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<p>(54) Title: METHOD OF DETECTING COMPOUNDS UTILIZING CHEMICALLY MODIFIED LAMBDOID BACTERIOPHAGE</p>		
<p>(57) Abstract</p> <p>Disclosed is a protein construct including a chemically modified lambdoid tail protein having a chemically reactive amino acid residue linked to a target molecule. Also disclosed is an infective lambdoid bacteriophage displaying on its outer surface the chemically modified tail protein. In addition, methods of detecting a molecule-of-interest in a solution and methods of detecting cells producing a molecule-of-interest which utilize the infective lambdoid bacteriophage having the chemically modified tail protein are disclosed.</p>		

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METHOD OF DETECTING COMPOUNDS UTILIZING
CHEMICALLY MODIFIED LAMBDOID BACTERIOPHAGEFIELD OF THE INVENTION

This invention relates to the detection of compounds, and more specifically to methods for detecting and assaying for a molecule-of-interest and for cells producing molecules-of-interest utilizing a genetically and chemically modified lambdoid bacteriophage.

BACKGROUND OF THE INVENTION

Bacteriophages have been used in strategies for detecting molecules-of-interest. For example, a method employing the bacteriophage M13 has been used to assay for various proteins of interest. In this method M13 phage displaying peptides fused to pIII, a minor M13 coat protein, have been used to screen for protein binding molecules and antibodies (Scott et al. (1990) *Science* 249:386; Devlin et al. (1990) *Science* 249:404). Special M13-derived systems have been used to express antibodies as fusion proteins on the surface of the phage, and techniques have been developed to enrich the population for phage expressing antibodies with desired affinities for an antigen (Garrard et al. (1991) *Bio/Technol.* 9:1373; Barbas et al. (1991) *Proc. Natl. Acad. Sci. (USA)* 88:7978). However, the use of M13 in assay methods is limited because M13 infection is not immediately ascertainable. This is because infection by M13 does not provide the cell with compounds required for cell growth and does not lyse the cells.

The bacteriophage T4 has also been used in assays for various proteins. For example, T4 has been used to detect nerve growth factor (NGF) (Olger et al. (1974) *Proc. Natl. Acad. Sci. (USA)* 71:1554-1558). In this assay, T4 was chemically

coupled to NGF using glutaraldehyde. Glutaraldehyde reacts with lysine and cysteine residues as well as with α -NH₂ groups (see *Laboratory Techniques in Biochemistry and Molecular Biology* (Elsevier Science Publishing Co., Amsterdam (1988)) Vol. 5 19). Antibodies against NGF were used to render the bacteriophage non-infective. When NGF was added to the medium, T4-NGF was displaced from the antibody and was free to infect *Escherichia coli* (*E. coli*).

Bacteriophage T4 has also been used to detect antibodies against a wide range of compounds. For example, Becker et al. (*Immunochem.* (1970) 7:741) used a T4 bacteriophage to detect antibodies against p-azobenzene arsonate. Hurwitz et al. (*Eur. J. Biochem.* (1970) 17:273) used a bacteriophage to detect and estimate levels of angiotensin-II-beta-amide and its antibodies. Gurari et al. (*Eur. J. Biochem.* (1972) 26:247) used bacteriophage T4 in the detection of antibodies to nucleic acids. These detection methods involve the chemical modification of the T4 phage resulting in the non-specific exposure on the phage surface of a compound to which the antibodies to be assayed are targeted. Such antibodies render the bacteriophage non-infective, thus enabling the decrease in plaque formation to be used as a measure of the level of antibody present.

The T4 system has also been used to measure hapten concentrations (see, e.g., Hurwitz et al. (1970) *Eur. J. Biochem.* 17:273-277) In this system, T4 is chemically modified such that it exposes the desired hapten non-specifically on its surface. The addition of anti-hapten antibody destroys the infectivity of the phage. Infectivity is restored in the presence of hapten.

Although both the M13 and T4 phage systems can be used to detect the presence of a compound by their ability to become infected in the presence of that compound, infection

by M13 is normally not immediately ascertainable, and T4 infection is lethal. Thus, these systems cannot be used where a quick screening or selection method based on the survival of the infected bacterial cell is desired, such as
5 where a particular cell type is being selected, or when the object of phage infection is to restore the ability of an auxotrophic bacterial cell to survive on its own under a given set of growth conditions. Another limitation is that none of these methods of detection allow the *in vitro* assembly
10 of phage, and none allow the display of non-proteinous target molecules on a particular area of a phage. Particular M13-derived phagemid systems such as the one employed by Barbas et al. (*Proc. Natl. Acad. Sci., USA* (1991) 88:7978) do carry genes which would endow an infected cell
15 with a selective growth advantage. However, these systems are limited to the expression of peptides or proteins. Furthermore, in all M13 systems where fusion proteins have been used to display proteins, the displayed protein has always been the molecule-of-interest and therefore not
20 useful for the detection of such molecules.

Thus, what is needed are efficient and accurate assay methods utilizing bacteriophage infection that give fast results and that do not have to result in bacterial cell
25 death. Additionally, assay methods utilizing bacteriophage infection are needed for non-proteinous molecules of interest and for living cells. In addition, novel selection and screening techniques are needed for the continuous production of desirable compounds by cell lines.

30

SUMMARY OF THE INVENTION

It has been discovered that a lambdoid bacteriophage having a target molecule chemically linked to one of its components, the gpV protein, can be successfully assembled
35 *in vitro* such that the target molecule is displayed on the outer surface of the phage. Furthermore, this chemically

modified lambdoid bacteriophage maintains its ability to infect *E. coli*. These findings have been exploited to develop the present invention, namely, a method of detecting a molecule-of-interest in a solution, and a method of selecting or screening for cell lines which continuously produce desirable compounds, both utilizing a genetically and chemically modified lambdoid bacteriophage.

As used herein, the term "lambdoid bacteriophage" is meant to encompass all lambda-related phages and all derivatives, genetically engineered derivatives, and hybrids thereof, such as, but not limited to, $\phi 80$, $\phi 81$, phages 21, 82, 424, 432, λ imm434, λ imm21, phagemids, λ EMBL, and λ gt.

In this method, a protein construct is provided which includes a modified gpV protein. As used herein, the term "gpV protein" is meant to encompass any major tail protein found in the lambdoid bacteriophages such as, but not limited to, gpV protein, gpV-related proteins, and equivalents of lambdoid gpV protein in the tails of other lambdoid viruses.

The gpV protein is modified by the presence of a chemically reactive amino acid residue replacing a native gpV amino acid residue and which is chemically linked to the target molecule. Preferably, the chemically reactive amino acid residue is histidine (His), cysteine (Cys), tyrosine (Tyr), or tryptophan (Trp). Most preferably, the reactive amino acid residue is cysteine because it can be selectively modified. These reactive residues may be anywhere in the gpV amino acid sequence provided the modification does not interfere with gpV protein function or in phage assembly and structure. In one embodiment the reactive residue is at the carboxy terminus of the gpV protein. In one preferred embodiment, the modified gpV protein has a cysteine residue instead of a serine residue

at its carboxy terminus. The target molecule is chemically coupled to the chemically reactive amino acid residue either directly or via a chemical cross-linker such as a bifunctional cross-linking agent.

5 In preferred embodiments of the invention, the target molecule is a protein, peptide, glycoprotein, lipoprotein, hormone, proteoglycan, proteolipid, lipid, lipopolysaccharide, nucleic acid, cofactor, toxin, carbohydrate glycolipid, terpene, antibiotic, or vitamin.
10 One preferred target molecule is a protein such as an enzyme, enzyme substrate, immunoglobulin, or binding portion thereof, ligand, toxin, growth factor, cytokine, receptor, or analog or fragment of any of these proteins.

 The method of the invention includes assembling an
15 infective lambdoid bacteriophage including the protein construct and having the target molecule displayed on the outer surface of the phage tail. In some embodiments, assembly is accomplished by providing the enzymes and components required for phage assembly in the form of a
20 packaging extract obtained from an *E. coli* cell infected with a mutant strain of lambdoid bacteriophage that requires exogenous gpV protein to assemble. As used herein, the term "packaging extract" includes at least that portion of the cytoplasm of the infected *E. coli* cell which contains the
25 enzymes and lambdoid components required for assembly.

 The target molecule on the bacteriophage surface is then processed such that the phage is rendered reversibly non-infective or inactive. The term "reversibly non-infective" refers to the ability of the temporarily
30 inactivated phage to become infective again. In some aspects of the invention, processing can be accomplished by treating the phage-linked target molecule with a binding molecule specific for the target molecule. In some embodiments, the binding molecule is an immunoglobulin, or

binding portion thereof, specific for an antigenic determinant on the target molecule, a receptor specific for a ligand-type target molecule, a ligand which binds to a receptor-type target molecule, or a matrix to which the phage is immobilized via the target molecule. Immobilization renders the phage non-infective because it cannot bind to the lambda cell receptor.

The non-infective bacteriophage is then treated with a solution-to-be-tested which potentially contains the molecule-of-interest. Preferred solutions-to-be-tested include cell culture media, cell lysates, or biological samples such as blood, urine, serum, semen, or lacrimal secretions. The molecule-of-interest in the solution renders the non-infective bacteriophage infective again.

The term "molecule-of-interest" is meant to encompass any molecule whose activity or presence is desired, and which can directly or indirectly render the non-infective phage infective again. This may be a protein, peptide, hormone, nucleic acid, carbohydrate, lipid, glycoprotein, proteoglycan glycolipid, proteolipid, lipopolysaccharide, vitamin, toxin, terpene, antibiotic, or cofactor.

In some embodiments, the molecule-of-interest is a protein such as an enzyme, enzyme substrate, immunoglobulin, or binding portion thereof, ligand, toxin, growth factor, cytokine, receptor, or analog or fragment of any of these proteins. In some aspects of the invention, the molecule-of-interest is an enzyme which cleaves the target molecule. Cleavage of the binding molecule-linked target molecule liberates the bacteriophage, thereby rendering it infective once again. In other embodiments, the molecule-of-interest is an unbound target molecule. Unbound target molecules present in the solution-to-be-tested displace the binding molecule on the phage-linked target molecule and bind with the binding molecule, thereby

liberating the phage and rendering it infective once again.

5 In another embodiment of the invention, the target molecule and the molecule-of-interest are the same and are ligands, and the binding molecule is a receptor specific for that ligand. In still another embodiment, the target
10 molecule and the molecule-of-interest are the same and are receptors, and the binding molecule is a ligand that binds that receptor. In yet another embodiment, the molecule-of-interest is not identical to the target molecule, but yet is capable of binding to the binding molecule, thus displacing the target molecule. In another embodiment, the target molecule and the molecule-of-interest contain the same antigenic determinant, and the binding molecule is an immunoglobulin, or portion thereof, that binds that
15 antigenic determinant. In yet another embodiment, the target molecule and the molecule-of-interest are the same immunoglobulin, or binding portion thereof, and the binding molecule contains an antigenic determinant bound by that immunoglobulin.

20 In the method of the invention, a bacterial cell such as an *E. coli* cell is contacted with the treated bacteriophage for a time sufficient for the bacteriophage to infect the cell. Infected cells are then detected, infection being indicative of the presence of the molecule-of-interest in
25 the solution.

In some embodiments, detection is accomplished by observing cell death in the form of cell lysis or plaque formation. Cell lysis results when the phage DNA successfully enters the cell, takes over the cell's protein
30 synthetic operations, thereby directing it to produce viral components at the expense of cellular components and to assemble them into phage particles, and causes the cell to rupture or lyse such that the assembled viral particles are released. Plaques result when multiple neighboring cells

plated on solid culture dishes lyse in this way, leaving clear or empty spots on the otherwise cloudy culture lawn.

5 Detection of infection is also accomplished by observing bacterial cell survival and/or growth in those embodiments of the invention where the bacterial cell infected by the treated phage is an auxotrophic mutant requiring a gene supplied by the phage. An example of one such gene is *trpE* carried by λ EMBL3, a vector well known to those knowledgeable in the art, and where the phage is a mutant temperate phage such as one that is temperature sensitive for lysogeny.

10 As used herein, the term "temperate phage" refers to a phage that can be both lytic or lysogenic. A lysogenic phage integrates its nucleic acid into the host cell genome and remains quiescent, replicating only when the host genome replicates. In its lytic or vegetative phase, the phage nucleic acid excises itself from the host genome, or does not integrate itself into the host cell genome, and takes over the protein synthetic machinery of the cell at the expense of cellular components and causes phage progeny to be assembled. New phages are released from the cell when the cell lyses. A temperate phage may contain a mutation conferring temperature sensitivity, i.e., it is lysogenic only at low growth temperatures (e.g., about 32°C and below) and is lytic at high growth temperatures (e.g., 15 20 25 37°C and above, such as about 42°C). Thus, at lower growth temperatures, lysogenic phage carrying a gene that will alleviate the auxotrophy of the cell integrates its DNA into the bacterial cell genome, providing it with the gene which the cell requires to survive.

30 Detection of infection is also accomplished by observing bacterial cell survival and/or growth in those embodiments of the invention where the phage, which is temperature sensitive as described in the above paragraph,

carries a gene encoding antibiotic resistance. Infection of *E. coli* by this phage permits the cell to survive and grow on media containing the antibiotic whose resistance is encoded by the gene carried by the phage.

5 Bacterial cell growth is also indicative of phage infection, and hence, of the presence of the molecule-of-interest in the solution-to-be-tested, in embodiments where the bacterial cell to be infected is an auxotroph which itself is producing and secreting the molecule-of-interest, and where the molecule-of-interest and the target molecule are the same or are otherwise capable of displacing one another from the binding molecule. In this method, the target molecule is chemically linked to the gpV protein and assembled into an infective phage carrying a bacterial gene encoding a protein required by the auxotrophic bacterial cell for survival. The phage is then inactivated being bound by a binding molecule (such as an antibody directed to the target molecule), and then contacted with the solution-to-be-tested (e.g., the medium in which the mutant bacterial cell had been growing and/or with the bacterial cell, itself). If the solution contains unbound molecule-of-interest, or if the cell is producing and secreting the molecule-of-interest, the binding molecule bound to the phage linked target molecule will be displaced and instead will bind to the unbound molecule-of-interest in the solution. The liberated phage then infects the bacterial cell, and at lower growth temperatures (e.g., about 32°C), provides it with the bacterial gene it needs for survival and growth.

30 In one aspect of the invention, a modified gpV protein is provided which encodes chemically reactive amino acid residues such as histidine, cysteine, tyrosine, and/or tryptophan. In another aspect, a nucleic acid is provided which encodes modified gpV protein. In yet other aspects, the invention includes the protein construct including the

modified gpV protein chemically linked via its chemically reactive amino acid residue to the target molecule.

5 In one embodiment the invention includes a genetically modified gpV protein having its carboxy terminal serine residue replaced with a chemically reactive amino acid residue such as cysteine. Preferably, this modified gpV protein has the amino acid sequence set forth in the Sequence Listing as SEQ ID NO:1.

10 Yet another embodiment of the invention is a chemically modified, infective lambdoid bacteriophage displaying the target molecule on its outer surface. In some aspects of the invention, this bacteriophage is assembled *in vitro*.

15 In another embodiment, the bacteriophage carries a gene encoding drug resistance. The bacteria to be infected are not able to grow in the presence of the first compound which is an antibiotic. Infection by the bacteriophage restores the ability of the bacteria to grow in the presence of the first compound.

20 Other methods of selecting or screening for a cell producing a molecule-of-interest are also provided herein. In these methods, the protein construct of the invention is assembled with other viral components *in vitro* into an infective lambdoid bacteriophage displaying the target
25 molecule on its outer surface. The target molecule is then processed such that the phage is rendered reversibly non-infective. A cell is contacted with the non-infective phage for a time sufficient to enable the molecule-of-interest produced by the cell to render the phage infective
30 once again and then to enable the phage to infect the cell. Infection is detected, infection being indicative of the presence of a cell producing the molecule-of-interest.

In one aspect, the molecule-of-interest and the target molecule are the same. In another aspect, the phage assembled *in vitro* is a temperature sensitive lambdoid bacteriophage which also carries a gene encoding a cellular component. The cell, auxotrophic for the cellular component, is contacted at or below about 32°C with the phage. At this low temperature, growth of the infective phage is detected as a measure of the presence of a cell producing the molecule-of-interest.

5

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects of the present invention, the various features thereof, as well as the invention itself may be more fully understood from the following description, when read together with the accompanying drawings in which:

FIG. 1A is a diagrammatic representation of the bacteriophage lambda;

FIG. 1B is a diagrammatic representation of the chemically modified bacteriophage lambda of the invention;

FIG. 2 is a schematic representation of the nucleic acid sequence and corresponding amino acid sequence of the modified gpV protein;

FIG. 3 is a schematic representation of the 3' and 5' primers used to provide the PCR fragment containing the full length, modified V gene fragment;

FIG. 4 is a schematic representation of the pSYM1 plasmid which contains the gene encoding the modified gpV protein of the invention;

FIG. 5A is a diagrammatic illustration of one embodiment of the method of the invention;

FIG. 5B is a diagrammatic illustration of another embodiment of the method of the invention;

FIG. 5C is a diagrammatic illustration of another embodiment of the method of the invention;

FIG. 6A is a diagrammatic illustration of another embodiment of the method of the invention;

FIG. 6B is a diagrammatic illustration of another embodiment of the method of the invention; and

FIG. 6C is a diagrammatic illustration of another embodiment of the method of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

It has been discovered that replacing an amino acid residue in the gpV protein with a chemically reactive amino acid residue and then chemically coupling this residue to
5 a target molecule yields a protein construct that may successfully be assembled *in vitro* into an infective lambdoid bacteriophage having the target molecule displayed on the outer surface of its tail. Furthermore, a lambdoid modified in this manner still retains its ability to infect
10 bacteria. Utilizing such a phage, a method of detecting a molecule-of-interest and of detecting a cell producing a molecule-of-interest have been developed. In these methods, either the death or growth of certain bacterial strains results from the presence of a molecule-of-interest
15 depending on the nature of the infecting lambdoid bacteriophage genome and any specific needs of the infected bacteria.

One type of lambdoid bacteriophage, the bacteriophage lambda, consists of a icosahedral head or capsid with a
20 radius of 30 nm and a flexible tail 150 nm long ending in a tapered basal part and a single tail fiber (FIG. 1A). The genome of this bacteriophage is linear DNA. The DNA is found in the capsid head and has cohesive ends, the right one of which, as defined by the genetic map, protrudes into
25 the upper third of the tail. The tail consists mainly of a tube of 32 disks of six gpV protein units, the product of the V gene.

In the present invention, the lambdoid bacteriophage is modified so as to expose or display a target molecule on
30 the outer surface of its tail (FIG. 1B). This is accomplished by chemically linking the target molecule to a genetically modified gpV protein, thereby forming a protein construct, and then assembling the bacteriophage *in*

vitro using this protein construct instead of or in combination with gpV protein.

The protein construct is prepared from a genetically altered gpV protein having relatively non-reactive native amino acid residue(s) replaced with chemically reactive amino acid residues. Such chemically reactive amino acid residues include histidine, cysteine, tyrosine, and tryptophan. These reactive amino acid residues may be located anywhere throughout the sequence of the gpV protein and more than one amino acid residue may be replaced with such a reactive residue. Cysteine is the preferred reactive amino acid residue because there are no cysteine residues present in the native gpV protein sequence, and this residue can be selectively modified. The native sequence also contains no histidine residues, but does have five tyrosine residues and six tryptophan residues. For example, SEQ ID NO:1 in the Sequence Listing sets forth the amino acid sequence of a gpV protein in which the carboxy terminal serine residue at position 246 has been replaced with a cysteine residue (FIG. 2). This altered gpV protein can be prepared as follows.

The nucleic acid sequence of the V gene is known (Sanger et al. (1982) *J. Mol. Biol.* 162:729). This gene was simultaneously cloned and modified by PCR methods (Scarf, "Cloning with PCR" in *PCR Protocols. A Guide to Methods and Applications* (Innis et al., eds.) Academic Press, San Diego, CA (1990) pp.84-91), resulting in a full length V gene with its carboxy terminal Ser²⁴⁶ codon replaced with a Cys codon TGT. The sequence for the modified V gene is set forth in the Sequence Listing as SEQ ID NO:2. The modified V gene has been cloned into an expression vector (pKK223-3, Pharmacia, Piscataway, NJ) resulting in the pSYM1 plasmid shown in FIG. 4. This plasmid is used to transform *E. coli*. The transformed strain is induced (Sambrook et al. in *Molecular*

Cloning: A Laboratory Manual (1989) p.17.13. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), then lysed as a source of modified gpV protein. If necessary, the gpV protein can be purified further as described by Katsura et al. (*Virology* 5 (1977) 76:129).

Partially purified, genetically modified gpV protein is then chemically coupled to a selected target molecule to form the protein construct. The target molecule can be any molecule that can be linked to the modified gpV protein via 10 its reactive amino acid residue(s) without abolishing phage assembly or infectivity. Such target molecules include proteins, peptides, carbohydrates, hormones, glycoproteins, proteoglycans, lipoproteins, proteolipids, lipids, lipopolysaccharides, toxins, cofactors, nucleic acids, 15 glycolipids, terpenes, antibiotics, or vitamins. Useful target proteins include immunoglobulins or portions thereof (such as Fab, Fv, (Fab')₂), receptors or portions thereof (such as the estrogen receptor and the insulin receptor), ligands (such as ciliary neuronotrophic factor and 20 luteinizing hormone), enzymes (such as beta-lactamase, triose phosphate isomerase, and hexokinase), enzyme substrates (such as pre-interleukin-1, proinsulin, and erythropoietin), cytokines (such as macrophage migration inhibition factor and the interleukins), growth factors 25 (such as fibroblast growth factor and granulocyte colony stimulating factor), or toxins (such as pertussis toxin and botulinum toxin A).

Coupling of the target molecule to the gpV protein may be accomplished by any chemical linkage method known in the 30 art, such as covalent bonding. For example, the sulfhydryl group in a cysteine residue of the gpV protein and a sulfhydryl group in any cysteine residues present in the target molecule may be oxidized to form a disulfide bond between them. The target molecule instead can be 35 chemically reacted with the imidazolyl group in histidine,

the hydroxyl group in tyrosine, or the indoyl group in tryptophan.

5 The target molecule may be chemically cross-linked to the reactive amino acid residue of the gpV protein. Cross-linking can be accomplished using any number of known cross-linking reagents, such as those set forth in U.S. Patent 5,112,615, herein incorporated by reference. One type of useful cross-linking reagent is a bifunctional reagent such as β -maleimidopropionic acid N-10 hydroxysuccinimide ester which can be employed according to methods known in the art (e.g., *Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Pub. Co., Amsterdam (1988) vol. 19).

15 To obtain the bacteriophage of the invention, the protein construct is contacted with other lambdoid phage components and enzymes required to assemble the phage *in vivo*. Methods of phage assembly are well known in the art (see, e.g., Hohn (1979) *Meth. Enzymol.* 68:299-309). *In vitro* assembly enables the ratio of unmodified gpV protein to modified gpV 20 protein to be controlled, thus allowing the introduction of a limited number of protein constructs per phage particle.

Lambdoid components and assembly enzymes can be provided in the form of a cell lysate (or packaging extract) from *E. coli* preinfected with lambdoid phage that is 25 defective in a step of morphogenesis. For example, when bacteria infected with a prophage mutant in gene V are induced, precursor phage particles accumulate. Induction may be accomplished by a shift in temperature if the infecting phage has a temperature sensitive genotype such as λ V *Clts Sam7*. To obtain a V derivative of a commercially 30 available lambda phage, such as λ C1857 *Sam7* (New England Biolabs, Beverly, MA), the phage are mutagenized (Katsura (1976) *Molec. Gen. Genet.* 148:31). *E. coli* JM105 (Stratagene, La

Jolla, CA) carrying the pSYM1 plasmid are infected with the mutagenized phage population. Colonies appearing at 32°C are replica plated to plates with or without IPTG, which induces gpV production. Colonies which grow on plates without IPTG, but do not grow on plates with IPTG, are selected as a source of V phage. The V gene mutation can be complemented by the addition of the missing gpV protein, which, in this invention, is chemically coupled to a target protein.

10 The assembled phage is then rendered non-infective. This may be accomplished by the addition of a binding molecule which is directed to the target molecule on the bacteriophage. Useful binding molecules include antibodies or, binding portions thereof such as Fv, Fab, or (Fab')₂ fragments. The production of such antibodies and biochemically or genetically produced fragments is well known in the art (see, e.g., *Antibodies: A Laboratory Manual* (Harlow and Lane, eds.) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 1988).

20 Other useful binding molecules include receptors and ligands. When the target molecule is a receptor ligand, the receptor serves as an immobilizing agent. Receptors as binding molecules may be presented in micelles or liposomes or on cell surfaces to keep their configuration. Such receptor-containing liposomes can be prepared using the method of Georgoussi et al. (*Biochem. Biophys. Acta* (1990) **1055:69**). Receptors which can be presented to the phage in this way include, but are not limited to, the nicotinic acetylcholine receptor (Chak et al. (1992) *Meth. Enzymol.* **207:546**), the inositol 1,4,5-triphosphate receptor (Kamata et al. (1992) *J. Biochem.* **111:546**), the hepatic vasopressin receptor (Georgoussi, *ibid.*) and the rat ovarian receptor for luteinizing hormone (Kusada et al. (1986) *J. Biol. Chem.* **261:16161**). Ligands include all molecules capable of

binding to the target molecule in a competitive fashion. When ligands are used as the binding molecule, they must be immobilized as described below.

5 Other useful binding molecules are enzyme substrates such as acetylcholine, γ -amino butyric acid, insulin, and glucagon.

10 Alternatively, the phage can be rendered non-infective by immobilization to a binding molecule such as a matrix via linkage to the target molecule. Such matrices include, but are not limited to, commercially available materials such as a gel consisting of dextran cross-linked with epichlorohydrin (Sephadex™) a special gel prepared from agarose (Sepharose™), and agarose. When the phage is immobilized to a matrix it is unable to bind to and infect
15 a cell. Immobilization to the matrix may be accomplished by chemical linkage such as one employing various chemical cross-linking methods (see, e.g. Wilchek et al. (1984) *Meth. Enzymol.* 104:3; and U.S. Patent No. 5,112,615, herein incorporated by reference).

20 The method of the invention may be designed such that the immobilized or inactivated phage is released or liberated from the matrix or binding molecule by the molecule-of-interest. In this way, the presence of the molecule-of-interest can be determined and quantitated by
25 the relative activity of the phage. For example, if the molecule-of-interest is an enzyme capable of cleaving the target molecule, the phage is rendered infective once again upon its liberation from the binding molecule (FIGs. 5A-5C). The target molecule may have an antigenic determinant
30 recognized by an antibody (FIG. 5A), be a ligand which is bound to a matrix (FIG. 5B), or be a ligand which binds a receptor (FIG. 5C). Any enzyme which cleaves the target molecule releases the phage which is now infective. The ability of these phage to infect bacteria is then assayed,

for example, using the plate method of Davis et al. (in *Advanced Bacterial Genetics* (1980) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 71).

5 If the molecule-of-interest has an antigenic determinant which the antibody molecule bound to the target molecule recognizes, the method is performed as delineated in FIGs. 6A or 6B. The bound target molecule (and hence the inactivated phage) is liberated by the presence of unbound molecule-of-interest in the solution which competes
10 for the binding molecule. If the binding molecule is a receptor which loses its three-dimensional confirmation upon purification, it may have to be presented on a liposome, micelle, or on the surface of a cell.

The method of the invention can also be used to select
15 a cell type that produces a desired product, such as a ligand or modified enzyme, from a population that does not produce that product. For example, in an *in vitro* assembly reaction partially purified, genetically modified gpV protein cross-linked to a target molecule is contacted with
20 the cell extract from an *E. coli* previously infected with a phage, such as $\lambda C1857 VtrpE^+$, obtained as described above. Either an antibody directed against the target molecule, or a ligand which can bind the receptor, or a cell receptor specific for the target molecule is utilized to render the
25 modified bacteriophage non-infective, or if the molecule-of-interest is an enzyme which cleaves its substrate, the target molecule can be chemically linked to a matrix, as described above.

A library that consists of a mutagenized population of
30 a cloned enzyme or antibody is transformed in an *E. coli* strain which is defective in the marker gene carried by the bacteriophage. By "cloned enzyme or antibody" is meant the enzyme or antibody which is produced by expression of the

cloned gene encoding the enzyme or antibody. The cloned enzyme or antibody gene can be mutagenized by one of a variety of methods such as those described by Foster (*Meth. Enzymol.* (1991) 204:114). The cloned enzyme or antibody also
5 must be excreted into the surrounding media. Several methods by which cloned enzymes are excreted are provided in the scientific literature (e.g., Chalmers et al. (1990) *Appl. Environ. Microbiol.* 56:104; Lazzaroni et al. (1981) *J. Bacteriol.* 145:1351; and Rampf et al. (1991) *FEBS Lett.* 280:27), as are
10 methods by which cloned antibody fragments are excreted (e.g., Ward et al. (1989) *Nature* 341:544).

In the case where mutagenized enzyme is employed, the target molecule is an analog of the natural substrate for the enzyme. This analog is not cleaved by the wild type
15 enzyme, but if a mutagenized enzyme present in the mutant library is capable of cleaving the analog, phage are released and infect the cell excreting the enzyme with the modified specificity thus permitting the cell to grow.

In the case where mutagenized antibody is employed,
20 the target molecule contains the epitope or antigenic determinant for the antibody. Although antibodies do not normally cleave their antigen, a mutagenized antibody present in the mutant library may be capable of cleaving the antigen. If so, phage are released and infect the cell
25 excreting the antibody with the modified activity, thus permitting the cell to grow. If the immobilizing agent is an antibody, it must recognize a different epitope on the antigen and must not interfere with the binding of the excreted antibody.

30 The method of the invention offers several advantages over other systems employing bacteriophages such as M13 or T4. First, lambdoid bacteriophage can be assembled *in vitro*, and thus the target molecule does not have to be a protein

or peptide. In fact, any molecule that can be linked to the gpV protein can be employed as long as it does not completely interfere with *in vitro* assembly or the ability of the resulting bacteriophage to infect bacteria.

5 Second, the method does not have to result in the death of the infected bacteria. Rather, it can be used to isolate cells that excrete/secret a desired compound, unlike the M13 and T4 systems. By using a temperature sensitive strain of lambdoid bacteriophage and a bacterial
10 cell population that requires for growth a particular gene product supplied by the bacteriophage, those cells that excrete/secret the desired compound will render infective an inactivated bacteriophage lambda which, in turn, will infect the cell, and at lower temperatures enable the cell
15 to grow. Likewise, the method can be used to isolate either a bacteria mutant or a genetically engineered bacteria that excretes or secretes a molecule-of-interest from a population of non-excretors.

 Third, this method enables the selective modification
20 of a specific protein, and hence the selective display of a target molecule, unlike the T4 system. With non-specific modifications, a large percentage of the modified phages are rendered permanently non-infective. For example, when nerve growth factor (NGF) was coupled to bacteriophage T4,
25 76% of the phage were rendered non-infective (Olger et al. (1974) *Proc. Natl. Acad. Sci. (USA)* 71:1554-1558).

 Fourth, as an extension of the method described in the previous paragraph, the method can also be used to screen
30 enzyme libraries for clones having the ability to cleave altered substrate. Immobilization of the bacteriophage via the altered substrate enables isolation of strains from a library that contain an enzyme with the altered specificity from the library.

The following examples illustrate the preferred mode of making and practicing the present invention, but is not meant to limit the scope of the invention since alternative methods may be utilized to obtain similar results.

5

EXAMPLES

1. Preparation of Modified V Gene and Its Expression

The V gene is simultaneously cloned into the expression vector pkk223-3 (Pharmacia, Piscataway, NJ) and modified using the PCR protocol of Scharf ("Cloning with PCR" in *PCR Protocols. A Guide to Method and Applications* (Innis et al., eds.) Academic Press, San Diego, CA (1990) pp. 84-91).
10 The resulting plasmid is shown in FIG. 4 (pSYM1). The primers used for the procedure are shown in FIG 3 and are set forth in the Sequence Listing as SEQ ID NOs:3 and 4.
15 The primer that anneals to the 5' end of the V gene (SEQ ID NO:3) is designed to include an EcoRI restriction endonuclease cleavage site. The primer that anneals to the 3' end of the V gene (SEQ ID NO:4) is designed to include HindIII and PstI restriction endonuclease cleavage sites.
20 In addition, this primer contains a single base substitution in the last codon of the V gene. This substitution results in the conversion of Ser²⁴⁶ to Cys²⁴⁶.

The plasmid is then used to transform *E. coli* strain JM109 (Yanish-Perron et al. (1985) *Gene* 33:103) and then
25 induced to express the modified gpV protein by addition of IPTG (Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual* p.17.13, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Cell extracts prepared by sonication serve as a source of modified gpV protein. If necessary, modified gpV
30 is further purified according to Katsura et al. (*Virology* (1977) 76:129).

2. Preparation of Antibody Column

A column having antibodies directed to the target molecule of the V-gene protein construct is prepared essentially as described in *Antibodies: A Laboratory Manual* (Harlow and Lane, eds.) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, (1988)). Briefly, specific antibodies are mixed with protein A beads (Sigma Chemical Company, St. Louis, MO) using 2 mg of antibody per milliliter of beads. The bead solution is mixed gently for 1 hour at room temperature. The beads are then washed and chemically cross-linked to the antibodies using a bifunctional cross-linking reagent such as dimethylpimelimidate (Sigma Chemical Company). Chemical cross-linking is accomplished by shaking the antibody-coated beads for 30 minutes in the presence of 20 mM dimethylpimelimidate. The cross-linking reaction is stopped by washing the beads in 0.2 M ethanolamine followed by a 2 hour incubation at room temperature in 0.2 M ethanolamine.

3. Detection of Ciliary Neurotrophic Factor

Rabbit ciliary neurotrophic factor (CNTF) is isolated from rabbit sciatic nerves as described by Lin et al. (*Science* (1989) **246**: 1023-1025). The CNTF is chemically cross-linked through a cysteine residue to the sole cysteine residue at the carboxy terminus of the modified gpV protein using 4-maleimidobenzophenone (Tao et al. (1986) *Biochemistry* **25**:7633-7639). An *in vitro* lambda packaging extract is prepared from *E. coli* carrying the λ CIts V⁻ temperature sensitive genotype (Hohn (1979) *Meth. Enzymol.* **68**:299-309). The CNTF-gpV protein construct is added to the packaging extract followed by the addition of unmodified gpV protein. The resulting *in vitro*-assembled phage containing CNTF exposed on their tail are purified by running the reaction mixture

over an anti-CNTF antibody column, prepared as describe above. Antibodies to CNTF are prepared as described by Lin et al. (*ibid.*). The CNTF-modified phage is inactivated using anti-CNTF antibodies, as described by Hurwitz et al. (*Eur. J. Biochem.* (1970) 17:273). The appropriate ratio of modified phage to antibody is determined experimentally using the method of Olger et al. (*Proc. Natl. Acad. Sci. (USA)* (1974) 71:1554). Inactivated phage are incubated with cell extracts suspected of containing CNTF (the solution-to-be-tested) under the conditions described by Olger et al. (*ibid.*). The infectivity of the phage is then assayed using the plate method of Davis et al. (in *Advanced Bacterial Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1980) p. 71). The increase in the number of infective phage is directly proportional to the amount of CNTF present in the original sample.

4. Detection of Interleukin-1 β Converting Enzyme

The precursor form of interleukin-1 β (pIL-1 β) has been cloned into *E. coli* (March et al. (1985) *Nature* 315:641-647). This precursor is isolated from the *E. coli*, using the method of Black et al. (*J. Biol. Chem.* (1988) 263:9437) and chemically cross-linked, through one of its cysteine residues, to the cysteine residue of the modified gpV, as described above. An *in vitro* lambda packaging extract is made from *E. coli* carrying λ CI_{ts} V, as described above. The pIL-1 β -gpV protein construct is added to the packaging extract followed by the addition of unmodified gpV protein. The resulting *in vitro* assembled phage containing pIL-1 β are purified by running the reaction mixture over an anti-IL-1 β antibody column prepared as described above. Antibodies to IL-1 β are obtained as described by Lin et al. (*ibid.*) using a synthetic peptide which spans Cys¹²⁴. Phage that bind antibodies to this region will most likely have pIL-1 β

cross-linked to modified gpV at a cysteine residue other than Cys¹²⁴. This is important because Cys¹²⁴ is located near the ICE cleavage site and cross-linking through this residue is likely to interfere with ICE activity. The pIL-1 β -modified phage are inactivated by cross-linking and thus immobilizing them to agarose (Wilchek et al. (1984) *Meth. Enzymol.* 104:3). The immobilized phage are then incubated with media suspected of containing IL-1 β converting enzyme (ICE) under the conditions described by Olger et al. (*Proc. Natl. Acad. Sci. (USA)* (1974) 71:1554). The infectivity of the phage is assayed using the plate method of Davis et al. (in *Advanced Bacterial Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1980) p.71). The increase in the number of infective phage is directly proportional to the amount of ICE present in the original sample.

5. Selection of *E. coli* Secreting Antibodies Capable of Cleaving Granulocyte Colony Stimulating Factor (G-CSF)

The Fv fragment of antibodies are the minimal structure for antigen-binding activity (Pluckthun et al. (1989) *Meth. Enzymol.* 178:497). The nucleotide sequence encoding the light chain variable region (V_L) and the heavy chain variable regions (V_H) of a murine antibody raised against G-CSF (Sigma Chemical Co., St. Louis, MO) are cloned using PCR as described by Chaudhary et al. (*Proc. Natl. Acad. Sci. (USA)* (1990) 87:1066). A hybridoma cell line producing monoclonal antibodies against G-CSF is prepared as described by Harlow and Lane (in *Antibodies: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1988) pp. 139-243). The V_L and V_H regions are cloned in the expression vector pASK22 (Pluckthun et al., *ibid.*). G-CSF is chemically cross-linked to the cysteine residue of the modified gpV, as described above. An *in vitro* lambdoid packaging extract is made from *E. coli* carrying λ Clts V⁻ trpE⁺,

as described above. G-CSF-gpV is added to the packaging extract followed by the addition of gpV. The resulting *in vitro* assembled phage containing G-CSF are purified by running the reaction mixture over an anti-G-CSF antibody column, prepared as described above. The G-CSF-modified phage are inactivated by cross-linking to the agarose as previously described. Immobilized (inactivated) phage are incubated with *E. coli* Sym3 (λ^- , F⁺ Δ *trpE recA hflA*) that have been transformed with a library consisting of a mutagenized population of the cloned antibody fragments. The cloned antibody fragments can be mutagenized by one of a variety of methods such as those described by Foster (*Meth. Enzymol.* (1991) 204:114). The transformed cells are plated on supplemented Davis minimal media lacking tryptophan (*Difco Manual*, 10th Edition. Difco Laboratories, Detroit, MI. (1984) p. 572). Because *E. coli* Sym3 requires tryptophan for growth, it will grow poorly unless infected by a lambdoid bacteriophage carrying *trpE+* which restores growth of *E. coli* Sym3 on medium lacking tryptophan. Therefore, a transformant of *E. coli* Sym3 that secretes an antibody fragment capable of cleaving G-CSF releases nearby lambdoid bacteriophage which will infect the cell, resulting in a great enhancement of the infected cell's growth rate relative to other cells on the plate. This cell gives rise to a visible colony. A second round of screening permits isolation of the strain producing the desired molecule-of-interest.

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- (i) APPLICANT: Ray, Bryan L.
Lin, Edmund C.C.
 - (ii) TITLE OF INVENTION: Method Of Detecting Compounds
Utilizing Chemically Modified
Lambdoid Bacteriophage
 - (iii) NUMBER OF SEQUENCES: 4
 - (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Lappin & Kusmer
 - (B) STREET: 200 State Street
 - (C) CITY: Boston
 - (D) STATE: MA
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 02109
 - (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
 - (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
 - (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Kerner, Ann-Louise
 - (B) REGISTRATION NUMBER: 33,523
 - (C) REFERENCE/DOCKET NUMBER: 93,075
 - (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 617/330-1300
 - (B) TELEFAX: 617/330-1311
- (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 246 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: peptide
 - (iii) HYPOTHETICAL: YES
 - (iv) ANTI-SENSE: NO

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 741 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

ATGCCTGTAC CAAATCCTAC AATGCCGGTG AAAGGTGCCG GGACCACCCT GTGGGTTTAT      60
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AAAGTTAAAG ACCTGACGCC CGGCGAACTG ACCGCTGAGT CCTATGACGA CAGCTATCTC     180
GATGATGAAG ATGCAGACTG GACTGCGACC GGGCAGGGGC AGAAATCTGC CGGAGATAACC     240
AGCTTCACGC TGGCGTGGAT GCCCGGAGAG CAGGGGCAGC AGGCGCTGCT GCGTGGTTTT     300
AATGAAGGCG ATACCCGTGC CTATAAAATC CGCTTCCCGA ACGGCACGGT CGATGTGTTT     360
CGTGGCTGGG TCAGCAGTAT CGGTAAGGCG GTGACGGCGA AGGAAGTGAT CACCCGCACG     420
GTGAAAGTCA CCAATGTGGG ACGTCCGTCG ATGGCAGAAG ATCGCAGCAC GGTAACAGCG     480
GCAACCGGCA TGACCGTGAC GCCTGCCAGC ACCTCGGTGG TGAAAGGGCA GAGCACCACG     540
CTGACCGTGG CCTTCCAGCC GGAGGGCGTA ACCGACAAGA GCTTTCGTGC GGTGTCTGCC     600
GATAAAACAA AAGCCACCGT GTCGGTCAGT GGTATGACCA TCACCGTGAA CGGCGTTGCT     660
GCAGGCAAGG TCAACATTCC GGTGTATCC GGTAAATGGT AGTTTGCTGC GGTTCAGAAA     720
ATTACCGTCA CCGCTGTTA A

```

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: misc. feature

(B) LOCATION:

(D) OTHER INFORMATION: standardname = "5' Primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGGGAATTCA ATGCCTGTAC CAAATCCTAC AATG

34

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(ix) FEATURE:

(A) NAME/KEY: misc. feature

(B) LOCATION:

(D) OTHER INFORMATION: standardname = "3' Primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCCGAAGCTT CCTGCAGTTA ACAGGCGGTG ACGGTAATTT CTGCAAC

47

What is claimed is:

1. An infective lambdoid bacteriophage having on its outer surface a modified gpV protein, the modified gpV protein comprising a chemically reactive amino acid residue and a target molecule
5 chemically coupled to the amino acid residue.
2. The lambdoid bacteriophage of claim 1 wherein the modified gpV protein comprises a chemically reactive amino acid residue selected from the group
10 consisting of histidine, cysteine, tryptophan, and tyrosine.
3. The lambdoid bacteriophage of claim 1 wherein the chemically reactive amino acid residue is cysteine.
- 15 4. The lambdoid bacteriophage of claim 1 wherein the chemically reactive amino acid residue is at the carboxy terminus of the modified gpV protein.
5. The lambdoid bacteriophage of claim 1 wherein the target molecule is selected from the group
20 consisting of a protein, peptide, lipoprotein, hormone, glycoprotein, proteoglycan, glycolipid, proteolipid, lipid, lipopolysaccharide, carbohydrate, cofactor, toxin, carbohydrate, nucleic acid, terpene, antibiotic, and vitamin.
- 25 6. The bacteriophage of claim 5 wherein the target molecule is a protein.

7. The lambdoid bacteriophage of claim 6 wherein
the target molecule is a protein selected from the
group consisting of an enzyme, enzyme substrate,
immunoglobulin, ligand, toxin, growth factor,
5 cytokine, hormone, and receptor.

8. The lambdoid bacteriophage of claim 1 wherein
the target molecule is chemically coupled to the
amino acid residue by means of a cross-linking
10 reagent.

9. The lambdoid bacteriophage of claim 1 which is assembled *in vitro*.
10. A protein construct comprising:
- 5 (a) a modified gpV protein having a chemically reactive amino acid residue; and
- (b) a target molecule chemically coupled to the chemically reactive amino acid residue.
11. The protein construct of claim 10 wherein the chemically reactive amino acid residue is selected from the group consisting of histidine, cysteine, tyrosine, and tryptophan.
12. The protein construct of claim 10 wherein the chemically reactive amino residue is at the carboxy terminus of the gpV protein.
- 15 13. The protein construct of claim 11 wherein the chemically reactive amino acid residue is a cysteine residue at the carboxy terminus of the gpV protein.
14. The protein construct of claim 10 wherein the target molecule is cross-linked to the chemically reactive amino acid residue.
- 20 15. The protein construct of claim 10 wherein the target molecule is selected from the group consisting of a protein, peptide, glycoprotein, lipoprotein, hormone, proteoglycan, proteolipid, glycolipid, lipid, lipopolysaccharide, cofactor,
- 25

toxin, carbohydrate, nucleic acid, terpene,
antibiotic, and vitamin.

16. The protein construct of claim 15 wherein the target molecule is protein selected from the group consisting of an immunoglobulin, ligand, receptor, enzyme, enzyme substrate, hormone, toxin, cytokine, and growth factor.
17. A nucleic acid encoding the modified gpV protein of the protein construct of claim 10.
18. A nucleic acid encoding the modified gpV protein of the protein construct of claim 11.
19. The nucleic acid of claim 18 encoding the amino acid sequence set forth in SEQ ID NO:1.
20. A method of detecting a molecule-of-interest in a solution comprising the steps of:
- (a) providing a protein construct comprising a modified gpV protein having a chemically reactive amino acid residue which is chemically coupled to a target molecule;
 - (b) assembling *in vitro* an infective lambdoid bacteriophage comprising the modified gpV protein and having the target molecule on the outer surface of the bacteriophage;
 - (c) processing the bacteriophage-linked target molecule such that the bacteriophage is rendered reversibly non-infective;

25

5 (d) treating the non-infective bacteriophage with a solution-to-be-tested, the solution-to-be-tested containing a molecule-of-interest which renders the non-infective bacteriophage infective;

(e) infecting a bacterial cell with the treated bacteriophage; and

(f) detecting the infected cell, infection being indicative of the presence of the molecule-of-interest in the solution.

21. The method of claim 20 wherein the providing
5 step (a) comprises providing a modified gpV protein having a chemically reactive amino acid residue selected from the group consisting of histidine, cysteine, tyrosine, and tryptophan.
22. The method of claim 20 wherein the providing
10 step (a) comprises providing a modified gpV protein having a chemically reactive amino acid residue at its carboxy terminus to which the target molecule is linked.
23. The method of claim 22 wherein the providing
15 step (a) comprises providing a modified gpV protein having a cysteine residue at its carboxy terminus to which is linked the target molecule.
24. The method of claim 20 wherein the providing
20 step (a) comprises providing a modified gpV protein having a chemically reactive amino acid residue which is cross-linked to the target molecule.
25. The method of claim 24 wherein the providing
25 step (a) comprises providing a modified gpV protein having a chemically reactive amino acid residue linked to the target molecule via a bifunctional cross-linking reagent.

26. The method of claim 20 wherein assembling step
(b) comprises contacting the gpV protein construct
with a packaging extract obtained from a bacterial
cell infected with a mutant lambdoid bacteriophage
5 that requires exogenous gpV protein to assemble, the
packaging extract containing lambdoid bacteriophage
components and enzymes required to assemble lambdoid
bacteriophage *in vitro*.

27. The method of claim 20 wherein processing step
(c) comprises treating the bacteriophage with a
binding molecule that binds the target molecule, the
binding of the target molecule rendering the
5 lambdaoid bacteriophage non-infective.

28. The method of claim 27 wherein processing step
(c) comprises treating the bacteriophage with a
binding molecule selected from the group consisting
of an immunoglobulin, receptor, and ligand.

10 29. The method of claim 20 wherein processing step
(c) comprises immobilizing the bacteriophage by
linking the target molecule to a matrix.

30. The method of claim 20 wherein treating step
(d) comprises treating the non-infective
15 bacteriophage with a solution-to-be-tested selected
from the group consisting of a culture medium,
blood, serum, saliva, semen, and lacrimal secretion.

31. The method of claim 20 wherein treating step
(d) comprises treating the non-infective
20 bacteriophage with a molecule-of-interest selected
from the group consisting of a protein, peptide,
hormone, nucleic acid, carbohydrate, lipid,
glycoprotein, proteoglycan glycolipid, proteolipid,
lipopolysaccharide, vitamin, toxin, terpene,
25 antibiotic, or cofactor.

32. The method of claim 27 wherein treating step
(d) comprises treating the non-infective
bacteriophage with the solution-to-be-tested, the
solution-to-be-tested containing a molecule-of-
5 interest which is an enzyme that cleaves the
bacteriophage-linked target molecule.

33. The method of claim 20 wherein treating step
(d) comprises treating the non-infective
bacteriophage with the solution-to-be-tested, the
solution-to-be-tested containing a molecule-of-
5 interest which is an unbound target molecule, the
unbound target molecule competing with the
bacteriophage-linked target molecules for the
binding molecule.

34. The method of claim 20 wherein treating step
10 (d) comprises treating the non-infective
bacteriophage with the solution-to-be-tested, the
solution-to-be-tested containing a molecule-of-
interest that displaces the target molecule from the
binding molecule.

15 35. The method of claim 27 wherein the target
molecule and the molecule-of-interest molecule are
ligands, and the binding molecule is a receptor
which binds the ligands.

20 36. The method of claim 27 wherein the target
molecule and the molecule-of-interest contain the
same antigenic determinant, and the binding molecule
is an immunoglobulin that binds the antigenic
determinant.

25 37. The method of claim 27 wherein the target
molecule and the molecule-of-interest are
immunoglobulins which bind an antigenic determinant
on the binding molecule.

38. The method of claim 20 wherein detecting step
(f) comprises detecting cell death, cell death being
indicative of the presence of the molecule-of-
interest which has rendered the bacteriophage
5 infective.

39. The method of claim 38 wherein detecting step
(f) comprises detecting cell lysis.

40. The method of claim 20 wherein assembling step
(b) comprises assembling *in vitro* a temperature
sensitive lambdoid bacteriophage that carries a gene
required by the cell to be infected in step (e);
5 infecting step (e) comprises infecting a bacterial
cell at or below about 32°C, the cell requiring the
gene carried by the bacteriophage for survival; and
detecting step (f) comprises detecting the growth of
the infected bacteria cell, growth being indicative
10 of the molecule-of-interest in the solution.

41. The method of claim 40 wherein the molecule-of-
interest and the target molecule are the same.

42. The method of claim 40 where assembling step
(b) comprises assembling *in vitro* a temperature
sensitive lambdoid bacteriophage that carries a gene
required by the cell to be infected in step (e), the
15 gene being selected from the group consisting of a
cellular gene required for cell biosynthesis and a
drug resistance gene.

20 43. A method of selecting for a cell producing a
molecule-of-interest comprising the steps of:

(a) providing a protein construct comprising a
modified gpV protein having a chemically
reactive amino acid residue and a target
25 molecule which is chemically coupled to the
amino acid residue;

(b) assembling *in vitro* of an infective lambdoid bacteriophage comprising the modified gpV protein and displaying the target molecule on the outer surface of the bacteriophage;

- 5 (c) processing the bacteriophage-linked target molecule such that the bacteriophage is rendered reversibly non-infective;

5 (d) contracting the cell with the processed bacteriophage for a time sufficient to enable the molecule-of-interest produced by the cell to render the non-infective bacteriophage infective and to enable the infective bacteriophage to infect the cell; and

(e) detecting the infected cell, infection being indicative of the presence of a cell which produces the molecule-of-interest.

10 44. The method of claim 42 wherein assembling step (b) comprises assembling *in vitro* a temperature sensitive lambdoid bacteriophage that carries a gene required for survival by the cell to be contacted in step (d); contacting step (d) comprises infecting
15 the cell at or below about 32°C, and detecting step (e) comprises detecting the growth of the infected bacteria cell, growth being indicative of the presence of a cell producing the molecule-of-interest.

20 45. The method of claim 42 wherein the molecule-of-interest and the target molecule are the same.

25 46. The method of claim 43 wherein assembling step (b) comprises assembling *in vitro* a temperature sensitive lambdoid bacteriophage that carries a gene required by the cell to be contacted in step (d), the gene being selected from the group consisting of a cell biosynthesis gene and a drug resistance gene.

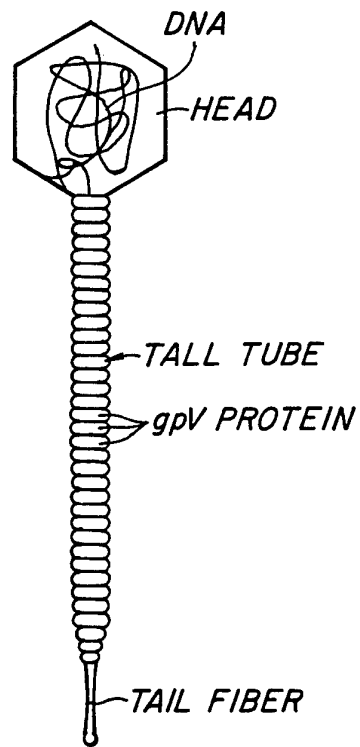


FIG. 1A

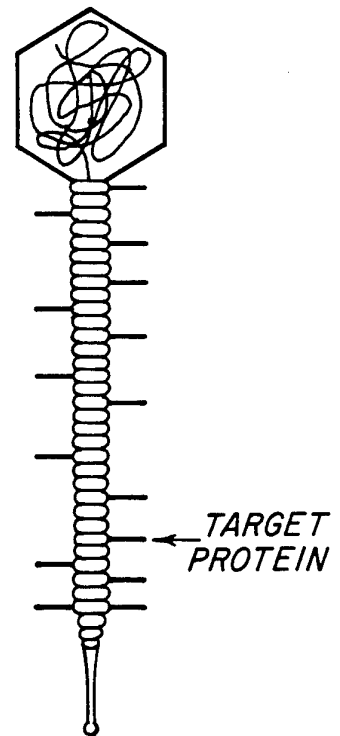


FIG. 1B

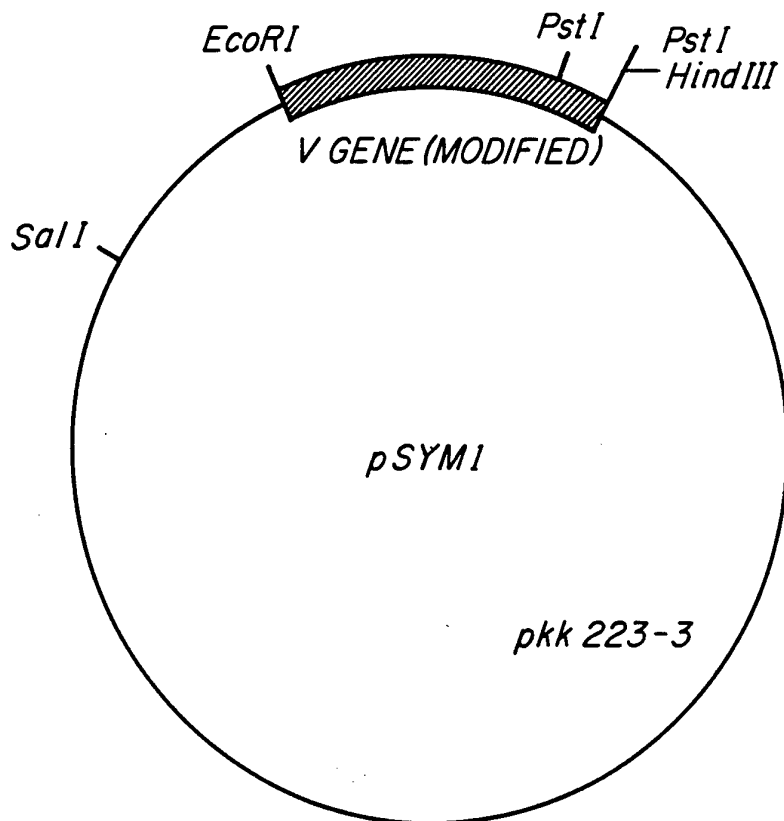


FIG. 4

M F V F N F T M P V K
 ATGCCTGTACCAAATCCTACAATGCCGGTGAA

 G A G T T L W V Y K G S G D P Y
 AGGTGCCGGCACCACCCTGTGGGTTTATAAGGGGAGCGGTGACCCTTACG

 A N P L S D V D W S R L A K V K D
 CGAATCCGCTTTCAGACGTTGACTCGTCGCGTCTGGCAAAAGTTAAAGAC

 L T P G E L T A E S Y D D S Y L D
 CTGACGCCCGGCGAACTGACCGCTGAGTCCATGACGACAGCTATCTCGA

 D E D A D W T A T G Q G Q K S A
 TGATGAAGATGCAGACTGGACTGCGACCGGGCAGGGGCAGAAATCTGCCG

 G D T S F T L A W M P G E Q G Q Q
 GAGATACCAGCTTCACGCTGGCGTGGATGCCCGGAGAGCAGGGGCAGCAG

 A L L A W F N E G D T R A Y K I R
 GCGCTGCTGGCGTGGTTAATGAAGGCGATACCCGTGCCTATAAAATCCG

 F P N G T V D V F R G W V S S I
 CTTCCCGAACGGCAGGCACATGTGTCCGTGGCTGGGTCAGCAGTATCG

 G K A V T A K E V I T R T V K V T
 GTAAGGCGGTGACGGCGAAGGAAGTGATCACCCGCACGGTGAAAGTCACC

 N V G R P S M A E D R S T V T A A
 AATGTGGGACGTCCGTTCGATGGCAGAAGATCGCAGCAGGTAACAGCGGC

 T G M T V T P A S T S V V K G Q
 AACCGGCATGACCGTGACGCCTGCCAGCACCTCGGTGGTGAAAGGGCAGA

 S T T L T V A F Q P E G V T D K S
 GCACCACGCTGACCGTGGCCTTCCAGCCGGAGGGCGTAACCGACAAGAGC

 F R A V S A D K T K A T V S V S G
 TTTGCTGCGGTGTCTGCGGATAAAAACAAAAGCCACCGTGTGCGGTCAAGTGG

 M T I T V N G V A A G K V N I P
 TATGACCATCACCGTGAACGGCGTTGCTGCAGGCAAGGTCAACATTCCGG

 V V S G N G E F A A V A E I T V T
 TTGTATCCGGTAATGGTGAGTTTGTGCGGTTGCAGAAATTACCGTCACC

 A C
 GCCTGT

FIG. 2

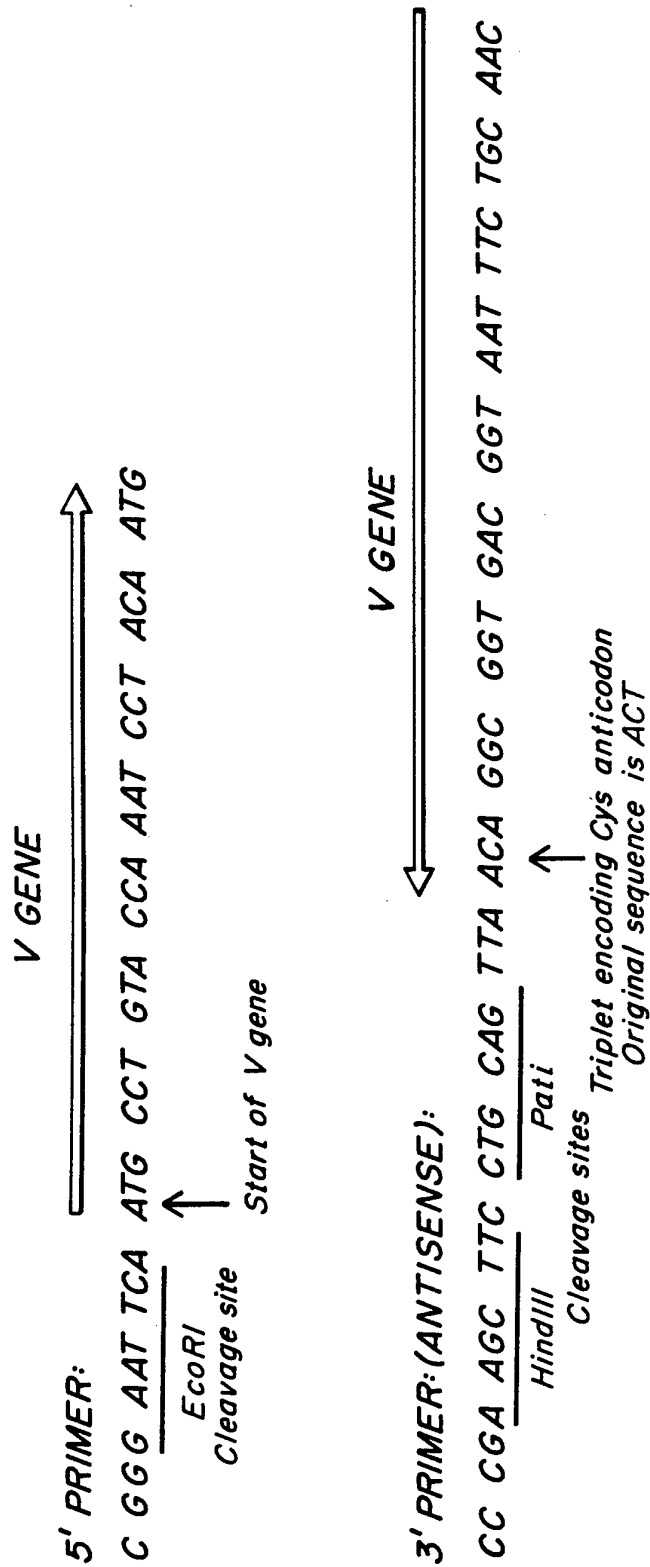


FIG. 3

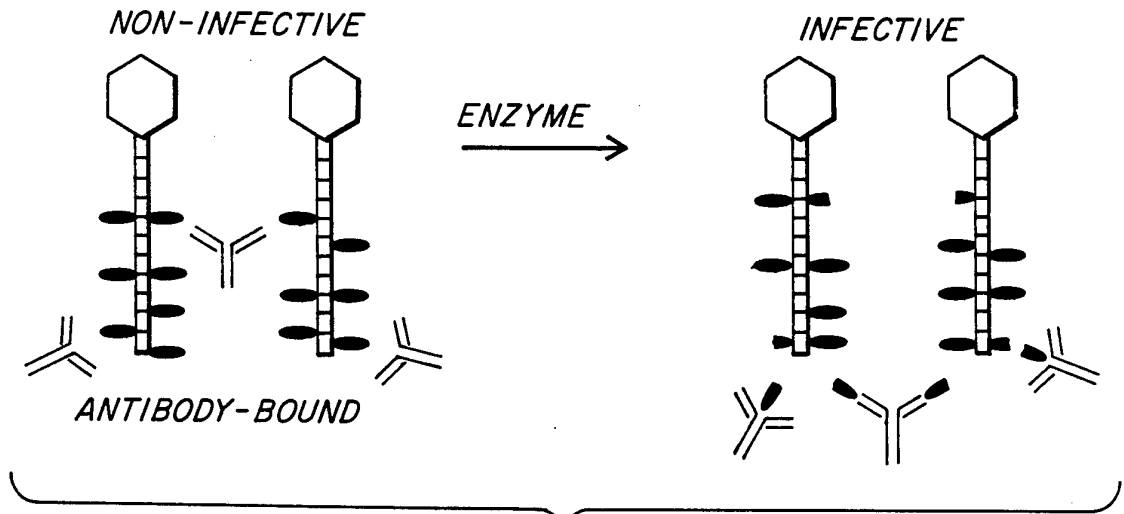


FIG. 5A

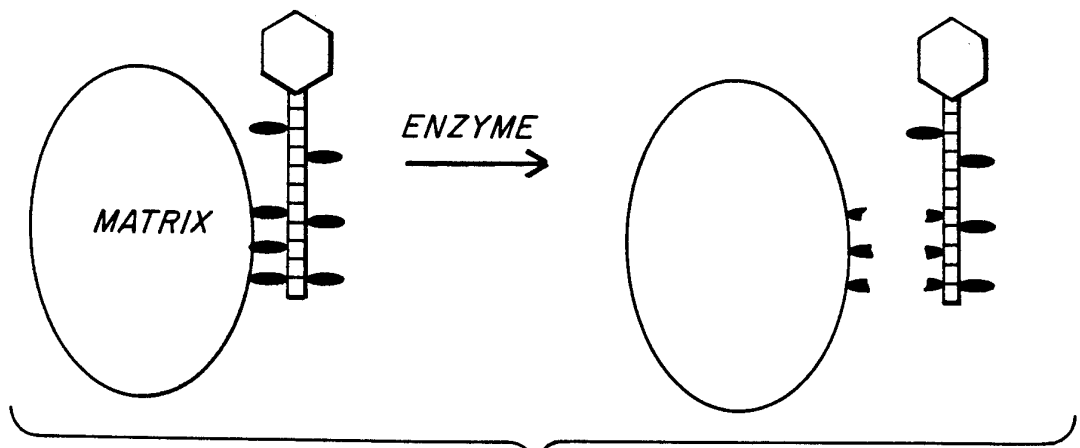


FIG. 5B

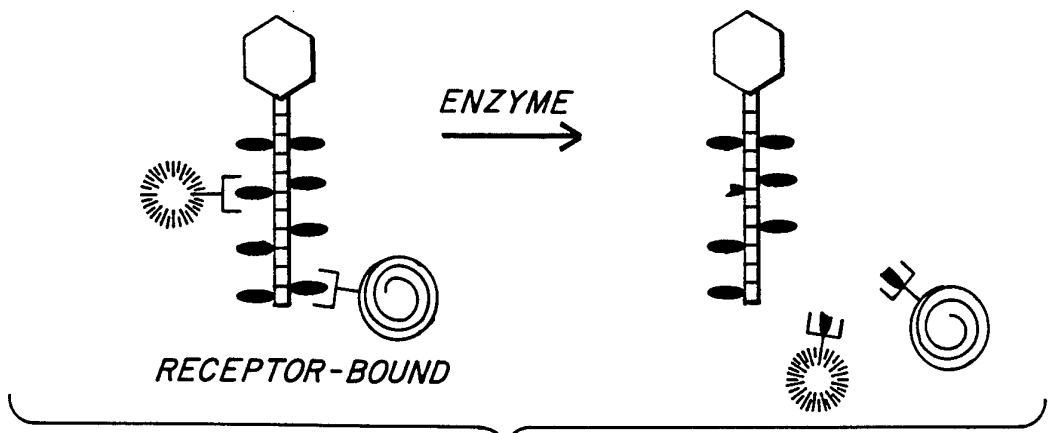


FIG. 5C

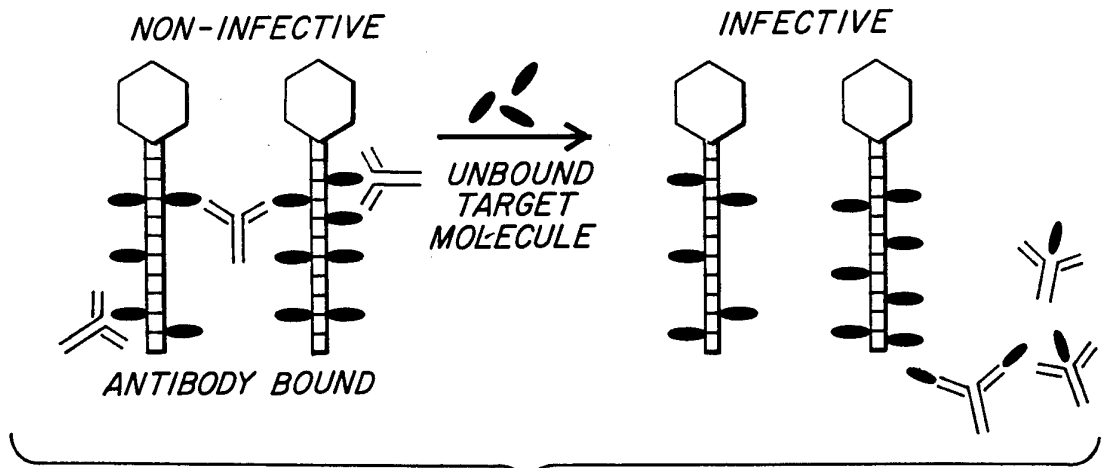


FIG. 6A

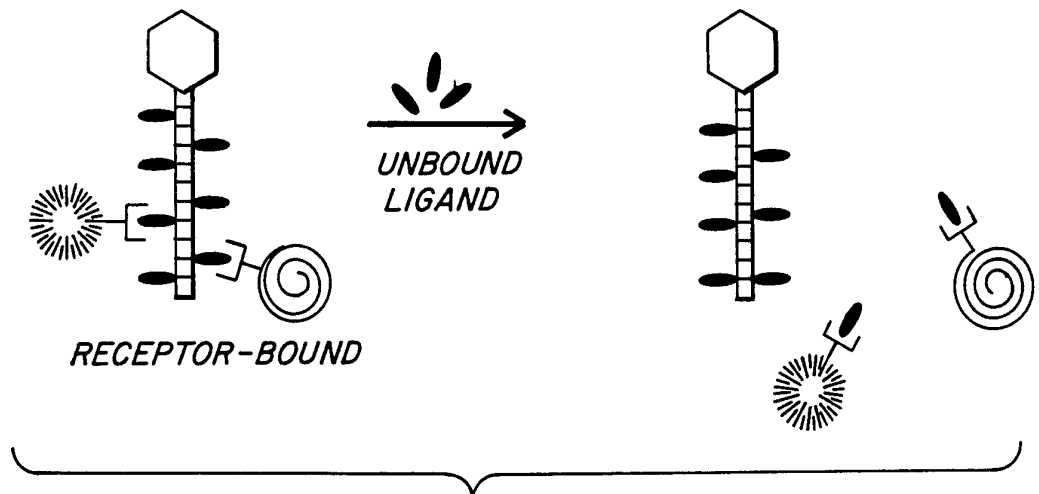


FIG. 6B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/04611

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/426; 435/7.21, 70.1, 172.1, 235, 320.1; 530/350, 351, 387, 398, 399; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, ONLINE SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. MOL. BIOL., VOLUME 146, NO. 4, ISSUED 1981, I. KATSURA, "STRUCTURE AND FUNCTION OF THE MAJOR TAIL PROTEIN OF BACTERIOPHAGE LAMBDA: MUTANTS HAVING SMALL MAJOR TAIL PROTEIN MOLECULES IN THEIR VIRION", PAGES 493-512, ESPECIALLY PAGE 493.	1-46
Y	EUR. J. BIOCHEM., VOLUME 17, ISSUED 1970, E. HURWITZ ET AL., "A SENSITIVE TECHNIQUE FOR DETECTING AND ESTIMATING THE PEPTIDE HORMONE ANGIOTENSIN-II-BETA-AMIDE AND ITS ANTIBODIES BY USING CHEMICALLY MODIFIED BACTERIOPHAGE AND ACTIVATED SEPHAROSE", PAGES 273-277, ESPECIALLY PAGE 273.	1-46

 Further documents are listed in the continuation of Box C.
 See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

08 JUNE 1994

Date of mailing of the international search report

JUL 15 1994

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/04611

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. MOL. BIOL., VOLUME 162, NO. 4, ISSUED 25 DECEMBER 1982, F. SANGER ET AL., "NUCLEOTIDE SEQUENCE OF BACTERIOPHAGE LAMBDA DNA", PAGES 729-773,SEE ENTIRE DOCUMENT.	1-46

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/04611

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

A61F 2/00; A61K 35/14, 37/24, 37/36, 37/38; C07H 17/00; C07K 3/00, 13/00, 15/00, 17/00; C12N 7/00, 15/00;
C12P 21/02; C12Q 1/00

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/426; 435/7.21, 70.1, 172.1, 235, 320.1; 530/350, 351, 387, 398, 399; 536/23.1