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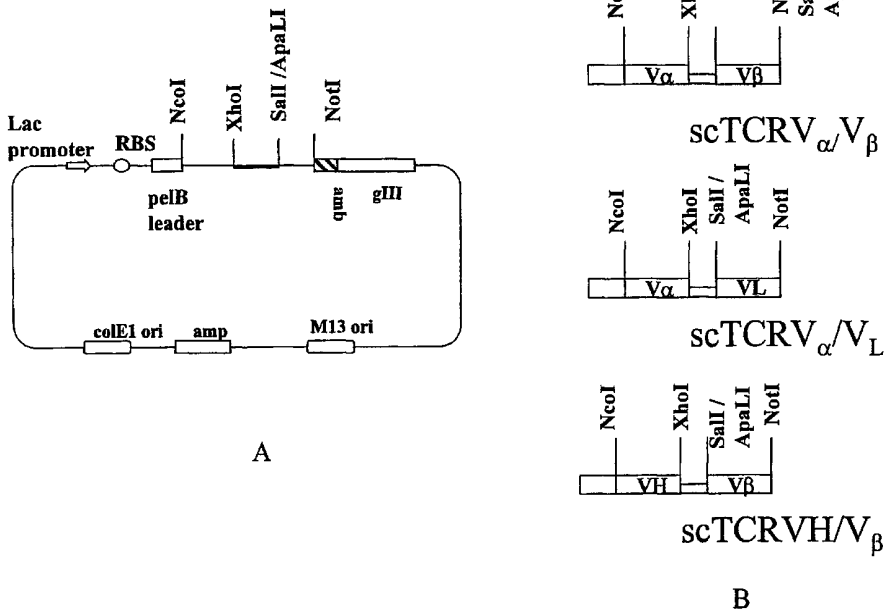
(54) Title: CHIMERIC AND TCR PHAGE DISPLAY LIBRARIES, CHIMERIC AND TCR REAGENTS AND METHODS OF USE THEREOF



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(57) Abstract: This invention provides a chimeric phage display library, TCR phage display library, recombinant chimeric reagents, methods of making the libraries, methods for identifying recombinant reagents, oligonucleotides, linkers, tags, methods of purification, methods of increasing the avidity of recombinant reagents, methods of diagnosing and treating a subject with a disease or a pathogenic condition.

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CHIMERIC AND TCR PHAGE DISPLAY LIBRARIES, CHIMERIC AND TCR REAGENTS AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

This invention provides a chimeric phage display library, TCR phage display library, recombinant chimeric reagents, methods of making the libraries, methods for identifing recombinant reagents, oligonucleotides, linkers, tags, methods of purification, methods of increasing the avidity of recombinant reagents, methods of diagnosing and treating a subject with a disease or a pathogenic condition.

BACKGROUND OF THE INVENTION

The immune system of vertebrates employs two principally different strategies to specifically combat foreign infectious agents or potentially harmful cells generated within the organism: the humoral and the cellular immune response. The essence of the humoral immune response is the mass production of antibodies (Ab) directed against the pathogen (typically, this will be of foreign origin), while within a cellular immune response, cytotoxic cells are generated having the ability to recognize and destroy pathogenic cells. Each of these two arms of the immune system is essential for its normal functioning. Disturbances of the balance between the two types of immune responses in humans leads to various types of diseases which are often life-threatening.

Although Ab, even within one organism, belong to diverse classes, they share a number of important features: they are typically soluble molecules, and consist of two types of chains, heavy (H) and light (L), which are constructed from structurally similar building blocks (domains). Furthermore, each chain consists of an N-terminal variable (V) domain, and a variable number of constant (C) domains. The V domains of H and L chains are designated V_H and V_L, respectively. In an intact Ab molecule, they are in close contact and are responsible for the antigen-binding property of the Ab. However, within a

given V domain, only certain sequences are actually contacting an antigen and exhibit extreme variability, the complemantary determining regions (CDR). For each V domain, there are three CDR called CDR1, CDR2, and CDR3, respectively.

Typically, the reactivity of an Ab is characterized by two important features: specificity and affinity. Because Ab consist of at least two identical antigen-binding sites (paratopes), it is possible that the overall affinity of an Ab molecule towards an antigen with multiple identical antigenic determinants (epitopes) must be defined by the combined affinities of the paratopes engaged in binding these epitopes. These combined affinities have also been referred to as avidity. This concept is particularly important in the case of IgM molecules, where the individual paratopes interact often only with low affinity. The avidity of the interaction is, nevertheless, high because an IgM molecule has theoretically ten antigen binding sites which could engage in parallel with a suitable antigen.

In theory, these properties should make Ab extremely versatile tools for diagnostic and therapeutic approaches. However, in the course of an immune response, an enormous variety of different Ab are produced, which makes standardization and continuous production of antisera with identical properties an impossible task. This principal difficulty has been solved by the invention of monoclonal antibodies (mAb)[1]. After immunization of an animal, typically a mouse, with an antigen, the Ab-producing cells are immortalized by fusing them with a myeloma cell line. A cell line secreting an Ab of the desired specificity can then be obtained using screening and cloning techniques. It has been possible to produce mAb against a wide variety of antigens, many of them also of diagnostic and therapeutic importance. However, mAb do have disadvantages, since their production relies on prior succesfull immunization. Another difficulty relates to their origin: mAb of animal origin are immunogenic after injection into humans. Furthermore, mAb have a molecular weight of at least 150.000, making penetration into solid tumours relatively inefficient [2]. Some, but not all, of these difficulties could be circumvented if mAb of human origin were available. However, even in trasgenic producing human Ab [3],

prior immunization is absolutely essential, thus limiting the general applicability of this technology.

The display of repertoires of Ab fragments on the surface of filamentous bacteriophage offers a new way of making mAb-like reagents, bypassing myeloma hybrid technology and even immunization [4-10]. Recombinant Ab fragments of human origin have been isolated not only with specificities against both foreign and self antigens, but with specificities that are difficult to make by the conventional mAb technology. The recombinant reagents are either expressed as single chain (sc) Fv fragments (V_H and V_L joined by a suitable linker) or Fab fragments (V_H and V_L joined to their respective constant domain fragments C_H1 and C_L). These reagents are considerably smaller than Ab, likely to be less immunogenic in humans than conventional mAb, and their production does not require any immunization and is usually rapidly achieved. The recombinant reagents are initially displayed as fusion proteins with a minor coat protein of filamentous phage particles. Each phage particle carries the genetic information for the recombinant reagent that it displays on its surface. This feature allows to identify the genetic information encoding an scFv fragment exhibiting a particular specificity by selecting that phage particle which carries it from a potentially very complex phage library. Since it is also possible to express the recombinant Ab fragment without being fused to another protein, molecules can be obtained which are principally suitable for a wide range of applications, including administration *in vivo*.

The affinities of antibody fragments derived from phage display libraries of the rearranged V-genes from immunised mice appear to be comparable with conventional mAb obtained after inducing secondary immune responses. However, if phage display libraries are employed which have not been generated from immunized human donors or animals ("naive" libraries), the affinities exhibited by scFv reagents typically fall in the range 10^6 - 10^7 M^{-1}, which is characteristic of primary immune responses. Therefore, potentially suitable reagents require further diversification by random mutation or by "chain shuffling" [11-12]. However, such procedures requires the additional

step of library formation and reselection. Methods for affinity maturation *in vitro*, allowing phages to be selected from a pool of reagents with very similar affinity or dissociation kinetics, have already been described. But it is *a priori* not clear whether they will meet with success for any given target molecules, because the affinities must typically be enhanced by a factor of 100 or more.

Like Ab, TCR belong to the immunoglobulin superfamily of proteins, and their building blocks share considerable structural similarity with those of Ab. Two types of TCR exist, TCR$\alpha\beta$ and TCR$\gamma\delta$. Each of the four chains consists of an N-terminal V domain ($V_{\alpha,\beta,\gamma,\delta}$) and a C domain ($C_{\alpha,\beta,\gamma,\delta}$). Like in Ab, V domains are also characterized by CDR, which are also designated CDR1, CDR2, and CDR3. Only very few attempts to generate recombinant reagents on the basis of TCR chains have been described [34-35]. This can be attributed to the difficulty of expressing these constructs in heterologous systems, which usually leads to the formation of insoluble inclusion bodies and no soluble protein [36-37]. These difficulties have so far also precluded the construction of phage libraries displaying TCR. Such libraries could e.g. be extremely useful for the recognition of MHC molecules complexed with tumour antigen-derived peptides or viral peptides.

Phage antibody technology as described in (W092/01047) offers the ability to isolate human reagents with Ab like reactivity directly. In this application, it is demonstrated for the first time that such reagents against self-antigens can be isolated from phage libraries derived from, for example, nonimmunised sources and from libraries prepared by synthetic recombination of V-gene sequences, preferably recombination of VH with, DH and JH, and VL with JL sequences. This application shows that single libraries derived in a manner described below can act as a source of reagents directed towards both foreign and self antigens, opening up the prospect of a large, universal library to isolate antibodies or/and recombinant reagents to any molecular target. It was

disclosed in patent application WO92/01047 that antibody fragments can be displayed on the surface of bacteriophage and that they will bind antigen.

It follows from the state of the art described above that advances are required in a number of areas to generate recombinant reagents with Ab- or TCR-like reactivity and suitable affinity and specificity. This is particularly obvious in the case of reagents which are intended to be used in a clinical setting. In particular, the areas needing improvement are the following:

To this end, constructs were developed that allow the expression of TCR variable chains. Furthermore, these constructs serve the development of TCR and chimeric Ab/TCR libraries, which combine elements of both, the humoral and cellular immune responses, so that chimeric recombinant reagents containing an Ab V domain as well as a TCR V domain can easily be prepared. The diversity of the types of phage display libraries (so far only antibody based libraries) is thus greatly expanded (TCR diversity), making it more probable that reagents of high specificity and affinity against any desired target molecule can be found within one of the libraries.

SUMMARY OF THE INVENTION

This invention provides a phage-display library for screening target molecules, comprising a plurality of recombinant phages, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for a T-cell receptor (TCR) recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an immunoglobulin (Ig) recognition element, and/or a mutation and variant thereof, and in which the vector expresses a recombinant chimeric TCR recognition element/immunoglobulin recognition element reagent from each of the recombinant phages.

In one embodiment, the TCR recognition element comprises a variable fragment of the TCR, or mutant and variant thereof. The variable fragment includes but is not limited to: one or more of TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains. In another embodiment the variable TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains comprises one or more of the CDR1, CDR2 or CDR3 segments. In another embodiment the TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to Cα, Cβ1, Cβ2, Cγ or Cδ.

In another embodiment, the immunoglobulin recognition element is an antibody comprising a variable domain. The antibody comprises a heavy chain and/or a light chain. Further, the heavy chain comprises one or more heavy chain variable fragments (VH). In another embodiment the heavy chain comprises one or more C_H1 constant domains. As contemplated herein, the light chain comprises one or more light chain variable fragments (VL). In one embodiment, the light chain comprises one or more Cκ (kappa) or Cλ(lambda) domains. In another embodiment, the variable domain comprises one or more of CDR1, CDR2 or CDR3 segments.

This invention provides a phage-display library for screening target molecules, comprising a plurality of recombinant phages, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for an Fv fragment. In one embodiment, the Fv fragment is a single chain Fv (scFv) fragment. In another

embodiment the vector has a polynucleotide which codes for a Fab fragment.

Further as provided herein, the recombinant phages comprise a vector having a polynucleotide, which codes for a TCR recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an Ig recognition element, and/or a mutation and variant thereof, wherein a linker region joins the TCR recognition element, and the Ig recognition element of the reagent. The linker region comprises a nucleic acid encoding an peptide or polypeptide which is characterized as: i) aiding in folding of the domains, ii) supporting the stabilization of the intact protein construct.

Further as provided herein, the recombinant phages comprise a vector having a polynucleotide, which codes for a TCR recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an Ig recognition element, and/or a mutation and variant thereof, wherein a novel tag region joins the TCR recognition element, or the Ig recognition element of the reagent to gIII protein of the phage. The linker region comprises a nucleic acid encoding an peptide or polypeptide which is characterized as: i) aiding in protein purification and detection.

Further, in one embodiment, the vector comprises a nucleic acid which codes for a second molecule that is linked to the reagent. The second molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target . The second molecule enhances the overall avidity of the interaction of the reagent with the target molecule.

This invention provides a phage-display library for screening target molecules, comprising a plurality of recombinant phages, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for a TCR recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an Ig recognition element, and/or a mutation and variant thereof, and in which the vector expresses a recombinant chimeric TCR recognition element/immunoglobulin recognition element reagent from each of the recombinant phages, such reagents, including mutants and/or variants thereof, include but are not limited to the following: a single chain TCRVα/VL, a single chain TCRVβ/VL, a single chain TCRVα/VH, a single chain TCRVβ/VH, a single chain VL/TCRVα, a single chain VL/TCRVβ, a single chain VH/TCRVα, a single chain VH/TCRVβ, a single chain TCRVγ/VL, a single chain TCRVδ/VL, a single chain TCRVγ/VH, a single chain TCRVδ/VH, a single chain VL/TCRVγ, a single chain VL/TCRVδ, a single chain VH/TCRVγ, and/or a single chain VH/TCRVδ, or mutants and/or variants thereof.

This invention provides a phage-display library for screening for target molecules, comprising a plurality of recombinant phages, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for a TCR recognition element, and/or a mutation and variant thereof; and in which the vector expresses a recombinant TCR recognition element from each of the recombinant phages.

In one embodiment the TCR recognition element comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not limited to: one or more of TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains. In another embodiment the variable TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ),

or TCR variable δ (TCRVδ) domains comprises one or more of the CDR1, CDR2 or CDR3 segments. In another embodiment the TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to Cα, Cβ1, Cβ2, Cγ or Cδ.

Such phage displayed reagents include but are not limited to the following: a single chain TCRVα/TCRVα, a single chain TCRVβ/TCRVβ, a single chain TCRVγ/TCRVγ, a single chain TCRVδ/TCRVδ, a single chain TCRVα/TCRVβ, a single chain TCRVα/TCRVγ, a single chain TCRVα/TCRVδ , a single chain TCRVβ/TCRVα , a single chain TCRVβ/TCRVγ, a single chain TCRVβ/TCRVδ, a single chain TCRVγ/TCRVα, a single chain TCRVγ/TCRVβ, a single chain TCRVγ/TCRVδ, a single chain TCRVδ/TCRVα, a single chain TCRVδ/TCRVβ, a single chain TCRVδ/TCRVγ and/or a mutation and variant thereof.

Further, in one embodiment the vector comprises a nucleic acid which codes for a second molecule that is linked to the reagent. The second molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target . The second

molecule enhances the overall avidity of the interaction of the reagent with the target.

This invention provides a soluble recombinant chimeric TCR recognition element/Ig recognition element reagent.

In one embodiment the TCR recognition element comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not limited to: one or more of TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains. In another embodiment the variable TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains comprises one or more of the CDR1, CDR2 or CDR3 segments. In another embodiment the TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to Cα, Cβ1, Cβ2, Cγ or Cδ.

In another embodiment the immunoglobulin recognition element is an antibody comprising a variable domain. The antibody comprises a heavy chain and/or a light chain. Further, the heavy chain comprises one or more heavy chain variable fragments (VH). In another embodiment the heavy chain comprises one or more C_H1 constant domains. As contemplated herein, the light chain comprises one or more light chain variable fragments (VL). In one embodiment, the light chain comprises one or more Cκ (kappa) or Cλ(lambda) domains. In another embodiment, the variable domain comprises one or more of the CDR1, CDR2 or CDR3 segments.

This invention provides a reagent which comprises a single chain Fv fragment. In another embodiment, the reagent comprises a Fab fragment

Further, as provided herein, the reagent has a linker region which comprises a nucleic acid encoding a peptide or polypeptide characterized as: i) aiding in folding of the domains, ii) supporting the stabilization of the intact protein construct. The nucleic acid sequence of the linker region is set forth in Figure 1.

Further, as provided herein, the reagent has a tag region which comprises a nucleic acid encoding a peptide or polypeptide characterized as: i) aiding in protein purification and detection. The nucleic acid sequence of the tag region is set forth in Figure 1.

Further, in one embodiment, the reagent comprises a second molecule that is linked to the reagent. The second molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target . The second molecule enhances the overall avidity of the interaction of the reagent with the target molecule or a multimeric target.

For example, the second molecule includes but is not limited to: a molecule with antibody or TCR like reactivity, a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or inorganic substance, ion, synthetic, or mimetic, thereof. An example, for a bispecific molecule contemplated herein is a reagent directed against a specific MHC/peptide complex coupled to CD8, or variant

thereof which exhibits low affinity to their respective target.

This invention provides a reagent which comprises the following soluble chimeric polypeptides: a single chain TCRVα/VL, a single chain TCRVβ/VL, a single chain TCRVα/VH, a single chain TCRVβ/VH, a single chain VL/TCRVα, a single chain VL/TCRVβ, a single chain VH/TCRVα, VH/TCRVβ, a single chain TCRVγ/VL, a single chain TCRVδ/VL, a single chain TCRVγ/VH, a single chain TCRVδ/VH, a single chain VL/TCRVγ, a single chain VL/TCRVδ, a single VH/TCRVγ, and/or a single chain VH/TCRVδ, or mutants and/or variants thereof.

The nucleic acids, which code for the chimeric reagents are discussed above.

This invention provides a soluble recombinant TCR recognition element reagent. In one embodiment the TCR recognition element comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not limited to: one or more of TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains. In another embodiment the variable TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains comprises one or more of the CDR1, CDR2 or CDR3 segments. In another embodiment the TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to Cα, Cβ1, Cβ2, Cγ or Cδ.

This invention provides a reagent which comprises the following soluble TCR polypeptides: See before a single chain TCRVα/TCRVα, a single chain TCRVβ/TCRVβ, a single chain TCRVγ/TCRVγ, a single chain TCRVδ/TCRVδ, a single chain TCRVα/TCRVβ, a single chain TCRVα/TCRVγ, a single chain TCRVα/TCRVδ , a single chain TCRVβ/TCRVα , a single chain TCRVβ/TCRVγ, a single chain TCRVβ/TCRVδ, a single chain TCRVγ/TCRVα, a single chain TCRVγ/TCRVβ, a single chain TCRVγ/TCRVδ, a single chain TCRVδ/TCRVα, a single chain TCRVδ/TCRVβ, a single chain TCRVδ/TCRVγ and/or a mutation and variant thereof.

This invention provides an expression vector comprising a polynucleotide which codes for a TCR recognition element, and/or a mutation and variant thereof; and polynuclotide which codes for an Ig recognition element, and/or a mutation and variant thereof. This invention provides a vector comprising a polynucleotide encoding the TCR and the Ig elements, fragments, domains and/or segments in a tail-to-head transcriptional orientation.

In one embodiment, the vector comprises a polynucleotide which encodes a TCR recognition element having a variable fragment of the TCR, mutant and variant thereof. The polynucleotide encoding the variable fragment includes but is not limited to: one or more of TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains. In another embodiment the polynucleotide encodes one or more of the CDR1, CDR2 or CDR3 segments of the variable domain. In another embodiment the vector comprises a polynucleotide which

encodes a TCR recognition element having a constant fragment. The polynucleotide encodes a constant fragment of the TCR which includes but is not limited to: to Cα, Cβ1, Cβ2, Cγ or Cδ.

In another embodiment, the vector comprises a polynucleotide which encodes a variable domain of an antibody. In another embodiment, the vector comprises a polynucleotide which codes for a heavy chain and/or a light chain of the antobody. In another embodiment the vector comprises a polynucleotide which codes for one or more VH fragments. In another embodiment the vector comprises a polynucleotide which codes for one or more C_H1 constant domains of the variable fragment. In another embodiment the vector comprises a polynucleotide which codes for one or more VL fragments. In another embodiment the vector comprises a polynucleotide which codes for one or more Cκ (kappa) or Cλ(lambda). In another embodiment, the vector comprises a polynucleotide which codes for one or more of CDR1, CDR2 or CDR3 segments of the variable segment.

Further, this invention provides a vector comprising a polynucleotide which codes for an Fv fragment. In one embodiment the Fv fragment is a scFv fragment. In another embodiment, the vector has a polynucleotide which codes for a Fab fragment

Further as provided herein, the vector comprises a polynucleotide which codes for a T-cell receptor (TCR) recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an immunoglobulin (Ig) recognition element, and/or a mutation and variant thereof; and a polynucleotide of a

linker region which when expressed joins the T-cell receptor (TCR) recognition element, and the Ig recognition element of the reagent. The polynucleotide of the linker region comprises a nucleic acid having a sequence as set forth in Fig.1.

Further, as provided herein, the reagent has a tag region which comprises a nucleic acid encoding a peptide or polypeptide characterized as: i) aiding in protein purification and detection. The nucleic acid sequence of the tag region is set forth in Figure 1.

Further, in one embodiment of the invention, the vector comprises a polynucleotide which codes for a second molecule that is linked to the reagent. The second molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target . The second molecule enhances the overall avidity of the interaction of the reagent with the target molecule or a multimeric target.

For example, the second molecule includes but is not limited to: a molecule with Ab-or TCR like reactivity, a nucleic acide, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or inorganic substance, ion, synthetic, or mimetic, thereof. An example, for a bispecific molecule contemplated herein is a reagent directed against a specific MHC/peptide complex coupled to CD8, or variant thereof which exhibits low affinity to their respective target.

This invention provides a vector comprising a polynucleotide which codes for TCR recognition element,

and/or a mutation and variant thereof; and a polynucleotide which codes for an Ig recognition element, and/or a mutation and variant thereof, and in which the vector expresses a recombinant chimeric TCR recognition element/Ig recognition element reagent from each of the recombinant phages, such vectors comprise a polynucleotide encoding: a single chain TCRVα/VL, a single chain TCRVβ/VL, a single chain TCRVα/VH, a single chain TCRVβ/VH, a single chain VL/TCRVα, a single chain VL/TCRVβ, a single chain VH/TCRVα, VH/TCRVβ, a single chain TCRVγ/VL, a single chain TCRVδ/VL, a single chain TCRVγ/VH, a single chain TCRVδ/VH, a single chain VL/TCRVγ, a single chain VL/TCRVδ, a single VH/TCRVγ, and/or a single chain VH/TCRVδ, or mutants and/or variants thereof.

This invention provides a method for creating a phage display chimeric TCR/Ig reagent comprising the steps of: obtaining a sample of cells; preparing mRNA of the cells, reverse transcribing mRNA of the cell population into cDNA sequences of TCR and Ig; amplifying the cDNA; cloning the population of DNA fragments into expression vectors; providing nucleic acid expression vectors which are capable of being packaged; combining (i) a genetically diverse repertoire of nucleic acid sequences in which each encode a unique or genetically diverse population first component part of the TCR elements with (ii) a genetically diverse repertoire of nucleic acid sequences which encodes a unique or genetically diverse population of the immunoglobulin elements, to form a library of nucleic acid sequences using said expression vectors encoding said TCR and Ab polypeptides; also with

the property of binding specifically to a target molecule of interest; expressing said library from said vectors in recombinant host organism cells, each of the said polypeptide chain components being expressed as a recombinant chimeric protein on its own or as part of phage particles which are components of the library; selecting from said expressed library by binding to a target molecule of interest said reagents binding specificifically to the target molecule, thereby producing a recombinant chimeric TCR /Ig reagent.

This invention provides a method for creating a phage display TCR reagent comprising the steps of: obtaining a sample of cells; preparing mRNA of cells; reverse transcribing mRNA of the cell population into cDNA sequences of T-cell receptor; amplifying the cDNA; cloning the population of DNA fragments into expression vectors; providing nucleic acid expression vectors which are capable of being packaged; combining a genetically diverse repertoire of nucleic acid sequences in which each encode a unique or genetically diverse population of the TCR elements, to form a library of nucleic acid sequences using said expression vectors encoding said TCR polypeptide; also with the property of binding specifically to a target molecule of interest; expressing said library from said vectors in recombinant host organism cells, said polypeptide chain components being expressed as a recombinant TCR protein on its own or or as part of phage particles which are components of the library; selecting from said expressed library by binding to a target molecule of interest said reagents binding specificifically to the target molecule, thereby producing a recombinant TCR reagent.

This invention provides a method for selecting recombinant reagents directed against a molecular target, said method comprising: contacting the phage display chimeric library or the phage display TCR library as hereinabove described, with the target molecule so as to form a complex, dissociating the specifically bound phage from the complex; amplifying the rescued phage in a bacterial host; repeating binding, dissociation and amplification steps; screening said library on a target molecule. In another embodiment, the method further comprises characterizing the the selected phage particles and their respective reagents.

The target is any composition: molecule, a complex, a nucleic acid sequence, a polypeptide, peptide fragment or any composition that can be assayed for its ability to function in given capacity or compound. The target molecule perhaps synthetic, recombinant or biological sample.

This invention provides a method for diagnosing a subject with a pathogenic condition for e.g. a malignant disease comprising the steps of: a)obtaining a sample from the subject, b) contacting the sample with a recombinant reagent as discussed above, wherein the reagent is specific for a specific target molecule so as to form a complex, c) detecting the complex, the presence of which is indicative of the subject having the disorder.

This invention provides use of a pharmaceutical composition comprising the reagent as described above, for the prevention or treatment of an infectious or autoimmune diseases, selected from the group consisting

of ankylosing spondylitis, Reiter's disease, psoriatic spondylitis, psoriasis vulgaris and Behcet disease, and rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, systemic lupus erythematosus, Sjogren's disease, IDDM, Addison disease, Graves disease, Hashimoto disease, coeliac disease, primary biliary cirrhosis, pemphigus vulgaris, epidermolysis bullosa acquisita, Hodgkin's disease, multiple sclerosis, optic neuritis, narcolepsy, myasthenia gravis, Goodpasture syndrome and alopecia areata.

This invention provides a method of treating a subject with a disease or a pathogenic conditions, comprising administering to the subject an effective amount of the reagent as described above, thereby treating the subject with the disease or pathogenic condition.

This invention provides a method for imaging a pathological condition for e.g. a neoplastic disorder in a subject comprising the steps of administering to the subject an amount of the recombinant reagent as described above, wherein the reagent is labeled, and detecting the label.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A. The phagmid vector used for cloning the libraries. It contains M13 origin of replication and plasmid origin of replication (M13 and colE1, respectively), ribosom binding site (RBS), hatch box includes six amino acides from 5'end of C domain, His and myc tags flanked by amber stop codon (amb), followed by DNA encoding the gIII protein of the bacteriophage. The expression is under the control of LacZ promotor. The linker between XhoI and SalI/ApaLI contains eight amino acids from the 5'end of the C domain followed by the classical (Gly4Ser)3 linker.

Figure 1B. The various assembly combinations of the current construct libraries are listed.

Figure 2: The primers used for characterization are indicated

Figure 3A. Representative Phage ELISA of TCRVα /V_L clones. Phage supermatants were detected by: Anti-M13 (Rows A,B), Protein A-HRP (C,D) and Protein L-HRP (E,F). The figure depicts the gray scale analysis of ELISA OD450-650.

Figure 3B. Representative Phage ELISA of TCRVH/Vβ clones. Phage supernatants were detected by: Anti-M13 (rows A,B), Protein A-HRP (C,D) and Protein L-HRP (E,F). The figure depicts the gray scale analysis of ELISA OD450-650. Column 7 is positive control from the Tomlison library containing DP47 (VH3) and DPK12.

Figure 3C. ELISA analysis of soluble single chain chimeric TCR clones. Number indicated ELISA OD450-650. Periplasmic extract prepared from HB2151 infected clones were detected by Protein A-HRP and Protein L-HRP.

Figure 4. Mutations in TCR molecule.

Figure 5: The linker and tag sequences and position in the phagmid vector is indicated

Figure 6: Nucleic sequence of the oligonucletoides used to amplify the TCRVα gene segments. Degenerated nucleotides are underline, and NcoI and NotI restriction site are in bold. M= A/C, V= A/G/C, R= A/G, H= A/C/T, W= A/T, D= A/G/T, S= G/C, B= G/C/T, Y= C/T, N=A/C/G/T, K= G/T. TCRVα genes in brackets indicates that these similar segments can also be amplified by the indicated degenerate primer. Cα-For-XhoI is the 3' oligonucleotide that aneal to the 5'end of the Cα gene segment.

Figure 7. Nucleic sequence of the primers used to amplify the TCRVβ genes. Degenerated nucleotides are underline. SalI and NotI restriction sites are indicated by bold letters. M= A/C, V= A/G/C, R= A/G, H= A/C/T, W= A/T, D= A/G/T, S= G/C, B= G/C/T, Y= C/T, N=A/C/G/T, K= G/T. TCRV genes that can be amplified by the same degenerated primers are listed in brackets. The 3' primers were designed to specifically aneal to Cβ1 and Cβ2 gene segment of TCR.

Figure 8. Sample sequencing of the various libraries.

Figure 9. Sequence analysis of clones originated from TG-1 bacteria infected by phage.

Figure 10. Fig10 shows sequence sampling of some of these selections.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a chimeric phage display library, TCR phage display library, recombinant chimeric reagents, methods of making the libraries methods for identifing recombinant reagents, oligonucleotides, linkers, tags, methods of purification, methods of increasing the avidity of recombinant reagents, methods of diagnosing and treating a subject with a disease or a pathogenic condition.

The scTCR and scTCR-Ig molecules as provided herein are extremely versatile, both for diagnosis as well as for treatment of various diseases, and will substantially complement the battery of dignostically and therapeutically applicable reagents. Furthermore, scTCR and scTCR-Ig molecules have an important application as diagnostic reagents for other pathological conditions like CD8, like urine or serum, since they are very important diagnostic markers e.g. in transplantation (transplant rejection) or for some diseases (e.g. nephrological problems), respectively. Moreover, use of such reagents could help e.g. to study the expression of peptide-devoid or peptide-filled HLA molecules in different lymphoid organs and cell subsets, since, the

mechanisms governing positive selection of T cell are up till now not understood.

Further, this invention provides therapy of malignant diseases using labeled or unlabeled constructs, eradication of infections by eliminating the infectious agents with labeled or unlabeled constructs, treatment of autoimmune diseases, and detection of molecules within cells.

I. Chimeric phage-display and TCR phage-display libraries

This invention provides chimeric phage-display, comprising a plurality of recombinant phages, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for a TCR recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an Ig recognition element, and/or a mutation and variant thereof, and in which the vector expresses a recombinant chimeric TCR recognition element/immunoglobulin recognition element reagent from each of the recombinant phages.

This invention provides TCR phage-display libraries, comprising a plurality of recombinant phages, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for a TCR recognition element, and/or a mutation and variant thereof; and in which the vector expresses a recombinant TCR recognition element from each of the recombinant phages.

The "Ig recognition element" includes heavy chain and light chain varibale domain (Ig-VH and Ig-VL) and natural or partly or wholly synthetically produced protein. The term also covers any protein having a binding domain

which is homologous to an immunoglobulin binding domain. These proteins can be derived from natural sources, or partly or wholly synthetically produced "Ig homologs" in this application include members of the Ig superfamily and homologs thereof. "Homolog" means that the polypeptides having the same or conserved residues at a corresponding position in their primary, secondary or tertiary structure. The term also extends to two or more nucleotide sequences encoding the homologous polypeptides.

A "domain" is a part of a protein that is folded within itself and independently of other parts of the same protein and independently of a complementary binding member.

As defined herein, a "library" is a collection of nucleotide sequences, e.g. DNA, within clones; or a genetically diverse collection of polypeptides, or specific binding pair members, or polypeptides displayed on phages capable of selection or screening to provide an individual polypeptide or a mixed population of polypeptides.

The "light chains" of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (K) and lambda (λ), based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, antibodies can be assigned to different classes of which there are five major classes: IgA, IgD, IgE, IgG and IgM. Several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, and IgG4; IgA1 and IgA2. The term "antibody

and antibody fragments" is used in the present specification and claims in the broadest sense and specifically covers single monoclonal antibodies as well as antibody fragments (e.g., Fab, F(ab')2, Fv and scFv), as long as they specifically recognize a molecular target. The invention thus relates to methods according to the invention wherein the antibody fragment is selected from the group consisting of Fab, F(ab')2, Fv, scFv and other molecular target-binding subsequences of an Ab.

This invention provides "mutants, variants and homologs" of the TCR and immunoglobulin polypeptides. Homolog means a polypeptides having the same or conserved residues at a corresponding position in their primary, secondary or tertiary structure. The term also extends to two or more nucleotide sequences encoding the homologous polypeptides. Examples for homologous peptides are the Ig isotypes. As contemplated herein, the TCR and/or Ig includes Ab or polypeptide components thereof, this is referring not only to diversity that can exist in the natural population of cells or organisms, but also diversity that can be created by artificial mutation *in vitro* or *in vivo*.

The polynucleotide comprises or encodes the Ig and the TCR includes RNA, cDNA, genomic DNA, fragments, isoforms, variants, mutants, alleles, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those skilled in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates,

etc.), charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids, etc.). Also included are synthetic molecules that mimic nucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule. Molecules substantially homologous to primary structural sequence but which include, e.g., *in vivo* or *in vitro* chemical and biochemical modifications or which incorporate unusual amino acids. The nucleic acid may be modified. Such modifications include, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquitinylation, labeling, e.g., with radionuclides, and various enzymatic modifications, as will be readily appreciated by those well skilled in the art.

Mutations can be introduced into a polynucleotide such that a particular codon is changed to a codon which codes for a different amino acid but the function is maintained. Such a mutation is generally made by making the fewest nucleotide changes possible. A substitution mutation of this sort can be made to change an amino acid in the resulting protein in a non-conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to another grouping) or in a conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to another amino acid belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting protein. A non-conservative change is more likely to alter the structure, activity or function of the resulting protein. The present invention should be considered to include sequences containing conservative changes which do not significantly alter the activity or binding characteristics of the resulting protein. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid

belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. Amino acids containing aromatic ring structures are phenylalanine, tryptophan, and tyrosine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such alterations will not be expected to affect apparent molecular weight as determined by polyacrylamide gel electrophoresis, or isoelectric point.

Particularly preferred substitutions are:
- Lys for Arg and vice versa such that a positive charge may be maintained;
- Glu for Asp and vice versa such that a negative charge may be maintained;
- Ser for Thr such that a free - hydroxyle group can be maintained; and
- Gln for Asn such that a free amino group can be maintained.

Synthetic DNA sequences allow convenient construction of genes which will express analogs or "muteins". A general method for site-specific incorporation of unnatural amino acids into proteins is described [Noren, et al. *Science*, 244:182-188 (April 1989)]. This method may be used to create analogs with unnatural amino acids.

A "nucleic acid" or "polynucleotide" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules") in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA

molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (*i.e.*, the strand having a sequence homologous to a mRNA). A "recombinant DNA" is a DNA that has undergone a molecular biological manipulation.

"Substantial identity" or "substantial sequence identity" mean that two sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap which share at least 65-99 percent sequence identity, share at least 75 percent sequence identity, share at least 80 percent sequence identity, share at least 90 percent sequence identity, preferably at least 95 percent sequence identity, more preferably at least 99 percent sequence identity or more. The following terms are used to describe the sequence relationships between two or more nucleic acid molecules or polynucleotides: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing or may comprise a complete cDNA or gene sequence.

Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman (1981) *Adv. Appl. Math.* 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. (USA)* 85:2444, or by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, WI).

Variant(s), as the term is used herein, are polynucleotides or polypeptides that differ from a reference polynucleotide or polypeptide respectively. Variants in this sense are described below and elsewhere in the present disclosure in greater detail. (1) A polynucleotide that differs in nucleotide

sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may be silent, i.e., they may not alter the amino acids encoded by the polynucleotide. Where alterations are limited to silent changes of this type a variant will encode a polypeptide with the same amino acid sequence as the reference polypeptide. Changes in the nucleotide sequence of the variant may alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Such nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. (2) A polypeptide that differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference and the variant are closely similar overall and, in many region, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions and truncations, which may be present in any combination. (3) A variant may also be a fragment of a polynucleotide or polypeptide of the invention that differs from a reference polynucleotide or polypeptide sequence by being shorter than the reference sequence, such as by a terminal or internal deletion. A variant of a polypeptide of the invention also includes a polypeptide which retains essentially the same biological function or activity as such polypeptide, e.g., proproteins which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide. (4) A variant may also be (i) one in which one or more of the amino acid residues are substituted with a conserved or non- conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. (5) A variant of the polynucleotide or polypeptide may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that

is not known to occur naturally. Such non-naturally occurring variants of the polynucleotide may be made by organic chemical synthesis, by mutagenesis techniques, including those applied to polynucleotides, cells or organisms, or may be made by recombinant means. Among polynucleotide variants in this regard are variants that differ from the aforementioned polynucleotides by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or non- coding regions or both. Alterations in the coding regions may produce conservative or non- conservative amino acid substitutions, deletions or additions. All such variants defined above are deemed to be within the scope of those skilled in the art from the teachings herein and from the art.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math., 4& 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package

(Devereux, J., et al., Nucleic Acids Research 12(I): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., J. Molec. Biol. 215: 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., J. Mol. Biol. 215: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity. Preferred parameters for polypeptide sequence comparison include the following:

1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970) Comparison matrix: matches = + 10, mismatch = 0 Gap Penalty: 50 Gap Length Penalty: 3 Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons. Preferred polymicleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to the reference sequence oas set forth in Figures 5, 6, and/or 7 or may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides as set forth in Figures 5,6, and/or 7 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides; in Figures 5,6, and/or 7, or: nn xn - (xn y), wherein nn is the number of nucleotide alterations, xn is the total number of nucleotides in SEQ ID NO: 1, y is 0.50 for 50 %, 0.60 for 60 %, 0.70 for 70 %, 0.80 for 80 %, 0.85 for 85 %, 0. 90 for 90 %, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and is the symbol for the multiplication operator, and wherein any non-integer product of xn and y is rounded down to the nearest integer prior to subtracting it from xn, Alterations of a polynucleotide sequence encoding the polypeptide may create

nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polymicleotide following such alterations.

The term "variable" refers to the fact that certain portions of the variable domains of Ig or TCR differ extensively in sequence among Ab or TCR and are used in the binding and specificity of each particular Ab or TCR for its particular molecular target. The variability is concentrated in three segments called complementarity determining regions (CDR) or hypervariable regions both in the light chain and the heavy chain variable domains (Ig or TCRα,or β,or γ,or δ).

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, "Molecular Cloning: A Laboratory Manual" (1989); "Current Protocols in Molecular Biology" Volumes I-III [Ausubel, R. M., ed. (1994)]; "Cell Biology: A Laboratory Handbook" Volumes I-III [J. E. Celis, ed. (1994))]; "Current Protocols in Immunology" Volumes I-III [Coligan, J. E., ed. (1994)]; "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription and Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

A "phage-display library" is a protein expression library, constructed in vectors, that expresses a

collection of cloned protein sequences as fusion with a phage coat protein. Thus, in the context of the invention, single-chain recombinant proteins having ligand-binding potential are expressed as fusion proteins on the exterior of the phage particle. This disposition advantageously allows contact and binding between the recombinant binding protein and a ligand. Those having ordinary skill in the art will recognize that phage clones expressing binding proteins specific for the ligand can be substantially enriched by serial rounds of phage binding to the ligand, especially when this is imobilised, dissociated from the ligand and amplified by growth of rescued phage in bacterial host cells. The phage may be a filamentous phage. The host may be E.coli.

As provided herein, "phage" may be a replicable genetic display package in which the particle is displaying a member of a specific binding pair at its surface. The package may be a bacteriophage which displays a binding domain at its surface. As provided herein, the binding domain on the surface is a chimeric TCR/Ig and/or a TCR and includes domains, fragments, subdomains, which may be connected by a synthetic linker.

"TCR recognition element" comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not limited to: one or more of TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains. In one embodiment, the TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains comprise one or more of the CDR1, CDR2 or CDR3 segments. In another embodiment, the TCR

recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to Cα, Cβ1, Cβ2, Cγ or Cδ.

"The Ig recognition element" is an Ab. The antibody comprises a heavy chain and/or a light chain. Further, the heavy chain comprises one or more heavy chain variable fragments (VH). In another embodiment, the heavy chain comprises one or more C_H1 constant domains. As contemplated herein, the light chain comprises one or more light chain variable fragments (VL). In one embodiment, the light chain comprises one or more Cκ (kappa) or Cλ (lambda) domains. In another embodiment, the variable domain comprises one or more of CDR1, CDR2 or CDR3 segments.

This invention provides a phage-display library for screening target molecules, comprising a plurality of recombinant phage, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for an Fv fragment. In one embodiment, the Fv fragment is a single chain Fv fragment. In another embodiment, the vector includes a polynucleotide which codes for a Fab fragment

Further as provided herein, the recombinant phages comprise a vector having a polynucleotide which codes for a TCR recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an Ig recognition element, and/or a mutation and variant thereof, wherein a linker region joins the TCR recognition element, and the Ig recognition element of the reagent. The linker region comprises a nucleic acid which is characterized as: i) aiding in folding of the

domains, ii) supporting the stabilization of the intact protein construct; wherein said nucleic acid has a sequence as set forth in Figure 1. Based on the information provided herein, one skilled in the art can make other linker sequences which aid in folding of the domains, and support the stabilization of the intact protein construct. In another embodiment, the linker comprises a nucleic acid having the sequence as set forth in Figure 5, including mutations, homologs, and variants thereof.

As shown herein, the novel linkers which were developed aid in folding of the domains, and support the stabilization of the intact protein construct, allowing for the first time the expression of scTCR protein constructs consisting only of two TCR V-domains on the surface of phage particles from a phage display library. The linkers contain a small number of the N-terminal amino acids of a constant TCR domains followed by the conventional linker sequence $(Gly_4Ser)_3$. The novel linker has the additional advantage that the corresponding DNA sequence is complementary to the two primers allowing universal amplification of the respective TCR gene.

Furthermore, the novel linker provides a means to purify and detect the recombinant protein in an entirely novel way. The junctional regions between the C-terminal $(Gly_4Ser)_3$ and any of the two N-terminally located TCR C-domain-derived peptides provide novel epitopes for reagents like Ab, which can recognize them with high specificity and affinity. Such reagents are not expected to react with TCR molecules on T cells, since these do not contain the junctional region between the peptides. The linkers should also be extremely useful tools to detect phage particles carrying recombinant proteins. Finally, it is contemplated herein, that the TCR C-domain-derived sequences are combined not only with the conventional $(Gly_4Ser)_3$ linker, but with other linking sequences as well, e.g. to provide recognition elements for effector molecules.

Further as provided herein, the novel tag which were developed contain a small number of the N-terminal amino acids of a constant TCR or Ig domains followed by the conventional his myc tags. The novel tag has the additional advantage that the corresponding DNA sequence is complementary to the two primers allowing universal amplification of the respective TCR or Ig gene. The tag should also be extremely useful tools to detect phage particles carrying recombinant proteins. Finally, it is contemplated herein, that the TCR C-domain-derived sequences are combined not only with the conventional his myc tags, but with other sequences as well, e.g. to provide elements for effector molecules.

The production of recombinant reagents against a particular MHC/peptide complex is provided herein. This interaction could be stabilized by linking another molecule with reactivity towards invariant parts of the MHC class I molecule e.g. CD8, to the first reagent, thereby increasing the avidity. However, care would have to exercised to prevent reactivity of these reagents on their own, because this would lead to the recognition of all HLA class I molecules by the construct. Therefore, the CD8 molecule would have to be mutated as to reduce its affinity towards MHC class I molecules. Since the structure of an HLA class I/CD8 complex has been solved [56], the interacting amino acids are precisely known, and decreasing the affinity of the interaction by site-directed mutagenesis will be simple.

For example, the the second molecule includes but is not limited to: a molecule with antibody or TCR like reactivity, a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or inorganic substance, ion, synthetic, or mimetic, thereof. An example, for a bispecific molecule contemplated herein is a reagent directed against a specific MHC/peptide complex coupled to CD8, or variant

thereof which exhibits low affinity to their respective target.

A. Phage Display Libraries

This invention provides a phage-display library for screening target molecules, comprising a plurality of recombinant phages, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for a TCR recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an (Ig) recognition element, and/or a mutation and variant thereof, and in which the vector expresses a recombinant chimeric TCR recognition element/Ig recognition element reagent from each of the recombinant phages, such reagents, including mutants and/or variants thereof, include but are not limited to the following: a single chain TCRVα/VL, a single chain TCRVβ/VL, a single chain TCRVα/VH, a single chain TCRVβ/VH, a single chain VL/TCRVα, a single chain VL/TCRVβ, a single chain VH/TCRVα, a single chain VH/TCRVβ, a single chain TCRVγ/VL, a single chain TCRVδ/VL, a single chain TCRVγ/VH, a single chain TCRVδ/VH, a single chain VL/TCRVγ, a single chain VL/TCRVδ, a single chain VH/TCRVγ, and/or a single chain VH/TCRVδ, or mutants and/or variants thereof.

In one embodiment, the TCR recognition element comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not limited to: one or more of TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains. In another embodiment the variable TCR variable α (TCRVα), TCR variable β (TCRVβ),

TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains comprises one or more of the CDR1, CDR2 or CDR3 segments. In another embodiment the TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to Cα, Cβ1, Cβ2, Cγ or Cδ.

Such phage displayed reagents include but are not limited to the following: single chain TCRVα/TCRVα, a single chain TCRVβ/TCRVβ, a single chain TCRVγ/TCRVγ, a single chain TCRVδ/TCRVδ,a single chain TCRVα/TCRVβ, a single chain TCRVα/TCRVγ, a single chain TCRVα/TCRVδ , a single chain TCRVβ/TCRVα , a single chain TCRVβ/TCRVγ, a single chain TCRVβ/TCRVδ, a single chain TCRVγ/TCRVα, a single chain TCRVγ/TCRVβ, a single chain TCRVγ/TCRVδ, a single chain TCRVδ/TCRVα, a single chain TCRVδ/TCRVβ, a single chain TCRVδ/TCRVγ and/or a mutation and variant thereof.

II. Reagents:

This invention provides a recombinant chimeric TCR recognition element/ Ig recognition element reagent. In the preferred embodiment, the reagent is soluble.

In one embodiment, the TCR recognition element comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not limited to: TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains. In another embodiment the variable TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains comprises one or more of the CDR1, CDR2 or CDR3 segments. In another

embodiment the TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to Cα, Cβ1, Cβ2, Cγ or Cδ..

In another embodiment the Ig recognition element is an antibody comprising a variable domain. The antibody comprises a heavy chain and/or a light chain. Further, the heavy chain comprises one or more heavy chain variable fragments (VH). In another embodiment the heavy chain comprises one or more CH1 constant domains. As contemplated herein, the light chain comprises one or more light chain variable fragments (VL). In one embodiment, the light chain comprises one or more Cκ (kappa) or Cλ(lambda) domains. In another embodiment, the variable domain comprises one or more of CDR1, CDR2 or CDR3 segments.

This invention provides a reagent which comprises a single chain Fv fragment. In another embodiment the reagent comprises a Fab fragment

Further, as provided herein, the reagent has a linker region comprises a nucleic acid which is characterized as: i) aiding in folding of the domains, ii) supporting the stabilization of the intact protein construct. The nucleic acid sequence of the linker region was described above. Further, in one embodiment the reagent comprises a second molecule that is linked to the reagent. The second molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target . The second molecule enhances the overall avidity of the interaction of the reagent with the target molecule or a multimeric target with the proviso that the affinity of the second molecule to its

interaction site is in itself insufficient to allow interaction with the target molecule or multimeric target under physiologic conditions.

For example, the second molecule includes but is not limited to: a molecule with antibody or TCR like reactivity, a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or inorganic substance, ion, synthetic, or mimetic, thereof. An example, for a bispecific molecule contemplated herein is a reagent directed against a specific MHC/peptide complex coupled to CD8, or variant thereof which exhibits low affinity to their respective target.

This invention provides a reagent which comprises the following soluble chimeric polypeptides: a single chain TCRVα/VL, a single chain TCRVβ/VL, a single chain TCRVα/VH, a single chain TCRVβ/VH, a single chain VL/TCRVα, a single chain VL/TCRVβ, a single chain VH/TCRVα, VH/TCRVβ, a single chain TCRVγ/VL, a single chain TCRVδ/VL, a single chain TCRVγ/VH, a single chain TCRVδ/VH, a single chain VL/TCRVγ, a single chain VL/TCRVδ, a single VH/TCRVγ, and/or a single chain VH/TCRVδ, or mutants and/or variants thereof. The nucleic acid which codes for the chimeric reagents are discussed above.

This invention provides a soluble recombinant TCR recognition element reagent. In one embodiment, the TCR recognition element comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment

includes but is not limited to: one or more of TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains. In another embodiment, the variable TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domain comprise one or more of the CDR1, CDR2 or CDR3 segments. In another embodiment the TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to Cα, Cβ1, Cβ2, Cγ or Cδ.

This invention provides a reagent which comprises the following soluble TCR polypeptides: single chain TCRVα/TCRVα, a single chain TCRVβ/TCRVβ, a single chain TCRVγ/TCRVγ, a single chain TCRVδ/TCRVδ, a single chain TCRVα/TCRVβ, a single chain TCRVα/TCRVγ, a single chain TCRVα/TCRVδ , a single chain TCRVβ/TCRVα , a single chain TCRVβ/TCRVγ, a single chain TCRVβ/TCRVδ, a single chain TCRVγ/TCRVα, a single chain TCRVγ/TCRVβ, a single chain TCRVγ/TCRVδ, a single chain TCRVδ/TCRVα, a single chain TCRVδ/TCRVβ, a single chain TCRVδ/TCRVγ and/or a mutation and variant thereof.

In one embodiment the TCR recognition element comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not limited to: TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains. In another embodiment the variable TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains comprise one or more of the CDR1, CDR2 or CDR3 segments. In another embodiment the

TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to Cα, Cβ1, Cβ2, Cγ or Cδ.

In another embodiment the Ig recognition element is an Ab. The antibody comprises a heavy chain and/or a light chain. Further, the heavy chain comprises one or more heavy chain variable fragments (VH). In another embodiment the heavy chain comprises one or more C_H1 constant domains. As contemplated herein, the light chain comprises one or more light chain variable fragments (VL). In one embodiment, the light chain comprises one or more Cκ (kappa) or Cλ(lambda) domains. In another embodiment, the variable domain comprises one or more of CDR1, CDR2 or CDR3 segments.

This invention provides a reagent which comprises a phage particle displaying single chain Fv fragment. In another embodiment the reagent comprises phage particle displaying Fab fragment

This invention provides a phage display reagent which display the following soluble chimeric polypeptides: a single chain TCRVα/VL, a single chain TCRVβ/VL, a single chain TCRVα/VH, a single chain TCRVβ/VH, a single chain VL/TCRVα, a single chain VL/TCRVβ, a single chain VH/TCRVα, a single chain VH/TCRVβ, a single chain TCRVγ/VL, a single chain TCRVδ/VL, a single chain TCRVγ/VH, a single chain TCRVδ/VH, a single chain VL/TCRVγ, a single chain VL/TCRVδ, a single chain VH/TCRVγ, and/or a single chain VH/TCRVδ, or mutants and/or variants thereof. The nucleic acid which codes for the chimeric reagents are discussed above.

This invention provides a soluble recombinant TCR recognition element reagent. In one embodiment the TCR recognition element comprises a variable fragment of the TCR, mutant and variant thereof. The variable fragment includes but is not limited to: one or more of TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains. In another embodiment the variable TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains comprises one or more of CDR1, CDR2 or CDR3 segments. In another embodiment the TCR recognition element comprises a constant fragment. The constant fragment of the TCR includes but is not limited to Cα, Cβ1, Cβ2, Cγ or Cδ.

This invention provides phage display reagent which comprises the following soluble TCR polypeptides: single chain TCRVα/TCRVα, a single chain TCRVβ/TCRVβ, a single chain TCRVγ/TCRVγ, a single chain TCRVδ/TCRVδ, a single chain TCRVα/TCRVβ, a single chain TCRVα/TCRVγ, a single chain TCRVα/TCRVδ , a single chain TCRVβ/TCRVα , a single chain TCRVβ/TCRVγ, a single chain TCRVβ/TCRVδ, a single chain TCRVγ/TCRVα, a single chain TCRVγ/TCRVβ, a single chain TCRVγ/TCRVδ, a single chain TCRVδ/TCRVα, a single chain TCRVδ/TCRVβ, a single chain TCRVδ/TCRVγ and/or a mutation and variant thereof.

III. Expression vectors:

This invention provides an expression vector comprising a polynucleotide which codes for a TCR recognition element, and/or a mutation and variant thereof; and polynuclotide

which codes for an Ig recognition element, and/or a mutation and variant thereof.

This invention provides for a replicable vector comprising the isolated nucleic acid molecule of the DNA virus. The vector includes, but is not limited to: a plasmid, cosmid, phage or yeast artificial chromosome (YAC) which contains at least a portion of the isolated nucleic acid molecule. As an example to obtain these vectors, insert and vector DNA can both be exposed to a restriction enzyme to create complementary ends on both molecules which base pair with each other and are then ligated together with DNA ligase. Alternatively, linkers can be ligated to the insert DNA which correspond to a restriction site in the vector DNA, which is then digested with the restriction enzyme which cuts at that site. Other means are also available and known to an ordinary skilled practitioner.

The term "vector", refers to viral expression systems, autonomous self-replicating circular DNA (plasmids), and includes both expression and nonexpression plasmids. Where a recombinant microorganism or cell culture is described as hosting an "expression vector," this includes both extrachromosomal circular DNA and DNA that has been incorporated into the host chromosome(s). Where a vector is being maintained by a host cell, the vector may either be stably replicated by the cells during mitosis as an autonomous structure, or is incorporated within the host's genome.

Expression vectors which can be used other than adenovirus include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus, swinepox virus, pox virus, rhabdovirus, herpes simplex virus, baculovirus, herpes simplex virus, adeno-associated virus, retrovirus, cytomegalovirus, human cytomegalovirus, papillomavirus, Epstein Barr virus (EBV), mouse mammary tumor virus (MMTV), Moloney

murine leukemia virus and plasmid and cosmid DNA vectors, to name but a few.

In one embodiment, the adenoviral vector is deficient in at least one essential gene function of the El region of the adenoviral genome, particularly the Ela region, more preferably, the vector is deficient in at least one essential gene function of the El region and part of the E3 region (e.g., an Xbal deletion of the E3 region) or, alternatively, the vector is deficient in at least one essential gene function of the El region and at least one essential gene function of the E4 region. Aadenoviral vectors deficient in at least one essential gene function of the E2a or E2b region and adenoviral vectors deficient in all of the E3 region also are contemplated here and are well known in the art. Furthermore, the viral vector's coat protein can be modified so as to incorporate a specific protein binding sequence.

The term "plasmid" refers to an autonomous circular DNA molecule capable of replication in a cell, and includes both the expression and nonexpression types. Where a recombinant microorganism or cell culture is described as hosting an "expression plasmid", this includes latent viral DNA integrated into the host chromosome(s). Where a plasmid is being maintained by a host cell, the plasmid is either being stably replicated by the cells during mitosis as an autonomous structure or is incorporated within the host's genome.

Regulatory elements required for expression include promoter or enhancer sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well-known in the art, for example the methods described above for constructing vectors in general. Enhancers were originally detected as genetic

elements that increased transcription from a promoter located at a distant position on the same molecule of DNA. This ability to act over a large distance had little precedent in classic studies of prokaryotic transcriptional regulation. Subsequent work showed that regions of DNA with enhancer activity are organized much like promoters. That is, they are composed of many individual elements, each of which binds to one or more transcriptional proteins.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell. A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence. A nucleic acid sequence is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the

correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front of the gene.

Below is a list of viral promoters, cellular enhancers and inducible enhancers that may be used, which include but are not limited to the following: ventricular myocyte specific promoter, cytomegalovirus, human cytomegalovirus, inflammatory promoters, TNF promoter, Rous Sarcoma Virus, Prostate Specific Antigen, Probasin, Immunoglobulin Heavy Chain, Immunoglobulin Light Chain, T-Cell Receptor, HLA, Interferon, Interleukin-2, Interleukin-2 Receptor, MHC Class II, Actin, Muscle Creatine Kinase, Proalbumin (Transthyretin), Elastase I, Metallothionein, Collagenase, Albumin Gene, Fetoprotein, Globin, c-fos, c-Ha-ras, Insulin, Neural Cell Adhesion Molecule (NCAM), antirypole, 2B (TH2B) Histone, Muse or Type I Collagen, Glucose-Regulated Proteins (GRP94 and GRP78), Human Serum Amyloid A (SAA), Troponin I (TN I), Platelet-Derived Growth Factor, Duchenne Muscular Dystrophy, SV40, Polyoma, Retroviruses, Papilloma Virus, Hepatitis B Virus, or Gibbon Ape Leukemia Virus.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

The basic distinction between enhancers and promoters is operational. An enhancer region as a whole must be able to stimulate transcription at a distance; this need not be true of a promoter region or its component elements. On the other hand, a promoter must have one or more elements

that direct initiation of RNA synthesis at a particular site and in a particular orientation, whereas enhancers lack these specificities. Promoters and enhancers are often overlapping and continguous, often seeming to have a very similar modular organization.

A DNA sequence is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front of the gene.

In one embodiment the vector comprises a polynucleotide which encodes a TCR recognition element having a variable fragment of the TCR, mutant and variant thereof. The polynucleotide encoding the variable fragment includes but is not limited to: one or more of TCR variable α (TCRVα), TCR variable β TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains. In another embodiment the polynucleotide encodes one or more of the complementarity determining residues (CDR) 1, CDR2 or CDR3 segments of the variable domain. In another embodiment the vector comprises a polynucleotide which encodes a TCR recognition element having a constant fragment. The polynucleotide encodes a constant fragment of the TCR includes but is not limited to: to Cα, Cβ1, Cβ2, Cγ or Cδ..

In another embodiment the vector comprises a polynucleotide which encodes a variable domain of an antibody. In another embodiment, the vector comprises a polynucleotide which codes for a heavy chain and/or a light chain of the antobody. In another embodiment the vector comprises a polynucleotide which codes for one or more heavy chain variable fragments (VH). In another embodiment the vector comprises a polynucleotide which codes for one or more C_H1 constant domains of the variable fragment. In another embodiment the vector comprises a polynucleotide which codes for one or more light chain variable fragments (VL). In another embodiment the vector comprises a polynucleotide which codes for one or more Cκ (kappa) or Cλ(lambda)domains. In another embodiment the vector comprises a polynucleotide which codes for one or more of CDR1, CDR2 or CDR3 segments of the variable segment.

Further, this invention provides a vector comprising a polynucleotide which codes for a Fv fragment. In one embodiment the Fv fragment is a single chain Fv fragment. In another embodiment the vector has a polynucleotide which codes for a Fab fragment

Further as provided herein, the vector comprises a polynucleotide which codes for a T-cell receptor (TCR) recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an immunoglobulin (Ig) recognition element, and/or a mutation and variant thereof; and a polynucleotide of a linker region which when expressed join the T-cell receptor (TCR) recognition element, and the immunoglobulin (Ig) recognition element of the reagent.

The polynucleotide of the linker region comprises a nucleic acid having a sequence as follows:

Further, in one embodiment of the invention, the vector comprises a polynucleotide which codes for a second molecule that is linked to the reagent. The second molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target . The second molecule enhances the overall avidity of the interaction of the reagent with the target molecule or a multimeric target.

For example, the second molecule includes but is not limited to: a molecule with antibody or TCR like reactivity, a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or inorganic substance, ion, synthetic, or mimetic, thereof. An example, for a bispecific molecule contemplated herein is a reagent directed against a specific MHC/peptide complex coupled to CD8, or variant thereof which exhibits low affinity to their respective target.

This invention provides a vector comprising a polynucleotide which codes for a T-cell receptor (TCR) recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an immunoglobulin (Ig) recognition element, and/or a mutation and variant thereof, and in which the vector expresses a recombinant chimeric TCR recognition element/immunoglobulin recognition element reagent from each of the recombinant phages, such vectors comprise a polynucleotide encoding: a single chain TCRVα/VL, a

single chain TCRVβ/VL, a single chain TCRVα/VH, a single chain TCRVβ/VH, a single chain VL/TCRVα, a single chain VL/TCRVβ, a single chain VH/TCRVα, VH/TCRVβ, a single chain TCRVγ/VL, a single chain TCRVδ/VL, a single chain TCRVγ/VH, a single chain TCRVδ/VH, a single chain VL/TCRVγ, a single chain VL/TCRVδ, a single VH/TCRVγ, and/or a single chain VH/TCRVδ, or mutants and/or variants thereof.

This invention provides a vector comprising a polynucleotide encoding the T-cell receptor (TCR) and the immunoglobulin elements, fragments, domains and/or segments in a tail-to-head transcriptional orientation. In one embodiment the vector comprises transcription and translation control sequences. In another embodiment the vector comprimises transcription control sequence which is selected from the group consisting of a promoter, an RNA polymerase initiation site, an RNA polymerase termination site, a TATA box, a CAT box, a poly A addition site, an enhancer and a part or combination thereof. In another embodiment, this invention provides the translation control sequence which are selected from the group consisting of a ribosome binding site, a leader sequence and a part or combination thereof.

Further, the vector comprises but is not limited to a reported gene. The following reporter genes mast be used: luciferase, β-galactosidase, or β-lacatamase. Other reporter genes include but are not limited to: β -lactamase and other antibiotic resistant gene, a cell surface marker as MHC I or II sub-types, a receptor for growth factor or cell adhesion and any gene of interest for therapeutic reasons. Additionally, the vector may comprise a marker inserted may provide for prototrophy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals, such as copper, or the like. The

selectable marker gene sequence can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements may also be needed for optimal . These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama, Molec. CeIZ. Biol. 3:280(1983).

This invention provides for an oligonucleotide comprising a nucleic acid having the sequence as set forth in Figure 6, including mutants, and variants thereof.

In addition, this invention provides a primer comprising the nucleic acid having the sequence as set forth in Figure 7, including mutants, and variants thereof.

Oligonucleotides which are complementary may be obtained as follows: The polymerase chain reaction is then carried out using the two primers. See *PCR Protocols: A Guide to Methods and Applications* [74]. Following PCR amplification, the PCR-amplified regions of a viral DNA can be tested for their ability to hybridize to the three specific nucleic acid probes listed above. Alternatively, hybridization of a viral DNA to the above nucleic acid probes can be performed by a Southern blot procedure without viral DNA amplification and under stringent hybridization conditions as described herein. Oligonucleotides for use as probes or PCR primers are chemically synthesized according to the solid phase phosphoramidite triester method first described by Beaucage and Carruthers [19] using an automated synthesizer, as described in Needham-VanDevanter [69]. Purification of oligonucleotides is by either native acrylamide gel electrophoresis or by anion-exchange HPLC

as described in Pearson, J.D. and Regnier, F.E. [75A]. The sequence of the synthetic oligonucleotide can be verified using the chemical degradation method of Maxam, A.M. and Gilbert, W. [63].

High stringent hybridization conditions are selected at about 5? C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is at least about 0.02 molar at pH 7 and the temperature is at least about 60?C. As other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of the complementary strands, the presence of organic solvents, ie. salt or formamide concentration, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one. For Example high stringency may be attained for example by overnight hybridization at about 68?C in a 6x SSC solution, washing at room temperature with 6x SSC solution, followed by washing at about 68?C in a 6x SSC in a 0.6x SSX solution.

Hybridization with moderate stringency may be attained for example by: 1) filter pre-hybridizing and hybridizing with a solution of 3x sodium chloride, sodium citrate (SSC), 50% formamide, 0.1M Tris buffer at Ph 7.5, 5x Denhardt's solution; 2.) pre-hybridization at 37?C for 4 hours; 3) hybridization at 37?C with amount of labelled probe equal to 3,000,000 cpm total for 16 hours; 4) wash in 2x SSC and 0.1% SDS solution; 5) wash 4x for

1 minute each at room temperature at 4x at 60?C for 30 minutes each; and 6) dry and expose to film.

The phrase "selectively hybridizing to" refers to a nucleic acid probe that hybridizes, duplexes or binds only to a particular target DNA or RNA sequence when the target sequences are present in a preparation of total cellular DNA or RNA. By selectively hybridizing it is meant that a probe binds to a given target in a manner that is detectable in a different manner from non-target sequence under high stringency conditions of hybridization. in a different "Complementary" or "target" nucleic acid sequences refer to those nucleic acid sequences which selectively hybridize to a nucleic acid probe. Proper annealing conditions depend, for example, upon a probe's length, base composition, and the number of mismatches and their position on the probe, and must often be determined empirically. For discussions of nucleic acid probe design and annealing conditions, see, for example, Sambrook *et al.*, [81] or Ausubel, F., *et al.*, [8].

It will be readily understood by those skilled in the art and it is intended here, that when reference is made to particular sequence listings, such reference includes sequences which substantially correspond to its complementary sequence and those described including allowances for minor sequencing errors, single base changes, deletions, substitutions and the like, such that any such sequence variation corresponds to the nucleic acid sequence of the pathogenic organism or disease marker to which the relevant sequence listing relates.

II Methods of creating phage display libraries:

In one embodiment, the nucleic acids are derived from pools of blood. The method provides the generation of diverse libraries. The cells are obtained from a human being or beings. In another embodiment the cells, are obtained from a nonhuman vertebrate species.

This invention provides a method for creating a phage display chimeric TCR/Ig reagent comprising the steps of:: obtaining a sample of cells; mRNA preparation; reverse transcribing mRNA of the cell population into cDNA sequences of T-cell receptor and immunoglobulin; amplifying the cDNA, providing nucleic acid expression vectors which are capable of being packaged; cloning the population of DNA fragments into expression vectors; combining (i) a genetically diverse repertoire of nucleic acid sequences in which each encode a unique or genetically diverse population of the TCR-cell receptor elements with (ii) a genetically diverse repertoire of nucleic acid sequences which encodes a unique or genetically diverse population of the immunoglobulin elements, to form a library of nucleic acid sequences using said expression vectors encoding said TCR and antibody polypeptide; expressing said library from said vectors in recombinant host organism cells, each of the said polypeptide chain components being expressed as a recombinant chimeric protein on its own or as part of phage particles which are components of the library, also with the property of binding specifically to a target molecule of interest; selecting from said expressed library by binding to a target molecule a unique or restricted population of said reagents binding specificity, thereby producing a recombinant chimeric TCR/Ig reagent.

This invention provides a method for creating a phage display T-cell receptor reagent comprising the steps of: obtaining a sample of cells; mRNA preparation; reverse transcribing mRNA of the cell population into cDNA sequences of T-cell receptor; amplifying the cDNA; providing nucleic acid expression vectors which are

capable of being packaged; cloning the population of DNA fragments into expression vectors; combining a genetically diverse repertoire of nucleic acid sequences in which each encode a unique or genetically diverse population of the TCR-cell receptor elements, to form a library of nucleic acid sequences using said expression vectors encoding said TCR polypeptide; expressing said library from said vectors in recombinant host organism cells, said polypeptide chain components being expressed as a recombinant TCR protein on its own or or as part of phage particles which are components of the library, also with the property of binding specifically to a target molecule of interest; selecting from said expressed library by binding to a target molecule of interest a unique or restricted population of said reagents binding specificity, thereby producing a recombinant TCR reagent.

PCR reaction conditions should be chosen which optimize amplified product yield and specificity, and, additionally, produce amplified products of lengths which may be resolved utilizing standard gel electrophoresis techniques. Such reaction conditions are well known to those of skill in the art, and important reaction parameters include, for example, length and nucleotide sequence of oligonucleotide primers as discussed above, and annealing and elongation step temperatures and reaction times.

III. Methods for selection of recombinant reagent:

This invention provides a method for selecting recombinant reagents directed against a molecular target, said method comprising: contacting the phage display chimeric library or the phage display TCR library as hereinabove described, with the target molecule so as to

form a complex, dissociating the specifically bound phage from the complex; amplifying the rescued phage in a bacterial host; repeating binding, dissociation and amplification steps; isolating the bound phage thereby selecting said library against a target molecule. In another embodiment the method further comprises characterizing the selected phage particles and their respective reagents.

As used herein, a "target" or "ligand" is a molecule that can bind a recombinant TCR protein or a chimeric recombinant reagent according to the invented method. A target as used herein, is any composition: molecule, a complex, a nucleic acid sequence, a polypeptide, peptide fragment or any composition that can be assayed for its ability to function in given capacity or compound. The target molecule perhaps synthetic, recombinant or biological sample derived for e.g. from body fluid such as serum or urine, part of organ or cells which conceivably derived from a destinct MHC subtype. The cell pupulation may be selected from the group consisting a population of eukariotic cells, such as murine cells, ovine cells, porcine cells, primate cells, human cells, plant cells transformed cells, fused cells and combinations thereof.

The target molecule may be coupled to a solid-phase support, e.g., nitrocellulose, nylon, column packing materials (e.g., Sepharose beads), magnetic beads, glass wool, plastic, metal, polymer gels, cells, or other substrates. Such supports may take the form, for example, of beads, wells, dipsticks, or membranes.

By "solid phase support or carrier" is intended any support capable of binding an target molecules, phage

particle or reagent. Well- known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros (?), and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding target molecules or phage or reagents, or will be able to ascertain the same by use of routine experimentation.

IV. Methods for screening of recombinant reagent

Screening assays can be conducted in a variety of ways which are known to those skilled in the art. The principle assays used to identify compounds that bind to the component parts of library involves binding the target molecule to a support, such as microtiter plates, nitrocellulose, or other solid support that is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable media, e.g. buffers followed by treatment with, the selected component of the libraries, or reagents for a time sufficient to interact and bind, thus forming a complex. After washing away unbound phage particles or reagent, bound material representing the binding partner of a target molecule can be detected by number of ways. Where

the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the previously non-immobilized component.e.g anti-M13 HRP antibodies.

This invention provides a method of detecting a reagent of the recombinant phage library as described above. As provided herein, the junctional regions between the variable domain of the single chain TCR/Ig chimera or single chain TCR contains Cα-TCR or Cβ-TCR N-terminal domain-derived peptides joint to $(Gly_4Ser)_3$ peptide linker. The combination of $(Gly_4Ser)_3$ and Cα-TCR or Cβ-TCR N-terminal domain-derived peptides provide novel epitopes for reagents like Ab, which can recognize them with high specificity and affinity. In addition, Cα-TCR or Cβ-TCR sequences at the C terminal end of the single chain TCR/Ig chimera or single chain TCR provide with novel tag for additional epitopes for reagents like Ab. Bound phage or reagents can therafter analyzed for e.g. amino acid sequence by well-known methods. Reagent so identified can be produced using recombinant DNA technology.

Likewise, a bioluminescent compound may be used to label the target molecule phage or reagents of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is

determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

"Specifically binding to " or "specifically immunoreactive with", when referring to the recombinant reagent contenplated in the present invention refers to the binding of a molecular target or a cell to the recombinant reagent as provided herein. A variety of immunoassay formats may be used to select recombinant reagents specifically immunoreactive with a particular molecular target. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow and Lane [32] for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

The target molecule phage or reagents may be labelled with a detectable marker including, but not limited to: a radioactive label, or non redioactive isoltopic lable, a colorimetric, a luminescent, or a fluorescent marker, or heavy metal. Isotopic labels include, but are not limited to: ^{3}H, ^{14}C, ^{32}P, ^{33}P; ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{59}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re, as well as ^{55}Mn, ^{56}Fe. Fluorescent markers include but are not limited to: fluorescein, isothiocyanate, a rhodamine, a phycoerythrin, a phycocyanin, an allophycocyanin, O-phthaldehyde, a fluorescamine, etc.) Other labels may include peridinin chlorophyll protein (PerCP), chemiluminescent labels, enzyme labels (e.g., alkaline phosphatase, horse radish peroxidase, etc.), protein labels, and labels useful in radioimaging and

radioimmunoimaging. Colorimetric markers include, but are not limited to: biotin, and digoxigenin.

Since specific enzymes may be coupled to other molecules by covalent links, the possibility also exists that they might be used as labels for the production of tracer materials. Suitable enzymes include alkaline phosphatase, beta-galactosidase, glucose-6-phosphate dehydrogenase, maleat dehydrogenase, and peroxidase. Two principal types of enzyme immunoassay are the enzyme-linked immunosorbent assay (ELISA), and the homogeneous enzyme immunoassay, also known as enzyme-multiplied immunoassay (EMIT, Syva Corporation, Palo Alto, CA). In the ELISA system, separation may be achieved, for example, by the use of antibodies coupled to a solid phase. The EMIT system depends on deactivation of the enzyme in the tracer-antibody complex; the activity can thus be measured without the need for a separation step.

Additionally, chemiluminescent compounds may be used as labels. Typical chemiluminescent compounds include luminol, isoluminol, aromatic acridinium esters, imidazoles, acridinium salts, and oxalate esters. Similarly, bioluminescent compounds may be utilized for labelling, the bioluminescent compounds including luciferin, luciferase, and aequorin. Once labeled, the reagent may be employed to identify and quantify ligand (e.g. antibody or antigenic polypeptide) utilizing techniques well-known to the art.

A description of a radioimmunoassay (RIA) may be found in *Laboratory Techniques in Biochemistry and Molecular Biology* [52], with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and

Related Techniques" by Chard, T., incorporated by reference herein. A description of general immunometric assays of various types can be found in the following U.S. Pat. Nos. 4,376,110 (David *et al.*) or 4,098,876 (Piasio).

In a further embodiment, of this invention, commercial test kits suitable for use by a medical specialist may be prepared to determine the presence or absence of predetermined binding activity or predetermined binding activity capability to suspected target cells.

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Further, as used herein, the term "label" refers to a molecule, which may be conjugated or otherwise attached (i.e., covalently or non-covalently) to a binding protein as defined herein. Particularly suitable labels include those, which permit analysis by flow cytometry, e.g., fluorochromes. Preferred fluorochromes include phycoerythrin (P.E., Coulter Corp., Hialeah, FL), phycoerythrin-cyanin dye 5 (PECy5, Coulter), and fluorescein isothiocyanate (FITC, International Biological Supplies, Melbourne, FL). Other suitable detectable labels include those useful in colorimetric enzyme systems, e. g., horseradish peroxidase (HRP) and alkaline phosphatase (AP). Other proximal enzyme systems are known to those of skill in the art, including hexokinase in conjunction with glucose-6-phosphate dehydrogenase. Chemiluminescent labels, such as green fluorescent proteins, blue fluorescent proteins, and variants thereof are known. Also bioluminescence or chemiluminescence can be detected using, respectively, NAD oxidoreductase with luciferase and substrates NADH and FNIN or peroxidase with luminol and substrate peroxide. Other suitable label systems useful in the present invention include radioactive compounds or elements, or immunoelectrodes.

V Diagnostic and therapeutic applications:

This invention provides use of a pharmaceutical composition comprising the reagent as described above, for the prevention or treatment of a autoimmune disease selected from the group consisting of ankylosing

spondylitis, Reiter's disease, psoriatic spondylitis, psoriasis vulgaris and Behcet disease) rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, systemic lupus erythematosus, Sj6gren disease, IDDM, Addison disease, Graves disease, Hashimoto disease, celiac disease, primary biliary cirrhosis, pemphigus vulgaris, epidermolysis bullosa acquisita, Hodgkin's disease, cervical squamous cell carcinoma, multiple sclerosis, optic neuritis, narcolepsy, myasthenia gravis, Goodpasture syndrome and alopecia areata).

In one embodiment the tumor cells are selected e.g. from a group consisting of: melanoma; lymphoma; leukemia; and prostate, colorectal, pancreatic, breast, brain, or gastric carcinoma. Examples of tumors include but are not limited to: sarcomas and carcinomas such as, but not limited to: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's sarcoma, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, germ tumor, non-small cell lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

This invention provides a method for diagnosing a subject with a tumor, comprising the steps of: a) Obtaining a

sample from the subject, b) Contacting the sample with a recombinant reagent as discussed above, wherein the reagent is specific for a specific tumor antigen so as to form a complex, c) Detecting the complex, the presence of which is indicative of a subject having the tumor.

This invention provides a method of detecting a reagent of the recombinant phage library as described above. As provided herein, the junctional regions between the variable domain of the single chain TCR/Ig chimera or single chain TCR contains Cα-TCR or Cβ-TCR N-terminal domain-derived peptides joint to $(Gly_4Ser)_3$. This combined $(Gly_4Ser)_3$ and Cα-TCR or Cβ-TCR N-terminal domain-derived peptides provide novel epitopes for reagents like Ab, which can recognize them with high specificity and affinity, but do not cross react with the TCR molecules on T cells. In addition, Cα-TCR or Cβ-TCR N-terminal domain-derived peptides provide peptide tag at the C-terminal end of the scFv, as a novel epitope for reagents like Ab. However, these reagents are expected to react with with in vivo Cα-TCR or Cβ-TCR and thus with TCR molecules on T cells and can not be used for in vivo imaging.

This invention provides a method for imaging a neoplastic disorder in a subject comprising the steps of administering to the subject an amount of the recombinant reagent as described above, wherein the reagent is labeled, and detecting the label.

For example, reagents such as those described, above may be used to quantitatively or qualitatively determine a subject with a disorder or pathogenic condition. This can

be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled reagent in a subject, and detecting the presense, amount, and/or distrubution but also its localization into the examined tissue.

This invention provides a method of detecting a reagent of the recombinant phage library as described above. This invention provides a method of treating a subject with a disease or a pathogenic conditions, comprising administrating to the subject an effective amount of the reagent as described above, thereby treating the subject with the disease or pathogenic condition.

As used herein, "pharmaceutical composition" means therapeutically effective amounts of the reagent of the invention as described above together with suitable diluents, preservatives, solubilizers, emulsifiers, adjuvant and/or carriers. A "therapeutically effective amount" as used herein refers to that amount which provides a therapeutic effect for a given condition and administration regimen. Such compositions are liquids or lyophilized or otherwise dried formulations and include diluents of various buffer content (e.g., Tris-HCl., acetate, phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts). solubilizing agents (e.g., glycerol, polyethylene glycerol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (e.g., lactose, mannitol), covalent attachment of polymers such as polyethylene glycol to the protein, complexation with metal ions, or incorporation of the material into or onto particulate preparations of polymeric compounds such as polylactic acid, polglycolic acid, hydrogels, etc, or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation

enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral. In one embodiment the pharmaceutical composition is administered parenterally, intratumorally, paracancerally, transmucosally, transdermally, intramuscularly, intravenously, intradermally, intravascularly, subcutaneously, intraperitonealy, intraventricularly, intracranially.

Further, as used herein "pharmaceutically acceptable carrier" are well known to those skilled in the art and include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobial, antioxidants, collating agents, inert gases and the like.

The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the antigen and also as a lymphoid system activator that non-specifically enhances the immune response (Hood et al., *Immunology, Second Ed.*, 1984, Benjamin/Cummings: Menlo Park, California, p. 384). Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail to elicit a humoral or cellular immune response. Adjuvant include, but are not limited to, complete Freud's adjuvant, incomplete Freud's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, dinitrophenol. Preferably, the adjuvant is pharmaceutically acceptable.

Controlled or sustained release compositions include formulation in lipophilic depots (e.g. fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g. poloxamers or poloxamines) and the compound coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

An active component can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The pharmaceutically acceptable form of the composition includes a pharmaceutically acceptable carrier. In the therapeutic methods and compositions of the invention, a therapeutically effective dosage of the active component is provided. A therapeutically effective dosage can be determined by the ordinary skilled medical worker based on patient characteristics (age, weight, sex, condition, complications, other diseases, etc.), as is well known in the art. Furthermore, as further routine studies are conducted, more specific information will emerge regarding appropriate dosage levels for treatment of various conditions in various patients, and the ordinary skilled worker,

considering the therapeutic context, age and general health of the recipient, is able to ascertain proper dosing. Generally, for intravenous injection or infusion, dosage may be lower than for intraperitoneal, intramuscular, or other route of administration. The dosing schedule may vary, depending on the circulation half-life, and the formulation used. The compositions are administered in a manner compatible with the dosage formulation in the therapeutically effective amount. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous. infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

The present invention provides a kit comprising all the essential materials and reagents required for the library. This generally will comprise selected expression constructs. Such kits will comprise distinct containers for each individual reagent. When the components of the kit are provided in one or more liquid solutions, the liquid solution preferably is an aqueous solution, with a sterile aqueous solution being particularly preferred.

The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXPERIMENTAL DETAILS SECTION

EXAMPLE 1: Construction of Phage display single chain libraries (scTCRVα/TCRVβ, scTCRVα/VL and scTCRVH/TCRVβ).

Set of primers for TCR variable domain amplification: The TCRVα domain is encoded in the human genome by three consecutive gene segments: 5'Vα-Jα-Cα-3'. The gene segments of the TCRVβ domain contain an additional segment, D, that is located between the Vβ and Jβ segments, thus the gene organization is as follows: 5'-Vβ-D-Jβ-Cβ1 or Cβ2.

In order to amplify all known TCR variable regions from a given human mRNA sample, universal oligonucleotides were generated. There are as many as 42 TCRVα and 47 TCRVβ segments and 61 Jα and 13 Jβ gene fragments, and the goal was to amplify as many gene combinations with as few primers as possible.

The oligonucleotides for PCR amplification of the Vα and Vβ gene fragments were designed based on the sequence information published by Arden et al 1995 (1). The 5' end sequences of all described variable TCR gene segments were aligned and grouped by their similarity. Conserved nucleotide sequences were included in the primer sets, and only non conserved ones were exchanged by degenerated sequences as summarized in **Figure 6 and Figure 7** for the Vα and Vβ genes, respectively. In some cases, however, the 5' V-TCR sequence could not be grouped and the oligonucleotides were designed only for a specific TCR V gene segment. In addition, the primers included at their 5'end NcoI or SalI restriction sites for cloning. To facilitate the PCR reactions, the 3' primers were designed to anneal to the 5' end of Cα or Cβ gene segments. As a result, the amplified TCRVα segments contained a stretch encoding seven amino acids from the Cα segment, and the amplified TCRVβ segments contained a stretch encoding six amino acids at the 3'-end, originated from the Cβ1 or Cβ2 segments. Both Cα and Cβ primers include XhoI and NotI restriction sites, respectively, which are needed for cloning.

Amplification of TCR gene segments: The total RNA used for TCR gene amplification was obtained from $1.2x10^9$ pooled white blood cells, freshly collected. RNA and messenger RNA (mRNA) were prepared according to standard protocols using Promega RNAgents Total RNA Isolation System and PolyATtract mRNA Isolation System (Promega, Madison, USA). The quality of total RNA prepared was then eval ed by formaldehyde gels according to standard procedures (Maniatis et al., 2).

RT-PCR was prepared by employing the Access RT-PCR system and the Access RT-PCR Introductory system from Promega following the manufacturer's instructions (Promega, Madison, USA). The Access PCR system includes an optimized single-buffer system that permits extremely sensitive detection of RNA transcripts, without a requirement for buffer additions between the reverse transcription and PCR amplification steps. Thus, combination of TCR-specific oligonucleotides for Vα or Vβ, together with their respective primers Cα or Cβ1 or Cβ2 were used for each RT-PCR reaction. The PCR reaction was done as in Nissim et al (3). Briefly, 1μl of DNA from the RT-PCR reaction was individually amplified using amplified using Vα and Cα or Vβ and Cβ1 or Cβ2 primers in a volume of 50μl with 250μM dedeoxynucleotide triphosphates (dNTPs), 10mM KCl, 10mM $(NH_4)_2SO_4$, 20mM TrisHCl pH8.8, 2mM $MgCl_2$, 100μg/ml bovine serum albumin (BSA) and 1μl (1 unit) of Taq DNA polymerase. 30 cycles (94°C-2 min, 55°C-1 min and 72°C-2 min) were used.

Preparation of Immunoglobulin (Ig) VH and VL segments: As it is the first time that phage display chimeric Ig/TCR is constructed, it was necessary to use known feature of several Ig variable domain proteins as a marker to test the integritiy of this novel construct; for example, the fact that VH3 familiy bind to protein A (4) and DPK12 to protein L (5). Thus we first tested our construct by fusing our TCR gene fragments to either VH3 or DPK12. The source of VH3 could originate from VH3 amplified PCR originated from the Nissim library, or from individual selected clones using this library bearing VH3 gene segments (e.g. anti-NIP or anti-phOx clones bearing DP-47 VH gene segment). Alternatively the commercially available antibody phage display libraries

"Tomlinson A" and "Tomlinson B" (MRC, Cambridge UK, unpublished) were employed. These libraries contain the DP47 gene segment belonging to the VH3 heavy chain gene family (4). As a source for VL we had two alternatives: either DPK12 gene from "Tomlinson A" and "Tomlinson B" or DPK12 from several other libraries available (Nissim et al, unpublished). The expression of the TCR/Ig chimera could then be evaluated by an ELISA using horseradish peroxidase (HRP)-coupled protein A and HRP-conjugated protein L.

To obtain the VH and VL inserts, PCR reaction using V-family based oligonucleotides were imployed as described (Marke et al, 6; Coks et al, 7; Williams et al, 8). If "Tomlison" library were used pTI plasmid vectors containing a sample of the library were prepared by QIAGEN plasmid purification kit according to manufacturer's instructions (QIAGEN, Germany). Purified plasmids or PCR products were then digested with the restriction enzymes NcoI/XhoI to obtain the VH segment and SalI/NotI (all from Boehringer, Mannheim, Germany) to obtain the VL gene segment (see Fig. 1). After overnight digestion, the V segments were gel purified according to manufacturer's instructions using the QIAGEN DNA purification kit. The sizes of the V gene segments obtained from these digests were about 350 bp.

Currently, to construct the chimeric TCR/Ig library, the Ig V gene segments are amplified from the same RNA pool used for the amplification of the TCR gene segments. RT-PCR is performed using VH and VL family-based primers in combination with their respective JH, JK and Jλ primers. Depending on the vector used to consytruct the libraries, the VL oligonucleotides were used as published or diversified to exchange ApaLI with SalI (Marke et al, 6; Coks et al, 7; Williams et al, 8). RT-PCR and PCR are performed as described above in case of the TCR V genes (section Ba).

Vectors: The following vectors were used: phage vectors, that encode the pIII-fusion protein and all functions required for replication, packaging and infection of bacteria, and phagemid vectors which require "rescue" with a helper phage. Phagemid vectors comprise the pIII-fusion, plasmid and phage origins of replication and antibiotic resistance markers; the helper phage provides the functions necessary for single strand DNA replication and

packaging. Helper phages are poorly packaged in comparison with agemids due to a defective M13 origin of replication (Vieira and Messing, 9).

The basic vector for the library construction is pHEN1 phagemid vector (REF). In pHEN1, the production of the pIII-fusion protein is under the control of the lacZ promotor which is inhibited with glucose and induced with isopropyl-D-thiogalactoside (IPTG) (De Bellis and Schwartz, 10). pHEN1 can also be used directly for expression of the recombinant antibodies as soluble fragments since an amber stop codon is located at the junction of the antibody gene and gIII. When grown in suppressor strains of *E. coli* such as TG1 (Gibson, 11), the gIII-fusion protein is produced and packaged into phage, while growth in non-suppressor strains such as HB2151 (Carter et al., 12) allows the secretion of soluble antibody fragments into the bacterial periplasm, and into the culture broth.

To check the feasibility of our concept it was convenient to us to use one of the pHEN1 modified vector named pTI (kindly donated by I. Tomlinson, MRC, Cambridge, UK) or pAN (Nissim et al, unpublished). As seen in Fig. 2, the phagmid vector is the same vector as the pHEN1 vector which includes NcoI and XhoI restriction sites for cloning the VH/L or TCR$\alpha/\beta/\gamma/\delta$ gene segments at the 5' end. However, it include additional SalI or ApaLI and NotI sites for cloning VH/L or TCR$\alpha/\beta/\gamma/\delta$ at the 3' end. Both pTI and pAN vectors, provide a myc tag (13) and a His tag (14) flanking the NotI site for detection and purification, respectively, and (Gly4Ser)3 linker between the V segments.

Cloning of TCR and Ig variable gene segments: For the first generation of constructs we were focused on TCR V genes in combination with VH3 and DPK12. After checking that for each of the PCR-amplified samples a band of the appropriate size was obtained on agarose gel electrophoresis, the PCR products of each of the amplifications encoding the different V segments were pooled as follows: pool (a) TCR-Vα ; pool (b) TCR-Vβ1; pool (c) TCR-Vβ2; pool (d) V_H-DP47; pool (e) VL-DPK12. The individual pools were purified by a PCR purification kit (Boehringer, Mannheim, Germany). After over night digestions with NcoI and XhoI restriction enzymes for Vα or VH, and SalI and

NotI restriction enzymes for Vβ1, Vβ2, or VL, respectively, fragments were gel purified and cloned into separate phagmide vectors (Fig2):

1. scTCRVα/VL: Vα between NcoI/XhoI and VL between SalI/NotI restriction sites;

2. ScTCRVH/Vβ: VH between NcoI/XhoI and Vβ between SalI/NotI restriction sites; 3. scTCRVαVβ: Vα between NcoI/XhoI and Vβ between SalI/NotI restriction sites

After ligation of 1µg of each plasmids and inserts, *E.coli* TG-1 bacteria were electroporated (15). The diversity of about 10^8 different clones were obtained for each scTCRVα/VL, or scTCRVH/Vβ, or scTCRVα/TCRVβ. Trasformed bacteria were scraped from plate and kept in 15% glycerol in 2XTY at -70^0C. Thereafter, 50µl bacteria of each library were used to rescue phage particles with helper phage VCS-M13 (Stratagene).

As previously described (Marks et al., 1991, 16; Hoogenboom and Winter, 1992, 17; Griffiths et al., 1993, 18), phage were rescued from the entire library or from single ampicillin-resistent colonies of infected TG-1 cells (K12, Δ(lac-pro), supE, thi, hsdD5/F'traD36, proA+B+, laclq, lacZDM15) using helper phage VCS-M13 (Stratagene). Soluble fragments were induced from single colonies of infected HB2151 bacteria ((K12, ara, Δ(lac-pro), thi/F'proA+B+, laclqZDM15) (Hoogenboom et al., 1991, 19)) by IPTG. Bacterial supernatants containing phage or sclg/TCR or scTCR fragments were screened for their s / phage titration PCR, ELISA and SDS gel electophoresis (take ref from Mark).

*Phage titration: P*hage were rescued from the entire library or from single ampicillin-resistant colonies of infected suppressor TG-1 bacteria using the helper phage VCS-M13 as described above. Bacterial supernatants containing phage fragments were used to re-infect new TG-1 bacteria that

were grown to the log phase ((OD at 600 nm of 0.4-0.5 (Phage/phagemid infect F+-E. coli via the sex pili. For sex pili production and efficient infection *E. coli* must be grown at 37^{0}C and be in log phase)). Finally, the titer of the phage particles of the various libraries as well as that of individual colonies was evaluated.

PCR and Sequencing: In order to assess the completeness of the integration of the individual TCR chains of scTCRVα/VL, scTCRVH/Vβ, and scTCRVαVβ constructs, single ampicillin-resistant colonies of infected *E. coli* TG-1 were used as template for PCR screening using the following primer combinations (Fig 3):

LMB3 (5'-CAGGAAACAGCTATGAC) and fdSEQ (5'-GAATTTTCTGTATGAGG), to measure the size of the entire insert which is expected to be about 900bp;

LMB3 and Cα–For-XhoI (Fig.6) to prime the Vα segment (500bp fragment only when priming the scTCRVα/VL or the scTCRVαVβ constructs);

LMB3 and Cβ–For-NotI (Fig.7) to prime the Vβ segments (900bp fragment only when priming the scVH/TCRVβ or scTCRVαVβ constructs).

Different clones were sequenced by the dideoxy method (Sanger et al., 1977, 20) using DyeDeoxy chain termination (Applied Biosystems Inc.) and an Applied Biosystems 373A DNA sequencer. The sequences were analysed by SeqEd (Applied Biosystems Inc.) and MacVector 3.5 (IBI Kodak, New Haven, CT).

Phage ELISA: ELISA plates were coated with phage supernatant for two hours at 37°C. Plates were then washed three times with Phosphate Buffered Saline (PBS), and 2% marvel in PBS was added for additional two hours of incubation in order to block the free plastic surface. Plates were re-washed three times with PBS-0.05% Tween 20, followed by 3 washes with PBS alone and horseradish peroxidase (HRP)-conjugated - anti-M13 polyclonal antibodies were added for an additional hour. After a further washing step, the

ELISA was developed with 100µl substrate solution (100 µg/ml 3,3',5,5'-tetra methylbenzidine (TMB) in 100 mM sodium acetate, pH 6.0, and 10 µl of 30% hydrogen peroxide per 50 ml of this solution directly before use). The reaction was stopped by adding 50 µl of 1 M sulphuric acid to each well. Plates were read at OD 450 and OD650; the final readout was the difference between the two values (OD650 – OD450). To detect the display of scTCRVα/VL and scTCRVH/Vβ on the surface of the phage particles, HRP-conjugated protein L and HRP-conjugated protein A were employed: (i)scTCRVα/VL should interact with Protein L but not protein A, (ii) scTCRVH/Vβ should interact with Protein A but not protein L, and (iii) scTCRVαVβ should not interact neither with Protein L nor with protein A, and (iv) sclgDP47-DPK12 should react with both reagents.

Expression of Ig-TCR and TCR fragments : Soluble fragments were induced from single colonies of infected non-suppressor HB2151 bacteria (Hoogenboom et al., 1991, 19) by IPTG. Briefly, HB2151 were infected with phage produced from individual TG-1 clones which had been screened as described above. Individual colonies were picked into 100 µl 2xTY-AMP-GLU in 96-well plates, grown overnight, transferred in a small inoculum (about 2 µl or using a 96-prong transfer device) to a second 96-well plate containing 200 µl 2xTY-AMP-0.1% GLU and grown to OD 0.9, IPTG to give a final concentration of 1 mM IPTG was then added and incubation was continued for further 16 to 24 hr by shaking at 30°C. Plates were then centrifuged at 1,800xg for 10 min and 100 µl of the supernatant was used for ELISA. In addition, bacterial periplasm (as described below) was prepared and tested in both ELISA and SDS gels.

Harvesting Fragments from the Periplasm: Induced bacterial cultures were centrifuged at 10,800xg for 15 min, resuspended in 1/20 the original volume with 30 mM Tris, pH 7.0-20% sucrose-1 mM EDTA and left for 20 min on ice. Tubes were centrifuged at 10,800xg for 15 min and supernatants collected (periplasmic fraction) to a new tube. Pellets were resuspended in 1/20 the original volume with 5 mM $MgSO_4$ and incubated further for 20 min on ice.

After centrifugation at 10,800xg for 15 min, supernatant (osmotic shock fraction) was combined with the periplasmic fraction.

Western blotting: Phage was purified using polyethylene glycol (PEG) precipitation of supernatants collected from infected bacteria by adding 1/5 volume PEG/NaCl (20% PEG 6000-2.5 M NaCl) and incubation for 1 hr at 4°C. Tubes were centrifuged at 10,800xg for 30 min. and pellets were resuspended in 1/10 of the original volume in water. Western blots of M-13 gIII and scFv-gIII or scIg/TCR-gIII or scTCR-gIII fusion proteins were conducted as follows: 20µl of each purified phage or periplasmic preparation were separated on a 10% SDS–acrylamide gel and then electroblotted. The nitrocellulose membran (Schleicher&Schuell, Dassel, Germany) was blocked overnight at 4°C in 10% marvel in PBS/0.5% Tween 20. After washing with PBS/0.5% Tween 20, gIII and scFv-gIII or scIg-TCR-gIII or scTCR-gIII fusion proteins were detected with HRP-conjugated anti-M13 gIII conjugate, or protein A-HRP or protein L-HRP. Peroxidase activity was detected using the ECL kit from Amersham Amersham Life Science, Little Chalfont, UK).

Target: Selection is performed against any target which include purified proteins, peptides, cells, body fluid, organ etc.

Selection of recombinant scTCR and scTCR-Ig display: Phage display libraries was enriched for antigen-binding scTCR and scTCR-Ig clones by subjecting the phages to repetitive rounds of selection including binding, washing and elution steps, re-infection into bacteria and growth to re-express the scTCR or scTCR-Ig molecules on the phage surface. A combination of procedures which have already been shown to work for the selection of scFv antibody fragments was applied (9, 10). Briefly, to select for phage particle(s) expressing scTCR or scTCR, immunotubes (Nunc-Immuno Tubes, MaxiSorp, Nunc, Denmark) was coated with the target protein by adding 10-100µg/ml of protein in PBS, and incubated overnight at 4^0. The uncoated sites on the immunotubes were then be blocked by 2% skimmed milk in PBS for two hours. After washing with PBS tubes will be exposed to 10^{12}-10^{13} transforming units (t.u.) of the phage library in 2% skimmed milk in PBS. After two hours incubation the library was washed free of unbound phage and the bound

phage will be eluted by alkali (100 mM triethylamine) and neutralized with 1M Tris, pH7.4. For enrichment of the target bound phage, the eluate was used to infect exponential E.coli TG-1 and after growing them over night, phage particles was rescued with VCS-M13 helper phage (Stratagene, USA) as described (9, 10). The process was repeated two to five times and final phage preparation was used to infect TG-1 bacteria to screen for individuals TG-1 colonies bearing specific phage.

To take the selection technology a step further towards the situation of interactions in vivo, the selection will be performed in solution by binding to biotinylated target, followed by capture of bound phage with streptavidin-coated paramagnetic beads. Selection will be performed as in case of immunotubes however, if biotin disulphide is used to link the antigen to biotin, the phage antigen complex can be eluted by use of dithiothreitol (DTT) which disrupts the disulphide linkage between biotin and antigen.

Additional step will include cell selection, since the ultimate goal must be the production of reagents, which are able to detect their targets in native state on tumor cell surfaces. In case of HLA/peptide target peptide transporter-deficient cell lines like BM36.1 (HLA-A1,Cw4,B35,Bw6) (11) or T2 (HLA-A2) will be employed. These cells can be grown for one day at 26°C and then loaded with the desired peptide (e.g. a MAGE-1-derived, HLA-A1-binding peptide). Moreover, counterselection involving cell selection technology in combination with selection against purified complexes will be employed. For example, selection for scTCR or scTCR-Ig molecules against HLA-A1/MAGE-1 peptide complexes will first involve two consecutive steps in which the MAGE peptide is represented in the context of two different HLA molecules, such as HLA-A2 and HLA-B44 molecules. This counterselection will eliminate those phage particles reacting with "public" determinants on HLA class I. Further selections will then be carried out using a mixture of HLA-A1/MAGE1 complexes as targets or HLA-A1 typed BM36.1 cells loaded with MAGE1 peptide. Variations on this theme are obviously possible and will be carried out.

Screening assays and specificity/affinity measurement of scTCR and scTCR-Ig molecules: The progress of selection can be readily monitored after each round by two simple tests. Firstly, the number of infective phage (transforming units; t.u.) eluted after each round provides a first hint of progress. Typically,the numbers are low in the early rounds (about $10^5/10^6$ t.u.), increasing in later rounds to about 109 t.u. Secondly, the binding of the phage population (or individual clones) can be detected by ELISA, detecting the bound phage with anti-phage enzyme-linked antisera (eg. anti-M13 horseradish peroxidase (HRP)). Depending on the power of selection, binding is usually detected after two to five rounds of selection. The selection is usually terminated when most of the phage clones are found to bind by ELISA. At this stage soluble fragments will be expressed and secreted directly using HB2151 non-suppressor strain. The expression of antibody fragments is driven from lacz promoter, and is therefore induced by IPTG. The myc peptide tag appended to the antibody fragment facilitate their detection in ELISA by anti-myc tag antibodies (Sigma). ELISA will be done with various antigens coated onto the wells of microtiter plates or being presented on fixed cells. However, since the ultimate goal must be the production of reagents which are able to detect their targets in native state on tumor cell surfaces, flow cytometric analysis of BM36.1 loaded with the target peptide (e.g. a MAGE-1-derived, HLA-A1-binding peptide) will be performed. The affinity of the binders for their antigens can be measured by Fluorescence Quenching (12), Competition ELISA (13), or most accurately, by Surface Plasmon Resonance (BIACore) (14).

The specificity of the recombinant scTCRs or scTCR-Ig to be produced will then be rigorously assessed by screening against a large panel of HLA-typed cell lines, their capacity to inhibit the lytic activity of CTL clones specific for various antigens, their capacity to inhibit the production of TNF or IFNg by stimulated CTL, and immunoprecipitation and Western blotting of tissue extracts.

Assessment of the diagnostic potential of recombinant scTCR molecules: Recombinant scTCR or scTCR-Ig molecules showing specificity for a defined

HLA/peptide combination are expected to be extremely versatile tools in tumor diagnostics. So far, the presence of a given antigen on the surface of tumor cells is deduced from the HLA type of the patient and from RT-PCR analysis of the expression of the relevant gene in the sample. It is obvious, though, that the expression of the gene does not necessarily mean that the antigenic peptide will be present in sufficient amount at the surface of the cells. This problem is becoming more important now that pilot vaccination studies have started with cancer patients selected on the basis of the criteria mentioned above. It is quite possible that some patients are included in these studies although the actual expression of the antigen at the surface of the tumor cells in vivo is extremely low and consequently irrelevant. In tissue sections, suitable recombinant scTCRs and scTCR-Ig would allow to define precisely the proportion and distribution of antigenic tumor cells within a solid tumor, an information relevant to anti-tumor vaccine development and as yet impossible to obtain. Furthermore, such recombinant reagents would react with their target not only when it is expressed on the tumor cell surface, but presumably also when it is shed into body fluids.

The latter may allow monitoring of tumor progression, remission or relapse,comparable to the detection of the products of certain HLA class I alleles which are shed by cells from transplanted organs during rejection episodes.

Assessment of the therapeutic potential of selected recombinant scTCR and scTCR-Ig molecules in SCID mice: Selected recombinant scTCR or scTCR-Ig molecules will be engineered into fusion proteins with a truncated form of Pseudomonas exotoxin (PE38) (15), which will be expressed in E. coli followed by isolation from intracellular inclusion bodies by refolding and purification. The specificity and sensitivity of the immunotoxin fusion-proteins will be tested using several cellular model systems, in vitro and in vivo. Immunodeficient SCID mice transplanted with three different human tumors (~5mm diameter) will be employed to assess the functional activity of scTCR-PE38 or scTCR-Ig-PE38 constructs in vivo in comparison with other therapeutic agents such as CTL with specificity for HLA/peptide complexes.

RESULTS

Construction of Libraries: Amplification of the TCR Vα and Vβ gene segments was done using specifically designed TCRV gene-based primers and RNA prepared from lymphocytes .

The subgroup-specific TCR primers gave single RT-PCR bands of varying intensities. After PCR re-amplification of each RT-PCR reaction, the various TCR Vα, Vβ, VH and VL were cloned into the phagmid vector, building three different libraries: (i) TCRVα/TCRVβ, (ii) chimaeric TCRVα/VL and finally (iii) chimaeric TCRVH/Vβ (Fig1). The diversity of each of these libraries was between 10^7 to 10^8 different clones, as estimated by counting colonies plated after electroporation. From each library, several samples were sequenced and analyzed using the TCR nucleotide database. This database contains human sequences of 87 Vα regions, 148 Vβ regions, 61 Jα regions, 17 Jβ regions and 2 Dβ. Sequences for Vα and Vβ were retrieved from the database that was submitted to the EMBL nucleotide sequence database by Arden et al. (1) and assigned the alignment number DS23485. The sequences for the Jα regions, Jβ regions and the two Dβ were retrieved from the IMGT database. Alignment for the cloned sequences was carried out against this local database using MacVector 6.1 (Oxford Molecular Ltd. Sequence Analysis Software for Macintosh).

Sequenced samples from the libraries generated as described above have shown a random representation of different TCRVα and TCRVβ gene segments. In addition, the various Jα and Jβ segments were also represented in the library (Fig8).

Evaluation of constructed libraries: Libraries were evaluated for phage display and soluble scTCR/Ig production by: phage titer, sequence analysis, ELISA, SDS gel electrophoresis and Western blotting.

Phage Display: Several TG-1 clones harbouring the pTI vector which includes either TCRV$_\alpha$/V$_L$ or TCRVH/V$_\beta$ and TCRVα/ TCRVβ were studied.

Phage titers of the various libraries: The titers of theTCRVα/VL, TCRVH/Vβ, chimaeric TCRVα/ TCRV$_\beta$ libraries were in the range of 10^9 for each of the library, and for individual clones originating from them as well. This result must be taken as an indication that the TCR and the chimaeric fusion proteins are not toxic and that phage with these fusion proteins can be propagated.

Screening for relevant inserts: Individual clones from chimaeric TCRVα/VL and TCRVH/V$_\beta$ were first screened by PCR for their relevant inserts using the following primers: LMB3 and Cα-For-XhoI for the TCRVα/V$_L$ library (Fig.3, 6) and LMB3 together with C$_\beta$-For-NotI for TCRVH/Vβ library (Fig.3, 7). Positive PCR clones were re-amplified by LMB3 and fdSEQ for sequence analysis and the presence of random combinations of various TCRV$_\alpha$-J$_\alpha$ and TCRV$_\beta$-J$_\beta$ genes was demonstrated (Fig8, 9).

Analysis of TCR and Ig-TCR phage expression: In order to estimate the functionality of the TCR and the chimaeric scIg/TCR proteins displayed on phage, ELISA was performed using phage supernatant. Bound phage were detected with anti-M13-HRP, Protein A-HRP and Protein L-HRP. However, it was important to use the same phage supernatant for the parallel analysis of TCR and Ig V domain proteins existing in the newly constructed libraries. Phage originating from the TCRVα/VL library reacted with anti-M13 and Protein L-HRP, but not with Protein A-HRP (Fig 3A). Phage originating from the TCRVH/Vβ library reacted with anti-M13 and Protein A-HRP, but not with Protein L-HRP (Fig 3B). These results demonstrate the integrity of the various chimaeric domain combinations at the level of phage display. Soluble TCRVα/TCRVβ or phage displayed TCR did not react with neither Protein A or L. Antibody reagent specifically against the new linker and tag is currently prepared which will be applied in the future ELISA.

SDS gel analysis and Western blotting: SDS gel analysis confirmed that the size of gIII and gIII-sc fusion were 60kD and 100kD, respectively, as expected. The same results were obtained by immunoblotting using anti-M13-HRP or Protein L-HRP (in case of TCRVα/VL) or Protein A-HRP (in case of TCRVH/Vβ) that detected mainly bands of 100kD. Similar results were obtained when purified phage originating from the library pool or from individual clones were analyzed.

Analysis of soluble scTCR and scIg-TCR : Soluble (protein fragments detached from phage) scTCRV$_\alpha$/TCR V$_\beta$, and and chimaeric scIg/TCR expression analysis was performed exactly as for the evaluation of the constructed libraries (see above). However, anti-M13 antibody was omitted. HB2151 clones harbouring the pTI vector which included either TCRV$_\alpha$/TCRV$_\beta$, chimaeric TCRVα/VL and TCRVH/Vβ were first screened by PCR for the presence of their relevant inserts using the same primer combinations as in (1). Only the positive PCR clones were taken for further sequence analysis. Periplasmic extracts originating from the same clones were used in parallel for ELISA with Protein A-HRP and Protein L-HRP, and SDS-PAGE analysis. As expected, scTCRVα/VL reacted with Protein L-HRP and scTCRVH/Vβ with Protein A-HRP in ELISA (Fig.3C). A major band of 30kD which corresponds to the scTCR/Ig and TCRV$_\alpha$/TCRV$_\beta$, fragment was detected in SDS-PAGE.

Linkers: TCRV gene amplification was done by a set gene-based primers annealing to the 5' end of TCRV genes together with a 3' primer, which hybridises to the 5' end of Cα or Cβ, resulting in RT-PCR amplification of each of the TCRV gene segments with a 3' tail originating from the constant region of the TCR genes. These tails are maintained in the library at two positions: (i) linker between Vα and Vβ and (ii) as additional 3' tag (Figure 5) As demonstrated above, these new linkers did not interfere with the expression of the intact scTCR molecules (Fig 4). This is not the first time that a non-Gly4Ser linker is used. However, usually they are employed in commercially available antibodies or linkers, facilitating recombination. By this

approach, not only the number of PCR reactions for the TCRV gene amplification has been reduced by two logs, but for the first time specific linkers and a tag have been developed which can be specifically used to detect our novel constructs.

Modification of TCR Libraries Based on the Structure of the TCR: These results indicate that the difficulties in obtaining expression of intact and soluble scTCR as well as chimaeric constructs could be overcome. Still, additional improvements could be included in further constructed libraries. For example, mutations in scTCR genes which incorporate stabilizing motives based on the structural analysis of both TCR and antibodies can be included. There are two regions within the TCR molecule that drew our attention based on the work of Kieke et al. (21), in which a mutated scTCR was expressed in yeast . One region refers to the "elbow-like" structure in Cβ chain that stabilizes the scTCR. VβA13 and VβG17 (Figure 5) belong to the FR1 region. In a TCR molecule, this region is positioned in proximity (7-10Å) to a negatively charged "elbow" generated by Glu221, Glu 222 and Asp 223 of the C_{β}domain (Figure 6, white). This charged "elbow" does not exist in antibodies and is unique to TCR molecules. It is stabilized by the high positive charge that characterizes the Vβ FR1 of the TCR. (The net charge of residues 8-17 of Vβ is +1.6, as calculated from Vβ sequences from the Kabat database). The positive charge of the Vβ FR1 is stabilized as a result of electrostatic interactions with the negatively charged "elbow" of TCR Cβ. In the absence of this "elbow", as in scTCR, the presence of negatively charged amino acids and more hydrophobic buried residues, as achieved by VβG17E and VβA13V mutations, respectively, stabilizes the positively charged region of Vβ FR1 and thus stabilizes scTCR folding. This assumption is confirmed by the fact that the FR1 sequence in this region in TCRVα, Ab-VH, Ab-Vκ and Ab-Vλ chains have a significantly lower positive charge (mean net charge of +0.4), and that scFv can be displayed without problem.

The second refers to the interface between Vα and Vβ which might be a target for further development: VαL43, VαL104 and VβT105 are involved in the

Vα/Vβ interface. VαL43 belongs to the Vα FR2. The VαL43P mutation generates a leucine-proline pair at the Vα/Vβ interface (instead of leucine-leucin pair). This pair is highly conserved in antibodies and stabilizes the Vα/Vβ interface and increases stability of the mutant, facilitating its secretion and display on yeast cells (Kieke et al., 21). It is interesting to note that VβA13V,VβG17E and VαL43P mutations are naturally found in many VH and VL sequences (21). In addition, VαL104 and VβT105 belong to the CDR3 loop. It should be noted that the VαL104P mutation reduces the affinity of the scTCR, probably since proline introduces conformational changes in the V_{α}CDR3 that is involved in the binding of the target. These mutations as described above can be included in construction of TCR libraries.

EXAMPLE 2 **To test the performance of the newly constructed library selection were performed against several targets as described in the methods section: recombinant IgE receptor (IgER), Epidermal growth factor (EGF), a** human epidermoid carcinoma cells (A431) which over express a very high number of EGF receptors, FITC-BSA,and HLA-A2/MAG3 and HLA-A2/MAGE10 complex. All selections have been performing very well, and specific clones were selected. Fig10 shows sequence sampling of some of these selections.

What is claimed is:

1. A phage-display library for screening target molecules, comprising a plurality of recombinant phages, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for a T-cell receptor (TCR) recognition element, and/or a mutation and variant thereof; and a polynucleotide which codes for an immunoglobulin (Ig) recognition element, and/or a mutation and variant thereof, and in which the vector expresses a recombinant chimeric TCR recognition element/immunoglobulin recognition element reagent from each of the recombinant phages.

2. The library of claim 1, wherein the TCR recognition element comprises a variable fragment of the TCR.

3. The library of claim 2, wherein the variable fragment of the TCR comprises one or more of TCR variable α(TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains.

4. The library of claim 3, wherein the TCRVα comprises one or more of the complementarity determining residues (CDR) 1, CDR2 or CDR3 segments.

5. The library of claim 3, wherein the TCRVβ comprises one or more of the CDR1, CDR2 or CDR3 segments.

6. The library of claim 3, wherein the TCRVγ comprises one or more of the CDR1, CDR2 or CDR3 segments.

7. The library of claim 3, wherein the TCRVδ comprises one or more of the CDR1, CDR2 or CDR3 segments.

8. The library of claim 1, wherein the TCR recognition element comprises a constant fragment.

9. The library of claim 8, wherein the constant fragment of the TCR is Cα, Cβ1, Cβ2, Cγ or Cδ domain.

10. The library of claim 1, wherein the immunoglobulin recognition element is an antibody comprising a variable domain.

11. The library of claim 10, wherein the antibody comprises a heavy chain and/or a light chain

12. The library of claim 11, wherein the antibody comprises a heavy chain .

13. The library of claim 10, wherein the antibody comprises a light chain.

14. The library of claim 12, wherein the heavy chain comprises one or more heavy chain variable fragments (VH).

15. The library of claim 13, wherein the antibody comprises one or more light chain variable fragments (VL).

16. The library of claim 12, wherein the heavy chain comprises one or more C_H1 constant domains.

17. The library of claim 13, wherein the light chain comprises one or more Cκ (kappa) or Cδlambda) domains.

18. The library of claims 14 or 15, wherein the variable domain comprises one or more of CDR1, CDR2 or CDR3 segments.

19. The library of claim 1, wherein the reagent is a Fv fragment.

20. The library of claim 19, wherein the Fv fragment is a single chain Fv fragment.

21. The library of claim 1, wherein the reagent is a Fab ~~Fv~~ fragment.

22. A linker which joins the T-cell receptor (TCR) recognition element, and the immunoglobulin (Ig) recognition element of the reagent comprising a nucleic acid which is characterized as: i) aiding in folding of the domains, ii) supporting the stabilization of the intact protein construct.

23. The linker of claim 22, wherein the nucleic acid comprises the sequence as set forth in Figure 5 .

24. The library of claim 1, wherein the vector comprises a nucleic acid which codes for a second molecule that is linked to the TCR and/or the chimeric TCR/Ig reagent.

25. The library of claim 24, wherein the second molecule is a molecule which interacts with a second,

nonoverlapping determinant of the target molecule or a multimeric target .

26. The library of claim 25, wherein with the second molecule enhances the overall avidity of the interaction of the TCR and/or the chimeric TCR/Ig fragment with the target molecule or a multimeric target.

27. The library of claim 26, wherein the TCR and/or the chimeric TCR/Ig fragment joint to the second molecule is a bispecific molecule.

28. The library of claim 26, wherein the second molecule is a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or inorganic substance, ion, synthetic, or mimetic, thereof.

29. The library of claim 28, wherein the second molecule is is a reagent directed against a specific MHC/peptide complex coupled to CD8, or variant thereof which exhibits low affinity to their respective target.

30. A tag which joins the T-cell receptor (TCR) reagent, and the chimeric reagent with gIII protein of the bacteriophage of the reagent comprising a nucleic acid which is characterized as: i) aiding in purification and detection of the reagent.

31. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVα/VL and/or mutation and variant thereof.

32. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVβ/VL and/or mutation and variant thereof.

33. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVα/VH and/or mutation and variant thereof.

34. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVβ/VH and/or mutation and variant thereof.

35. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VL/TCRVα and/or mutation and variant thereof.

36. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VL/TCRVβ and/or mutation and variant thereof.

37. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VH/TCRVα and/or mutation and variant thereof.

38. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VH/TCRVβ and/or mutation and variant thereof.

39. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVγ/VL and/or mutation and variant thereof.

40. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVδ/VL and/or mutation and variant thereof.

41. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVγ/VH and/or mutation and variant thereof.

42. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain TCRVδ/VH and/or mutation and variant thereof.

43. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VL/TCRVγ and/or mutation and variant thereof.

44. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VL/TCRVδ and/or mutation and variant thereof.

45. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VH/TCRVγ and/or mutation and variant thereof.

46. The library of claim 1, wherein the phage displayed chimeric TCR/Ig fragment is a single chain VH/TCRVδ and/or mutation and variant thereof.

47. A phage-display library for screening for target molecules, comprising a plurality of recombinant phages, wherein each of the recombinant phages comprise a vector having a polynucleotide which codes for a T-cell receptor (TCR) recognition element, and/or a mutation and variant

thereof; and in which the vector expresses a recombinant TCR recognition element from each of the recombinant phages.

48. The library of claim 47, wherein the TCR recognition element comprises a variable fragment of the TCR.

49. The library of claim 48, wherein the variable fragment of the TCR comprises one or more of TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains.

50. The library of claim 49, wherein the TCRVα comprises one or more of the complementarity determining residues (CDR) 1, CDR2 or CDR3 segments.

51. The library of claim 49, wherein the TCRVβ comprises one or more of the CDR1, CDR2 or CDR3 segments.

52. The library of claim 49, wherein the TCRVγ comprises one or more of the CDR1, CDR2 or CDR3 segments.

53. The library of claim 49, wherein the TCRVδ comprises one or more of the CDR1, CDR2 or CDR3 segments.

54. The library of claim 47, wherein the TCR recognition element comprises a constant fragment.

55. The library of claim 54, wherein the constant fragment of the TCR is Cα, Cβ1, Cβ2, Cγ or Cδ domain.

56. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVα/TCRVβ and/or mutation and variant thereof.

57. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVα/TCRVγ and/or mutation and variant thereof.

58. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVα/TCRVδ and/or mutation and variant thereof.

59. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVβ/TCRVα and/or mutation and variant thereof.

60. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVβ/TCRVγ and/or mutation and variant thereof.

61. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVβ/TCRVδ and/or mutation and variant thereof.

62. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVγ/TCRVα and/or mutation and variant thereof.

63. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVγ/TCRVβ and/or mutation and variant thereof.

64. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVγ/TCRVδ and/or mutation and variant thereof.

65. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVδ/TCRVα and/or mutation and variant thereof.

66. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVδ/TCRVβ and/or mutation and variant thereof.

67. The library of claim 47, wherein the phage displayed recombinant TCR fragment is a single chain TCRVδ/TCRVγ and/or mutation and variant thereof.

68. The library of claim 47, wherein the vector comprises a nucleic acid which codes for a second molecule that is linked to the reagent

69. The library of claim 68, wherein the second molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target .

70. The library of claim 69, wherein the second molecule enhances the overall avidity of the interaction of the reagent with the target molecule or a multimeric target.

71. The library of claim 68, wherein the TCR fragment joint to the second molecule is a bispecific molecule.

72. The library of claim 68, wherein the second molecule is a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or inorganic substance, ion, synthetic, or mimetic, thereof.

73. A phage displayed recombinant chimeric TCR recognition element/Ig recognition element reagent.

74. A soluble reagent detached from phage which includes recombinant chimeric TCR recognition element/immunoglobulin recognition element reagent.

75. The reagent of claim 74, wherein the TCR recognition element comprises a variable fragment of the TCR.

76. The reagent of claim 75, wherein the variable fragment of the TCR comprises one or more of TCR variable α (TCRVα), TCR variable β (TCRVβ), TCR variable γ (TCRVγ), or TCR variable δ (TCRVδ) domains.

77. The reagent of claim 76, wherein the TCRVα comprises one or more of the complementarity determining residues (CDR) 1, CDR2 or CDR3 segments.

78. The reagent of claim 76, wherein the TCRVβ comprises one or more of the CDR1, CDR2 or CDR3 segments.

79. The reagent of claim 76, wherein the TCRVγ comprises one or more of the CDR1, CDR2 or CDR3 segments.

80. The reagent of claim 76, wherein the TCRVδ comprises one or more of the CDR1, CDR2 or CDR3 segments.

81. The reagent of claim 74, wherein the TCR recognition element comprises a constant fragment.

82. The reagent of claim 81, wherein the constant fragment of the TCR is Cα, Cβ1, Cβ2, Cγ or Cδ domain.

83. The reagent of claim 74, wherein the immunoglobulin recognition element is an antibody comprising a variable domain.

84. The reagent of claim 83, wherein the antibody comprises a heavy chain and/or a light chain

85. The reagent of claim 84, wherein the antibody comprises a heavy chain.

86. The reagent of claim 84, wherein the antibody comprises a light chain.

87. The reagent of claim 84, wherein the heavy chain comprises one or more heavy chain variable fragments (VH).

88. The reagent of claim 84, wherein the antibody comprises one or more light chain variable fragments (VL).

89. The reagent of claim 84, wherein the heavy chain comprises one or more C_H1 constant domains.

90. The reagent of claim 84, wherein the light chain comprises one or more Cκ (kappa) or Cδ (lambda) domains.

91. The reagent of claim 87, wherein the variable domain comprises one or more of CDR1, CDR2 or CDR3 segments.

92. The reagent of claim 83, wherein the reagent is a Fv fragment.

93. The reagent of claim 92, wherein the Fv fragment is a single chain Fv fragment.

94. The reagent of claim 83, wherein the reagent is a Fab fragment.

95. The reagent of claim 94, wherein the vector comprises a nucleic acid which codes for a second molecule that is linked to the reagent.

96. The reagent of claim 95, wherein the second molecule is a molecule which interacts with a second, nonoverlapping determinant of the target molecule or a multimeric target.

97. The reagent of claim 96, wherein the second molecule enhances the overall avidity of the interaction of the reagent with the target molecule or a multimeric target.

98. The reagent of claim 93 wherein the single chain Fv fragment is displayed on phage or wherein the Fab fragment is displayed on phage.

99. The reagent of claim 97, wherein the second molecule is a nucleic acid, DNA, RNA, peptide, polypeptide, enzyme, single chain polypeptide, carbohydrate, glycosphingolipid, fatty acid, organic or inorganic substance, ion, synthetic, or mimetic, thereof.

100. The reagent of claim 99, wherein the second molecule is is a reagent directed against a specific MHC/peptide complex coupled to CD8, or variant thereof which exhibits low affinity to their respective targetCD8 or anti-β_2m.

101. The reagent of claim 74, wherein the reagent is a single chain TCRVα/VL and/or mutation and variant thereof.

102. The reagent of claim 74, wherein the reagent is a single chain TCRVβ/VL and/or mutation and variant thereof.

103. The reagent of claim 74, wherein the reagent is a single chain TCRVα/VH and/or mutation and variant thereof.

104. The reagent of claim 74, wherein the reagent is a single chain TCRVβ/VH and/or mutation and variant thereof.

105. The reagent of claim 74, wherein the reagent is a single chain VL/TCRVα and/or mutation and variant thereof.

106. The reagent of claim 74, wherein the reagent is a single chain VL/TCRVβ and/or mutation and variant thereof.

107. The reagent of claim 74, wherein the reagent is a single chain VH/TCRVα and/or mutation and variant thereof.

108. The reagent of claim 74, wherein the reagent is a single chain VH/TCRVβ and/or mutation and variant thereof.

109. The reagent of claim 74, wherein the reagent is a single chain TCRVγ/VL and/or mutation and variant thereof.

110. The reagent of claim 74, wherein the reagent is a single chain TCRVδ/VL and/or mutation and variant thereof.

111. The reagent of claim 74, wherein the reagent is a single chain TCRVγ/VH and/or mutation and variant thereof.

112. The reagent of claim 74, wherein the reagent is a single chain TCRVδ/VH and/or mutation and variant thereof.

113. The reagent of claim 74, wherein the reagent is a single chain VL/TCRVγ and/or mutation and variant thereof.

114. The reagent of claim 74, wherein the reagent is a single chain VL/TCRVδ and/or mutation and variant thereof.

115. The reagent of claim 74, wherein the reagent is a single VH/TCRγ and/or mutation and variant thereof.

116. The reagent of claim 74, wherein the reagent is a single chain VH/TCRVδ and/or mutation and variant thereof.

117. A recombinant TCR recognition element reagent and mutant and /or variant thereof.

118. The reagent of claim 117, wherein the reagent is a single chain TCRVα/TCRVβ.

119. The reagent of claim 117, wherein the reagent is a single chain TCRVα/TCRVγ.

120. The reagent of claim 117, wherein the reagent is a single chain TCRVα/TCRVδ.

121. The reagent of claim 117, wherein the reagent is a single chain TCRVβ/TCRVα.

122. The reagent of claim 117, wherein the reagent is a single chain TCRVβ/TCRVγ.

123. The reagent of claim 117, wherein the reagent is a single chain TCRVβ/TCRVδ.

124. The reagent of claim 117, wherein the reagent is a single chain TCRVγ/TCRVα.

125. The reagent of claim 117, wherein the reagent is a single chain TCRVγ/TCRVβ.

126. The reagent of claim 117, wherein the reagent is a single chain TCRVγ/TCRVδ.

127. The reagent of claim 117, wherein the reagent is a single chain TCRVδ/TCRVα.

128. The reagent of claim 117, wherein the reagent is a single chain TCRVδ/TCRVβ.

129. The reagent of claim 117, wherein the reagent is a single chain TCRVδ/TCRVγ.

130. An expression vector comprising a polynucleotide which codes for a T-cell receptor (TCR) recognition element, and/or a mutation and variant thereof; and polynuclotide which codes for an immunoglobulin (Ig) recognition element, and/or a mutation and variant thereof.

131. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRVα/VL.

132. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRVβ/VL.

133. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRVα/VH.

134. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRVβ/VH.

135. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VL/TCRVα.

136. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VL/TCRVβ.

137. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VH/TCRVα.

138. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VH/TCRVβ.

139. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRVγ/VL.

140. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRVδ/VL.

141. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRVγ/VH.

142. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRVδ/VH.

143. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VL/TCRVγ.

144. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VL/TCRVδ.

145. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VH/TCRVγ.

146. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VH/TCRVδ.

147. The vector of claim 130, wherein the polynucleotide encoding the T-cell receptor (TCR) and the immunoglobulin elements, fragments, domains and/or segments are in a tail-to-head transcriptional orientation.

148. The vector of claim 130, wherein the vector is selected from the group consisting of plasmids, phages, phagemids, viral vectors and combinations thereof.

149. The vector of claim 130, further comprising transcription and translation control sequences.

150. The vector of claim 130, wherein the transcription control sequence is selected from the group consisting of a promoter, an RNA polymerase initiation site, an RNA polymerase termination site, a TATA box, a CAT box, a poly A addition site, an enhancer and a part or combination thereof.

151. The vector of claim 130, wherein the translation control sequence is selected from the group consisting of a ribosome binding site, a leader sequence and a part or combination thereof.

152. The vector of claim 130, wherein the vector is selected from the group consisting of pNMV, pCDV, p1PP12, PUC19-CK-CH1, PUM 19-CK-CHI and pJS.

153. An oligonucleotide comprising a nucleic acid having the sequence as set forth in Figure 1.

154. A method for creating a phage display chimeric T-cell receptor/immunoglobulin reagent comprising the steps of:

(i) obtaining a sample of cells;

(ii) preparing mRNA

(iii) reverse transcribing mRNA of the cell population into cDNA sequences of T-cell receptor and immunoglobulin;

(iv) amplifying the cDNA;

(v) providing nucleic acid expression vectors which are capable of being packaged;

(vi) cloning the population of DNA fragments into expression vectors;

(vii) combining (i) a genetically diverse repertoire of nucleic acid sequences in which each encode a unique or genetically diverse population first component part of the TCR-cell receptor elements with (ii) a genetically diverse repertoire of nucleic acid sequences which encodes a unique or genetically diverse population of the immunoglobulin elements, to form a library of nucleic acid sequences using said expression vectors encoding said TCR and antibody polypeptide; also with the property of binding specifically to a target molecule of interest;

(viii) expressing said library from said vectors in recombinant host organism cells, each of the said polypeptide chain components being expressed as a recombinant chimeric protein on its own or or as part of phage particles which are components of the library;

(ix) selecting from said expressed library, by binding to a target molecule of interest, for

example with a MHC-peptide complex, a unique or restricted population of said reagents binding specificity, thereby producing a recombinant chimeric T-cell receptor/immunoglobulin reagent.

155. A method for creating a phage display T-cell receptor reagent comprising the steps of:

(i) obtaining a sample of cells;

(ii) preparing mRNA

(iii) reverse transcribing mRNA of the cell population into cDNA sequences of T-cell receptor;

(iv) amplifying the cDNA;

(v) providing nucleic acid expression vectors which are capable of being packaged;

(vi) cloning the population of DNA fragments into expression vectors;

(vii) combining a genetically diverse repertoire of nucleic acid sequences in which each encode a unique or genetically diverse population first component part of the TCR-cell receptor elements, to form a library of nucleic acid sequences using said expression vectors encoding said TCR polypeptide; also with the property of binding specifically to a target molecule of interest;

(viii) expressing said library from said vectors in recombinant host organism cells, said polypeptide chain components being expressed as a recombinant TCR protein on its own or as part of phage particles which are components of the library;

(ix) selecting from said expressed library, by binding to a target molecule of interest, for example with a MHC-peptide, a unique or restricted population of said reagents binding specificity, thereby producing a recombinant T-cell receptor reagent.

156. A primer comprising the nucleic acid having the sequence as set forth in Figure 6, 7.

157. A method for selection against a target molecule, said method comprising:

i) contacting the library of claims 1 or 45 with the target molecule so as to form a complex,
ii) dissociating the bound phage from the complex;
iii) amplifying bound phage by growth in bacterial host;
iv) repeating round of binding, dissociation and amplification; and
v) screening said selected library on a target molecule.

158. The method of claim 157, further comprising characterizing the selected phage.

159. The method of claim 157, wherein the target and/or library are labeled.

160. The method of claim 157, wherein the library is attached to a target molecule bound to a support matrix.

161. The method of claim 160, wherein the support is a plastic dish, a virus particle, or a cell culture.

162. The method of claim 157, wherein the target molecule comprises cells such as tumor cells, viral infected cells, cells originated from tissue or organs.

163. A method for diagnosing a subject with a tumor, comprising the steps of:

i) Obtaining a sample from the subject,

ii) Contacting the sample with a recombinant reagent of claims 72-128, wherein the reagent is specific for a specific tumor antigen so as to form a complex,

iii) Detecting the complex, the presence of which is indicative of a subject having the tumor.

164. Use of a pharmaceutical composition according to any of claims 56-90 for the prevention or treatment of a disease selected from the group consisting of HLA class I associated diseases (ankylosing spondylitis, Reiter disease, psoriatic spondylitis, psoriasis vulgaris and Behcet disease) and rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, systemic lupus erythematosus, Sj6gren disease, IDDM, Addison disease, Graves disease, Hashimoto disease, celiac disease, primary biliary cirrhosis, pemphigus vulgaris, epidermolysis bullosa acquisita, Hodgkin disease, cervical squamous cell carcinoma, multiple sclerosis, optic neuritis, narcolepsi, myasthenia gravis, Goodpasture syndrome and alopecia areata).

165. A method of treating a subject with a disease or a pathogenic conditions, comprising administrating to

the subject an effective amount of the reagent of claims 74-130, thereby treating the subject with the disease or pathogenic condition.

166. A method for imaging a neoplastic disorder in a subject comprising the steps of administrating to the subject an amount of the recombinant reagent of claims 74-130, wherein the reagent is labeled, and detecting the label.

167. A method of purifying and detecting the reagent of the recombinant phage library of claim 1, wherein the reagent comprises a linker region.

168. A method of purifying and detecting the reagent of the recombinant phage library of claim 1, wherein the reagent comprises a tag.

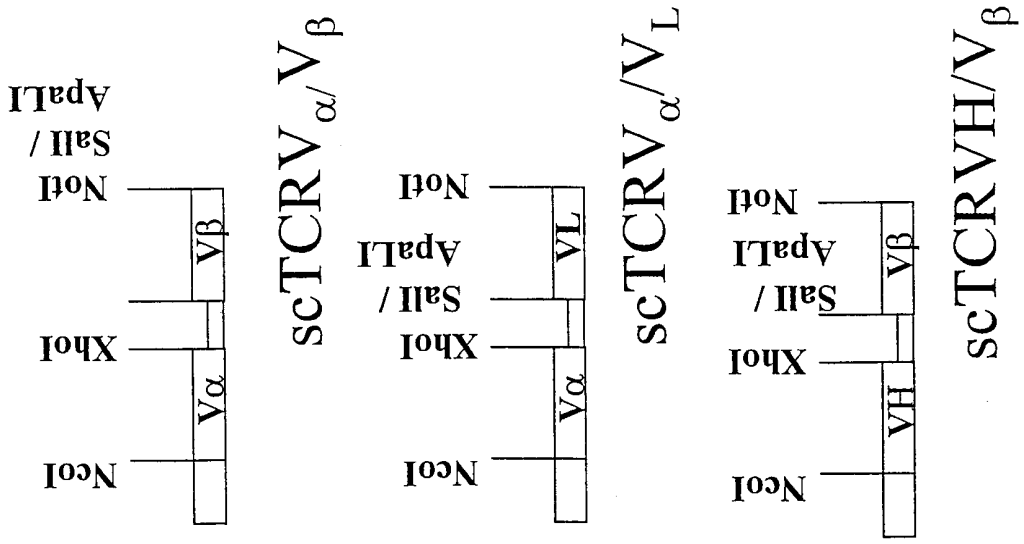

scTCRV$_\alpha$/V$_\beta$
NcoI
Vα
XhoI
Vβ
SalI / ApaLI
NotI
scTCRV$_\alpha$/V$_L$
NcoI
Vα
XhoI
VL
SalI / ApaLI
NotI
scTCRVH/V$_\beta$
NcoI
VH
XhoI
Vβ
SalI / ApaLI
NotI

FIG .1B

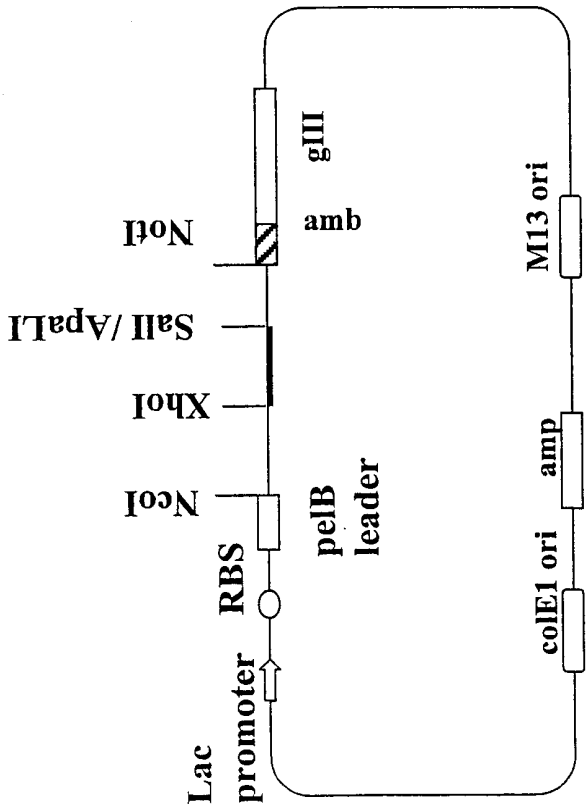

Lac promoter
RBS
NcoI
pelB leader
XhoI
SalI /ApaLI
NotI
gIII
amb
colE1 ori
amp
M13 ori

FIG .1A

FIG .2A

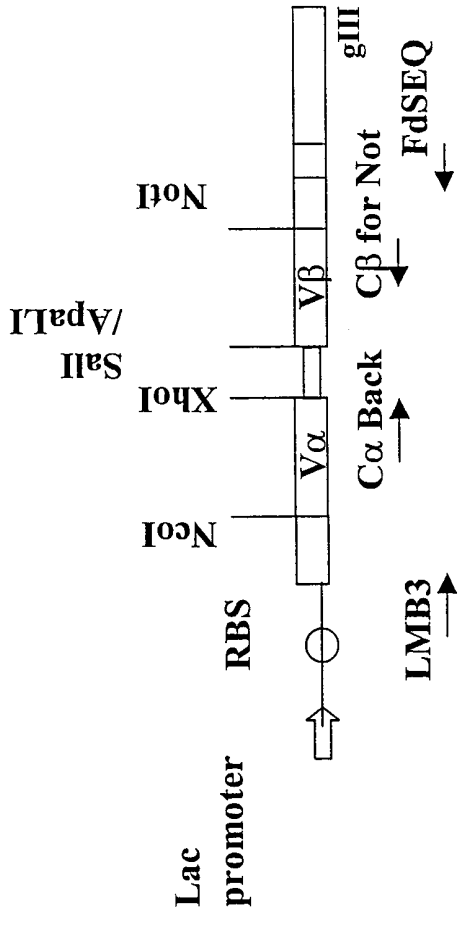


FIG .2B

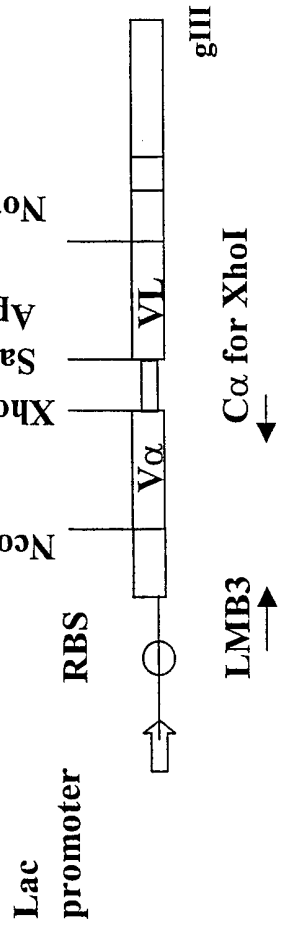
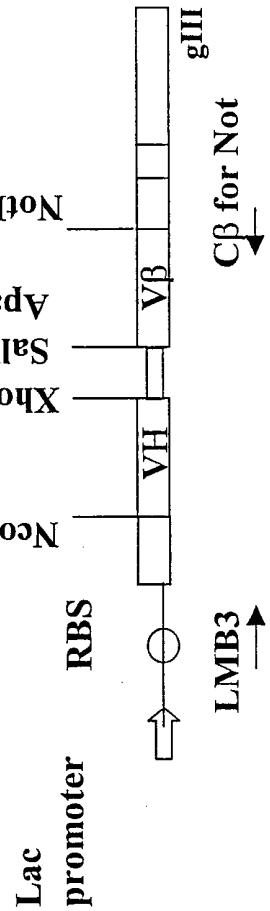


FIG .2C



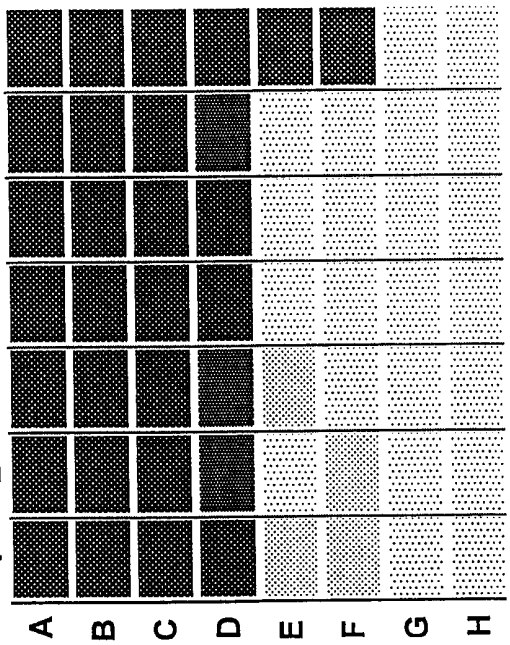


FIG.3B

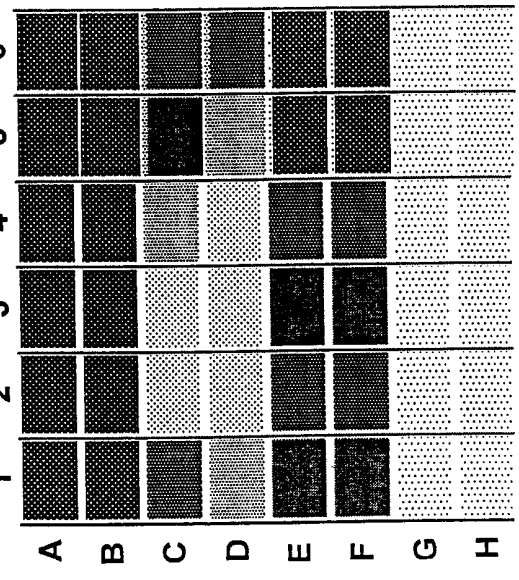


FIG.3A

ScTCRVα/VL	ScTCRVH/Vβ
Protein A/L	Protein A/L
0.055/1.078	0.550/0.012
0.043/0.431	0.319/0.011
0.078/1.486	0.266/0.010
0.074/0.249	0.168/0.009

FIG.3C

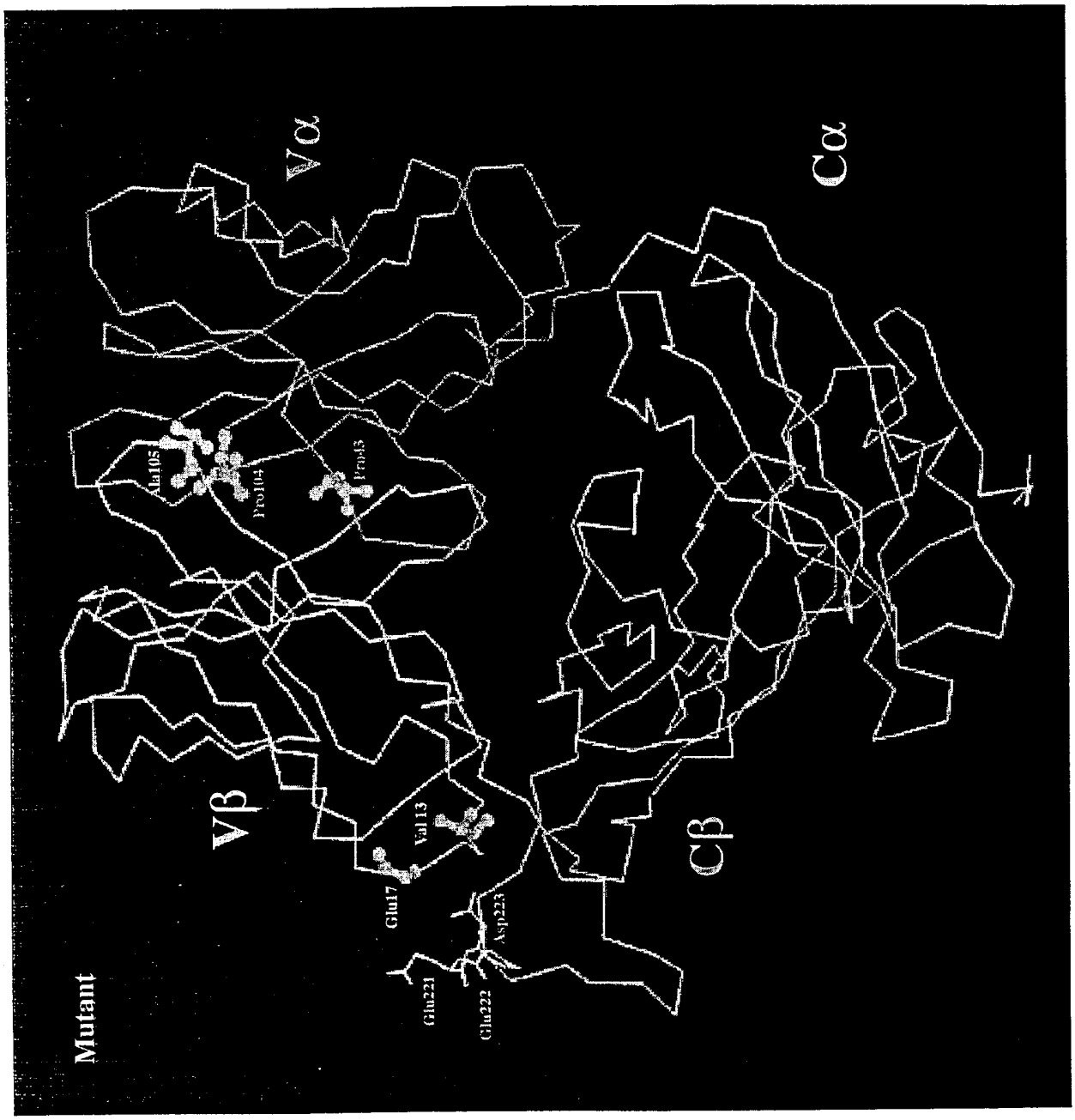

Mutant
Vα
Cα
Vβ
Cβ
Ala105
Pro104
Pro43
Val 13
Glu17
Glu221
Glu222
Asp223

FIG.4A

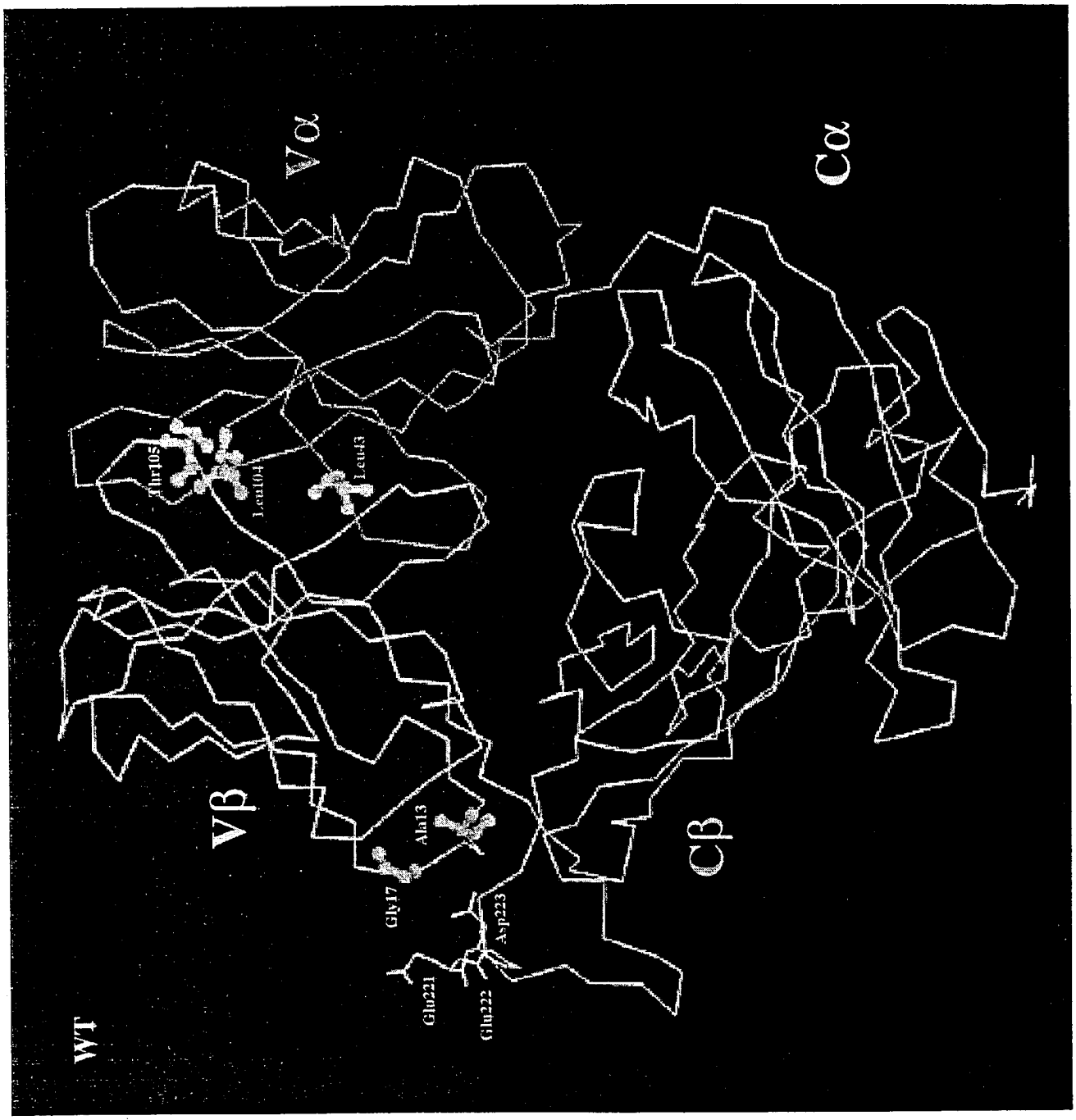

Vα
Cα
Vβ
Cβ
Thr105
Leu104
Leu43
Ala13
Gly17
Glu221
Glu222
Asp223
WT

FIG.4B

XhoI

NcoI- - Vα gene- - GAC TCT AAA TCC AGT GAC AAG TCT GTC TCG AGC GGT GGA GGC GGT TCA GGC GGA GGT GGC AGC GGC

D S K S S D K S V S S G G G G S G G G G S G

Sal I Vβ NotI

GGT GGC GGG TCG ACG - gene - - GAG GAC CTG AAC AAG GTG GCG GCC GCA

G G G S T E D L N K V A A A

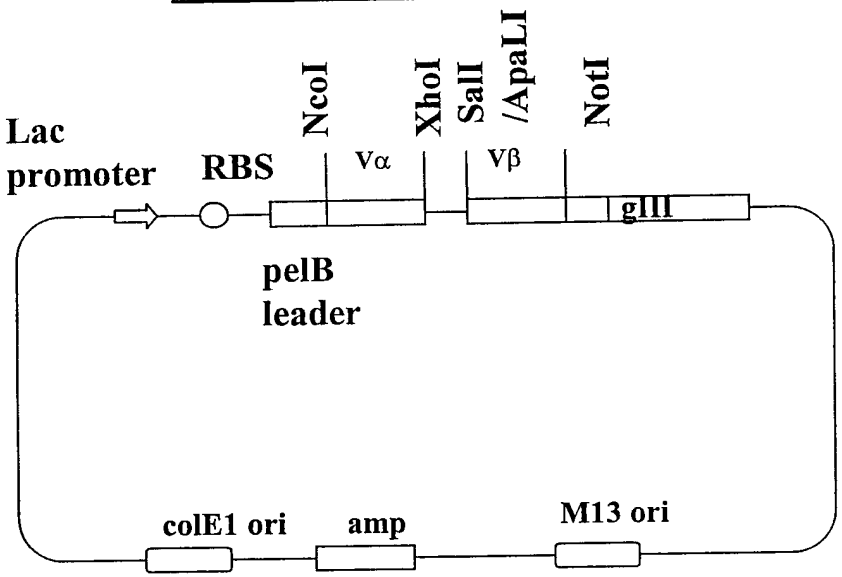


FIG .5A

LINKER: D S K S S D K S V S S G G G G S G G G G S G G G G S T

Cα seq

Tag E D L N K V A A A H H H H H H H----myc tag

Cβ seq

Vα- - - - - GAC TCT AAA TCC AGT GAC AAG TCT GTC TCG AGC GGT GGA GGC GGT TCA GGC GGA GGT GGC AGC GGC
D S K S S D K S V S S G G G G S G G G G S G

Sal I NotI
AGC GGC GGT GGC GGG TCG ACG - - - - - - - - - - GAG GAC CTG AAC AAG GTG GCG GCC GCA
G G G S T E D L N K V A A A

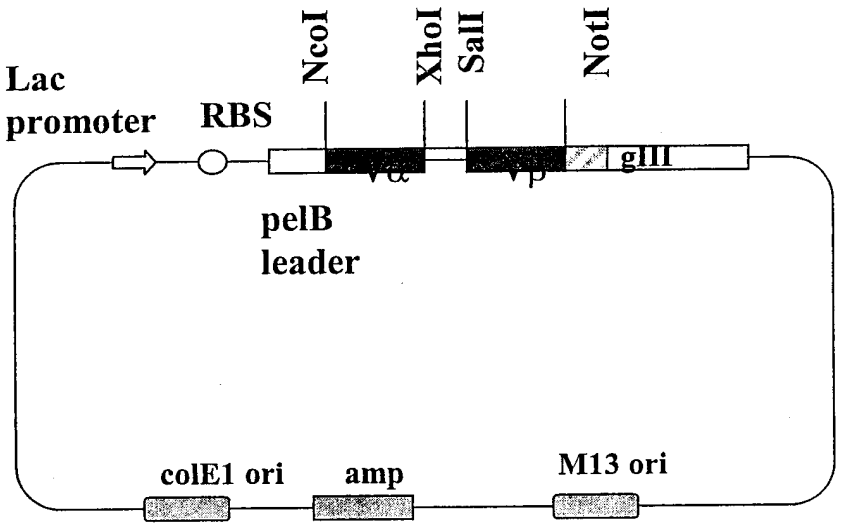


FIG .5B

Vα-gene	Oligo
AV1S1 (AVS2-5)	GCG GCC CAG CCG GCC **ATG G**CC CAG TCK GTG ASC CAG CWT
AV2S1 (AV2S2-3)	GCG GCC CAG CCG GCC ATG GCC CAG GAG CTG GAG CAG RAT
AV3S1	GCG GCC CAG CCG GCC ATG GCC CAA CAG GGA GAA GAG GAT
AV4S1-2, AV20S1	GCG GCC CAG CCG GCC ATG GCC GCT AAG ACC ACC ACC CAG CCC
AV6S1	GCG GCC CAG CCG GCC ATG GCC CAG AAG ATA ACT CAA ACC
AV7S1 (AV7S2	GCG GCC CAG CCG GCC ATG GCC CAA ARC MTT GAS CAG CCC
AV8S1	GCG GCC CAG CCG GCC ATG GCC GAG ART GTG GRG CWG CAT
AV9S1	GCG GCC CAG CCG GCC ATG GCC CAG AGA GTG ACT CAG CCC
AV10S1	GCG GCC CAG CCG GCC ATG GCC CAG CTG CTG GAG CAG AGC
AV11S1	GCG GCC CAG CCG GCC ATG GCC GAC CAA GTG TTT CAG CCT
AV12S1	GCG GCC CAG CCG GCC ATG GCC CAG AAG GTA ACT CAA GCG
AV14S1-2	GCG GCC CAG CCG GCC ATG GCC CAG ACA GTC ACT CAG TCT
AV16S1	GCG GCC CAG CCG GCC ATG GCC CAG TCA GTG GCT CAG CCG
AV18S1 (AV15S1)	GCG GCC CAG CCG GCC ATG GCC GTG RAY GTG GAG CAA MGT
AV19S1 (AV24S1,AV13S1	GCG GCC CAG CCG GCC ATG GCC AWH SAA GTG GAG CAG AGT
AV21S1 (AV5S1)	GCG GCC CAG CCG GCC ATG GCC CAG MAA RTT AAG CAA AAT

FIG. 6A

AV22S1A1 (AV22S1A2)	GCG GCC CAG CCG GCC ATG GCC RAT TCA GTG ACC CAG ATG
AV23S1 (AV32S1	GCG GCC CAG CCG GCC ATG GCC CAR SAG GTR AYR CAR ATT
AV26S1 (AV25S1, AV17S1, AV30S1)	GCG GCC CAG CCG GCC ATG GCC SAV SAR STG RMD CAR AGT
AV27S1	GCG GCC CAG CCG GCC ATG GCC CTG AAA GTG GAA CAA AAC
AV28S1	GCG GCC CAG CCG GCC ATG GCC GAC AAG GTG GTA CAA AGC
AV29S1	GCG GCC CAG CCG GCC ATG GCC CAA CCA GTG CAG AGT CCT
AV31S1	GCG GCC CAG CCG GCC ATG GCC AAT TCA GTC AAG CAG ACG
Cα-For- Xho	GTGCATAGCTG**CTCGAG**ACAGACTTGTCACTGGATTTAGAGTC

FIG. 6B

Template TCRVβ gene	Sequence	
BV1S1A1N1 (BV11S1A1T, BV14S1, BV11S2OP)	CGC CGC AGT GTT AG**G TCG AC**G	SGM RTS WMA CAA AMC CCA
BV2S1A2	CGC CGC AGT GTT AGG TCG ACG	GTC GTC TCT CAA CAT CCG
BV3S1	CGC CGC AGT GTT AGG TCG ACG	AAA GTA ACC CAG AGC TCG
BV4S1A1T	CGC CGC AGT GTT AGG TCG ACG	GTC ATC TCT CAA AAG CCA
BV5S5P,6,3,7,2 (BV5S4)	CGC CGC AGT GTT AGG TCG ACG	GGA GTC ACM CAA AGT CCC
BV6S4A1,3,5 (BV6S4A2, BV6S5A1N1,BV6S5A2, BV26S1, BV6S6, 3, BV22S1A2N1,2)	CGC CGC AGT GTT AGG TCG ACG	GRA GTC WSC CAG DMY CCC
BV21S3A1,2 (BV21S1, BV16, BV6S5A1N2T) -	CGC CGC AGT GTT AGG TCG ACG	GGR GTC TYY CAR TCY CCA
BV6S8,9 (BV23S1A1,2T, BV5S1A1T, BV5S1A2T)	CGC CGC AGT GTT AGG TCG ACG	GGA GTC TCC CAG TCC CCT
BV6S2	CGC CGC AGT GTT AGG TCG ACG	GGA GTC TCC CAG TCC CTG
BV6S7P	CGC CGC AGT GTT AGG TCG ACG	GGA GTC TCC CAG TCC CTG
BV7S1A1N2T (BV27S1P, BV13S2A1T,S1)	CGC CGC AGT GTT AGG TCG ACG	GRW RTY ACB CAG ACM CCA
BV7S2A2T (BV30S1A1, BV31S1P,BV13S5)	CGC CGC AGT GTT AGG TCG ACG	GGR RTY ASG CAR RYA CCA
BV8S1-2 (BVS4, BVS5)	CGC CGC AGT GTT AGG TCG ACG	GGH RTT ATC CAG TCA CCC
BV8S3& (BV19S1P,2)	CGC CGC AGT GTT AGG TCG ACG	ARA GTC ACM CAG ACW CCA

FIG. 7A

BV9S1 (BV9S2)	CGC CGC AGT GTT AGG TCG ACG	GCY GTT TCC CAG ACT CCA
BV10S1P	CGC CGC AGT GTT AGG TCG ACG	AAG GTC ACC CAG AGA CCT
BV12S1A1N1 (BV12S2A1T, BV17S1A3T, BV29S1P)	CGC CGC AGT GTT AGG TCG ACG	GRM ATC WMY CAG WBS CCA
BV15S1	CGC CGC AGT GTT AGG TCG ACG	GAT GTT ACC CAG ACC CCA
BV20S1A	CGC CGC AGT GTT AGG TCG ACG	ACT ATT CAT CAA TGG CCA
BV21S2A1,2	CGC CGC AGT GTT AGG TCG ACG	GGA GTG GTT CAG TCT CCC
BV21S3A1,2 (BV21S1, BV16, BV6S5A1N2T)	CGC CGC AGT GTT AGG TCG ACG	GRA GTT DCC CAG TYY CCC
BV24S1A2T (BV18S1)	CGC CGC AGT GTT AGG TCG ACG	RKS GTC ATS CAG AAC CCA
BV25S1A1,2,3	CGC CGC AGT GTT AGG TCG ACG	GAA GAA GTC GCC CAG ACT
BV28S1P	CGC CGC AGT GTT AGG TCG ACG	GTA GTT ACA CAA TTC CCA
BV32S1P	CGC CGC AGT GTT AGG TCG ACG	GGG ATC ACC CAG ATG CCA
BV33S1P	CGC CGC AGT GTT AGG TCG ACG	GAA GCC ACC TAG ACT CTA
BV34S1P	CGC CGC AGT GTT AGG TCG ACG	AAA GTA ACA CAG ACC
Cβ1-F0R-Not	GAGCCCATGATGT**GCGGCCGC**CACCTTGTTCAGGTCCTC	
Cβ2-F0R-Not	CAGTGACCCATGT**GCGGCCGC**CACGTTTTTCAGGTCCTC	

FIG. 7B

Vα GENE	Jα GENE	Vα GENE	Jα GENE
AV28S1A3T	Tra42	**AV22S1A2N1**	TRA47
AV28S1A3T	TRA36	**AV22S1A2N1**	TRA4
AV28S1A3T	TRA37	**AV22S1A2N1**	Tra53
AV28S1A1T	TRA41		
AV28S1A3T	TRA26	AV1S4A1N1T	TRA44
AV28S1A1T	Tra43		
AV28S1A3T	TRA58	AV4S2A2T	TRA34
		AV4S2A2T	TRA49
AV6S1A1N1	Tra9		
AV6S1A2N2	Tra8	AV16S1A2PT	TRA13
AV6S1A2N2	Tra30		
AV6S1A1N1	Tra37	AV3S1	TRA40
AV6S1A2N1	Tra13	AV3S1	TRA16
AV6S1A2N2	Tra48	AV3S1	Tra9
AV6S1A2N2	Tra52		
AV6S1A1N1	Tra5	AV14S2A2N1	TRA10
AV6S1A2N2	TRA10	AV14S1	TRA50
AV6S1A1N1	TRA26	AV14S2A1T	TRA50
		AV14S2A1T	TRA50
AV21S1A1N	TRA6	AV14S2A1T	TRA50
AV21S1A1N1	TRA57	AV14S1	TRA50
AV21S1A1N1	TRA52		
AV21S1A1N	TRA36	AV20S1	TRA4
AV21S1A1N	TRA36	AV10S1A2	TRA49
		AV9S1	TRA9
AV27S1	TRA43		
AV27S1	TRA54	AV19S1	Tra 22
AV27S1	TRA31		
AV27S1	Tra50	AV19S1	Tra 54
AV27S1	Tra33	AV23S1	Tra 58
AV27S1	Tra56	AV23S1	Tra 21
AV27S1	TRA20		
AV27S1	TRA20		
AV27S1	TRA48		
AV27S1	TRA45		
AV27S1	TRA41		

FIG. 8A

TCRVβ1 gene	Jβ gene	TCRVβ2 gene	TCR-Jβ
BV2S1A1	TRBJ1_1	BV2S1A1	2-7
BV2S1A1	trbj1-4	BV2S1A1	2-2
		BV2S1A1	2-3
BV3S1	TRB1-5	BV2S1A1	2-4
BV3S1	TRB1-4	BV3S1	2-7
		BV3S1	2-5
BV4S1A1T	TRB1-5	BV3S1	2-4
BV4S1A1T	TRB1-1	BV3S1	2-1
BV4S1A3T	TRB1-2	BV4S1A1T	2-5
		BV4S1A1T	2-3
BV5S4A2T	TRB1-1	BV4S1A1T	2-2
		BV4S1A1T	2-1
BV6S5A1N1	TRBJ 1-5	BV5S1A1T	2-7
BV6S5A1N1	TRBJ1_1	BV6S2A1N1T	2-5
		BV6S2A1N1T	2-3
BV8S2A1T	TRB1-1	BV6S4A1	2-1
BV8S1	TRB1-5	BV6S4A1	2-3
BV8S1	TRB1-2	BV6S4A1	2-7
		BV6S5A1N1	2-5
		BV6S5A1N1	2-3
BV9S1A1T	TRB1-5	BV7S1A1N2T	2-3
BV9A1A1T	TRBJ1_2 (172)	BV7S3A2T	2-7
		BV9S1A1T	2-7
		BV13S6A2T	2-2
BV13S1	TRBJ1_1	BV15S1	2-1
BV13S6A2T	TRBJ1_4	BV18S1	2-3
BV13S1	TRB1-1	BV19S1P	2-3
		BV19S1P	2-4
		BV19S1P	2-7
BV22S1A2N1	TRB1-5	BV21S2A2	2-2
		BV21S2A2	2-1
		BV22S1A2N1	1-1
		BV22S1A2N1	2-3

FIG. 8B

TCRVα	Jα
AV14S2A2N	TRA37
AV22S1A2N	TRA9
AV3S1	TRA47
ADV6S1A1N	NC
AV23S1	TRA22
ADV6S1A1N	TRA39
AV22S1A1N	TRA20
AV16S1A2P	TRA4
AV22S1A2N	TRA13

FIG. 9

	TCR-Vα	TCR-Jα
Anti-IgER clones		
	AV21S1A1N	TRA57
	AV21S1A1N	TRA49
	AV21S1A1N	TRA30
	AV27S1	TRA20
	AV27S1	TRA27
	AV27S1	TRA27
	AV21S1A1N	TRA53
	AV6S1A2N2	TRA21
Anti-EGF clones		
	AV6S1A2N1	TRA5
	AV6S1A2N2	TRA22
	AV28S1A3T	TRA11
	AV6S1A2N2	TRA39
	AV16S1A2PT	TRA5
	AV22S1A2N1	TRA11
	AV27S1	TRA54
	AV8S1A1	TRA9
Anti-A431 clones	AV27S1	TRA57
	AV27S1	TRA49
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	AV21S1A2PT	TRA49
	AV27S1	TRA52

FIG. 10

SEQUENCE LISTING

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