1**, comprising:

- (1) preparing a unilamellar liposome enclosing:
  - (a) a DNA comprising a promoter sequence, a translational initiation sequence, and a sequence encoding a membrane protein;
  - (b) an RNA polymerase;
  - (c) a ribonucleotide; and
  - (d) a cell-free protein synthesis system; and
- (2) treating the unilamellar liposome prepared in (1) with a nuclease,

wherein the nuclease is selected from the group consisting of a ribonuclease and a deoxyribonuclease.

**12.** A method of producing the unilamellar liposome of claim **2**, comprising:

- (1) preparing a unilamellar liposome enclosing:
  - (a) a DNA comprising a promoter sequence, a translational initiation sequence, and a sequence encoding a membrane protein that is a transporter;
  - (b) an RNA polymerase;
  - (c) a ribonucleotide;
  - (d) a cell-free protein synthesis system; and
  - (e) a factor that binds to a ligand transported by the membrane protein; and
- (2) treating the unilamellar liposome prepared in (1) with a nuclease,

wherein the nuclease is selected from the group consisting of a ribonuclease and a deoxyribonuclease.

**13.** The method of claim **11** or **12**, wherein the nuclease is a ribonuclease.

**14.** A method of producing the unilamellar liposome of claim **7**, comprising:

- (1) preparing a unilamellar liposome enclosing:
  - (a) an RNA comprising a translational initiation sequence, and a sequence encoding a membrane protein; and
  - (b) a cell-free protein synthesis system; and

- (2) treating the unilamellar liposome prepared in (1) with a nuclease,

wherein the nuclease is a ribonuclease.

**15.** A method of producing the unilamellar liposome of claim **8**, comprising:

- (1) preparing a unilamellar liposome enclosing:
  - (a) an RNA comprising a translational initiation sequence, and a sequence encoding a membrane protein that is a transporter;
  - (b) a cell-free protein synthesis system; and
  - (c) a factor that binds to a ligand that is transported by the membrane protein; and
- (2) treating the unilamellar liposome prepared in (1) with a nuclease,

wherein the nuclease is a ribonuclease.

**16.** A screening method using a library of unilamellar liposomes, comprising:

- (i) providing the library of any one of claims **4** to **6**;
- (ii) selecting a unilamellar liposome having a desired feature from the library;
- (iii) amplifying an DNA included in the unilamellar liposome to obtain an amplified DNA; and
- (iv) isolating the amplified DNA of (iii).

**17.** A screening method using a library of unilamellar liposomes, comprising:

- (i) providing the library of either claim **9** or claim **10**;
- (ii) selecting a unilamellar liposome having a desired feature from the library;
- (iii) generating a DNA by operating a reverse transcriptase on an RNA included in the unilamellar liposome to obtain a generated DNA;
- (iv) amplifying the generated DNA of (iii) to obtain amplified DNA; and
- (v) isolating the amplified DNA of (iv).

\* \* \* \* \*