

WHAT IS CLAIMED IS:

1. A composition comprising a polynucleotide, wherein the polynucleotide comprises:
 - a. first and second homology arms homologous to first and second target nucleic acid sequences;
 - b. a TCR gene sequence positioned between the first and second homology arms;
 - c. a first P2A-coding sequence positioned upstream of the TCR gene sequence and a second P2A-coding sequence positioned downstream of the TCR gene sequence, wherein the first and second P2A-coding sequences code for the same amino acid sequence that are codon-diverged relative to each other;
 - d. a sequence coding for the amino acid sequence Gly Ser Gly positioned immediately upstream of the P2A-coding sequences; and
 - e. a sequence coding for a Furin cleavage site positioned upstream of the second P2A-coding sequence.
2. The composition of claim 1, wherein the first and second homology arms of the polynucleotide are each from about 300 bases to about 2,000 bases in length.
3. The composition of claim 1, wherein the first and second homology arms of the polynucleotide are each about 600 bases to about 1,000 bases in length.
4. The composition of claim 1, wherein the polynucleotide further comprises a human growth hormone signal sequence positioned between the first P2A-coding sequence and the TCR gene sequence.
5. The composition of claim 1, wherein the polynucleotide further comprises a second TCR gene sequence positioned between the second P2A-coding sequence and the second homology arm.
6. The composition of claim 5, wherein the polynucleotide further comprises:
 - a. a first human growth hormone signal sequence positioned between the first P2A-coding sequence and the first TCR gene sequence; and
 - b. a second human growth hormone signal sequence positioned between the second P2A-coding sequence and the second TCR gene sequence,
wherein the first and the second human growth hormone signal sequences are codon diverged relative to each other.

7. The composition of claim 1, wherein the polynucleotide further comprises an exogenous sequence of interest.
8. The composition of claim 7, wherein the exogenous sequence of interest encodes for a protein useful in autologous cell therapy.
9. The composition of claim 1, wherein the polynucleotide is a circular DNA.
10. The composition of claim 1, wherein the polynucleotide is a linear DNA.
11. The composition of claim 1, wherein the TCR gene sequence encodes for a TCR that recognizes a cancer antigen.
12. The composition of claim 11, wherein the cancer antigen is a neoantigen.
13. The composition of claim 11, wherein the cancer antigen is a patient specific neoantigen.
14. The composition of claim 1, wherein the TCR gene sequence is a patient specific TCR gene sequence.
15. The composition of claim 1, further comprising a nuclease.
16. The composition of claim 15, wherein the nuclease is a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) family nuclease or derivative thereof.
17. The composition of claim 16, further comprising an sgRNA.
18. The composition of claim 15, wherein the nuclease targets an endogenous TCR locus.
19. The composition of claim 15, wherein the nuclease targets a TCR-alpha and a TCR-beta loci.
20. The composition of claim 1, wherein the first and second target nucleic acid sequences are positioned within an endogenous TCR locus.
21. The composition of claim 20, wherein the endogenous TCR locus is a TCR-alpha locus.
22. The composition of claim 1, wherein the polynucleotide is non-viral.
23. The composition of claim 1, wherein the polynucleotide is a gene therapy vector.
24. A composition comprising a polynucleotide, wherein the polynucleotide comprises:
 - a. first and second homology arms homologous to first and second target nucleic acid sequences;
 - b. a TCR gene sequence positioned between the first and second homology arms;
 - c. a first P2A-coding sequence positioned upstream of the TCR gene sequence and a second P2A-coding sequence positioned downstream of the TCR gene sequence, wherein the first and second P2A-coding sequences code for the same amino acid sequence that are codon-diverged relative to each other;

- d. a sequence coding for a flexible linker positioned immediately upstream of the P2A-coding sequences; and
 - e. a sequence coding for a protease cleavage sequence positioned upstream of the second P2A-coding sequence.
25. A composition comprising a circular polynucleotide, wherein the circular polynucleotide comprises:
- a. first and second homology arms homologous to first and second target nucleic acid sequences;
 - b. a TCR gene sequence positioned between the first and second homology arms;
 - c. a first P2A-coding sequence positioned upstream of the TCR gene sequence and a second P2A-coding sequence positioned downstream of the TCR gene sequence, wherein the first and second P2A-coding sequences code for the same amino acid sequence that are codon-diverged relative to each other;
 - d. a sequence coding for the amino acid sequence Gly Ser Gly positioned immediately upstream of the P2A-coding sequences; and
 - e. a sequence coding for a Furin cleavage site positioned upstream of the second P2A-coding sequence.