CLAIMS

1. A method of nucleic acid synthesis comprising:

(a1) providing a nucleic acid, a first nucleoside triphosphate, and a first terminal deoxynucleotidyl transferase (TdT), wherein the first nucleoside triphosphate comprises a modified base comprising a photocleavable carbon chain moiety having a length of at least 60 Å;

(b1) contacting (i) the nucleic acid and (ii) the first nucleoside triphosphate with the first TdT to generate a first modified nucleic acid comprising the nucleic acid incorporated with one first nucleotide comprising the modified base from the first nucleoside triphosphate; and

(c1) photocleaving the photocleavable carbon chain moiety of the modified base of the first nucleotide in the first modified nucleic acid to remove the photocleavable carbon chain moiety from the first modified nucleic acid.

2. The method of claim 1, further comprising:

(a2) providing a second nucleoside triphosphate and a second TdT, wherein the second nucleoside triphosphate comprises a modified base comprising a photocleavable carbon chain moiety having a length of at least 60 Å;

(b2) contacting (i) the first modified nucleic acid with the photocleavable carbon chain moiety of the modified base of the first nucleotide removed and (ii) the second nucleoside triphosphate with the second TdT to generate a second modified nucleic acid comprising the first modified nucleic acid incorporated with one second nucleotide comprising the modified base from the second nucleoside triphosphate; and

(c2) photocleaving the photocleavable carbon chain moiety of the modified base of the second nucleotide in the second modified nucleic acid to remove the photocleavable carbon chain moiety from the second modified nucleic acid.

3. A method of nucleic acid synthesis comprising:

(a) providing a nucleic acid and a plurality of nucleoside triphosphates, wherein each of the plurality of nucleoside triphosphates comprises a modified base comprising a photocleavable carbon chain moiety having a length of at least 60 Å;

(b1) contacting (i) the nucleic acid and (ii) a first nucleoside triphosphate of the

plurality of nucleoside triphosphates with a first terminal deoxynucleotidyl transferase (TdT) to generate a first modified nucleic acid comprising the nucleic acid incorporated with one first nucleotide comprising the modified base from the first nucleoside triphosphate;

(c1) photocleaving the photocleavable carbon chain moiety of the modified base of the first nucleotide in the first modified nucleic acid to remove the photocleavable carbon chain moiety from the first modified nucleic acid;

(b2) contacting (i) the first modified nucleic acid with the photocleavable carbon chain moiety of the modified base of the first nucleotide removed and (ii) a second nucleoside triphosphate of the plurality of nucleoside triphosphates with a second TdT to generate a second modified nucleic acid comprising the first modified nucleic acid incorporated with one second nucleotide comprising the modified base from the second nucleoside triphosphate; and

(c2) photocleaving the photocleavable carbon chain moiety of the modified base of the second nucleotide in the second modified nucleic acid to remove the photocleavable carbon chain moiety from the second modified nucleic acid.

4. The method of any one of claims 1-3, wherein a concentration of the nucleic acid is at least 10 nM.

5. The method of any one of claims 1-4, wherein the nucleic acid comprises:

i) a single-stranded (ss) nucleic acid; and/or

ii) a deoxyribonucleic acid (DNA) or at least one ribonucleotide.

6. The method of any one of claims 1-5, wherein the nucleic acid is attached to a solid support; optionally wherein:

i) the solid support comprises a flow cell; and/or

ii) the method further comprises: detaching the modified nucleic acid from the solid support.

7. The method of any one of claims 1-6, wherein a concentration of the first nucleoside

triphosphate and/or the second nucleoside triphosphate is at least 0.1 μM.

8. The method of any one of claims 1-7, wherein the modified base comprises:

i) a modified cytosine, a modified uracil, a modified thymine, a modified adenine, or a modified guanine; and/or

ii) a propargylamino group, an aminoallyl group, a propargylhydroxyl group or a combination thereof.

9. The method of any one of claims 1-8, wherein the photocleavable carbon chain moiety comprises a saturated or unsaturated, substituted or unsubstituted, straight or branched carbon chain; optionally wherein:

i) the carbon chain has a length of at least 60 Å; and/or

ii) the photocleavable carbon chain moiety comprises at least 54 carbon, oxygen, nitrogen, and/or sulfur atoms in a main chain of the carbon chain.

10. The method of any one of claims 1-9, wherein the photocleavable carbon chain moiety comprises a plurality of repeat units; optionally wherein:

i) the plurality of repeating units comprises identical repeating units; and/or

ii) one of the plurality of repeating units comprises at least three carbon, oxygen, nitrogen, and/or sulfur atoms in a main chain of the repeating unit; and/or

iii) the plurality of repeating units comprises a polyethylene glycol (PEG); and/or

iv) a repeating unit of the plurality of repeating units comprises no aromatic group or a repeating unit of the plurality of repeating units comprises an aromatic group; and/or

v) a number of the plurality of repeating units is at least 18.

11. The method of any one of claims 1-10, wherein the photocleavable carbon chain moiety comprises an photocleavable moiety selected from a group consisting of a carbonyl group, an arylcarbonylmethyl group, a phenacyl group, an o-alkylphenacyl group, a p-hydroxyphenacyl group, a benzoin group, a benzyl group, a nitroaryl group, a nitrobenzyl group, an o-nitrobenzyl group, an o-nitro-2-phenethyloxycarbonyl group, an o-nitroanilide, a coumarin-4-ylmethyl group, an arylmethyl group, a coumaryl group, an o-hydroxyarylmethyl group, a metal-containing group, a pivaloyl group, an ester of a carboxylic acid, an arylsulfonyl group, a ketone group, a carbanion-mediated group, a sisyl

group, a silicon-based group, a 2-hydroxycinnamyl group, an α-keto amide group, an α,β-unsaturated anilide, a methyl(phenyl)thiocarbamic acid group, a thiochromone S,S-dioxide group, 2-pyrrolidino-1,4-benzoquinone group, a triazine group, an arylmethyleneimino group, a xanthene group, a pyronin group, a 7-hydroxy-1,1-dimethylnaphthalenone group, a carboxylic acid group, a phosphate group, a phosphite group, a sulfate group, an acid group, an alcohol group, a thiol group, a N-oxide group, a phenol group, an amine group, a derivative of any of the proceeding, or a combination thereof.

12. The method of any one of claims 1-11, wherein a concentration of the first TdT and/or the second TdT is at least 10 nM.

13. The method of any one of claims 1-12, wherein:

i) the first TdT and/or the second TdT comprises a recombinant TdT;

ii) the first TdT and the second TdT are identical;

iii) the first TdT and the second Tdt comprise identical molecules of a TdT;

iv) the first TdT and the second Tdt comprise different molecules of a TdT; or

v) the first TdT and the second TdT are different TdTs.

14. The method of any one of claims 1-13, further comprising: removing the first TdT after the step (b1) and before the step (c1); and removing the second TdT after the step (b2) and before the step (c2); optionally wherein:

i) the first TdT is attached to a first magnetic bead, wherein removing the first TdT comprises magnetically removing the first TdT after the step (b1) and before the step (c1), and/or

ii) the second TdT is attached to a second magnetic bead, and wherein removing the second TdT comprises magnetically removing the second TdT after the step (b2) and before the step (c2);

further optionally wherein the first magnetic bead and the second magnetic bead are identical.

15. The method of any one of claims 1-14, further comprising: inactivating the first TdT after the step (b1) and before the step (c1); and inactivating the second TdT after the step (b2) and before the step (c2); optionally wherein inactivating the first TdT comprises

heat inactivating the first TdT, and wherein inactivating the second TdT comprises heat inactivating the second TdT.

16. The method of any one of claims 1-15, wherein the contacting in step (b1) is performed for about 5 minutes to about 20 minutes, and wherein the contacting in step (b2) is performed for about 5 minutes to about 20 minutes.

17. The method of any one of claims 1-16, wherein the contacting in step (b1) is performed at about 16 °C to about 58 °C, and wherein the contacting step (b2) is performed at about 16 °C to about 58 °C.

18. The method of any one of claims 1-17, wherein the first modified nucleic acid in step (b1) comprises at least 95% of the nucleic acid, and wherein the second modified nucleic acid in step (b2) comprises at least 95% of the first modified nucleic acid.

19. The method of any one of claims 1-18, wherein at least 95% of the first modified nucleic acid in step (b1) comprises the first modified nucleic acid comprising the nucleic acid incorporated with a single first nucleotide from the first nucleoside triphosphate, and wherein at least 95% of the second modified nucleic acid in step (b2) comprises the second modified nucleic acid comprising the first modified nucleic acid incorporated with a single second nucleotide from the second nucleoside triphosphate.

20. The method of any one of claims 1-19, wherein:

i) the photocleaving in step (c1) is performed with a first radiation, and wherein the photocleaving in step (c2) is performed with a second radiation; and/or

ii) the first radiation and/or the second radiation has a wattage of about 5 watts to about 20 watts.

21. The method of claim 20, wherein:

i) the first radiation and/or the second radiation comprises a radiation with an ultraviolet radiation; and/or

ii) the first radiation and/or the second radiation comprises a radiation with a

wavelength of about 300 nm to about 400 nm; and/or

iii) the first radiation and/or the second radiation is generated using a ultraviolet (UV) lamp with a wattage of about 10 watts to about 60 watts.

22. The method of any one of claims 1-21, wherein:

i) the photocleaving in step (c1) and/or the photocleaving in step (c2) is performed for about 1 minute to about 20 minutes; and/or

ii) the photocleaving in the step (c1) and/or the step (c2) has an efficiency of at least 90%; and/or

iii) the photocleaving in step (c1) and the photocleaving in step (c2) each is completed in about 1 minute; and/or

iv) the contacting in step (b1) and the contacting in step (b2) each is completed in about 7 minutes; and/or

v) the contacting in step (b1) and the photocleaving in step (c1) are completed in about 10 minutes, and wherein the contacting in step (b2) and the photocleaving in step (c2) are completed in about 10 minutes; and/or

vi) the method further comprises generating a reverse complement of the modified nucleic acid using a polymerase.

23. A method of nucleic acid synthesis, comprising:

(a1) providing a nucleic acid,

iteratively,

(a2) providing a nucleoside triphosphate of a plurality of nucleoside triphosphates and a terminal deoxynucleotidyl transferase (TdT), wherein the nucleoside triphosphate comprises a modified base comprising a photocleavable carbon chain moiety having a length of at least 60 Å;

(b) contacting (i) the nucleic acid in (a1) for a first iteration, or the modified nucleic acid in (c) from an immediate prior iteration for any iteration other than the first iteration, and (ii) the nucleoside triphosphate with the TdT to generate a modified nucleic acid comprising the nucleic acid in (a1) for the first iteration, or the modified nucleic acid in (c) from an immediate prior iteration for any iteration other than the first iteration, incorporated with one nucleotide comprising the modified base from the nucleoside

triphosphate; and

(c) photocleaving the photocleavable carbon chain moiety of the modified base of the nucleotide in the modified nucleic acid to remove the photocleavable carbon chain moiety from the modified nucleic acid,

thereby generating the modified nucleic acid comprising a predetermined sequence.

24. The method of claim 23, wherein:

i) at least 95% of the modified nucleic acid generated after a plurality of iterations comprises the predetermined sequence, optionally wherein the plurality of iterations comprises at least 200 iterations; and/or

ii) the method further comprises: receiving the predetermined sequence.

25. A plurality of nucleoside triphosphates for nucleotide synthesis a terminal deoxynucleotidyl transferase (TdT) each comprising a modified base, wherein the modified base comprises a photocleavable carbon chain moiety having a length of at least 60 Å.

26. The plurality of nucleoside triphosphates of claim 25, wherein:

i) the modified base comprises a modified cytosine, a modified uracil, a modified thymine, a modified adenine, or a modified guanine; and/or

ii) the modified base comprises a propargylamino group, an aminoallyl group, or a combination thereof; and/or

iii) the photocleavable carbon chain moiety comprises a saturated or unsaturated, substituted or unsubstituted, straight or branched carbon chain, optionally wherein a) the carbon chain has a length of at least 60 Å, and/or b) the photocleavable carbon chain moiety comprises at least 54 carbon, oxygen, nitrogen, and/or sulfur atoms in a main chain of the carbon chain.

27. The plurality of nucleoside triphosphates of claim 25 or claim 26, wherein the photocleavable carbon chain moiety comprises a plurality of repeat units, optionally wherein:

i) the plurality of repeating units comprises identical repeating units; and/or

ii) one of the plurality of repeating units comprises at least three carbon, oxygen,

nitrogen, and/or sulfur atoms in a main chain of the repeating unit; and/or

iii) the plurality of repeating units comprises a polyethylene glycol (PEG); and/or

iv) a repeating unit of the plurality of repeating units comprises no aromatic group or a repeating unit of the plurality of repeating units comprises an aromatic group; and/or

v) a number of the plurality of repeating units is at least 18.

28. The plurality of nucleoside triphosphates of claim 27, wherein the photocleavable carbon chain moiety comprises an photocleavable moiety selected from a group consisting of a carbonyl group, an arylcarbonylmethyl group, a phenacyl group, an o-alkylphenacyl group, a p-hydroxyphenacyl group, a benzoin group, a benzyl group, a nitroaryl group, a nitrobenzyl group, an o-nitrobenzyl group, an o-nitro-2-phenethyloxycarbonyl group, an o-nitroanilide, a coumarin-4-ylmethyl group, an arylmethyl group, a coumaryl group, an o-hydroxyarylmethyl group, a metal-containing group, a pivaloyl group, an ester of a carboxylic acid, an arylsulfonyl group, a ketone, a carbanion-mediated group, a sisyl group, a silicon-based group, a 2-hydroxycinnamyl group, an α-keto amide, an α,β-unsaturated anilide, a methyl(phenyl)thiocarbamic acid, a thiochromone S,S-dioxide, 2-pyrrolidino-1,4-benzoquinone group, a triazine group, an arylmethyleneimino group, a xanthene group, a pyronin group, a 7-hydroxy-1,1-dimethylnaphthalenone, a carboxylic acid, a phosphate, a phosphite, a sulfate, an acid, an alcohol, a thiol, a N-oxide, a phenol, an amine, a derivative of any of the proceeding, or a combination thereof; optionally wherein:

i) the photocleavable moiety is photocleavable by a radiation with a wattage of about 5 watts to about 20 watts in about 1 minute to about 20 minutes with an efficiency of at least 90%, optionally wherein the radiation comprises an ultraviolet (UV) radiation; and/or

ii) the radiation has a wavelength of about 300 nm to about 400 nm.