

CLAIMS

1. A polynucleotide molecule comprising, in a 5' to 3' orientation, a sequence insert comprising stop codons in each of the different reading frames, a Beta 2 Microglobulin (β 2M) sequence, and a Multiple Histocompatibility Complex (MHC) allele sequence.
2. The polynucleotide of claim 1, wherein the sequence insert is flanked by a first universal target sequence and/or a second universal target sequence.
3. The polynucleotide of claim 1 or 2 further comprising a nucleotide sequence encoding an antigenic peptide.
4. A polynucleotide molecule comprising, in a 5' to 3' orientation, a first universal target sequence, a nucleotide sequence encoding an antigenic peptide, a second universal target sequence that is distinct from the first universal target sequence, a Beta 2 Microglobulin (β 2M) sequence, and a Multiple Histocompatibility Complex (MHC) allele sequence.
5. The polynucleotide of claim 3 or 4, wherein the antigenic peptide is a neoantigen.
6. The polynucleotide of any one of claims 1-5, wherein the MHC allele is a human HLA allele.
7. The polynucleotide of claim 6, wherein the HLA allele is selected from the group consisting of: HLA-A*01:01, HLA-A*02:01, HLA-A*03:01, HLA-A*24:02, HLA-A*30:02, HLA-A*31:01, HLA-A*32:01, HLA-A*33:01, HLA-A*68:01, HLA-A*11:01, HLA-A*23:01, HLA-A*30:01, HLA-A*33:03, HLA-A*25:01, HLA-A*26:01, HLA-A*29:02, HLA-A*68:02, HLA-B*07:02, HLA-B*14:02, HLA-B*18:01, HLA-B*27:02, HLA-B*39:01, HLA-B*40:01, HLA-B*44:02, HLA-B*46:01, HLA-B*50:01, HLA-B*57:01, HLA-B*58:01, HLA-B*08:01, HLA-B*15:01, HLA-B*15:03, HLA-B*35:01, HLA-B*40:02, HLA-B*42:01, HLA-B*44:03, HLA-B*51:01, HLA-B*53:01, HLA-B*13:02, HLA-B*15:07, HLA-B*27:05, HLA-B*35:03, HLA-B*37:01, HLA-B*38:01, HLA-B*41:02, HLA-B*44:05, HLA-B*49:01, HLA-B*52:01, HLA-B*55:01, HLA-C*02:02, HLA-C*03:04, HLA-C*05:01, HLA-C*07:01, HLA-C*01:02, HLA-C*04:01, HLA-C*06:02, HLA-C*07:02, HLA-C*16:01, HLA-C*03:03, HLA-C*07:04, HLA-C*08:01, HLA-C*08:02, HLA-C*12:02, HLA-C*12:03, HLA-C*14:02, HLA-C*15:02, and HLA-C*17:01.

8. The polynucleotide of claim 6, wherein the HLA allele comprises a sequence selected from the group consisting of SEQ ID NOs: 109-174.
9. The polynucleotide of any one of claims 1-8, wherein the β 2M allele is a human β 2M allele.
10. The polynucleotide of any one of claims 2-9, wherein the first and second universal target sequences comprise a polymerase chain reaction (PCR) primer target site.
11. The polynucleotide of any one of claims 2-10, wherein the first and second universal target sequences comprise a restriction enzyme cleavage site.
12. The polynucleotide of any one of claims 1-10 further comprising a signal sequence.
13. The polynucleotide of any one of claims 1-11, wherein the 3' end of the polynucleotide sequence further comprises a purification cluster sequence comprising a first affinity tag sequence, a protease cleavage site sequence, and a second affinity tag sequence.
14. The polynucleotide of any one of claims 2-13, wherein the first universal target sequence comprises the sequence set forth in SEQ ID NO: 3 and the second universal target sequence comprises the sequence set forth in SEQ ID NO: 4.
15. An expression construct comprising the polynucleotide molecule of any one of claims 1-14.
16. A library comprising at least two polynucleotide molecules of any one of claims 1-14 or at least two expression constructs of claim 15.
17. The library of claim 16 comprising at least 66 distinct polynucleotide molecules or 66 distinct expression constructs.
18. A polypeptide encoded by any one of claims 1-14.
19. The polypeptide of claim 18, wherein the MHC allele encodes for an HLA peptide comprising a Y84A or a Y84C mutation.
20. A library comprising at least two polypeptides of claim 17 or 18.

21. A kit comprising a polynucleotide of any one of claims 1-14, an expression construct of claim 15, a library of polynucleotides of claims 16 or 17, a polypeptide of claim 18 or 19, or a library of polypeptides of claim 20 and instructions for use.
22. A method for isolating an antigen-specific T cell, the method comprising:
- a. preparing a plurality of particles, wherein each particle comprises a polypeptide of any one of claim 18 or 19;
 - b. obtaining a sample known or suspected to comprise one or more T cells;
 - c. contacting the plurality of particles with the sample, wherein the contacting comprises providing conditions sufficient for a single T cell to bind the polypeptide attached to the particle, and
 - d. isolating the single T cell associated with the particle.
23. A method for assaying a T cell interaction with an antigen, the method comprising:
- a. preparing a plurality of particles, wherein each particle comprises a polypeptide of any one of claims 18 or 19;
 - b. obtaining a sample known or suspected to comprise one or more T cells;
 - c. contacting the plurality of particles with the sample, wherein the contacting comprises providing conditions sufficient for a single T cell to bind the polypeptide attached to the particle, and
 - d. treating the particles such that the labeled polypeptides are released and
 - e. detecting release of the labeled polypeptides from the T cells to assay the T cell interaction with the antigen sequence.