

CLAIMS

1. A polynucleotide comprising:
 - a) a polynucleotide sequence encoding a DNA-dependent DNA polymerase
 - 5 that enables replication of the polynucleotide;
 - b) a polynucleotide sequence encoding a pore that enables secretion of DNA from eukaryotic cells; and
 - c) a payload sequence.
- 10 2. The polynucleotide of claim 1, comprising:
 - a) an origin of replication; and
 - b) a polynucleotide sequence encoding:
 - i) a protelomerase; or
 - ii) a terminal protein and a DNA-binding protein required for
 - 15 plasmid replication in eukaryotic cells.
3. The polynucleotide of claim 2, wherein the DNA-dependent DNA polymerase is from adenovirus, optionally wherein:
 - a) the DNA-dependent DNA polymerase is encoded by the gene E2B;
 - 20 b) the DNA-dependent DNA polymerase comprises SEQ ID NO: 18, or an amino acid sequence that is at least 90% identical to SEQ ID NO: 18 that retains the ability to function as a DNA-dependent DNA polymerase;
 - c) the polynucleotide sequence encoding the DNA-dependent DNA polymerase comprises SEQ ID NO: 19, or a polynucleotide sequence that is at least 90% identical to SEQ ID NO: 19 that retains the ability to function as a DNA-dependent DNA
 - 25 polymerase when expressed;
 - d) the terminal protein and DNA-binding protein are terminal protein pTP encoded by the gene E2B and DNA-binding protein encoded by the gene E2A from adenovirus;
 - 30 e) the terminal protein comprises SEQ ID NO: 20, or an amino acid sequence that is at least 90% identical to SEQ ID NO: 20 that retains the ability to function as a terminal protein;

- f) the polynucleotide sequence encoding the terminal protein comprises SEQ ID NO: 21, or a polynucleotide sequence that is at least 90% identical to SEQ ID NO: 21 that retains the ability to function as a terminal protein when expressed;
- g) the DNA-binding protein comprises SEQ ID NO: 22, or an amino acid
5 sequence that is at least 90% identical to SEQ ID NO: 22 that retains the ability to function as a DNA-binding protein; and/or
- h) the polynucleotide sequence encoding the DNA-binding protein comprises SEQ ID NO: 23, or a polynucleotide sequence that is at least 90% identical to SEQ ID NO: 23 that retains the ability to function as a DNA-binding protein when expressed.
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4. The polynucleotide of claim 2, wherein:
- a) the origin of replication is from bacteriophage N15;
- b) the DNA-dependent DNA polymerase is from bacteriophage N15;
- c) the DNA-dependent DNA polymerase is encoded by the repA gene;
- 15 d) the DNA-dependent DNA polymerase comprises SEQ ID NO: 1, or an amino acid sequence that is at least 90% identical to SEQ ID NO: 1 that retains the ability to function as a DNA-dependent DNA polymerase;
- e) the protelomerase is TelN from bacteriophage N15;
- f) the protelomerase comprises SEQ ID NO: 3, or an amino acid sequence that
20 is at least 90% identical to SEQ ID NO: 3 that retains the ability to function as a protelomerase; and/or
- g) the polynucleotide further comprises a *telRL* site, optionally wherein the *telRL* site polynucleotide sequence comprises SEQ ID NO: 5.
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5. The polynucleotide of claim 2, wherein the DNA-dependent DNA polymerase is from bacteriophage Phi29, optionally wherein:
- a) the DNA-dependent DNA polymerase is encoded by gene 2; and/or
- b) the DNA-dependent DNA polymerase comprises SEQ ID NO: 10, or an amino acid sequence that is at least 90% identical to SEQ ID NO: 10 that retains the ability
30 to function as a DNA-dependent DNA polymerase.
6. The polynucleotide of claim 5, wherein:

- a) the protelomerase is TelN from bacteriophage N15;
- b) the protelomerase comprises SEQ ID NO: 3, or an amino acid sequence that is at least 90% identical to SEQ ID NO: 3 that retains the ability to function as a protelomerase;
- 5 c) the polynucleotide further comprises a *telRL* site, optionally wherein the *telRL* site polynucleotide sequence comprises SEQ ID NO: 5;
- d) the terminal protein and DNA-binding protein are terminal protein TP and DNA-binding proteins p5 and p6 from bacteriophages of the Phi29 group of *Bacillus subtilis*; and/or
- 10 e) the terminal protein comprises SEQ ID NO: 12, or an amino acid sequence that is at least 90% identical to SEQ ID NO: 12 that retains the ability to function as a terminal protein,
- optionally wherein the DNA-binding proteins p5 and p6 comprise SEQ ID NOs: 14 and 16, respectively, or amino acid sequences that are at least 90% identical to SEQ ID
- 15 NOs: 14 and 16, respectively, that retain the ability to function as DNA-binding proteins.
7. The polynucleotide of any one of the preceding claims, wherein the pore is TraB from *Streptomyces* spp.
- 20 8. The polynucleotide of claim 7, wherein the pore comprises SEQ ID NO: 24, or an amino acid sequence that is at least 90% identical to SEQ ID NO: 24 that retains the ability to function as a pore.
9. The polynucleotide of claim 7 or 8, further comprising a *clt* locus, optionally
- 25 wherein the *clt* locus comprises SEQ ID NO: 26, or a variant of SEQ ID NO: 26 that differs by 1, 2, 3, 4 or 5 nucleotides, wherein the variant maintains the ability to function as a *clt* locus.
10. The polynucleotide of any one of claims 1 to 6, wherein the pore is TdtA from
- 30 *Thermus* spp., optionally wherein the pore expressed by the polynucleotide sequence comprises SEQ ID NO: 28, or an amino acid sequence that is at least 90% identical to SEQ ID NO: 28 that retains the ability to function as a pore.

11. The polynucleotide of any one of the preceding claims, further comprising a promoter.
- 5 12. The polynucleotide of claim 1, wherein the payload sequence is:
- a) a therapeutic gene;
 - b) a CRISPR RNA-guided nuclease, optionally including CRISPR donor DNA;
 - c) a zinc finger nuclease or TALEN;
 - 10 d) a gene encoding an immunogenic protein or protein from a pathogen or a tumour, or an antibiotic, antifungal or antiviral compound, or an antibody, or a chimeric antigen or T-cell receptor, or a B-cell receptor; or
 - e) a gene encoding a therapeutic RNA, optionally wherein the therapeutic RNA is an antisense oligonucleotide (ASO) or a small-interfering RNA (siRNA).
- 15 13. A circular or linear plasmid comprising the polynucleotide as defined in any one of claims 1 to 12.
14. A pharmaceutical composition comprising the polynucleotide as defined in any one
20 of claims 1 to 12, or the plasmid as defined in claim 13, and a pharmaceutically acceptable excipient.
15. The polynucleotide as defined in any one of claims 1 to 12, the plasmid as defined in claim 13, or the pharmaceutical composition as defined in claim 14, for use in the
25 treatment of the human or animal body by therapy.