CLAIMS:

1. An engineered ligase comprising a polypeptide sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the reference sequence of SEQ ID NOs: 2, 6, 32, 34, and/or 38, wherein the engineered ligase comprises a 451K or 451G substitution in its polypeptide sequence, wherein the amino acid positions of the polypeptide sequence are numbered with reference to SEQ ID NO: 6, and wherein said engineered ligase exhibits increased enzymatic activity of at least 1.1-fold, at least 2-fold or at least 5-fold the enzymatic activity of the wild-type T4 DNA ligase.

2. The engineered ligase of Claim 1, wherein the engineered ligase comprises at least one substitution or substitution set selected from:

(a) 58K/451K, 63R/88R/451K, 63R/89K/226E/440K/451K, 63R/89K/451K, 63R/89K/451K, 88R/225A/440K/451K, and 88R/306A/440K/451K, wherein the amino acid positions are numbered with reference to SEQ ID NO: 6;

(b) 13K/89K/183K/232K/386K/451G, 13K/183K/232K/386K/451G, 13K/232K/385K/451G, 89K/183K/329G/451G/453R, and 183K/427R/451G, wherein the amino acid positions are numbered with reference to SEQ ID NO: 38; or

(c)13K/19K/63T/88R/127K/183K/225A/232K/233A/237R/329L/371W/440K/451K/452P/453G/466G, 13K/19K/63T/88R/127K/183K/225A/232K/233A/237R/371W/386K/440K/451G/452P, 19K/63T/88R/127K/183K/225A/231K/233A/237R/371W/427R/440K/451K/452P/466G, 19K/63T/88R/127K/183K/225A/233A/237R/371W/373A/386K/440K/451K/452P, 19K/63T/88R/127K/225A/233A/237R/371W/385K/440K/451K/452P/453R/466G, 19K/63T/88R/127K/225A/233A/237R/371W/440K/451K/452P, 63T/88R/127K/149R/225A/240P/371W/440K/451K/452P, 86R/88R/89K/127K/149R/225A/233A/237N/240P/440K/451K, 88R/89K/127K/225A/233A/237R/240P/440K/448A/451K/453G/454L, 88R/127K/149R/225A/233A/237N/440K/451K/454L, 88R/127K/225A/440K/451K, and 88R/225A/440K/451K, wherein the amino acid positions are numbered with reference to SEQ ID NO: 6.

3. The engineered ligase of Claim 1, wherein said engineered ligase:

(a) comprises a polypeptide sequence set forth in SEQ ID NOs: 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52 or 54; or

(b) consists of the sequence of SEQ ID NO: 38.

4. The engineered ligase of any of Claims 1-3, wherein:

(a) said engineered ligase has at least one improved property, as compared to wild-type T4 DNA ligase, wherein said improved property is selected from: exhibiting greater activity with low DNA substrate concentrations, and production of fewer adapter dimers;

(b) said engineered ligase is more thermostable than wild-type T4 DNA ligase;

(c) said engineered ligase exhibits a broader range of pH stability than wild-type T4 DNA ligase; and/or

(d) said polypeptide is purified.

5. A polynucleotide sequence encoding at least one engineered ligase of any of Claims 1-4.

6. The polynucleotide sequence of Claim 5, wherein said polynucleotide sequence:

(a) comprises SEQ ID NOs: 31, 33, and/or 37;

(b) is operably linked to a control sequence; and/or

(c) is codon-optimized.

7. An expression vector comprising at least one polynucleotide sequence of Claim 5 or 6.

8. An *in vitro* host cell transformed with the expression vector of Claim 7.

9. A method of producing an engineered ligase polypeptide in a host cell comprising culturing a host cell of Claim 8, under suitable culture conditions, such that at least one engineered ligase is produced, optionally wherein the method further comprises recovering at least one engineered ligase from the culture and/or host cells; for example wherein the method further comprises the step of purifying said at least one engineered ligase.

10. A composition comprising at least one engineered ligase of any of Claims 1-4.

11. An *in vitro* method for producing:

(a) a ligation product, comprising providing at least one engineered ligase of Claims 1-4, a substrate comprising at least two nucleic acid fragments, and a reaction mixture; combining said at least one engineered ligase, said substrate, and said reaction mixture under conditions such that ligation of said nucleic acid fragments occurs and at least one ligation product is produced;

(b) a DNA library, comprising providing at least one engineered ligase of Claims 1-4, a substrate comprising input double-stranded DNA, and a reaction mixture composition comprising at least one T-tailed adapter oligonucleotide, adenosine, and a reaction buffer; combining said at least

one engineered ligase, said substrate, and said reaction mixture under conditions such that an adenosine is added to the 3' ends of both strands of said DNA, and ligation of said T-tailed adapter to the ends of the input DNA, to producing a product comprising a DNA library, optionally wherein said input double-stranded DNA comprises blunt ended DNA fragments;

(c) a plurality of DNA fragments suitable for sequencing, comprising providing at least one engineered ligase of Claim 1-4, a substrate comprising input double-stranded DNA, and a reaction mixture comprising an oligonucleotide comprising single-base deoxyadenine 3' overhangs and 5' monophosphate ends, an adapter oligonucleotide comprising a 5' deoxythymidine overhang and 5' phosphate at the ligation compatible end; and combining said at least one engineered ligase, said substrate, and said reaction mixture under conditions such that ligation of the oligonucleotide, adapter oligonucleotide, and input double-stranded DNA occurs, producing a product comprising plurality of DNA fragments suitable for sequencing; or

(d) a plurality of DNA fragments suitable for sequencing, comprising providing at least one engineered ligase of Claim 1-4, a substrate comprising input double-stranded DNA, and a reaction mixture comprising an oligonucleotide comprising single-base deoxyadenine 3' overhangs and 5' monophosphate ends, an adapter oligonucleotide comprising a 5' deoxythymidine overhang and 5' phosphate at the ligation compatible end; and combining said at least one engineered ligase, said substrate, and said reaction mixture under conditions such that ligation of the oligonucleotide, adapter oligonucleotide, and input double-stranded DNA occurs, producing a product comprising plurality of DNA fragments suitable for sequencing, wherein the concentration of said adapter oligonucleotide in said reaction mixture is less than a 20-fold molar excess of the substrate concentration.

12. The method of Claim 11, wherein:

(a) said exposing is performed in the presence of a crowding agent;

(b) the product is transformed into *E. coli* after heat inactivation of the product;

(c) the product is used to generate a library of DNA molecules, optionally wherein said library of DNA molecules is subjected to sequencing;

(d) said reaction mixture comprises at least one compound that inhibits ligation, optionally wherein said at least one compound that inhibits ligation comprises a sub-optimal buffer for ligation;

(e) said method produces more product than the same methods comprising wild-type T4 DNA ligase;

(f) said reaction mixture comprises at least one enzyme, optionally wherein said enzyme is selected from polymerases, polynucleotide kinases, exonucleases, endonucleases, and cytidine deaminases;

(g) said input double-stranded DNA concentration is less than 100nM, less than 50nM, less than 10nM, less than 1nM, or less than 100pM;

(h) the adapter concentration is less than 10-fold, less than 5-fold, less than 3-fold, or less than

2-fold excess of the concentration of inserts in the reaction;

(i) the method further comprises the step of amplifying the product;

(j) the method further comprises the step of sequencing the product;

(k) the method further comprises the steps of amplifying and sequencing the product;

(l) no steps are taken to remove adapter dimer from the product; and/or

(m) said substrate is selected from isolated cell-free DNA, circulating tumor DNA, DNA isolated from leukemia cells, DNA isolated from lymphoma cells, DNA isolated from circulating tumor cells, DNA isolated from virally-infected cells, circulating fetal DNA, and fine-needle aspirates.

13. The method of any of Claims 11 or 12, wherein:

(a) said substrate comprises input double-stranded DNA provided in a crude sample; or

(b) said substrate comprises input double-stranded DNA that is purified prior to its inclusion in the reaction mixture.

14. The method of any of Claims 11-13, wherein:

(a) said method is conducted under conditions utilizing microfluidic devices and/or droplets, optionally wherein the volume of the combination of said reaction mixture and said engineered ligase is less than 5000pL, less than 1000pL, less than 100pL, less than 10pL, or less than 1pL;

(b) said substrate comprises immobilized double-stranded input DNA;

(c) said engineered ligase is immobilized;

(d) said substrate comprising double-stranded input DNA and said engineered ligase are immobilized;

(e) at least one compound in said reaction mixture is immobilized;

(f) the product is used to generate libraries for DNA sequencing, high-throughput screening, genetic selections, phage display, yeast display, ribosomal display, cell-based assays, biochemical assays, imaging-based high-content screening, or chromatin conformation capture (C3);

(g) the time length of said combining is less than 30 minutes;

(h) the time length of said combining is less than 15 minutes, optionally wherein said time length of said combining is less than 10, 9, 8, 7, 6, 5, 4, 3 or 2 minutes, for example, wherein said time length of said combining is less than 5 minutes;

(i) said product comprises fewer adapter dimers than ligation methods in which the time length of combining is 15 minutes or more;

(j) said method is cell-free;

(k) the substrate is cell-free DNA extracted from a fluid obtained from a patient, optionally wherein said fluid comprising serum or plasma; and/or

(l) said substrate comprises nucleic acids with differing sequences at their 3’ and 5’ ends,

optionally wherein the methods achieve low bias in the ligation.

15. The methods of any of Claims 11-14, wherein said combining:

(a) is conducted at a temperature in the range between 10°C to 40°C, optionally wherein the temperature range is 16° to 37°C; and/or

(b) is conducted at a pH in the range of pH 7 to pH 10, optionally wherein said combining is conducted at a pH between 7.5 and 9.