

CLAIMS

What is claimed is:

1. A method

of modifying a population of human cells, comprising contacting the population with one or more polynucleic acids comprising:

(a) a mobile genetic element, wherein the mobile genetic element comprises a sequence encoding LINE-1 polypeptides comprising:

(i). a human ORF1p or a functional fragment thereof, and

(ii). a human ORF2p or a functional fragment thereof, wherein the human ORF2p or functional fragment thereof comprises two NLSs that are the same or different;

(b) an insert sequence, wherein the insert sequence is a reverse complement of a sequence comprising a gene or a fragment thereof, wherein the polypeptides encoded by the sequence of the mobile genetic element promotes integration of the sequence comprising the gene or the fragment thereof into a genome of a cell of the population of human cells via target-primed reverse transcription (TPRT),

wherein the one or more polynucleic acids comprise one or more mRNA molecules, and wherein the sequence comprising the gene or fragment thereof integrates into the genome at a locus that is not a ribosomal RNA locus.

2. The method of claim 1, wherein the sequence comprising the gene or the fragment thereof encodes an exogenous therapeutic polypeptide selected from the group consisting of a ligand, an antibody, a receptor, an enzyme, a transport protein, a structural protein, a hormone, a contractile protein, a storage protein and a transcription factor.
3. The method of any one of claims 1-2, wherein at least one of the two NLSs comprises an SV40 NLS comprising the sequence PKKKRKV, or a nucleoplasmin NLS comprising the sequence KRPAATKKAGQAKKKK.
4. The method of any one of claims 1-3, wherein the human ORF2p comprises an endonuclease domain and a reverse transcriptase domain; and wherein the human ORF2p comprises a mutation that inactivates the ORF2p endonuclease activity, or reduces the DNA target recognition of ORF2p endonuclease compared to the nuclease domain without the mutation.
5. The method of claim 4, wherein the mutation comprises a D205 mutation of the endonuclease domain.
6. The method of any one of claims 1-5, wherein the one or more mRNA molecules comprise a sequence encoding a heterologous endonuclease domain from a heterologous protein other than human ORF2p.
7. The method of claim 6, wherein the heterologous endonuclease domain from the heterologous protein is a Cas 9 endonuclease.

8. The method of claim 7, wherein the Cas 9 endonuclease is a Cas 9 nickase.
9. The method of any one of claims 1-8, wherein the one or more polynucleic acids comprise a guide RNA sequence, or a polynucleic acid encoding the guide RNA sequence, wherein the guide RNA sequence targets a DNA target site of the genome and the sequence comprising the gene or the fragment thereof is integrated into the genome at the DNA target site of the genome.
10. The method of any one of claims 6-9, wherein the sequence comprising the gene or the fragment thereof is integrated into the genome by cleavage of a DNA strand of a target site by the heterologous endonuclease encoded by the one or more polynucleic acids, and via TPRT.
11. The method of claim 1, wherein the one or more polynucleic acids comprises
 - (i). a recombinant RNA molecule comprising:
 - (a) a 5'UTR sequence comprising the sequence encoding the LINE-1 polypeptides, wherein the 5'UTR sequence comprises a sequence that binds to a target sequence by homology that is between 2-15 nucleotides long or a genomic DNA priming sequence;
 - (b) the insert sequence, wherein the insert sequence is downstream of the sequence encoding the LINE-1 polypeptides; and
 - (c) a 3'UTR sequence downstream of the insert sequence, and wherein the 3'UTR sequence comprises a sequence that binds to a target sequence by homology or a genomic DNA priming sequence; and
 - (ii). one or more guide RNA sequences or a polynucleic acid encoding the one or more guide RNA sequences, and wherein the one or more guide RNA sequences target a DNA target site of a genome and the insert sequence is integrated into the genome at the DNA target site of the genome.
12. A composition comprising one or more polynucleic acids comprising one or more mRNA molecules, comprising:
 - (a) a 5'UTR sequence;
 - (b) a mobile genetic element, wherein the mobile genetic element comprises a sequence encoding LINE-1 polypeptides comprising:
 - (i). a human ORF1p or a functional fragment thereof, and
 - (ii). a human ORF2p or a functional fragment thereof, wherein the human ORF2p or functional fragment thereof comprises 2 NLSs that are the same or different;
 - (c) an insert sequence, wherein the insert sequence is a reverse complement of a sequence comprising a gene or a fragment thereof; and
 - (d) a 3'UTR sequence downstream of the insert sequence.
13. The composition of claim 12, wherein

- (a) the 5'UTR sequence comprises the sequence encoding the LINE-1 polypeptides, wherein the 5'UTR sequence comprises a sequence that binds to a target sequence by homology that is between 2-15 nucleotides long or a genomic DNA priming sequence; and
 - (b) the 3'UTR sequence comprises a sequence that binds to a target sequence by homology or a genomic DNA priming sequence.
14. The composition of claim 12 or 13, wherein the composition further comprises one or more guide RNA sequences or a polynucleic acid encoding the one or more guide RNA sequences, and wherein the one or more guide RNA sequences target a DNA target site of a genome and the insert sequence is integrated into the genome at the DNA target site of the genome.
 15. The composition of any one of claims 12-14, wherein the human ORF2p or a functional fragment thereof comprises an endonuclease domain and a reverse transcriptase domain, wherein the human ORF2p includes a mutation in the endonuclease domain that inactivates the ORF2p endonuclease.
 16. The composition of any one of claims 12-15, wherein the human ORF2p comprises a D205 mutation.
 17. The composition of any one of claims 12-16, wherein the one or more mRNA molecules comprise a sequence encoding a heterologous endonuclease domain from a heterologous protein other than human ORF2p, wherein the heterologous endonuclease domain from a heterologous protein is a Cas endonuclease.
 18. The composition of any one of claims 12-17, further comprising one or more siRNAs capable of knocking down any one of ADAR1, ADAR2 (ADAR1B), APOBEC3C, BRCA1, let-7 miRNA, RNase L, TASHOR (HUSH complex) and/or RAD51.
 19. The composition of any one of claims 12-18, wherein the composition further comprises lipid nanoparticles (LNPs) encapsulating the one or more mRNA molecules.
 20. A method of expressing an exogenous human therapeutic polypeptide encoded by an exogenous sequence from a site-specifically integrated genomic DNA sequence of a target human cell, the method comprising:
 - (a) contacting a composition to the target human cell, the composition comprising an RNA molecule encoding a Cas nickase and one or more RNA molecules, wherein the target human cell uptakes the one or more RNA molecules, and wherein the one or more RNA molecules comprise a first RNA molecule comprising an RNA sequence encoding a Cas nickase and a second RNA molecule comprising an RNA sequence that comprises:
 - (i). a sequence that is a reverse complement of a DNA sequence that encodes the exogenous human therapeutic polypeptide; and
 - (ii). a mobile genetic element, wherein the mobile genetic element comprises an RNA sequence encoding a polypeptide with target-primed reverse transcription (TPRT)

- activity, wherein the polypeptide with TPRT activity comprises an endonuclease with a mutation that abrogates endonuclease activity of the endonuclease domain;
- (b) translating the RNA sequence encoding the polypeptide with TPRT activity and translating the RNA sequence encoding the Cas nickase;
 - (c) nicking the genomic DNA site of the target human cell via the Cas nickase translated in (b);
 - (d) reverse transcribing the sequence that is the reverse complement of the DNA sequence that encodes the human therapeutic polypeptide via the TPRT activity of the polypeptide with TPRT activity translated in (b), thereby producing a DNA sequence encoding the exogenous human therapeutic polypeptide;
 - (e) integrating the DNA sequence encoding the exogenous human therapeutic polypeptide produced in (d) into a genomic DNA site of the target human cell; and
 - (f) expressing the exogenous human therapeutic polypeptide in the target human cell, wherein the exogenous human therapeutic polypeptide is expressed from the DNA sequence integrated into the genomic DNA of the target human cell in (e).
21. The method of claim 20, wherein the ratio of the first RNA molecule to the second RNA molecule in the composition is at least 2:1 and at most 5:1.
 22. The method of claim 20 or 21, wherein the exogenous human therapeutic polypeptide is expressed in at least 2% of target human cells that are contacted with the composition.
 23. The method of any one of claims 20-22, wherein the second RNA molecule comprises a homology arm complementary to a sequence comprising the target site in the genomic DNA.
 24. A composition comprising:
 - (i). an RNA molecule encoding a Cas nickase and
 - (ii). one or more RNA molecules, wherein the target human cell uptakes the one or more RNA molecules, and wherein the one or more RNA molecules comprise a first RNA molecule comprising an RNA sequence encoding a Cas nickase and a second RNA molecule comprising an RNA sequence that comprises:
 - (a) a sequence that is a reverse complement of a DNA sequence that encodes the exogenous human therapeutic polypeptide; and
 - (b) a mobile genetic element, wherein the mobile genetic element comprises an RNA sequence encoding a polypeptide with target-primed reverse transcription (TPRT) activity, wherein the polypeptide with TPRT activity comprises an endonuclease with a mutation that abrogates endonuclease activity of the endonuclease domain.