pMHC tetramer combinations directly from a human blood sample with astonishing sensitivity, although this technology is presently limited to ~100 such combinations per assay [12]. Each of these approaches has contributed valuable insights into T-cell epitopes; however, these methods are slow, labor-intensive and require a high degree of user skill.

[0007] The present invention addresses this need for new and improved technologies for the efficient and systematic identification of the repertoire of T-cell epitopes.

SUMMARY ~~OF THE INVENTION~~

[0008] The present disclosure ~~This invention~~ provides an isolated suspension-adapted cell transduced by or transfected with a heterologous nucleic acid comprising, in 5' to 3' order:

a leader oligonucleotide sequence, contiguous with

an oligonucleotide sequence encoding an 8, 9, 10, 11 or 12 amino acid peptide, contiguous with

an oligonucleotide sequence encoding a first linker, contiguous with

an oligonucleotide sequence encoding a beta 2 microglobulin sequence, contiguous with

an oligonucleotide sequence encoding a second linker, contiguous with

an oligonucleotide sequence encoding a Major Histocompatibility Complex heavy chain sequence, contiguous with

an oligonucleotide sequence encoding a third linker, contiguous with

an oligonucleotide sequence encoding a fluorescent protein, contiguous with

an oligonucleotide sequence encoding a fourth linker, contiguous with

an oligonucleotide sequence encoding a Major Histocompatibility Complex heavy chain transmembrane domain.

[0009] The present disclosure ~~This invention~~ also provides isolated suspension-adapted cell expressing an expression product of a heterologous nucleic acid transduced or transfected therein, which expression product comprises, in N-terminal to C-terminal order:

an 8, 9, 10, 11 or 12 amino acid peptide, contiguous with

a first linker peptide sequence, contiguous with

a beta 2 microglobulin sequence, contiguous with

a second linker peptide sequence, contiguous with

a Major Histocompatibility Complex heavy chain sequence, contiguous with

a third linker peptide sequence, contiguous with

a fluorescent protein, contiguous with

[0015] Also provided is a method of identifying a T-cell epitope comprising
contacting a T-cell with a plurality of isolated suspension-adapted cells comprising at least two cells, each expressing an expression product of a heterologous nucleic acid transduced or transfected therein, each of which expression products comprises an 8, 9, 10, 11 or 12 amino acid peptide, contiguous with first linker peptide sequence, contiguous with a beta 2 microglobulin sequence, contiguous with a second linker peptide sequence, contiguous with a Major Histocompatibility Complex heavy chain sequence, contiguous with a third linker peptide sequence, contiguous with a Major Histocompatibility Complex heavy chain transmembrane domain, wherein the plurality of isolated suspension-adapted cells expresses at least two different encoded 8, 9, 10, 11 or 12 amino acid peptides among the cells thereof under conditions permitting T-cells to conjugate with the 8, 9, 10, 11 or 12 amino acid peptides;
recovering T-cell(s) which have formed a conjugate with a suspension-adapted cell;
recovering DNA from the recovered T-cell(s);
sequencing the recovered DNA;
identifying the 8, 9, 10, 11 or 12 amino acid peptide(s) encoded for in the DNA,
so as to thereby identify a T-cell epitope.

[0016] In one aspect of the present disclosure, there is ~~Also~~ provided ~~is~~ an isolated suspension-adapted cell transduced by or transfected with a heterologous nucleic acid comprising, in 5' to 3' order:
a leader oligonucleotide sequence, contiguous with
an oligonucleotide sequence encoding a 5 to 20 amino acid peptide, contiguous with
an oligonucleotide sequence encoding a first linker, contiguous with
an oligonucleotide sequence encoding a beta 2 microglobulin sequence, contiguous with
an oligonucleotide sequence encoding a second linker, contiguous with
an oligonucleotide sequence encoding a Major Histocompatibility Complex heavy chain sequence, contiguous with
an oligonucleotide sequence encoding a third linker, contiguous with
an oligonucleotide sequence encoding a fluorescent protein or encoding an immunoglobulin Fc domain, contiguous with
an oligonucleotide sequence encoding a fourth linker, contiguous with
an oligonucleotide sequence encoding a mammalian transmembrane domain.

[0016a] In another aspect of the present disclosure, there is provided a membrane-bound portion expressing the expression product of a cell as disclosed herein.

[0017] In another aspect of the present disclosure, there is ~~Also~~ provided ~~is~~ an isolated suspension-adapted cell expressing an expression product of a heterologous nucleic acid transduced or transfected therein, or a membrane-

bound portion of such cell expressing the expression product, which expression product comprises, in N-terminal to C-terminal order:
a 5 to 20 amino acid peptide, contiguous with

a first linker peptide sequence, contiguous with

a beta 2 microglobulin sequence, contiguous with

a second linker peptide sequence, contiguous with

a Major Histocompatibility Complex heavy chain sequence, contiguous with

a third linker peptide sequence, contiguous with

a fluorescent protein or a sequence of an immunoglobulin Fc domain, contiguous with

a fourth linker peptide sequence, contiguous with

a mammalian transmembrane domain.

[0018] Also provided is an isolated suspension-adapted cell expressing an expression product of a heterologous nucleic acid transduced or transfected therein, or a membrane-bound portion of such cell expressing the expression product, which expression product comprises, in N-terminal to C-terminal order:

a 5 to 20 amino acid peptide, contiguous with

a first linker peptide sequence, contiguous with

a beta 2 microglobulin sequence, contiguous with

a second linker peptide sequence, contiguous with

a Major Histocompatibility Complex heavy chain sequence, contiguous with

a third linker peptide sequence, contiguous with

a fluorescent protein or a sequence of an immunoglobulin Fc domain, contiguous with

a fourth linker peptide sequence, contiguous with

a mammalian transmembrane domain.

[0019] In a further aspect of the present disclosure, there is provided a~~A~~ plurality of the isolated suspension-adapted cells or a plurality of membrane-bound portions of such cells expressing the expression product, wherein the plurality comprises at least two different encoded 5 to 20 amino acid peptides, is also provided.

[0020] In another aspect of the present disclosure, there is provided a~~A~~ (i) virus-like particle or (ii) virus, produced by an isolated suspension-adapted cell as described herein is provided, which virus like particle or a virus is physically associated via a cell membrane portion having attached thereto, by a mammalian transmembrane domain, an expression product comprising in N-terminal to C-terminal order:

a 5 to 20 amino acid peptide, contiguous with

a first linker peptide sequence, contiguous with
a beta 2 microglobulin sequence, contiguous with
a second linker peptide sequence, contiguous with
a Major Histocompatibility Complex heavy chain sequence, contiguous with
a third linker peptide sequence, contiguous with
a fluorescent protein or a sequence of an immunoglobulin Fc domain, contiguous with
a fourth linker peptide sequence, contiguous with
the mammalian transmembrane domain, contiguous with
a viral packaging sequence.

[0021] In a further aspect of the present disclosure, there is provided a~~A~~ plurality of the virus-like particles described, or of the viruses described, is also provided.

[0022] In another aspect of the present disclosure, there is ~~Also~~ provided ~~is~~ a recombinant nucleic acid comprising, in 5' to 3' order:
a sequence encoding a leader oligonucleotide sequence, contiguous with
an oligonucleotide sequence encoding a 5 to 20 amino acid peptide, contiguous with
an oligonucleotide sequence encoding a first linker, contiguous with
an oligonucleotide sequence encoding a beta 2 microglobulin sequence, contiguous with
an oligonucleotide sequence encoding a second linker, contiguous with
an oligonucleotide sequence encoding a Major Histocompatibility Complex heavy chain sequence, contiguous with
an oligonucleotide sequence encoding a third linker peptide sequence, contiguous with
an oligonucleotide sequence encoding a fluorescent protein or an immunoglobulin Fc domain, contiguous with
an oligonucleotide sequence encoding a fourth linker peptide sequence, contiguous with
an oligonucleotide sequence encoding a mammalian transmembrane domain.

[0023] In a further aspect of the present disclosure, there is ~~Also~~ provided ~~is~~ an isolated suspension-adapted cell transduced by or transfected with a heterologous nucleic acid comprising, in 5' to 3' order:
an oligonucleotide sequence encoding a first B2M leader sequence,
contiguous with an oligonucleotide sequence encoding a preselected 5 to 20 amino acid peptide,
contiguous with an oligonucleotide sequence encoding a first amino acid linker sequence,
contiguous with an oligonucleotide sequence encoding a sequence of amino acids identical to a human native B2M peptide sequence,
contiguous with an oligonucleotide sequence encoding a second amino acid linker sequence,

contiguous with an oligonucleotide sequence encoding a preselected second peptide sequence,
contiguous with an oligonucleotide sequence encoding a third amino acid linker,
contiguous with an oligonucleotide sequence encoding a second B2M leader sequence,
contiguous with an oligonucleotide sequence encoding a sequence of amino acids identical to a MHC heavy chain,
contiguous with an oligonucleotide sequence encoding a fourth amino acid linker,
contiguous with an oligonucleotide sequence encoding a sequence of amino acids identical to an immunoglobulin Fc domain,
contiguous with an oligonucleotide sequence encoding a fifth linker,
contiguous with an oligonucleotide sequence encoding a mammalian transmembrane domain.

[0024] In another aspect of the present disclosure, there is ~~Also~~ provided ~~is~~ an isolated suspension-adapted cell expressing an expression product of a heterologous nucleic acid transduced or transfected therein, or a membrane-bound portion of such cell expressing the expression product, which expression product comprises a recombinant polypeptide construct comprising (i) a preselected 5 to 20 amino acid peptide bound by a first amino acid linker sequence contiguous with a sequence of amino acids comprising a sequence identical to a human native B2M peptide sequence contiguous with a second amino acid linker sequence contiguous with a preselected second peptide sequence, wherein (i) is bound by one, or more than one, disulfide bond to (ii) a sequence of amino acids having the sequence of a MHC heavy chain contiguous with a fourth amino acid linker sequence contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain contiguous with a fifth amino acid linker, contiguous with a mammalian transmembrane domain.

[0025] In a further aspect of the present disclosure, there is ~~Also~~ provided ~~is~~ an isolated suspension-adapted cell transduced by or transfected with a virus, plasmid or viral vector comprising a heterologous nucleic acid comprising, in 5' to 3' order:
an oligonucleotide sequence encoding a first B2M leader sequence,
contiguous with an oligonucleotide sequence encoding a preselected 5 to 20 amino acid peptide, contiguous with an oligonucleotide sequence encoding a first amino acid linker sequence, contiguous with an oligonucleotide sequence encoding a sequence of amino acids identical to a human native B2M peptide sequence,

contiguous with an oligonucleotide sequence encoding a second amino acid linker sequence,
contiguous with an oligonucleotide sequence encoding a preselected second peptide sequence,
contiguous with an oligonucleotide sequence encoding a third amino acid linker,
contiguous with an oligonucleotide sequence encoding a second B2M leader sequence,
contiguous with an oligonucleotide sequence encoding a sequence of amino acids identical to a MHC heavy chain,
contiguous with an oligonucleotide sequence encoding a fourth amino acid linker,
contiguous with an oligonucleotide sequence encoding a sequence of amino acids identical to an immunoglobulin Fc domain,
contiguous with an oligonucleotide sequence encoding a fifth linker,
contiguous with an oligonucleotide sequence encoding a mammalian transmembrane domain,
contiguous with an oligonucleotide encoding a viral packaging sequence.

[0026] In another aspect of the present disclosure, there is provided a~~A~~ (i) virus like particle or (ii) virus, produced by ~~the~~ a cell disclosed herein~~of Claim 46~~, which virus like particle or a virus is physically associated via a cell membrane portion having attached thereto, by a mammalian transmembrane domain, an expression product comprising in N-terminal to C-terminal order:
a recombinant polypeptide construct comprising (i) a preselected 5 to 20 amino acid peptide bound by a first amino acid linker sequence contiguous with a sequence of amino acids comprising a sequence identical to a human native B2M peptide sequence contiguous with a second amino acid linker sequence contiguous with a preselected second peptide sequence, wherein (i) is bound by one, or more than one, disulfide bond to (ii) a sequence of amino acids having the sequence of a MHC heavy chain contiguous with a fourth amino acid linker sequence contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain contiguous with a fifth amino acid linker, contiguous with a mammalian transmembrane domain, contiguous with a viral packaging sequence.

[0027] In a further aspect of the present disclosure, there is ~~Also~~ provided ~~is~~ a recombinant nucleic acid comprising, in 5' to 3' order:
an oligonucleotide sequence encoding a first B2M leader sequence,
contiguous with an oligonucleotide sequence encoding a preselected 5 to 20 amino acid peptide,
contiguous with an oligonucleotide sequence encoding a first amino acid linker sequence,
contiguous with an oligonucleotide sequence encoding a sequence of amino acids identical to a human native B2M peptide sequence,

contiguous with an oligonucleotide sequence encoding a second amino acid linker sequence,
contiguous with an oligonucleotide sequence encoding a preselected second peptide sequence,
contiguous with an oligonucleotide sequence encoding a third amino acid linker,
contiguous with an oligonucleotide sequence encoding a second B2M leader sequence,
contiguous with an oligonucleotide sequence encoding a sequence of amino acids identical to a MHC heavy chain,
contiguous with an oligonucleotide sequence encoding a fourth amino acid linker,
contiguous with an oligonucleotide sequence encoding a sequence of amino acids identical to an immunoglobulin Fc domain,
contiguous with an oligonucleotide sequence encoding a fifth linker,
contiguous with an oligonucleotide sequence encoding a mammalian transmembrane domain.

[0028] In another aspect of the present disclosure, there is provided a~~A~~ method of identifying a T-cell epitope comprising

contacting a T-cell with a plurality of isolated suspension-adapted cells comprising at least two cells, or a membrane-bound portion of such cells expressing the expression product, each cell or membrane bound portion expressing an expression product of a heterologous nucleic acid transduced or transfected therein, each of which expression products comprises a 5 to 20 amino acid peptide, contiguous with first linker peptide sequence, contiguous with a beta 2 microglobulin sequence, contiguous with a second linker peptide sequence, contiguous with a Major Histocompatibility Complex heavy chain sequence, contiguous with a third linker peptide sequence, contiguous with a fluorescent protein or an immunoglobulin Fc domain, contiguous with a fourth linker peptide sequence, contiguous with a mammalian transmembrane domain, wherein the plurality of isolated suspension-adapted cells or membrane-bound portions expresses at least two different encoded 5 to 20 amino acid peptides among the cells or membrane-bound portions under conditions permitting T-cells to conjugate with the 5 to 20 amino acid peptides;

recovering T-cell(s) which have formed a conjugate with a suspension-adapted cell or membrane-bound portions;

recovering DNA from the suspension-adapted cell(s);

sequencing the recovered DNA;

identifying the 5 to 20 amino acid peptide(s) encoded for in the DNA,

so as to thereby identify a T-cell epitope.

[0029] In a further aspect of the present disclosure, there is provided a~~A~~ method of identifying a T-cell epitope comprising

contacting a T-cell with a plurality of isolated suspension-adapted cells comprising at least two cells, or membrane-bound portions thereof, each expressing an expression product of a heterologous nucleic acid transduced or transfected therein, each of which expression products comprises (i) a preselected 5 to 20 amino acid peptide bound by a first amino acid linker sequence contiguous with a sequence of amino acids comprising a sequence identical to a human native B2M peptide sequence contiguous with a second amino acid linker sequence contiguous with a preselected second peptide sequence,

wherein (i) is bound by one, or more than one, disulfide bond to (ii) a sequence of amino acids having the sequence of a MHC heavy chain contiguous with a fourth amino acid linker sequence contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain contiguous with a fifth amino acid linker, contiguous with a mammalian transmembrane domain, wherein the plurality of isolated suspension-adapted cells or membrane-bound portions thereof expresses at least two different encoded 5 to 20 amino acid peptides among the cells or portions under conditions permitting T-cells to conjugate with the 5 to 20 amino acid peptides;

recovering T-cell(s) which have formed a conjugate with a suspension-adapted cell or membrane-bound portion;

recovering DNA from the suspension-adapted cell(s);

sequencing the recovered DNA;

identifying the 5 to 20 amino acid peptide(s) encoded for in the DNA,

so as to thereby identify a T-cell epitope.

[0030] In another aspect of the present disclosure, there is provided a~~A~~ method of identifying a T-cell epitope comprising

contacting a T-cell with a plurality of isolated suspension-adapted cells comprising at least two cells, or virus-like particle or viruses associated with a membrane portion of such cells, the cells or membrane bound portion expressing an expression product of a heterologous nucleic acid transduced or transfected therein, each of which expression products comprises a 5 to 20 amino acid peptide, contiguous with first linker peptide sequence, contiguous with a beta 2 microglobulin sequence, contiguous with a second linker peptide sequence, contiguous with a Major Histocompatibility Complex heavy chain sequence, contiguous with a third linker peptide sequence, contiguous with a fluorescent protein or an immunoglobulin Fc domain, contiguous with a fourth linker peptide sequence, contiguous

with a mammalian transmembrane domain, contiguous with a viral packaging sequence, wherein the plurality of isolated suspension-adapted cells or of virus-like particles or viruses associated with the membrane portion of the cells, expresses at least two different encoded 5 to 20 amino acid peptides among the cells or virus-like particles or viruses under conditions permitting T-cells to conjugate with the 5 to 20 amino acid peptides;

recovering T-cell(s) which have formed a conjugate with a suspension-adapted cell, virus-like particle or virus of the plurality;

recovering DNA from the suspension-adapted cell or RNA from the virus-like particle or virus;

sequencing the recovered DNA or RNA;

identifying the 5 to 20 amino acid peptide(s) encoded for in the DNA or RNA,

so as to thereby identify a T-cell epitope.

[0031] In a further aspect of the present disclosure, there is provided a~~A~~ method of identifying a T-cell epitope comprising

contacting a T-cell with a plurality of isolated suspension-adapted cells comprising at least two cells, or virus-like particles or viruses associated with a membrane portion of such a cells, each expressing an expression product of a heterologous nucleic acid transduced or transfected therein, each of which expression products comprises (i) a preselected 5 to 20 amino acid peptide bound by a first amino acid linker sequence contiguous with a sequence of amino acids comprising a sequence identical to a human native B2M peptide sequence contiguous with a second amino acid linker sequence contiguous with a preselected second peptide sequence, wherein (i) is bound by one, or more than one, disulfide bond to (ii) a sequence of amino acids having the sequence of a MHC heavy chain contiguous with a fourth amino acid linker sequence contiguous with a sequence of amino acids identical to an immunoglobulin Fc domain contiguous with a fifth amino acid linker, contiguous with a mammalian transmembrane domain, wherein the plurality of isolated suspension-adapted cells, virus-like particles or viruses expresses at least two different encoded 5 to 20 amino acid peptides among the cells thereof under conditions permitting T-cells to conjugate with the 5 to 20 amino acid peptides;

recovering T-cell(s) which have formed a conjugate with a suspension-adapted cell, virus-like particle or virus associated membrane portion;

recovering DNA from the suspension-adapted cell or RNA from the virus-like particle or virus;