



(12) **United States Patent**
Cheng et al.

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(54) **RECOMBINANT NUCLEOTIDE SEQUENCE, CELL OR VECTOR CONTAINING THE SAME AND METHOD FOR USING CELL CONTAINING THE SAME TO ENCODE ANTI-POLYETHYLENE GLYCOL MONOCLONAL ANTIBODIES**

C12N 15/64 (2006.01)
C12N 15/00 (2006.01)
C07H 21/04 (2006.01)

(52) **U.S. Cl.**
USPC **435/7.1**; 435/6.1; 435/6.19; 435/91.4;
435/320.1; 536/23.53

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

(73) Assignee: **Kaohsiung Medical University**, Kaohsiung (TW)

(56) **References Cited**

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 468 days.

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* cited by examiner

(21) Appl. No.: **12/766,930**

Primary Examiner — Anne Gussow

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Assistant Examiner — Channing S Mahatan

(65) **Prior Publication Data**

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(30) **Foreign Application Priority Data**

Sep. 17, 2009 (TW) 98131334 A

(57) **ABSTRACT**

(51) **Int. Cl.**
G01N 33/53 (2006.01)
C12Q 1/68 (2006.01)

The invention provides a recombinant nucleotide sequence, including the sequence of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3 or SEQ ID No. 4, wherein the recombinant nucleotide sequence encodes an anti-polyethylene glycol recombinant single chain membrane antibody.

5 Claims, 13 Drawing Sheets

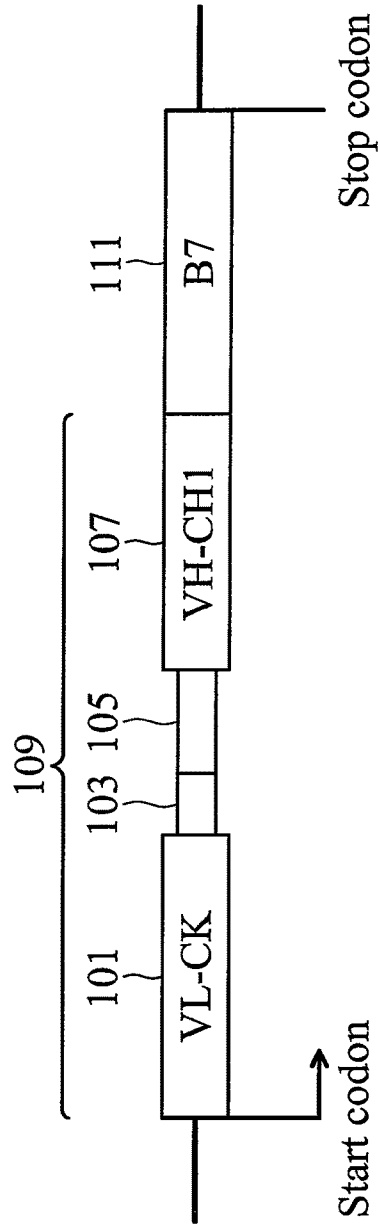


FIG. 1

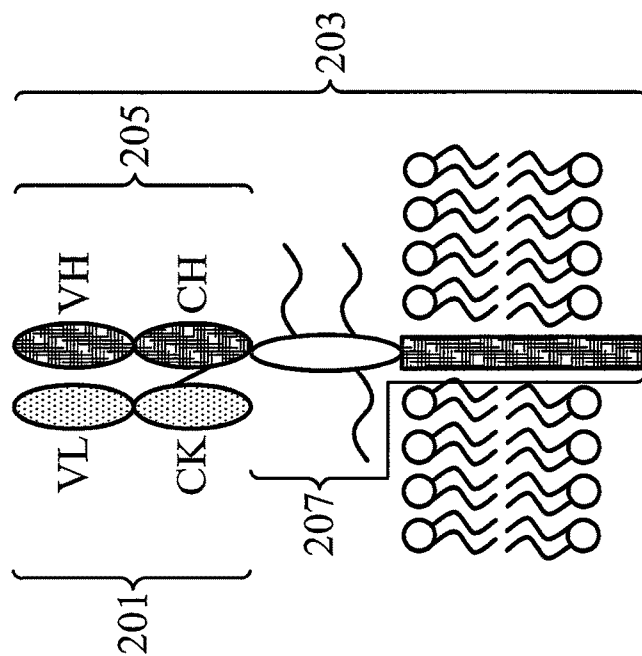


FIG. 2

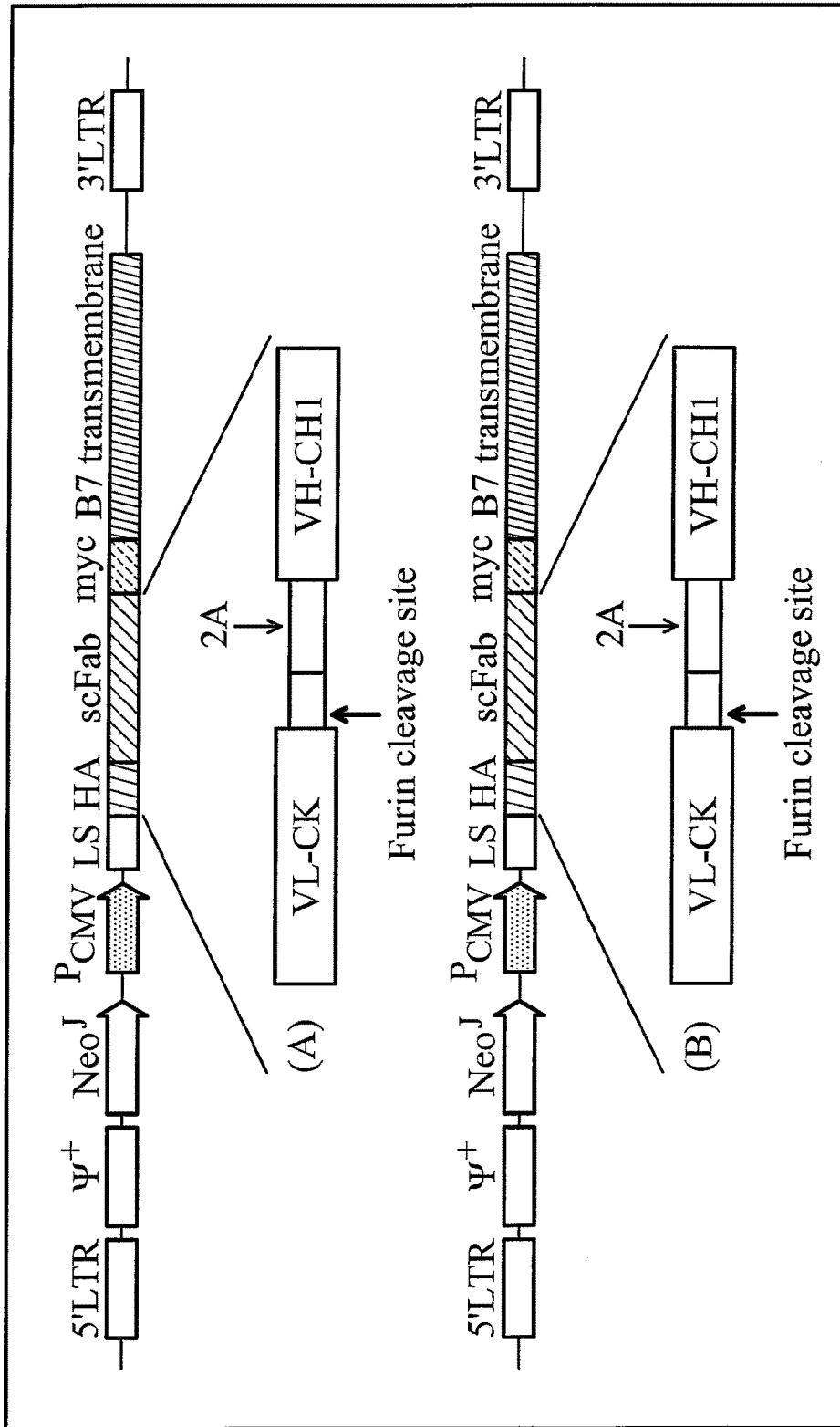


FIG. 3

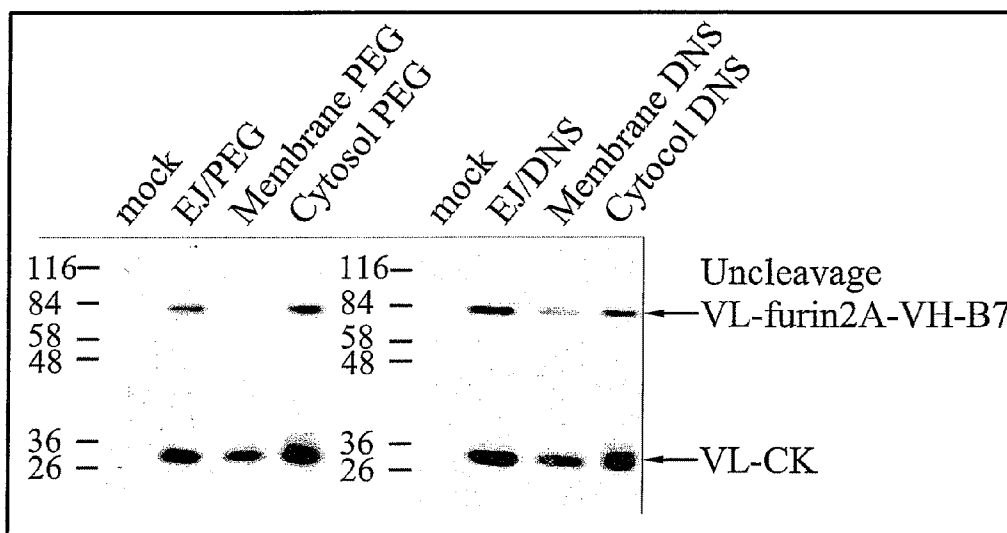


FIG. 4A

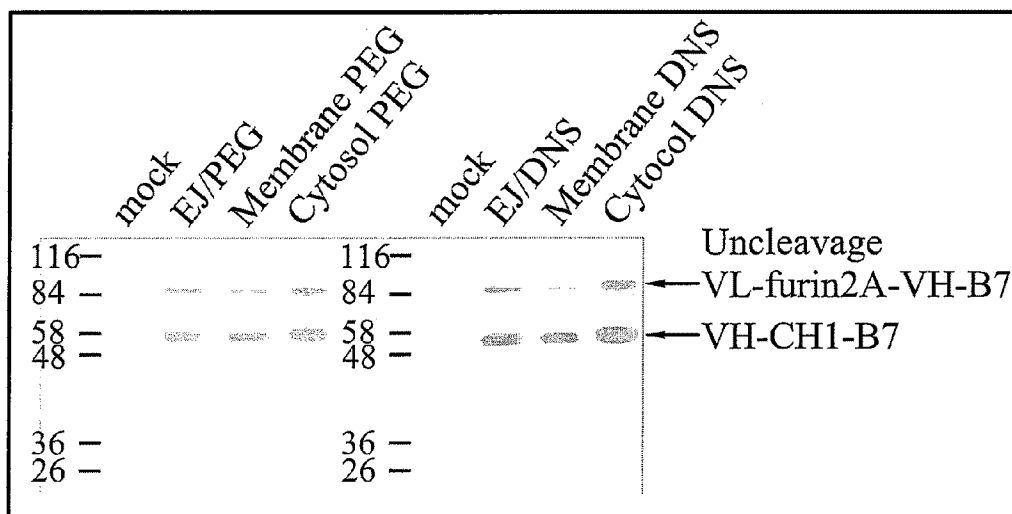


FIG. 4B

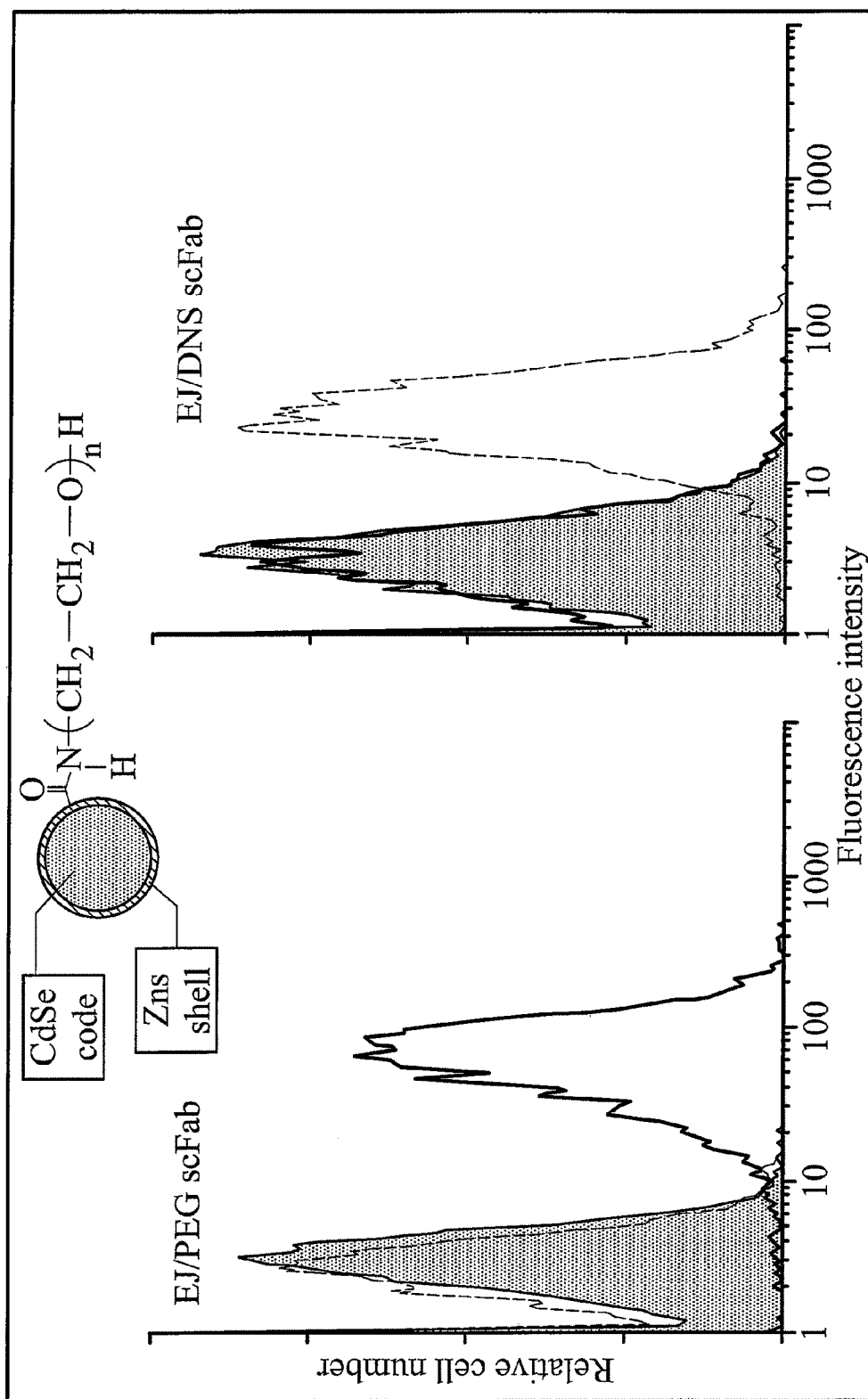


FIG. 5A

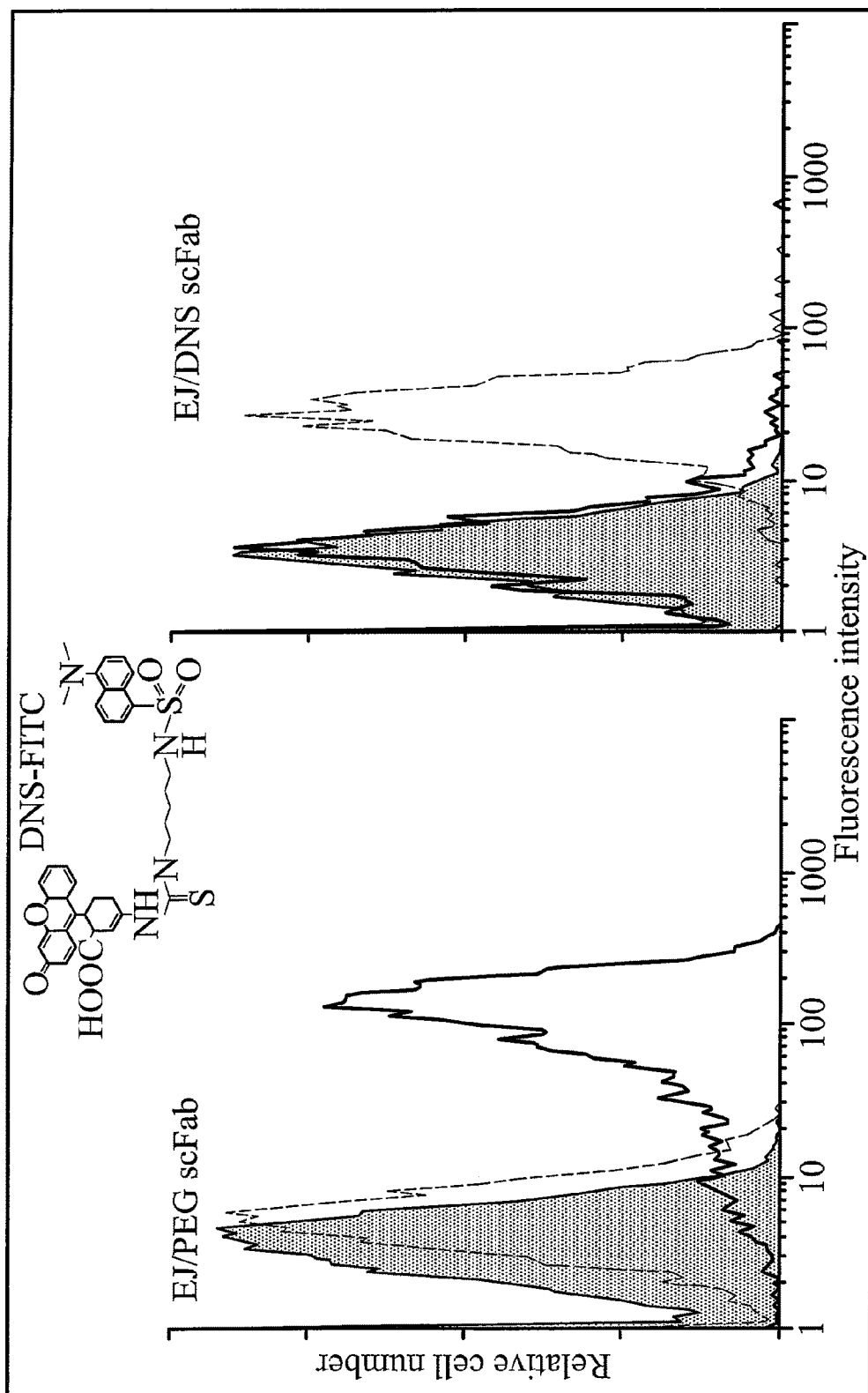


FIG. 5B

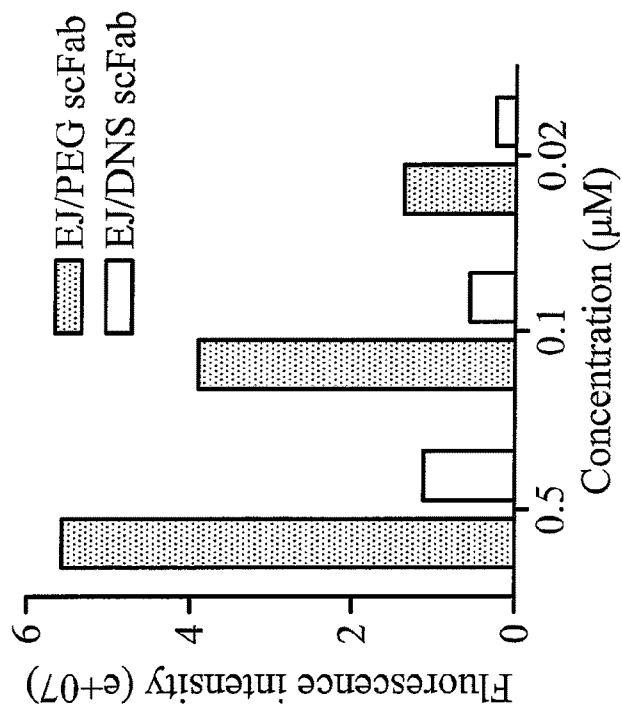


FIG. 6B

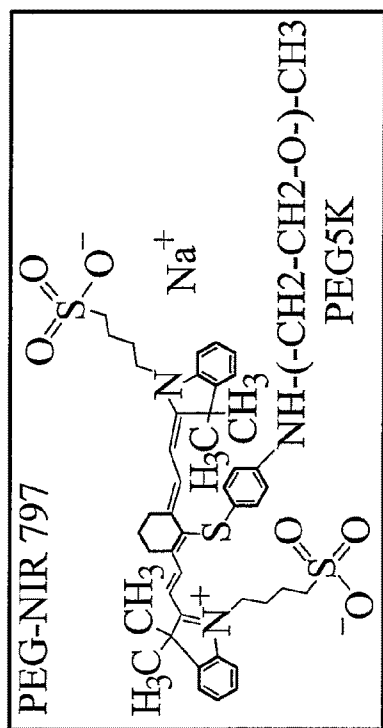


FIG. 6A

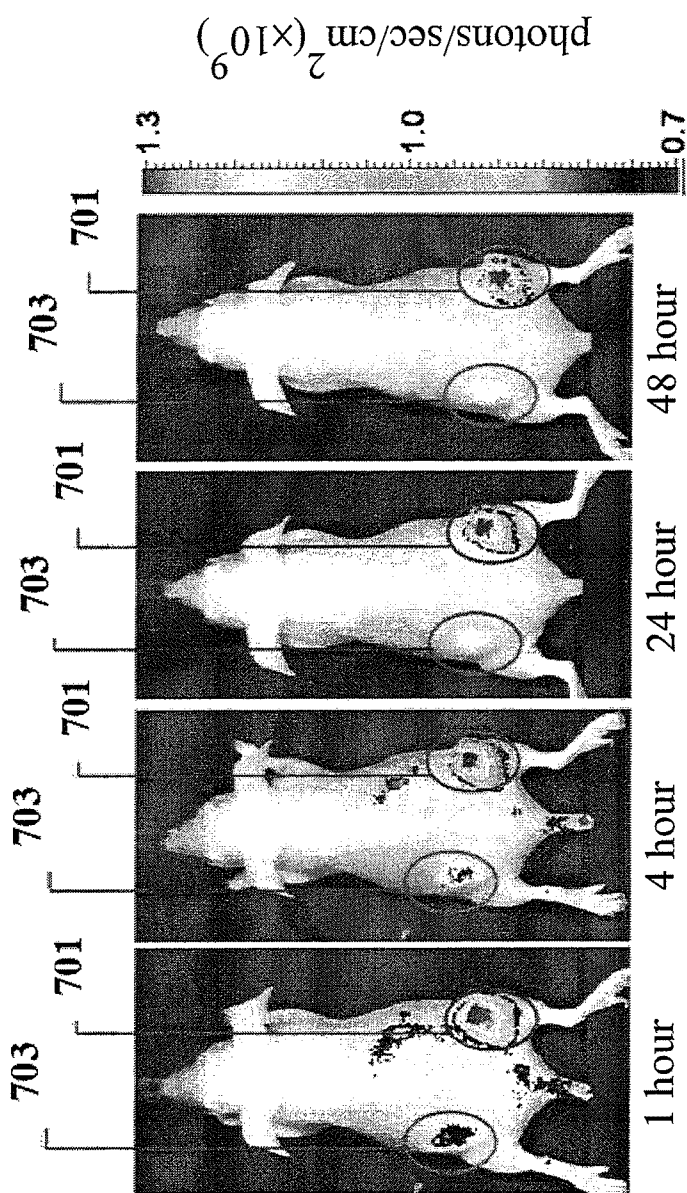


FIG. 7

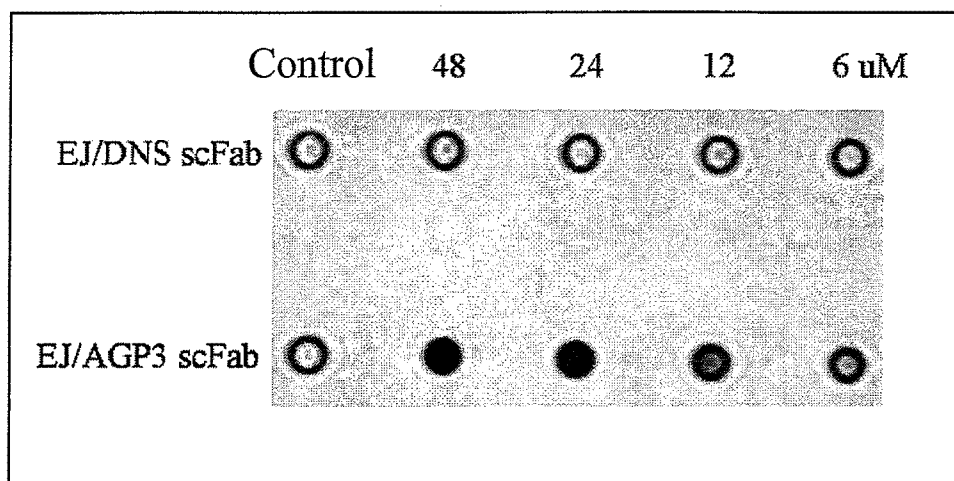


FIG. 8

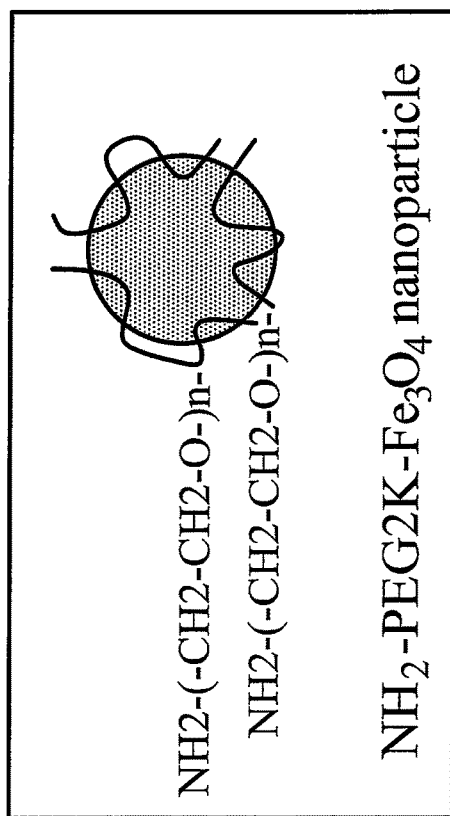


FIG. 9A

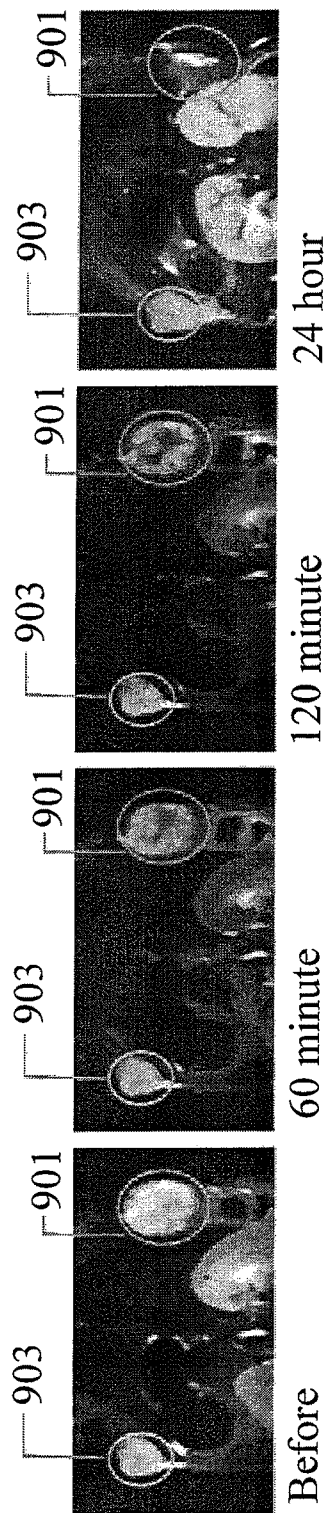


FIG. 9B

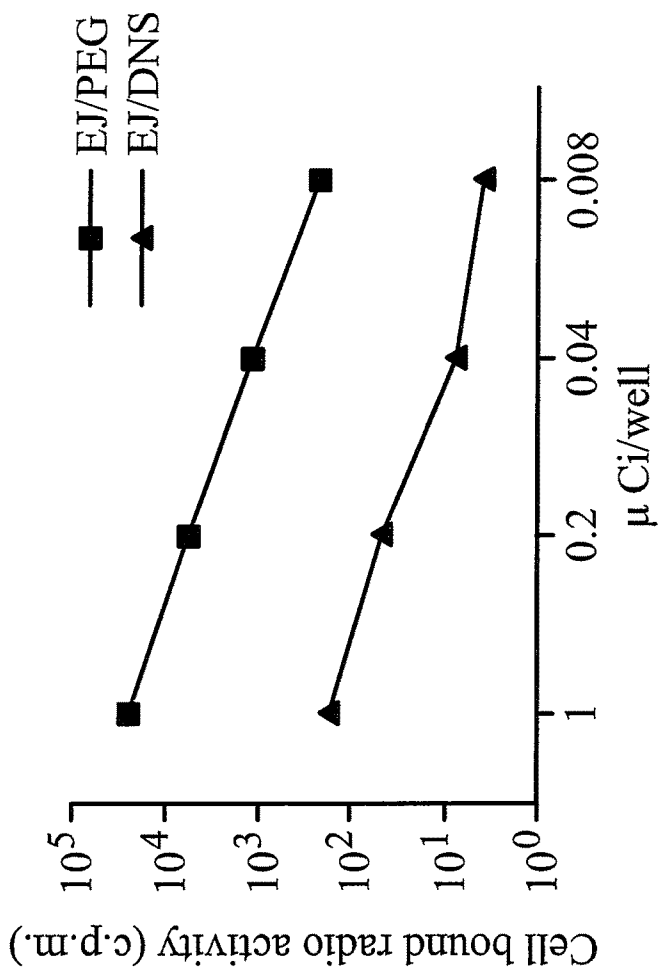


FIG. 10

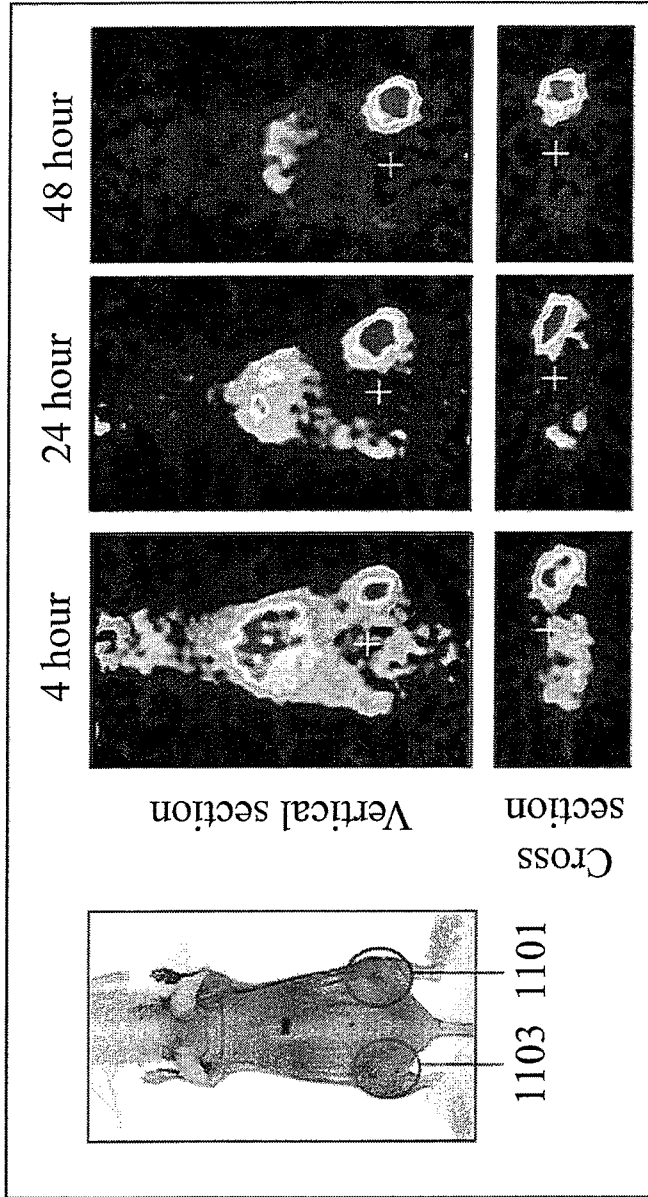


FIG. 11

US 8,507,207 B2

1

**RECOMBINANT NUCLEOTIDE SEQUENCE,
CELL OR VECTOR CONTAINING THE SAME
AND METHOD FOR USING CELL
CONTAINING THE SAME TO ENCODE
ANTI-POLYETHYLENE GLYCOL
MONOCLONAL ANTIBODIES**

**CROSS REFERENCE TO RELATED
APPLICATIONS**

This Application claims priority of Taiwan Patent Application No. 098131334, filed on Sep. 17, 2009, the entirety of which is incorporated by reference herein.

**INCORPORATION BY REFERENCE OF
SEQUENCE LISTING**

A sequence listing submitted as a text file via EFS-Web is incorporated herein by reference. The text file containing the sequence listing is named "0911-A21209-US_Seq_Listing.txt"; its date of creation is Feb. 2, 2010; and its size is 24,505 bytes.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a novel reporter gene system, and in particular relates to a method for using a recombinant nucleotide sequence encoding an anti-polyethylene glycol recombinant single chain membrane antibody as a reporter gene to monitor presence and distribution of a gene and a cell.

2. Description of the Related Art

Developing non-immunogenic and specific reporter genes to monitor expressions and distributions of genes and cells in vivo is very important for the optimization of gene or cell therapy.

Presently, there are two types of reporter genes for non-invasive imaging: (1) exogenous reporter genes: mainly from bacterium or virus of non-mammal systems, such as the gene of the herpesvirus thymidine kinase, the gene of the bacterial cytosine deaminase and the gene of the green fluorescent protein. Although exogenous reporter genes have specificities, the products thereof usually induce immune responses that result in tissue injury. Thus, exogenous reporter genes limit the continuous expression (long term) and orientation imaging of reporter genes, limiting clinical application; and (2) endogenous reporter genes: from such as human dopamine D2 and transferrin. Although endogenous reporter genes do not easily induce immune responses, dopamine D2 and transferrin are widely expressed in normal human body systems. Thus, endogenous reporter genes lack specificity and application thereof is limited. Therefore, developing low immunogenic and highly specific reporter genes is desired. As such, a gene of an anti-polyethylene glycol membrane antibody which belongs to the exogenous reporter genes and meets the low immunogenicity and high specificity features and requirements of reporter genes is disclosed herein.

BRIEF SUMMARY OF THE INVENTION

The invention provides a recombinant nucleotide sequence, comprising the sequence of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3 or SEQ ID No. 4, wherein the recombinant nucleotide sequence encodes an anti-polyethylene glycol recombinant single chain membrane antibody.

2

The invention also provides a vector comprising the recombinant nucleotide sequence of the invention.

The invention also provides a cell comprising the recombinant nucleotide sequence of the invention.

5 The invention provides another cell comprising the vector of the invention.

The invention further provides an anti-polyethylene glycol recombinant single chain membrane antibody, comprising: a first part, comprising a variable light chain-kappa constant (VL-CK) region, and an amino acid sequence thereof comprising the sequence of SEQ ID No. 5 or SEQ ID No. 6; and a second part, comprising a variable heavy chain-heavy chain first constant (VH-CH1) region, and an amino acid sequence thereof comprising the sequence of SEQ ID No. 7 or SEQ ID No. 8, wherein the first part and the second part is connected by a disulfide bond.

The invention further provides a method for using a cell, comprising: (a) providing a living being having the cell claimed in claim 6, wherein the cell expresses the anti-polyethylene glycol recombinant single chain membrane antibody on a cell membrane thereof; (b) injecting a polyethylene glycol imaging probe into the living being, wherein the polyethylene glycol imaging probe binds to the anti-polyethylene glycol recombinant single chain membrane antibody, and the polyethylene glycol imaging probe is formed by a polyethylene glycol connecting to an imaging agent; and (c) using an imaging system to observe a region presenting the anti-polyethylene glycol recombinant single chain membrane antibody in the living being.

A detailed description is given in the following embodiments with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention can be more fully understood by reading the subsequent detailed description and examples with references made to the accompanying drawings, wherein:

FIG. 1 shows a construction illustration for a recombinant nucleotide sequence, the sequence of SEQ ID No. 1, of the invention;

FIG. 2 shows a structure illustration for an anti-polyethylene glycol recombinant single chain membrane antibody encoded by the recombinant nucleotide sequence of the invention.

FIG. 3A shows a construction illustration for a recombinant nucleotide sequence, the sequence of SEQ ID No. 4, of the invention which encodes an anti-polyethylene glycol recombinant single chain membrane antibody;

FIG. 3B shows a construction illustration for a recombinant nucleotide sequence which encodes an anti-dansyl recombinant single chain membrane antibody.

FIG. 4A shows the expression results and the molecular weights of the light chains after performing western blotting with anti-HA antigen monoclonal antibodies;

FIG. 4B shows the expression results and the molecular weights of the heavy chains after performing western blotting with anti-myc antigen monoclonal antibodies;

FIG. 5A shows the specificity testing results for in vitro binding between the PEG-Ouquantum dots to anti-polyethylene glycol recombinant single chain membrane antibodies;

FIG. 5B shows the specificity testing results for in vivo binding between the PEG-Ouquantum dots to anti-polyethylene glycol recombinant single chain membrane antibodies;

FIG. 6A shows the structure of a polyethylene glycol-fluorescent imaging agent (PEG₅₀₀₀-NIR979);

FIG. 6B shows the analyzed and digitized results of an IVIS imaging system for cells expressing the anti-polyethylene glycol recombinant single chain membrane antibodies or cells of a control group after reacting with different concentrations of PEG-fluorescent imaging agents;

FIG. 7 shows the imaging results of an IVIS imaging system at different time points for tumor cells of expressing the anti-polyethylene glycol recombinant single chain membrane antibodies (EJ/PEG scFab) and tumor cells of a control group (EJ/DNS scFab) in a mouse after the mouse being injected with PEG-fluorescent imaging agents;

FIG. 8 shows imaging results of a magnetic resonance imaging system for cells expressing the anti-polyethylene glycol recombinant single chain membrane antibodies (EJ/PEG scFab) or cells of a control group (EJ/DNS scFab) after reacting with different concentrations of polyethylene glycol-iron oxide nanoparticles;

FIG. 9A shows the structure of a polyethylene glycol-iron oxide nanoparticle (PEG-Fe₃O₄);

FIG. 9B shows the imaging results of an IVIS imaging system at different time points for tumor cells of a mouse expressing the anti-polyethylene glycol recombinant single chain membrane antibodies (EJ/PEG scFab) and tumor cells of a control group (EJ/DNS scFab) in a mouse after the mouse being injected with polyethylene glycol-iron oxide nanoparticles;

FIG. 10 shows the gamma counter detecting results for cells expressing the anti-polyethylene glycol recombinant single chain membrane antibodies (EJ/PEG scFab) or cells of a control group (EJ/DNS scFab) after reacting with different concentrations of polyethylene glycol-¹³¹I; and

FIG. 11 shows the imaging results of a micro-PET imaging system at different time points for tumor cells of a mouse expressing the anti-polyethylene glycol recombinant single chain membrane antibodies (EJ/PEG scFab) and tumor cells of a control group (EJ/DNS scFab) in a mouse after the mouse being injected with polyethylene glycol-¹²⁴I.

DETAILED DESCRIPTION OF THE INVENTION

The following description is of the best-contemplated mode of carrying out the invention. This description is made for the purpose of illustrating the general principles of the invention and should not be taken in a limiting sense. The scope of the invention is best determined by reference to the appended claims.

FIG. 1 shows a construction illustration for a recombinant nucleotide sequence, the sequence of SEQ ID No. 1, of the invention. First, polymerase chain reactions (PCRs) are performed using total RNA of a hybridoma secreting anti-polyethylene glycol monoclonal antibody as a template and a universal primer pair which is able to obtain a gene sequence of a variable light chain-kappa constant (VL-CK) region for all antibodies of mice and using total RNA of a hybridoma secreting anti-polyethylene glycol monoclonal antibody as a template and a universal primer pair which is able to obtain a gene sequence of a variable heavy chain-heavy chain first constant (VH-CH1) region for all antibodies of mice, respectively, to obtain a gene sequence of a variable light chain-kappa constant (VL-CK) region of the hybridoma secreting anti-polyethylene glycol monoclonal antibodies (the sequence of SEQ ID No. 9) 101 and a gene sequence of a variable heavy chain-heavy chain first constant (VH-CH1) region of the hybridoma secreting anti-polyethylene glycol monoclonal antibodies (the sequence of SEQ ID No. 12) 107, respectively.

A nucleotide sequence encoding furin-cleavage site 103 is the sequence of SEQ ID No. 10 and a nucleotide sequence encoding foot and mouth disease virus 2A processing sequence 105 is the sequence of SEQ ID No. 11.

Then, 101-105, the sequences of SEQ ID Nos. 9-12 are fused in order to form 109, the sequence of SEQ ID No. 13 by a method, such as a polymerase chain reaction method.

Next, a nucleotide sequence encoding a transmembrane region of B7 protein (the sequence of SEQ ID No. 14) 111 is fused to a 3' end of the sequence of SEQ ID No. 13 to form a recombinant nucleotide sequence of the invention, the sequence of SEQ ID No. 1, which may encode an anti-polyethylene glycol recombinant single chain membrane antibody.

In another embodiment, a nucleotide sequence encoding hemagglutinin (HA) (the sequence of SEQ ID No. 15) is fused to a 5' end of the sequence of SEQ ID No. 1 to form the sequence of SEQ ID No. 2 which may also encode an anti-polyethylene glycol recombinant single chain membrane antibody.

In further another embodiment, first a nucleotide sequence encoding myc protein (the sequence of SEQ ID No. 16) is fused to a 3' end of the sequence of SEQ ID No. 1 and then a nucleotide sequence encoding a transmembrane region of B7 protein (the sequence of SEQ ID No. 14) is fused to a 3' end of the sequence of SEQ ID No. 16 which has been connected to the sequence of SEQ ID No. 1 to form the sequence of SEQ ID No. 3 which may also encode an anti-polyethylene glycol recombinant single chain membrane antibody.

Moreover, a nucleotide sequence encoding hemagglutinin (HA) (the sequence of SEQ ID No. 15) may be fused to a 5' end of the sequence of SEQ ID No. 3 to form the sequence of SEQ ID No. 4 which may also encode an anti-polyethylene glycol recombinant single chain membrane antibody.

The recombinant nucleotide sequence of the invention may be used as a reporter gene by fusing with a promoter of a gene that needs to be monitored. Specifically, a polyethylene glycol imaging probe may specifically bind to an anti-polyethylene glycol recombinant single chain membrane antibody, so that gene expressions and distributions of the gene that needs to be monitored may be observed. The anti-polyethylene glycol recombinant single chain membrane antibody encoded by the recombinant nucleotide sequence may be expressed on a cell membrane of a cell. In one embodiment, the cell mentioned above may comprise a stem cell, tumor cell or immune cell. A cell or a vector may comprise the recombinant nucleotide sequence of the invention or a cell may comprise a vector containing the recombinant nucleotide sequence of the invention. The cell may comprise a stem cell, tumor cell or immune cell and the vector may comprise a plasmid, viral vector or non-viral vector.

After the recombinant nucleotide sequence of the invention is translated by a cell, the 2A processing sequence encoded from the recombinant nucleotide sequence of the invention is cleaved to separate a light chain amino sequence of the anti-polyethylene glycol recombinant single chain membrane antibody from a heavy chain amino sequence of the anti-polyethylene glycol recombinant single chain membrane antibody. Following, the separated light chain and heavy chain amino sequences of the membrane antibody of the invention is assembled as the membrane antibody in an endoplasmic reticulum (ER). Furthermore, since the furin cleavage site is between the light chain sequence of the antibody and 2A processing sequence, after the membrane antibody enters a Golgi apparatus, the remaining 2A processing sequence after the light chain sequence is completely cleaved by a Furin protease to express a functional membrane anti-

body on a cell membrane of a cell. In one embodiment, the cell mentioned above may comprise a stem cell, tumor cell or immune cell. FIG. 2 shows a structure illustration for an anti-polyethylene glycol recombinant single chain membrane antibody encoded by the recombinant nucleotide sequence of the invention. The anti-polyethylene glycol recombinant single chain membrane antibody may comprise a first part 201 comprising a variable light chain-kappa constant (VL-CK) region, and a second part 203 comprising a variable heavy chain-heavy chain first constant (VH-CH1) region 205 and a transmembrane region 207, wherein the first part 201 and the second part 203 is connected by a disulfide bond.

In one embodiment, the anti-polyethylene glycol recombinant single chain membrane antibody is encoded by the sequence of SEQ ID No. 1, and thus an amino sequence of the first part thereof is the sequence of SEQ ID No. 5 and an amino sequence of the second part thereof is the sequence of SEQ ID No. 7.

In another embodiment, the anti-polyethylene glycol recombinant single chain membrane antibody is encoded by the sequence of SEQ ID No. 2, and thus an amino sequence of the first part thereof is the sequence of SEQ ID No. 6 and an amino sequence of the second part thereof is the sequence of SEQ ID No. 7.

In another embodiment, the anti-polyethylene glycol recombinant single chain membrane antibody is encoded by the sequence of SEQ ID No. 3, and thus an amino sequence of the first part thereof is the sequence of SEQ ID No. 5 and an amino sequence of the second part thereof is the sequence of SEQ ID No. 8.

In further another embodiment, the anti-polyethylene glycol recombinant single chain membrane antibody may be encoded by the sequence of SEQ ID No. 4 and thus an amino sequence of the first part thereof is the sequence of SEQ ID No. 6 and an amino sequence of the second part thereof is the sequence of SEQ ID No. 8.

In addition, a method for using a cell containing the recombinant nucleotide sequence of the invention may comprise providing a living being having the cell of the invention, wherein the cell expresses the anti-polyethylene glycol recombinant single chain membrane antibody on the cell membrane thereof. After that, a polyethylene glycol imaging probe is injected into the living being and the polyethylene glycol imaging probe will specifically bind to the anti-polyethylene glycol recombinant single chain membrane antibody, wherein the polyethylene glycol imaging probe is formed by a polyethylene glycol connecting to an imaging agent. Finally, an imaging system is used to observe a region presenting the anti-polyethylene glycol recombinant single chain membrane antibodies in the living being, as the polyethylene glycol imaging probe specifically binds to the anti-polyethylene glycol recombinant single chain membrane antibody.

The living being mentioned above may comprise a mammal. The cell may comprise an endogenous cell of the living being or an exogenous cell. Furthermore, the imaging agent of the invention may comprise any imaging agent able to be connected to the polyethylene glycol, such as a fluorescent imaging agent, iron oxide nanoparticle and radioactive imaging agent.

EXAMPLE

First, polymerase chain reactions (PCR) were performed using a total RNA of a hybridoma secreting anti-polyethylene glycol monoclonal antibody as a template and a primer pair

(forward primer: the sequence of SEQ ID. No. 17; reverse primer: the sequence of SEQ ID. No. 18) which was able to obtain a gene sequence of a variable light chain-kappa constant (VL-CK) region for all antibodies of mice and using a total RNA of a hybridoma secreting anti-polyethylene glycol monoclonal antibody as a template and a primer pair (forward primer: the sequence of SEQ ID. No. 19; reverse primer: the sequence of SEQ ID. No. 20) which was able to obtain a gene sequence of a variable heavy chain-heavy chain first constant (VH-CH1) region for all antibodies of mice, respectively, to obtain a gene sequence of a variable light chain-kappa constant (VL-CK) region of the hybridoma secreting anti-polyethylene glycol monoclonal antibody (the sequence of SEQ ID No. 9) and a gene sequence of a variable heavy chain-heavy chain first constant (VH-CH1) region of the hybridoma secreting anti-polyethylene glycol monoclonal antibody (the sequence of SEQ ID No. 12), respectively.

Next, a polymerase chain reaction was performed by using the sequence of SEQ ID No. 9 and the sequence of SEQ ID No. 12 as a first template and a second template, respectively, the sequence of SEQ ID No. 21 (forward) and the sequence of SEQ ID No. 22 (reverse) as the primer pair for the first template, and the sequence of SEQ ID No. 23 sequence (forward) and the sequence of SEQ ID No. 24 (reverse) as the primer pair for the second template to obtain the sequence of SEQ ID No. 13. The sequence of SEQ ID No. 22 contained the entire sequence of SEQ ID No. 10, also the sequence of SEQ ID No. 23 contained the entire sequence of SEQ ID No. 11, and the sequence of SEQ ID No. 22 and the sequence of SEQ ID No. 23 had an overlapping region.

The operation condition of the polymerase chain reaction was as follows:

- (1) 95° C., 2 minutes
- (2) 95° C., 30 seconds; 60° C., 30 seconds; 68° C., 2 minutes (for 3 cycles)
- (3) 95° C., 30 seconds; 58° C., 30 seconds; 68° C., 2 minutes (for 3 cycles)
- (4) 95° C., 30 seconds; 56° C., 30 seconds; 68° C., 2 minutes (for 3 cycles)
- (5) 95° C., 30 seconds; 54° C., 30 seconds; 68° C., 2 minutes (for 3 cycles)
- (6) 95° C., 30 seconds; 52° C., 30 seconds; 68° C., 2 minutes (for 3 cycles)
- (7) 95° C., 30 seconds; 50° C., 30 seconds; 68° C., 2 minutes (for 3 cycles)
- (8) 72° C., 8 minutes
- (9) 4° C. for storage

Then, the sequence of SEQ ID No. 13 was transplanted into a retroviral vector containing the sequences of SEQ ID No. 15, SEQ ID No. 16 and SEQ ID No. 14 by utilizing designed restriction enzyme sites (Sfi 1 and Sal 1), wherein the sequence of SEQ ID No. 13 was located between the sequence of SEQ ID No. 15 and the sequence of SEQ ID No. 16, as shown in FIG. 3A.

A similar method was used to construct a nucleotide sequence encoding the anti-polyethylene glycol recombinant single chain antibody in a vector (as a control), as shown in FIG. 3B.

Determining the Light Chain and Heavy Chain of the Membrane Antibodies

The vector (pLNCX) containing the recombinant nucleotide sequence encoding an anti-polyethylene glycol recombinant single chain membrane antibody (the sequence of SEQ ID No. 4) and the vector (pLNCX) containing the recombinant nucleotide sequence encoding an anti-dansyl recombinant single chain membrane antibody the sequence of (the sequence of SEQ ID No. 23) were transfected and expressed

in Balb-3T3 cells, respectively. Western blotting was used to determine whether the light chains and the heavy chains of the two kinds of the antibodies mentioned above were separated during the translation. FIG. 4A shows the light chains detected by performing western blotting with anti-HA antigen monoclonal antibodies and FIG. 4B shows the heavy chains detected by performing western blotting with anti-myc antigen monoclonal antibodies. FIGS. 4A and 4B show that after the genes of the two kinds membrane antibodies were translated, the FMDV 2A processing sequence was cleaved to separate the light chain from the heavy chain and then the separated light chain and the heavy chain were assembled as a functional membrane antibody. Because the light chains of the two kinds of the antibodies in FIG. 4A show the correct molecular weights, respectively, the remaining 2A processing sequences after the light sequences had been completely cleaved by Furin protease in a Golgi apparatus. Moreover, by isolating the proteins of the cell membrane and the cytoplasm and performing a western blotting, it was confirmed that the single chain membrane antibodies expressed on the cell membranes were completely assembled functional membrane antibodies and not immature proteins with a non-cleaved 2A processing sequence.

Stability of the Single Chain Membrane Antibody In Vivo or In Vitro

The vector (pLNCX) containing the recombinant nucleotide sequence encoding an anti-polyethylene glycol recombinant single chain membrane antibody (the sequence of SEQ ID No. 4) and the vector (pLNCX) containing the recombinant nucleotide sequence encoding an anti-anti-dansyl recombinant single chain membrane antibody (the sequence of SEQ ID No. 23) were transfected and expressed in EJ cells, respectively. Flow cytometry was used to analyze the ability for the polyethylene glycol-Quantum dots (PEG-Quantum dots) to bind to the cells expressing the anti-polyethylene glycol recombinant single chain membrane antibodies. The left part of FIG. 5A shows that in vitro, the PEG-Quantum dot had the ability for specifically binding to the cells expressing the anti-polyethylene glycol recombinant single chain membrane antibodies (EJ/PEG scFab) and FIG. 5B shows that in vivo, the PEG-Quantum dot also had the ability for specifically binding to the cells expressing the anti-polyethylene glycol recombinant single chain membrane antibodies (EJ/PEG scFab) isolated from the living being. As FIGS. 5A and 5B shows, the same amount of PEG-Quantum dots was able to specifically bind to the cells expressing the anti-polyethylene glycol recombinant single chain membrane antibodies (EJ/PEG scFab) both in vitro and in vivo, confirming that the anti-polyethylene glycol recombinant single chain membrane antibodies are able to be expressed stably in vitro and in vivo. A similar result was also shown for cells expressing the anti-dansyl recombinant single chain membrane antibodies (the right parts of FIGS. 5A and 5B).

Specificity for the Polyethylene Glycol-Fluorescent Imaging Agent (PEG-NIR979) In Vitro

PEG-fluorescent imaging agents (PEG5000-NIR797) (FIG. 6A) with different concentration (0.5 μm , 0.1 μm and 0.02 μm) were used as probes to reacted with cells expressing the anti-polyethylene glycol recombinant single chain membrane antibodies (EJ/PEG scFab) and control group cells expressing the anti-dansyl recombinant single chain membrane antibodies (EJ/DNS scFab). Following an IVIS imaging system (Xenogen IVIS Imaging System 50 Series) was used to analyze the results and then the results were digitized. FIG. 6B shows that the PEG-fluorescent imaging agents were able to specifically bind to the cells expressing the anti-poly-

ethylene glycol recombinant single chain membrane antibody (EJ/PEG scFab) and not the control group cells.

In Vitro Imaging by Polyethylene Glycol-Fluorescent Imaging Agent (PEG-NIR979)

A PEG-fluorescent imaging agent (PEG₅₀₀₀-NIR797) was injected into a mouse with tumors expressing the anti-polyethylene glycol recombinant single chain membrane antibody 701 (EJ/PEG scFab) and tumors of a control group expressing the anti-dansyl recombinant single chain membrane antibody 703 (EJ/DNS scFab) by intravenous injection. Following the mouse was imaged by an IVIS imaging system (Xenogen IVIS Imaging System 50 Series) at different times. The results were shown in FIG. 7. FIG. 7 shows that the PEG-fluorescent imaging agent was able to specifically bind to the tumors expressing the anti-polyethylene glycol recombinant single chain membrane antibody (EJ/PEG scFab) within 48 hours, and not to tumors of a control group.

Specificity for the Polyethylene Glycol-Iron Oxide Nanoparticle Imaging Agent (PEG-NIR979) In Vitro

FIG. 8 shows the imaging results for reacting PEG-Fe₃O₄ nanoparticles (T2 magnetic resonance imaging agent) with different concentrations with cells expressing the anti-polyethylene glycol recombinant single chain membrane antibodies (EJ/PEG scFab) and cells of a control group expressing the anti-dansyl recombinant single chain membrane antibodies (EJ/DNS scFab) by magnetic resonance imaging (MRI). As FIG. 8 shown, PEG-Fe₃O₄ nanoparticles with different concentrations (48, 24, 12 and 6) were able to specifically bind to the cells expressing the anti-polyethylene glycol recombinant single chain membrane antibodies (EJ/PEG scFab) (a dark signal represents the binding of the PEG-Fe₃O₄ nanoparticle to cells expressing the anti-polyethylene glycol recombinant single chain membrane antibodies), and not the control group cells.

In Vitro Imaging by Polyethylene Glycol-Iron Oxide Nanoparticle (PEG-Fe₃O₄ Nanoparticle)

PEG-Fe₃O₄ nanoparticle (PEG5000-NIR797) (FIG. 9A) was injected into a mouse with tumors expressing the anti-polyethylene glycol recombinant single chain membrane antibody 901 (EJ/PEG scFab) and tumors of a control group expressing the anti-dansyl recombinant single chain membrane antibody 903 (EJ/DNS scFab) by intravenous injection. Following, the mouse was imaged by a T2 MRI imaging system at different times. The results are shown in FIG. 9B. FIG. 9B shows that the PEG-Fe₃O₄ nanoparticle was able to specifically bind to tumors expressing the anti-polyethylene glycol recombinant single chain membrane antibody (EJ/PEG scFab) (a dark signal represents the binding of the PEG-Fe₃O₄ nanoparticle to cells expressing the anti-polyethylene glycol recombinant single chain membrane antibodies), and not to tumors of a control group.

Specificity for the Polyethylene Glycol-¹³¹Iodine In Vitro

FIG. 10 shows the gamma counter detecting results for cells expressing the anti-polyethylene glycol recombinant single chain membrane antibodies (EJ/PEG scFab) or cells of a control group expressing the anti-dansyl recombinant single chain membrane antibodies (EJ/DNS scFab) after reacting with different concentrations of polyethylene glycol-¹³¹iodine. As FIG. 10 shows, polyethylene glycol-¹³¹iodine with different concentrations (1, 0.2, 0.04 and 0.08 $\mu\text{Ci}/50 \mu\text{l}/\text{well}$) were able to specifically bind to the cells expressing the anti-polyethylene glycol recombinant single chain membrane antibodies (EJ/PEG scFab) (The radioactive intensity increased with increased concentrations), and not to the cells of the control group.

US 8,507,207 B2

9

In Vitro Imaging by Polyethylene Glycol-¹²⁴Iodine
Polyethylene glycol-¹²⁴iodine was injected into a mouse with tumors expressing the anti-polyethylene glycol recombinant single chain membrane antibodies 1101 (EJ/PEG scFab) and tumors of a control group expressing the anti-dansyl recombinant single chain membrane antibodies 1103 (EJ/DNS scFab) by intravenous injection. Following, the mouse was imaged by a micro-PET imaging system at different times. The results are shown in FIG. 11. FIG. 11 shows that the polyethylene glycol-¹²⁴iodine was able to specifically bind to the tumors expressing the anti-polyethylene glycol

10

recombinant single chain membrane antibodies (EJ/PEG scFab) (The radioactive intensity increased with increased concentrations), and not to tumors of a control group.

While the invention has been described by way of example and in terms of the preferred embodiments, it is to be understood that the invention is not limited to the disclosed embodiments. To the contrary, it is intended to cover various modifications and similar arrangements (as would be apparent to those skilled in the art). Therefore, the scope of the appended claims should be accorded the broadest interpretation so as to encompass all such modifications and similar arrangements.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 25

<210> SEQ ID NO 1

<211> LENGTH: 1896

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant nucleotide sequence
encoding an anti-polyethylene glycol recombinant single chain
membrane antibody

<400> SEQUENCE: 1

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gatattgtgt tgacgcaggc tgcattctcc aatccagtca ctcttggaac atcagcttcc      60
atctcctgca ggtctagtaa gagtctccta catagtaatg gcatcactta tttgtattgg      120
tatctgcaga agccaggcca gtctcctcag ctctgattt atcagatgtc caaccttgcc      180
tcaggagtcc cagacaggtt cagtagcagt gggtcaggaa ctgatttcac actgagaatc      240
agcagagtgg aggctgagga tgtgggtggt tattactgtg ctcaaaatct agaactatct      300
acgttcggct cggggacaaa gttggaata aaacgggctg atgctgcacc aactgtatcc      360
atcttccac catccagtga gcagttaaca tctggaggty cctcagtcgt gtgcttcttg      420
aacaacttct accccaaaga catcaatgtc aagtggaaga ttgatggcag tgaacgacaa      480
aatggcgtcc tgaacagttg gactgatcag gacagcaaag acagcaccta cagcatgagc      540
agcaccctca cgttgaccaa ggacgagtat gaacgacata acagctatac ctgtgaggcc      600
actcacaaga catcaacttc acccattgtc aagagcttca acaggaatga gtgtcgagca      660
aaacgagcac cagtaaaaca aacactaaac ttcgacctac taaaactagc aggagacgta      720
gaatcaaaac caggaccaga agtgcagctg gtggagtctg ggggaggctt agtgaagcct      780
ggagggtccc tgaactctc ctgtgcagcc tctggattca ctttcagtga ctattacatg      840
tattgggttc gccagactcc ggaaaagagg ctggagtggg tcgcaacat tagtgatgat      900
ggtaactaca cctactatcc acacagtgtg aagggcgat tcaccatctc cagagacagt      960
gccaagaaca acctgtacct gcaattgagc agtctgaagt ctgaggacac agccatgtat     1020
tactgtgcaa gaaatgatgc taggggggac tactggggtc aaggaacctc agtcaccgtc     1080
tctcagaga gtcagtcctt cccaaatgtc tccccctcg tctcctgca gagccccctg     1140
tctgataaga atctgggtgc catgggctgc ctggccggg acttctctgcc cagcaccatt     1200
tccttcacct ggaactacca gaacaacact gaagtcatcc agggatcag aaccttccca     1260
aactgagga cagggggcaa gtacctagcc acctcgcagg tgttctgttc tcccaagagc     1320
atcctgaag gttcagatga atacctggtg tgcgaaatcc actacggagg caaaaacaga     1380
gatctgcatg tgcccattcc agctgctgac ttctctacce ccaacataac tgagtctgga     1440
aaccatctg cagacactaa aaggattacc tgctttgctt ccgggggttt cccaaagcct     1500

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US 8,507,207 B2

11

12

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cgcttctctt ggttggaaaa tggaagagaa ttacctggca tcaatacgac aatttcccag 1560
gatcctgaat ctgaattgta caccattagt agccaactag atttcaatac gactcgcgaac 1620
cacaccatta agtgtctcat taaatatgga gatgctcagc tgtcagagga cttcacctgg 1680
gaaaaacccc cagaagacc cctgatagc aagaacacac ttgtgctctt tggggcagga 1740
ttcggcgcag taataacagt cgtcgtcatc gttgtcatca tcaaatgctt ctgtaagcac 1800
agaagctggt tcagaagaaa tgaggcaagc agagaaacaa acaacagcct taccttcggg 1860
cctgaagaag cattagctga acagaccgtc ttcctt 1896

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<210> SEQ ID NO 2

<211> LENGTH: 1923

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Recombinant nucleotide sequence
encoding an anti-polyethylene glycol recombinant single chain
membrane antibody

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<400> SEQUENCE: 2

```

tatccatag atgttcacaga ttatgctgat attgtgttga cgcaggctgc atttccaat 60
ccagtcactc ttggaacatc agcttccatc tctgcaggt ctagtaagag tctcctacat 120
agtaatggca tcaacttatt gtattggtat ctgcagaagc caggccagtc tctcagctc 180
ctgatttacc agatgtccaa ccttgccctc ggagtcaccag acagggtcag tagcagtggg 240
tcaggaactg atttcacact gagaatcagc agagtggagg ctgaggatgt ggggttttat 300
tactgtgctc aaaatctaga actattcacg ttcggctcgg ggacaaaagt ggaataaaa 360
cgggctgatg ctgcaccaac tgtatccatc tcccacatc ccagtgcga gttaacatct 420
ggaggtgcct cagtctgtg cttcttgaac aacttctacc ccaagacat caatgtcaag 480
tggaagattg atggcagtga acgacaaaat ggcgtcctga acagttggac tgatcaggac 540
agcaaagaca gcacctacag catgagcagc accctcacgt tgaccaagga cgagtatgaa 600
cgacataaca gctatacctg tgaggccact cacaagacat caacttcacc cattgtcaag 660
agcttcaaca ggaatgagtg tgcagcaaaa cgagcaccag taaaacaaac actaaacttc 720
gacctactaa aactagcagg agacgtagaa tcaaaccag gaccagaagt gcagctggtg 780
gagctctggg gaggettagt gaagcctgga gggtcctga aactctctg tgcagcctct 840
ggattcactt tcagtgacta ttacatgat tgggttcgcc agactccgga aaagaggctg 900
gagtggtctg caaccattag tgatgatggt acttacacct actatccaca cagtgtgaag 960
gggcgattca ccatctccag agacagtgcc aagaacaacc tgtacctgca attgagcagt 1020
ctgaagtctg aggacacagc catgtattac tgtgcaagaa atgatgctag gggggactac 1080
tggggtcaag gaacctcagt caccgtctcc tcagagagtc agtccctccc aaatgtcttc 1140
ccccctgctc cctgcgagag cccccgtctc gataagaatc tgggtggccat gggctgcctg 1200
gccccggact tctgcccag caccatttcc ttcacctgga actaccagaa caaactgaa 1260
gtcatccagg gtatcagaac cttcccacaa ctgaggacag ggggcaagta cctagccacc 1320
tcgcagggtg tgctgtctcc caagagcatc cttgaaggtt cagatgaata cctggatgac 1380
gaaatccact acggaggcaa aacagagat ctgcatgtgc ccattccagc tgctgacttc 1440
tctaccccc aataactga gtctggaac ccatctgcag aactaaaag gattacctgc 1500
tttctctccg ggggttccc aaagcctcgc ttctcttggg tggaaaatgg aagagaatta 1560
cctggcatca atacgacaat tcccaggat cctgaatctg aattgtacac cattagtagc 1620

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US 8,507,207 B2

13

14

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caactagatt tcaatacgac tcgcaaccac accattaagt gtctcattaa atatggagat 1680
gctcacgtgt cagaggactt cacctgggaa aaacccccag aagaccctcc tgatagcaag 1740
aacacacttg tgctctttgg ggcaggattc ggcgcagtaa taacagtcgt cgtcacgtt 1800
gtcatcatca aatgcttctg taagcacaga agctgtttca gaagaaatga ggcaagcaga 1860
gaaacaaaca acagccttac cttcgggctt gaagaagcat tagctgaaca gaccgtcttc 1920
ctt 1923

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<210> SEQ ID NO 3
<211> LENGTH: 1926
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant nucleotide sequence
encoding an anti-polyethylene glycol recombinant single chain
membrane antibody

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<400> SEQUENCE: 3
gatattgtgt tgacgcagge tgcattctcc aatccagtea ctcttgaac atcagcttcc 60
atctcctgca ggtctagtaa gagtctccta catagtaatg gcatcactta tttgtattgg 120
tatctgcaga agccaggcca gtctcctcag ctctgattt atcagatgtc caaccttgcc 180
tcaggagtcc cagacagggt cagtagcagt gggtcaggaa ctgatttcac actgagaatc 240
agcagagtgg aggctgagga tgtgggtgtt tattactgtg ctcaaatct agaactattc 300
acgttcggct cggggacaaa gttggaaata aaacgggctg atgctgcacc aactgtatcc 360
atcttcccac catccagtga gcagtaaca tctggaggtg cctcagtcgt gtgcttcttg 420
aacaacttct accccaaga catcaatgtc aagtgaaga ttgatggcag tgaacgacaa 480
aatggcgtcc tgaacagttg gactgatcag gacagcaaag acagcaccta cagcatgagc 540
agcacctca cgttgaccaa ggacgagtat gaacgacata acagctatac ctgtgaggcc 600
actcacaaga catcaacttc acccattgtc aagagcttca acaggaatga gtgtcgagca 660
aaacgagcac cagtaaaaca aacactaac ttcgacctac taaaactagc aggagacgta 720
gaatcaaacc caggaccaga agtgcagctg gtggagtctg ggggaggctt agtgaagcct 780
ggagggtccc tgaactctc ctgtgcagcc tctggattca ctttcagtga ctattacatg 840
tattgggttc gccagactcc ggaaaagagg ctggagtggg tcgcaacct tagtgatgat 900
ggtacttaca cctactatcc acacagtgtg aagggcgcat tcaccatctc cagagacagt 960
gccaaagaaca acctgtacct gcaattgagc agtctgaagt ctgaggacac agccatgtat 1020
tactgtgcaa gaaatgatgc taggggggac tactggggtc aaggaacctc agtcaccgtc 1080
tcctcagaga gtcagtcctt cccaaatgtc tccccctcg tctcctgcga gagccccctg 1140
tctgataaga atctggtgac catgggctgc ctggccccgg acttctgccc cagcaccatt 1200
tccttcacct ggaactacca gaacaacact gaagtcatcc agggatcag aaccttccca 1260
acactgagga cagggggcaa gtacctagcc acctcgcagg tgttctgtgc tccaagagc 1320
atccttgaag gttcagatga atacctgta tgcgaaatcc actacggagg caaaaacaga 1380
gatctgcatg tgcccattcc agctgaacaa aaactcatct cagaagagga tctggctgac 1440
ttctctacc ccaacataac tgagtctgga aacctatctg cagacactaa aaggattacc 1500
tgctttgctt cgggggttt cccaaagcct cgcttctctt ggttgaaaa tggaagagaa 1560
ttacctggca tcaatacgac aatttccag gatcctgaat ctgaattgta caccattagt 1620
agccaactag atttcaatac gactcgaac cacaccatta agtgtctcat taaatatgga 1680

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US 8,507,207 B2

15

16

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gatgctcacg tgtcagagga cttcacctgg gaaaaacccc cagaagaccc tctgatagc 1740
aagaacacac ttgtgctcct tggggcagga ttcggcgcag taataacagt cgtcgtcatc 1800
gttgatcatc tcaaatgctt ctgtaagcac agaagctgtt tcagaagaaa tgaggcaagc 1860
agagaaacaa acaacagcct taccttcggg cctgaagaag cattagctga acagaccgtc 1920
ttcctt 1926

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<210> SEQ ID NO 4
<211> LENGTH: 1953
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant nucleotide sequence
        encoding an anti-polyethylene glycol recombinant single chain
        membrane antibody

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<400> SEQUENCE: 4

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```

tatccatag atgttcacaga ttatgctgat attgtgttga cgcaggetgc atttccaat 60
ccagtcactc ttggaacatc agcttccatc tctgcaggt ctagtaagag tctcctacat 120
agtaatggca tcaacttatt gtattggtat ctgcagaagc caggccagtc tcctcagctc 180
ctgatttate agatgtccaa ccttgctca ggagtcaccag acaggttcag tagcagtggg 240
tcaggaactg atttcacact gagaatcagc agagtggagg ctgaggatgt ggggtgtttat 300
tactgtgctc aaaatctaga actattcacg ttcggctcgg ggacaaaagt ggaataaaaa 360
cgggctgatg ctgcaccaac tgtatccatc tccccaccat ccagtgagca gttaacatct 420
ggaggtgcct cagtcgtgtg cttcttgaac aacttctacc ccaagacat caatgtcaag 480
tggaagattg atggcagtga acgacaaaat ggcgtcctga acagttggac tgatcaggac 540
agcaaagaca gcacctacag catgagcagc accctcacgt tgaccaagga cgagtatgaa 600
cgacataaca gctatacctg tgaggccact cacaagacat caacttcacc cattgtcaag 660
agcttcaaca ggaatgagtg tcgagcaaaa cgagcaccag taaaaaaaac actaaaactc 720
gacctactaa aactagcagg agacgtagaa tcaaacccag gaccagaagt gcagctgggt 780
gagtcctggg gaggtcttagt gaagcctgga gggtcctga aactctcctg tgcagcctct 840
ggattcactt tcagtacta ttacatgtat tgggttcgcc agactccgga aaagaggctg 900
gagtggtctg caaccattag tgatgatggt acttacacct actatccaca cagtgtgaag 960
ggcgattca ccatctccag agacagtgcc aagaacaacc tgtacctgca attgagcagt 1020
ctgaagtctg aggacacagc catgtattac tgtgcaagaa atgatgctag gggggactac 1080
tggggtcaag gaacctcagt caccgtctcc tcagagagtc agtccttccc aaatgtcttc 1140
ccccctgctc cctgcgagag cccctgtctc gataagaatc tgggtggccat gggctgcctg 1200
gcccgggact tctgcccag caccatttcc ttcacctgga actaccagaa caaactgaa 1260
gtcatccagg gtatcagaac cttcccaaca ctgaggacag ggggcaagta cctagccacc 1320
tcgcaggtgt tgctgtctcc caagagcadc cttgaaggtt cagatgaata cctggtatgc 1380
gaaatccact acggaggcaa aaacagagat ctgcatgtgc ccattccagc tgaacaaaaa 1440
ctcatctcag aagaggatct ggctgacttc tctaccccca acataactga gtctggaaac 1500
ccatctgcag aactaaaag gattacctgc tttgcttccg ggggttctcc aaagcctcgc 1560
ttctcttggt tggaaaaatg aagagaatta cctggcatca atacgacaat tcccaggat 1620
cctgaatctg aattgtacac cattagtagc caactagatt tcaatacgac tcgcaaccac 1680
accattaagt gtctcattaa atatggagat gtcacgtgt cagaggactt cacctgggaa 1740

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US 8,507,207 B2

17

18

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aaacccccag aagaccctcc tgatagcaag aacacacttg tgctctttgg ggcaggattc 1800
ggcgcagtaa taacagtcgt cgtcatcgtt gtcacatca aatgcttctg taagcacaga 1860
agctgtttca gaagaaatga ggcaagcaga gaaacaaaca acagccttac cttcgggcct 1920
gaagaagcat tagctgaaca gaccgtcttc ctt 1953

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<210> SEQ ID NO 5
<211> LENGTH: 218
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<223> OTHER INFORMATION: Light chain sequence of
anti-polyethylene glycol recombinant single chain membrane
antibody

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<400> SEQUENCE: 5

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

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<210> SEQ ID NO 6
<211> LENGTH: 227
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Light chain sequence
of anti-polyethylene glycol recombinant single chain membrane
antibody

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<400> SEQUENCE: 6

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```

Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Asp Ile Val Leu Thr Gln Ala
1           5           10           15
Ala Phe Ser Asn Pro Val Thr Leu Gly Thr Ser Ala Ser Ile Ser Cys
          20           25           30

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

<210> SEQ ID NO 7
 <211> LENGTH: 386
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Heavy chain and transmembrane region sequence
 of anti-polyethylene glycol recombinant single chain membrane
 antibody

<400> SEQUENCE: 7

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

US 8,507,207 B2

21

22

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145 150 155 160

Gln Asn Asn Thr Glu Val Ile Gln Gly Ile Arg Thr Phe Pro Thr Leu
 165 170 175

Arg Thr Gly Gly Lys Tyr Leu Ala Thr Ser Gln Val Leu Leu Ser Pro
 180 185 190

Lys Ser Ile Leu Glu Gly Ser Asp Glu Tyr Leu Val Cys Glu Ile His
 195 200 205

Tyr Gly Gly Lys Asn Arg Asp Leu His Val Pro Ile Pro Ala Ala Asp
 210 215 220

Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly Asn Pro Ser Ala Asp Thr
225 230 235

Lys Arg Ile Thr Cys Phe Ala Ser Gly Gly Phe Pro Lys Pro Arg Phe
 245 250 255

Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro Gly Ile Asn Thr Thr Ile
 260 265 270

Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr Ile Ser Ser Gln Leu Asp
 275 280 285

Phe Asn Thr Thr Arg Asn His Thr Ile Lys Cys Leu Ile Lys Tyr Gly
 290 295 300

Asp Ala His Val Ser Glu Asp Phe Thr Trp Glu Lys Pro Pro Glu Asp
305 310 315

Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly Ala Gly Phe Gly
 325 330 335

Ala Val Ile Thr Val Val Val Ile Val Val Ile Ile Lys Cys Phe Cys
 340 345 350

Lys His Arg Ser Cys Phe Arg Arg Asn Glu Ala Ser Arg Glu Thr Asn
 355 360 365

Asn Ser Leu Thr Phe Gly Pro Glu Glu Ala Leu Ala Glu Gln Thr Val
 370 375 380

Phe Leu
385

<210> SEQ ID NO 8
 <211> LENGTH: 396
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Heavy chain and transmembrane region sequence
 of anti-polyethylene glycol recombinant single chain membrane
 antibody

<400> SEQUENCE: 8

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
 20 25 30

Tyr Met Tyr Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
 35 40 45

Ala Thr Ile Ser Asp Asp Gly Thr Tyr Thr Tyr Tyr Pro His Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Ser Ala Lys Asn Asn Leu Tyr
65 70 75 80

Leu Gln Leu Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95

Ala Arg Asn Asp Ala Arg Gly Asp Tyr Trp Gly Gln Gly Thr Ser Val
 100 105 110

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Thr Val Ser Ser Glu Ser Gln Ser Phe Pro Asn Val Phe Pro Leu Val
 115 120 125

Ser Cys Glu Ser Pro Leu Ser Asp Lys Asn Leu Val Ala Met Gly Cys
 130 135 140

Leu Ala Arg Asp Phe Leu Pro Ser Thr Ile Ser Phe Thr Trp Asn Tyr
 145 150 155 160

Gln Asn Asn Thr Glu Val Ile Gln Gly Ile Arg Thr Phe Pro Thr Leu
 165 170 175

Arg Thr Gly Gly Lys Tyr Leu Ala Thr Ser Gln Val Leu Leu Ser Pro
 180 185 190

Lys Ser Ile Leu Glu Gly Ser Asp Glu Tyr Leu Val Cys Glu Ile His
 195 200 205

Tyr Gly Gly Lys Asn Arg Asp Leu His Val Pro Ile Pro Ala Glu Gln
 210 215 220

Lys Leu Ile Ser Glu Glu Asp Leu Ala Asp Phe Ser Thr Pro Asn Ile
 225 230 235 240

Thr Glu Ser Gly Asn Pro Ser Ala Asp Thr Lys Arg Ile Thr Cys Phe
 245 250 255

Ala Ser Gly Gly Phe Pro Lys Pro Arg Phe Ser Trp Leu Glu Asn Gly
 260 265 270

Arg Glu Leu Pro Gly Ile Asn Thr Thr Ile Ser Gln Asp Pro Glu Ser
 275 280 285

Glu Leu Tyr Thr Ile Ser Ser Gln Leu Asp Phe Asn Thr Thr Arg Asn
 290 295 300

His Thr Ile Lys Cys Leu Ile Lys Tyr Gly Asp Ala His Val Ser Glu
 305 310 315 320

Asp Phe Thr Trp Glu Lys Pro Pro Glu Asp Pro Pro Asp Ser Lys Asn
 325 330 335

Thr Leu Val Leu Phe Gly Ala Gly Phe Gly Ala Val Ile Thr Val Val
 340 345 350

Val Ile Val Val Ile Ile Lys Cys Phe Cys Lys His Arg Ser Cys Phe
 355 360 365

Arg Arg Asn Glu Ala Ser Arg Glu Thr Asn Asn Ser Leu Thr Phe Gly
 370 375 380

Pro Glu Glu Ala Leu Ala Glu Gln Thr Val Phe Leu
 385 390 395

<210> SEQ ID NO 9
 <211> LENGTH: 654
 <212> TYPE: DNA
 <213> ORGANISM: Mus musculus
 <220> FEATURE:
 <223> OTHER INFORMATION: Gene sequence of a variable light chain - kappa
 constant (VL-CM) region of the hybridoma secreting
 anti-polyethylene glycol monoclonal antibody

<400> SEQUENCE: 9

gatattgtgt tgacgcaggc tgcattctcc aatccagtca ctcttgaac atcagcttcc 60

atctcctgca ggtctagtaa gagtctccta catagtaatg gcatcactta tttgtattgg 120

tatctgcaga agccaggcca gtctctcag ctcctgattt atcagatgtc caaccttgcc 180

tcaggagtcc cagacaggtt cagtagcagt gggtcaggaa ctgatttcac actgagaatc 240

agcagagtgg aggctgagga tgtgggtgtt tattactgtg ctcaaaatct agaactatc 300

acgttcggct cggggacaaa gttgaaata aaacgggctg atgctgcacc aactgtatcc 360

atcttcccac catccagtga gcagttaaca tctggaggty cctcagtcgt gtgcttcttg 420

US 8,507,207 B2

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26

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```

aacaacttct accccaaga catcaatgtc aagtggaaga ttgatggcag tgaacgacaa 480
aatggcgctcc tgaacagttg gactgatcag gacagcaaag acagcaccta cagcatgagc 540
agcacctca cgttgaccaa ggacgagtat gaacgacata acagctatac ctgtgaggcc 600
actcacaaga catcaacttc acccattgtc aagagcttca acaggaatga gtgt 654

```

```

<210> SEQ ID NO 10
<211> LENGTH: 12
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Furin-cleavage site

```

```

<400> SEQUENCE: 10

```

```

cgagcaaac ga 12

```

```

<210> SEQ ID NO 11
<211> LENGTH: 72
<212> TYPE: DNA
<213> ORGANISM: Aphtae epizooticae
<220> FEATURE:
<223> OTHER INFORMATION: 2A self-processing sequence

```

```

<400> SEQUENCE: 11

```

```

gcaccagtaa aacaacact aaacttcgac ctactaaaac tagcaggaga cgtagaatca 60

```

```

aaccaggac ca 72

```

```

<210> SEQ ID NO 12
<211> LENGTH: 666
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<223> OTHER INFORMATION: Gene sequence of a variable heavy chain - heavy
chain first constant (VH-CH1) region of the hybridoma secreting
anti-polyethylene glycol monoclonal antibody

```

```

<400> SEQUENCE: 12

```

```

gaagtgcagc tgggtggagtc tgggggaggc ttagtgaagc ctggagggtc cctgaaactc 60

```

```

tctctgtcag cctctggatt cactttcagt gactattaca tgtattgggt tcgccagact 120

```

```

ccggaaaaga ggctggagtg ggtcgcaacc attagtgatg atggtactta cacctactat 180

```

```

ccacacagtg tgaaggggagc attcaccatc tccagagaca gtgccaagaa caacctgtac 240

```

```

ctgcaattga gcagtctgaa gtctgaggac acagccatgt attactgtgc aagaaatgat 300

```

```

gctagggggg actactgggg tcaaggaacc tcagtcaccg tctcctcaga gagtcagtc 360

```

```

ttcccaaatg tcttccccct cgtctcctgc gagagccccc tgtctgataa gaatctggtg 420

```

```

gccatgggct gcctggcccg ggacttctcg cccagcacca tttccttcac ctggaactac 480

```

```

cagaacaaca ctgaagtcat ccagggtatc agaaccttcc caacactgag gacagggggc 540

```

```

aagtacctag ccacctcgca ggtgttgtg tctcccaaga gcatcctga aggttcagat 600

```

```

gaatacttg tatgcgaaat ccactacgga ggcaaaaaca gagatctgca tgtgccatt 660

```

```

ccagct 666

```

```

<210> SEQ ID NO 13
<211> LENGTH: 1404
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Sequence containing SEQ ID. Nos. 9-12

```

```

<400> SEQUENCE: 13

```

US 8,507,207 B2

27

28

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gatattgtgt tgacgcagcgc tgcattctcc aatccagtcg ctcttggaac atcagcttcc 60
atctcctgca ggtctagtaa gagtctccta catagtaatg gcatcactta tttgtattgg 120
tatctgcaga agccagggca gtctctcag ctctgattt atcagatgtc caaccttgcc 180
tcaggagtcc cagacagggt cagtagcagt gggtcaggaa ctgatttcac actgagaatc 240
agcagagtgg aggctgagga tgtgggtgtt tattactgtg ctcaaatct agaactatc 300
acgttcggct cggggacaaa gttgaaata aaacgggctg atgctgcacc aactgtatcc 360
atcttcccac catccagtga gcagtaaca tctggagggt cctcagtcgt gtgcttcttg 420
aacaacttct acccacaaga catcaatgtc aagtggaaga ttgatggcag tgaacgacaa 480
aatggcgtcc tgaacagttg gactgatcag gacagcaaag acagcaccta cagcatgagc 540
agcacctca cgttgaccaa ggacgagtat gaacgacata acagctatac ctgtgaggcc 600
actcacaaga catcaacttc acccattgtc aagagcttca acaggaatga gtgtcgagca 660
aaacgagcac cagtaaaaca aacactaac ttcgacctac taaaactagc aggagacgta 720
gaatcaaacc caggaccaga agtgcagctg gtggagtctg ggggaggctt agtgaaccc 780
ggagggtccc tgaactctc ctgtgcagcc tctggattca ctttcagtga ctattacatg 840
tattgggttc gccagactcc ggaaaagagg ctggagtggg tcgcaacctat tagtgatgat 900
ggtaactaca cctactatcc acacagtgtg aagggcgcat tcaccatctc cagagacagt 960
gccagaaca acctgtacct gcaattgagc agtctgaagt ctgaggacac agccatgtat 1020
tactgtgcaa gaaatgatgc taggggggac tactggggtc aaggaacctc agtcaccgtc 1080
tcctcagaga gtcagtcctt cccaaatgct tccccctcg tctctcga gagccccctg 1140
tctgataaga atctggtggc catgggctgc ctggcccggg acttctctgc cagcaccatt 1200
tccttcacct ggaactacca gaacaacact gaagtcctcc agggatcag aaccttcca 1260
acactgagga cagggggcaa gtacctagcc acctcgcagg tgttctgtgc tcccaagagc 1320
atccttgaag gttcagatga atacctggta tgcgaaatcc actacggagg caaaaacaga 1380
gatctgcatg tgccattcc agct 1404

```

<210> SEQ ID NO 14

<211> LENGTH: 492

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<220> FEATURE:

<223> OTHER INFORMATION: Nucleotide sequence encoding a transmembrane region of B7 protein

<400> SEQUENCE: 14

```

gctgacttct ctaccccaa cataactgag tctggaaacc catctgcaga cactaaaagg 60
attacctgct ttgcttcagg gggtttccca aagcctcgtc tctcttggtt ggaaatgga 120
agagaattac ctggcatcaa tacgacaatt tcccaggatc ctgaaatgga attgtacacc 180
attagtagcc aactagatct caatacgact cgcaaccaca ccattaagtg tctcattaaa 240
tatggagatg ctcacgtgtc agaggacttc acctgggaaa aacccccaga agaccctcct 300
gatagcaaga acacacttgt gctctttggg gcaggattcg gcgcagtaat aacagtcgtc 360
gtcatcgttg tcatcatcaa atgcttctgt aagcacagaa gctgtttcag aagaaatgag 420
gcaagcagag aaacaacaa cagccttacc ttcgggctcg aagaagcatt agctgaacag 480
accgtcttcc tt 492

```

<210> SEQ ID NO 15

<211> LENGTH: 27

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<212> TYPE: DNA
<213> ORGANISM: Influenza virus
<220> FEATURE:
<223> OTHER INFORMATION: Nucleotide sequence encoding hemagglutinin (HA)

<400> SEQUENCE: 15
tatccatgatg atgttccaga ttatgct 27

<210> SEQ ID NO 16
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Nucleotide sequence encoding myc protein

<400> SEQUENCE: 16
gaacaaaaac tcatctcaga agaggatctg 30

<210> SEQ ID NO 17
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward primer

<400> SEQUENCE: 17
gggagctcga yattgtgmts acmcarwctm ca 32

<210> SEQ ID NO 18
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse primer

<400> SEQUENCE: 18
aactcattc ctggtgaa 18

<210> SEQ ID NO 19
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: 15
<223> OTHER INFORMATION: Forward primer, n=a or g or c or t/u

<400> SEQUENCE: 19
ccggaattcs argtnmagct gsagsagtc 29

<210> SEQ ID NO 20
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse primer

<400> SEQUENCE: 20
agctggaatg ggcacatg 18

<210> SEQ ID NO 21
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward primer

<400> SEQUENCE: 21

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ggcccagccg gccgatattg tgttgacgca g 31

<210> SEQ ID NO 22
 <211> LENGTH: 45
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Reverse primer

<400> SEQUENCE: 22

ttgttttact ggtgctcggt ttgctcgaca ctcatctctg ttgaa 45

<210> SEQ ID NO 23
 <211> LENGTH: 90
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Forward primer

<400> SEQUENCE: 23

gcaccagtaa aacaaacact aaacttcgac ctactaaaac tagcaggaga cgtagaatca 60
 aaccaggac cagaagtgca gctggaggag 90

<210> SEQ ID NO 24
 <211> LENGTH: 24
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Reverse primer

<400> SEQUENCE: 24

gtcgacagct ggaatgggca catg 24

<210> SEQ ID NO 25
 <211> LENGTH: 1974
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant nucleotide sequence
 encoding an anti-dansyl recombinant single chain membrane antibody

<400> SEQUENCE: 25

tatccatag atgttccaga ttatgctgat gttgtgatga cccaaactcc actctccctg 60
 cctgtcagtc ttggaaatca agcctccatc tcttcagat ctagtcagag ccttgtacac 120
 agtaatggaa acacctatctt acattggtac ctgcagaagc caggccagtc tccaaagctc 180
 ctgatctaca aagtttccaa cggattttct ggggtcccag acaggttcag tggcagtgga 240
 tcaggacagc atttcacact caagatcagc agagtggagg ctgaggatct gggagtttat 300
 ttctgctctc aaagtacaca tgttccatc acgttcggct cggggacaaa gttggaata 360
 aaacgtgctg atgctgcacc aactgtatcc atcttcccac catccagtga gcagttaaca 420
 tctggaggty cctcagtcgt gtgcttcttg aacaacttct accccaaaga catcaatgct 480
 aagtgaaga ttgatggcag tgaacgacaa aatggcgtcc tgaacagttg gactgatcag 540
 gacagcaaa acagcaccta cagcatgagc agcaccctca cgttgaccaa ggacgagtat 600
 gaacgacata acagctatac ctgtgaggcc actcacaaga catcaacttc acccattgct 660
 aagagcttca acaggaatga gtgtcgagca aaacgagcac cagtaaaaca aacactaaac 720
 ttcgacctac taaaactagc aggagacgta gaatcaaacc caggaccaag tgaagtgaag 780
 cttgaggagt ctggaggagg cttggtgcaa cctggaggat ccatgaaact ctcttgtgct 840

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acttctggat tcaacttttag tgatgcctgg atggactggg tccgccagtc tccagagaag	900
gggcttgagt gggttgctga aattagaaac aaagctaata atcatgcaac atactatgct	960
gagtcctgtga aagggagggt caccatctca agagatgatt ccaaaaggag agtgtacctg	1020
caaatgaaca ccttaagagc tgaagacact ggcatTTatt actgtaccgg gatctactat	1080
cattaccctt ggtttgctta ctggggccaa gggactctgg tcaactgtctc tgcagagagt	1140
cagtccttcc caaatgtctt ccccctgtc tctgcgaga gccccctgtc tgataagaat	1200
ctggtggcca tgggctgcct ggcccgggac ttctgcccc gcaccatttc cttcacctgg	1260
aactaccaga acaacactga agtcatccag ggtatcagaa ccttcccaac actgaggaca	1320
gggggcaagt acctagccac ctgcgaggtg ttgtgtctc ccaagagcat cctgaaggt	1380
tcagatgaat acctggtatg cgaaatccac tacggaggca aaaacagaga tctgcatgtg	1440
cccattccag ctgaacaaaa actcatctca gaagaggatc tggtgactt ctetacccc	1500
aacataactg agtctggaaa cccatctgca gacactaaaa ggattacctg ctttgtctcc	1560
gggggtttcc caaagcctcg cttctcttgg ttggaaaatg gaagagaatt acctggcatc	1620
aatacgacaa tttcccagga tcttgaatct gaattgtaca ccattagtag ccaactagat	1680
ttcaatacga ctgcacaacca caccattaag tgtctcatta aatatggaga tgctcacgtg	1740
tcagaggact tcacctggga aaaaccccc gaagaccctc ctgatagcaa gaacacactt	1800
gtgctctttg gggcaggatt cgggcgagta ataacagtcg tegtcatcgt tgtcatcctc	1860
aaatgcttct gtaagcacag aagctgttcc agaagaaatg aggcaagcag agaacaaac	1920
aacagcctta ccttcgggcc tgaagaagca ttagctgaac agaccgtctt cctt	1974

What is claimed is:

1. A recombinant nucleotide sequence, comprising the sequence of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3 or SEQ ID No. 4, wherein the recombinant nucleotide sequence encodes an anti-polyethylene glycol recombinant single chain membrane antibody.

2. The recombinant nucleotide sequence as claimed in claim 1, wherein the recombinant nucleotide sequence is a reporter gene.

35 3. The recombinant nucleotide sequence as claimed in claim 1, wherein the recombinant nucleotide sequence encodes an anti-polyethylene glycol recombinant single chain membrane antibody expressed on a cell membrane of a cell.

40 4. The recombinant nucleotide sequence as claimed in claim 3, wherein the cell comprises a stem cell, tumor cell or immune cell.

5. A vector comprising the recombinant nucleotide sequence as claimed in claim 1.

* * * * *