CLAIMS

1. A population of deoxyribonucleic acid (DNA) sequences encoding data useful in the authentication of objects and for protection against counterfeiting (e.g., selected from DNA 1, et seq. and/or DNA 2, et seq.), comprising nucleic acid data packets ("nackets"), wherein each nacket is encoded by a plurality of DNA molecules encoding the same data, wherein the sequences of the DNA molecules are heterogeneous.

2. The population of DNA sequences of claim 1, wherein the DNA sequences are prepared using heterologous cassette data writing, wherein two or more cassette sequences are provided for a single bit or combination of bits in a machine-readable code, such that all or nearly all of the DNA molecules in the nacket encode the same data, but the sequences of the individual molecules exhibit extremely high variation, wherein the nackets comprise a plurality of heterologous cassettes.

3. The population of DNA sequences of claim 1 or 2, wherein the data is in *n*-bit code wherein *n* is greater than 1, e.g., binary or ternary code.

4. The population of DNA sequences of any foregoing claim, wherein the DNA sequences are prepared from heterologous cassettes encoding the same bit or bits of data, wherein the percent abundance of the different cassette variants used in writing the DNA provides a unique and distinguishable feature of the DNA.

5. The population of DNA sequences of any foregoing claim, wherein the data encoded in the DNA is a nonfungible token (NFT).

6. The population of DNA sequences of any foregoing claim, wherein the one or more DNA sequences and/or cassettes contain one or more topoisomerase recognition sequences, e.g., wherein the topoisomerase recognition sequence is 5'-CCCTT-3', 5'-TCCTT-3', 5'-CCCTG-3', or 5'-TGACT-3'.

7. The population of DNA sequences of any foregoing claim, wherein the DNA comprises cassettes, wherein each cassette comprises (i) an information domain having sequence which corresponds to one or more bits in a machine-readable code, and (ii) a topoisomerase recognition sequence, wherein the cassette is 18-25 nucleotides in length.

8. The population of DNA sequences of any foregoing claim, wherein the DNA is incorporated into or associated with goods for purposes of identifying and authenticating the goods.

9. The population of DNA sequences of any foregoing claim, wherein the DNA is adsorbed onto, incorporated into, or encapsulated by silica beads or particles.

10. The population of DNA sequences of any foregoing claim, wherein the DNA sequences comprise one or more PCR primer sequences.

11. The population of DNA sequences of any foregoing claim, wherein the DNA sequences comprise an end cap sequence, which contains information about the desired digital code or how to read the code.

12. The population of DNA sequences of any foregoing claim, wherein the heterogeneity of the sequences of the DNA molecules, expressed as a Heterogeneity Index, is greater than 100, wherein the Heterogeneity Index is defined as the ratio between the number of unique DNA sequences encoding a machine-readable code or data packet and the number of machine-readable codes or data packets.

13. A method of object authentication, comprising:

i. synthesizing a population of DNA sequences, e.g., according to claim 1, comprising nucleic acid data packets (“nackets”), wherein each nacket contains a plurality of DNA molecules encoding the same data, wherein the sequences of the DNA molecules are heterogeneous;
ii. incorporating said DNA sequences into or onto an object;
iii. extracting said DNA sequences from the object; and

iv. analyzing the extracted DNA sequences;

v. optionally, comparing the analyzed DNA sequences to a database of DNA sequences;

vi. optionally, confirming object authenticity, optionally wherein step (iv) includes amplifying and sequencing a representative sample of the extracted DNA sequences.

14. The method of claim 13 wherein the DNA sequences are synthesized by sequential addition of DNA cassettes to DNA receptor strands, wherein in each sequential addition step the cassettes comprise a heterologous population of synonymous cassettes, such that the cassettes have at least two different sequences encoding the same data in a machine-readable code (e.g., binary or ternary code), optionally wherein the cassettes are conjugated together using a topoisomerase enzyme.

15. The method of claim 13 or 14 wherein the nackets are synthesized by sequential addition of cassettes to DNA receptor strands using an inkjet printing head (e.g., a piezoelectric print head), wherein each cassette comprises multiple nucleotides, wherein in each sequential addition step the cassettes comprise a heterologous population of cassettes of at least two different sequences encoding the same data in a machine-readable code (e.g., binary or ternary code), and wherein the cassettes are dispensed by an inkjet writing print head on at least one writing spot on a wafer array, the head or nozzle writing the same code to a plurality of polymer memory strands dispensed on the at least one spot, e.g., comprising the following steps:

a) loading the desired spot to be written with a starter polymer or DNA attached at one end to the desired spot;

b) washing the surface of the spot;

c) positioning an inkjet nozzle having a heterologous population of cassettes wherein the population comprises cassettes having at least two different sequences, but all encoding the same information in one or more bits (e.g., 1 or 0, or 00, 01, 10, 11, etc. in binary code) over the desired spot to be written corresponding to the unique code;

d) causing the inkjet nozzle to release a droplet comprising the heterologous population of cassettes onto the spot, thereby writing a bit or portion of the unique code to the DNA or polymer memory strings (or strands) associated with the spot; and

e) washing the surface of the spot;

optionally further comprising steps f) – i):

f) causing the inkjet nozzle to release a droplet of deblock/adapter reagent onto the spot;

g) washing the surface of the spot;

h) repeating steps (c) through (g) until the unique code has been written in the memory string at the spot; and

i) removing the memory strings from the spot and flowing the memory strings from the spot into a collection or storage container for later incorporation into or onto an object.

16. The method of claim 15, wherein the cassettes are added by topoisomerase mediated ligation comprising:

(i) reacting double-stranded acceptor DNA strands with topoisomerases charged with double-stranded DNA cassettes from the heterologous population of cassettes covalently bound to the topoisomerases,

wherein a strand of the acceptor DNA has a 5’ overhang,
wherein each cassette comprises an informational sequence, a topoisomerase recognition sequence, and 5’ overhangs on both strands,
wherein the 5’ overhang of the strand of the oligomer that does not bear the topoisomerase (“bottom strand”) is complementary to the 5' overhang of the acceptor DNA but is not complementary to the 5’ overhang of the strand bearing the topoisomerase (“top strand”) of the cassette,
wherein the 5’ end of the strand bearing the topoisomerase (“top strand”) of the cassette and 5’ end of the acceptor DNA are not protected, e.g., not phosphorylated (i.e., 5’-OH), and
wherein the topoisomerase charged with a double-stranded DNA cassette is delivered to the location of the acceptor strand by a piezo-electric inkjet nozzle;

(ii) reacting the acceptor DNA thus extended in step (i) with a topoisomerase charged with a further double-stranded DNA cassette,

wherein the further cassette comprises an informational sequence that is the same as or is different from any informational sequence in the cassette of step (i), a topoisomerase recognition sequence, and 5’ overhangs on both strands,

wherein the 5’ overhang of the strand of the further cassette not bearing the topoisomerase (“bottom strand”) is complementary to the 5' overhang of the extended acceptor DNA but is not complementary to the 5’ overhang of the strand of the further cassette bearing the topoisomerase (“top strand”), and

wherein the 5’end of the strand bearing the topoisomerase (“top strand”) of the further cassette is not protected, e.g., not phosphorylated (i.e., 5’-OH); and

(iii) repeating steps (i) and (ii) until the desired nucleotide sequence is obtained; wherein there is optionally a washing step after step (i) and/or after step (ii); and

optionally, wherein the desired nucleotide sequence thus obtained is further reacted with a terminal sequence comprising one or more replication primers, such as one or more PCR primer sequences.

17. The method of claim 15, wherein the method comprises the following steps:

a) loading the desired spot to be written with a starter polymer or DNA attached at one end to the desired spot;

b) washing the surface of the spot;

c) positioning an inkjet nozzle having a heterologous population of cassettes wherein the population comprises cassettes having at least two different sequences, but all encoding the same information in one or more bits over the desired spot to be written corresponding to the unique code;

d) causing the inkjet nozzle to release a droplet comprising the heterologous population of cassettes onto the spot, thereby writing a bit or portion of the unique code to the DNA or polymer memory strings (or strands) associated with the spot; and

e) washing the surface of the spot;

f) causing the inkjet nozzle to release a droplet of deblock/adapter reagent onto the spot;

g) washing the surface of the spot;

h) repeating steps (c) through (g) until the unique code has been written in the memory string at the spot; and

i) removing the memory strings from the spot and flowing the memory strings from the spot into a collection or storage container for later incorporation into or onto an object.