

WHAT IS CLAIMED IS:

1. A method for nucleic acid synthesis and regeneration of a reusable initiator for ~~such the~~ nucleic acid synthesis, the method comprising:

5 exposing an initiator attached to a solid support for the nucleic acid synthesis to a linking nucleotide in the presence of a polymerase, so that the linking nucleotide is incorporated to the initiator, the linking nucleotide having a substrate base, a substrate sugar, and a 3' hydroxyl group;

10 exposing the initiator containing the linking nucleotide to nucleotide monomers in the presence of the polymerase, so that a nucleic acid is synthesized and is coupled to the initiator right after the linking nucleotide;

15 providing a mono-functional DNA glycosylase, the linking nucleotide with the substrate base being recognizable and excisable by the mono-functional DNA glycosylase;

20 subjecting the substrate base to an excision treatment with the mono-functional DNA glycosylase, so that the substrate base is excised by the mono-functional DNA glycosylase to generate an abasic site;

25 providing an abasic site endonuclease, the resulting abasic site being recognizable and the substrate sugar being cleavable by the abasic site endonuclease;

subjecting the abasic site to a cleavage treatment with the abasic site endonuclease, so that the substrate sugar and the backbone of the nucleic acid at the abasic site are both cleaved to release the nucleic acid from the initiator, so that a 3'-terminal nucleotide of the initiator has a 3' phosphate group, and so that a 5'-terminal nucleotide of the synthesized nucleic acid has a 5' phosphate group;

providing a 3' phosphatase activity-possessing enzyme; and

subjecting the 3'-terminal nucleotide of the initiator to a dephosphorylation treatment with the 3' phosphatase activity-possessing enzyme, so that the 3' phosphate group of the 3'-terminal nucleotide of the initiator is converted back to the original 3' hydroxyl group.

132. A method of regenerating a reusable initiator for nucleic acid synthesis, the method comprising:
providing a mono-functional DNA glycosylase;
providing an initiator for the nucleic acid synthesis and a synthesized nucleic acid, the initiator being attached to a solid support, the synthesized nucleic acid being linked to the initiator right after a linking nucleotide having a substrate base and a substrate sugar, the linking

nucleotide with the substrate base being recognizable and excisable by the mono-functional DNA glycosylase; subjecting the substrate base to an excision treatment with the mono-functional DNA glycosylase, so that the substrate base is excised by the mono-functional DNA glycosylase to generate an abasic site; providing an abasic site endonuclease, the resulting abasic site being recognizable and the substrate sugar being cleavable by the abasic site endonuclease;

subjecting the abasic site to a cleavage treatment with the abasic site endonuclease, so that the substrate sugar and the backbone of the nucleic acid at the abasic site are both cleaved to release the synthesized nucleic acid from the initiator, so that a 3'-terminal nucleotide of the initiator has a 3' phosphate group, and so that a 5'-terminal nucleotide of the synthesized nucleic acid has a 5' phosphate group;

providing a 3' phosphatase activity-possessing enzyme; and

subjecting the 3'-terminal nucleotide of the initiator to a dephosphorylation treatment with the 3' phosphatase activity-possessing enzyme, so that the 3' phosphate group of the 3'-terminal nucleotide of the initiator is converted back to an original 3' hydroxyl group.

223. A method of synthesizing a nucleic acid, the method comprising the following steps of:

5 a) providing an initiator attached to a solid support, wherein the initiator is being coupled with a linking nucleotide having a substrate base, a substrate sugar, and a 3' hydroxyl group;

10 b) exposing the initiator coupled with the linking nucleotide to nucleotide monomers in the presence of a polymerase, so that the nucleic acid is synthesized and coupled to the initiator by reacting one of the nucleotide monomers with the 3' hydroxyl group of the linking nucleotide;

15 c) subjecting the substrate base to an excision treatment with a mono-functional DNA glycosylase, so that the substrate base is excised by the mono-functional DNA glycosylase to generate an abasic site;

20 d) subjecting the abasic site to a cleavage treatment with an abasic site endonuclease, so that the substrate sugar and a sugar phosphate the backbone of the nucleic acid at the abasic site are both cleaved to release the nucleic acid from the initiator, thereby forming a 3' phosphate group at a 3'-terminal nucleotide of the initiator; and

25 e) subjecting the 3'-terminal nucleotide having the 3' phosphate group to a dephosphorylation treatment with a 3' phosphatase activity-possessing

enzyme, so that a hydroxyl group is formed at the 3'-terminal nucleotide to regenerate the initiator.

364. A method of regenerating an initiator for nucleic acid synthesis, the method comprising—the following steps of:

a) providing an initiator attached to a solid support, wherein the initiator is being coupled with a linking nucleotide and a newly-synthesized nucleic acid, and the linking nucleotide has ving a substrate base and a substrate sugar;

b) subjecting the substrate base to an excision treatment with a mono-functional DNA glycosylase, so that the substrate base is excised by the mono-functional DNA glycosylase to generate an abasic site;

c) subjecting the abasic site to a cleavage treatment with an abasic site endonuclease, so that the substrate sugar and a sugar phosphate the backbone of the nucleic acid at the abasic site are both cleaved to release the newly-synthesized nucleic acid from the initiator, thereby forming a 3' phosphate group at a 3'-terminal nucleotide of the initiator; and

d) subjecting the 3'-terminal nucleotide having the 3' phosphate group to a dephosphorylation treatment with a 3' phosphatase activity-possessing enzyme, so that a hydroxyl group is formed at the

3'-terminal nucleotide to regenerate the initiator for being reused for further nucleic acid synthesis.

5 25. The method according to any one of Claims 1 to 4, wherein the mono-functional DNA glycosylase is selected from the group consisting of uracil-DNA glycosylase, alkyladenine DNA glycosylase, single-strand-selective monofunctional uracil DNA glycosylase 1, methyl-binding domain glycosylase 4, 10 thymine DNA glycosylase, mutY homolog DNA glycosylase, alkylpurine glycosylase C, alkylpurine glycosylase D, 8-oxo-guanine glycosylase 1 without abasic site lyase activity, endonuclease III-like 1 without abasic site lyase activity, endonuclease VIII-like glycosylase 1 15 without abasic site lyase activity, endonuclease VIII-like glycosylase 2 without abasic site lyase activity, endonuclease VIII-like glycosylase 3 without abasic site lyase activity, and enzymatically active fragments thereof.

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36. The method according to Claim 25, wherein the mono-functional DNA glycosylase is one of uracil-DNA glycosylase and alkyladenine DNA glycosylase.

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47. The method according to any one of Claims 1 to 4~~Claim 1~~, wherein the abasic site endonuclease is selected from the group consisting of endonuclease

VIII, endonuclease III, and enzymatically active fragments thereof.

5 58. The method according to Claim 47, wherein the abasic site endonuclease is endonuclease VIII.

10 69. The method according to any one of Claims 1 to 4~~Claim 1~~, wherein the 3' phosphatase activity-possessing enzyme is selected from the group consisting of a polynucleotide kinase 3'-phosphatase, a 3'-phosphoesterase, and enzymatically active fragments thereof.

15 710. The method according to Claim 69, wherein the 3' phosphatase activity-possessing enzyme is selected from the group consisting of T4 polynucleotide kinase with 3' phosphatase activity and zinc finger DNA 3'-phosphoesterase.

20 811. The method according to any one of Claims 1 to 4~~Claim 1~~, wherein the substrate base of the linking nucleotide is selected from the group consisting of uracil, hypoxanthine, thymine, cytosine, guanine, 5-fluorouracil, 5-hydroxymethyluracil, 5-formylcytosine, 5-carboxylcytosine, 3-methyladenine, 3-methylguanine, 7-methyladenine, 7-methylguanine, N⁶-methyladenine, 8-oxo-7,8-dihydroguanine, 5-

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hydroxyl cytosine, 5-hydroxyl uracil, dihydroxyuracil, ethenocytosine, ethenoadenine, thymine glycol, cytosine glycol, 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine, a
5 formamidopyrimidine derivative of adenine, a formamidopyrimidine derivative of guanine, adenine opposite guanine, uracil opposite guanine, uracil opposite adenine, thymine opposite guanine, ethenocytosine opposite guanine, adenine opposite 8-
10 oxo-7,8-dihydroguanine, and 2-hydroxyladenine opposite guanine.

912. The method according to Claim 811, wherein the substrate base of the linking nucleotide is one of
15 uracil and hypoxanthine.

3113. The method according to Claim 223 or 4, wherein the substrate base is uracil, thereby forming deoxyuridine.
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3214. The method according to Claim 223 or 4, wherein the substrate base is hypoxanthine, thereby forming deoxyinosine.
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1015. The method according to any one of Claims 1 to 4~~Claim 1~~, wherein the initiator, the synthesized nucleic acid, and the linking nucleotide are each in

one of a template-independent form and a template-dependent form.

5 ~~11~~16. The method according to Claim 1 or 3, wherein the polymerase is selected from the group consisting of a family-A DNA polymerase, a family-B DNA polymerase, a family-C DNA polymerase, a family-D DNA polymerase, a family-X DNA polymerase, a family-Y DNA polymerase, a reverse transcriptase, and
10 enzymatically active fragments thereof.

~~12~~17. A kit for nucleic acid synthesis and regeneration of a reusable nucleic acid for such the nucleic acid synthesis, the kit comprising:
15 a polymerase and a linking nucleotide for the nucleic acid synthesis;
a mono-functional DNA glycosylase;
an abasic site endonuclease; and
a 3' phosphatase activity-possessing enzyme~~7~~₁
20 wherein the kit is used according to a the method as described in of Claim 1.

~~13. A method of regenerating a reusable initiator for nucleic acid synthesis, comprising:~~
25 ~~providing a mono functional DNA glycosylase;~~
~~providing an initiator for nucleic acid synthesis~~
~~and a synthesized nucleic acid, the initiator being~~

~~attached to a solid support, the synthesized nucleic acid being linked to the initiator right after a linking nucleotide having a substrate base and a substrate sugar, the linking nucleotide with the substrate base being recognizable and excisable by the mono functional DNA glycosylase;~~

~~subjecting the substrate base to an excision treatment with the mono functional DNA glycosylase, so that the substrate base is excised by the mono functional DNA glycosylase to generate an abasic site;~~

~~providing an abasic site endonuclease, the resulting abasic site being recognizable and the substrate sugar being cleavable by the abasic site endonuclease;~~

~~subjecting the abasic site to a cleavage treatment with the abasic site endonuclease, so that the substrate sugar and the backbone of the nucleic acid at the abasic site are both cleaved to release the synthesized nucleic acid from the initiator, so that a 3' terminal nucleotide of the initiator has a 3' phosphate group, and so that a 5' terminal nucleotide of the synthesized nucleic acid has a 5' phosphate group;~~

~~providing a 3' phosphatase activity possessing enzyme; and~~

~~subjecting the 3' terminal nucleotide of the initiator to a dephosphorylation treatment with the~~

~~3' phosphatase activity possessing enzyme, so that the 3' phosphate group of the 3' terminal nucleotide of the initiator is converted back to an original 3' hydroxyl group.~~

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~~14. The method according to Claim 13, wherein the mono-functional DNA glycosylase is selected from the group consisting of uracil-DNA glycosylase, alkyladenine DNA glycosylase, single-strand-selective monofunctional uracil DNA glycosylase 1, methyl-binding domain glycosylase 4, thymine DNA glycosylase, mutY homolog DNA glycosylase, alkylpurine glycosylase C, alkylpurine glycosylase D, 8-oxo-guanine glycosylase 1 without abasic site lyase activity, endonuclease III-like 1 without abasic site lyase activity, endonuclease VIII-like glycosylase 1 without abasic site lyase activity, endonuclease VIII-like glycosylase 2 without abasic site lyase activity, endonuclease VIII-like glycosylase 3 without abasic site lyase activity, and enzymatically active fragments thereof.~~

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~~15. The method according to Claim 14, wherein the mono-functional DNA glycosylase is one of uracil-DNA glycosylase and alkyladenine DNA glycosylase.~~

~~16. The method according to Claim 13, wherein the abasic site endonuclease is selected from the group consisting of endonuclease VIII, endonuclease III, and enzymatically active fragments thereof.~~

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~~17. The method according to Claim 16, wherein the abasic site endonuclease is endonuclease VIII.~~

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~~18. The method according to Claim 13, wherein the 3' phosphatase activity possessing enzyme is selected from the group consisting of a polynucleotide kinase 3'-phosphatase, a 3'-phosphoesterase, and enzymatically active fragments thereof.~~

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~~19. The method according to Claim 18, wherein the 3' phosphatase activity possessing enzyme is selected from the group consisting of T4 polynucleotide kinase with 3' phosphatase activity and zinc finger DNA 3'-phosphoesterase.~~

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~~20. The method according to Claim 13, wherein the substrate base of the linking nucleotide is selected from the group consisting of uracil, hypoxanthine, thymine, cytosine, guanine, 5-fluorouracil, 5-hydroxymethyluracil, 5-formylcytosine, 5-carboxylcytosine, 3-methyladenine, 3-methylguanine, 7-methyladenine, 7-methylguanine, N⁶-methyladenine,~~

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~~8-oxo-7,8-dihydroguanine, 5-hydroxyl cytosine, 5-hydroxyl uracil, dihydroxyuracil, ethenocytosine, ethenoadenine, thymine glycol, cytosine glycol, 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine, a formamidopyrimidine derivative of adenine, a formamidopyrimidine derivative of guanine, adenine opposite guanine, uracil opposite guanine, uracil opposite adenine, thymine opposite guanine, ethenocytosine opposite guanine, adenine opposite 8-oxo-7,8-dihydroguanine, and 2-hydroxyladenine opposite guanine.~~

~~21. The method according to Claim 13, wherein the initiator, the synthesized nucleic acid, and the linking nucleotide are each in one of a template-independent form and a template-dependent form.~~

~~22. A method of synthesizing a nucleic acid, the method comprising the following steps of:~~

~~a) providing an initiator attached to a solid support, wherein the initiator is coupled with a linking nucleotide having a substrate base, a substrate sugar, and a 3' hydroxyl group;~~

~~b) exposing the initiator coupled with linking nucleotide to nucleotide monomers in the presence of a polymerase, so that the nucleic acid is synthesized and coupled to the initiator by reacting one of the~~

~~nucleotide monomers with the 3' hydroxyl group of the linking nucleotide;~~

~~c) subjecting the substrate base to an excision treatment with a mono-functional DNA glycosylase, so that the substrate base is excised by the mono-functional DNA glycosylase to generate an abasic site;~~

~~d) subjecting the abasic site to a cleavage treatment with an abasic site endonuclease, so that the substrate sugar and a sugar phosphate backbone at the abasic site are both cleaved to release the nucleic acid from the initiator, thereby forming a 3' phosphate group at a 3' terminal nucleotide of the initiator; and~~

~~e) subjecting the 3' terminal nucleotide having the 3' phosphate group to a dephosphorylation treatment with a 3' phosphatase activity possessing enzyme, so that a hydroxyl group is formed at the 3' terminal nucleotide to regenerate the initiator.~~

~~23. The method according to Claim 22, wherein the mono-functional DNA glycosylase is uracil-DNA glycosylase, alkyladenine DNA glycosylase, single-strand-selective monofunctional uracil DNA glycosylase 1, methyl-binding domain glycosylase 4, thymine DNA glycosylase, mutY homolog DNA glycosylase, alkylpurine glycosylase C, alkylpurine glycosylase D, 8-oxo-guanine glycosylase 1 without abasic site lyase~~

~~activity, endonuclease III-like 1 without abasic site lyase activity, endonuclease VIII-like glycosylase 1 without abasic site lyase activity, endonuclease VIII-like glycosylase 2 without abasic site lyase activity, or endonuclease VIII-like glycosylase 3 without abasic site lyase activity.~~

~~24. The method according to Claim 22, wherein the mono-functional DNA glycosylase is uracil-DNA glycosylase or alkyladenine DNA glycosylase.~~

~~25. The method according to Claim 22, wherein the abasic site endonuclease is endonuclease VIII, endonuclease III, a enzymatically active fragment of endonuclease VIII, or a enzymatically active fragment of endonuclease III.~~

~~26. The method according to Claim 22, wherein the abasic site endonuclease is endonuclease VIII.~~

~~27. The method according to Claim 22, wherein the 3'-phosphatase activity-possessing enzyme is a polynucleotide kinase 3'-phosphatase or a 3'-phosphoesterase.~~

~~28. The method according to Claim 22, wherein the 3'-phosphatase activity-possessing enzyme is T4~~

~~polynucleotide kinase with 3' phosphatase activity
or zinc finger DNA 3'-phosphoesterase.~~

5 ~~29. The method according to Claim 22, wherein the
substrate base of the linking nucleotide is uracil,
hypoxanthine, thymine, cytosine, guanine, 5-
fluorouracil, 5-hydroxymethyluracil, 5-
formylcytosine, 5-carboxylcytosine, 3-methyladenine,
3-methylguanine, 7-methyladenine, 7-methylguanine,
10 ~~N⁶-methyladenine, 8-oxo-7,8-dihydroguanine, 5-
hydroxyl cytosine, 5-hydroxyl uracil,
dihydroxyuracil, ethenocytosine, ethenoadenine,
thymine glycol, cytosine glycol, 2,6-diamino-4-
hydroxy-5-N-methylformamidopyrimidine, a
15 ~~formamidopyrimidine derivative of adenine, a
formamidopyrimidine derivative of guanine, adenine
opposite guanine, uracil opposite guanine, uracil
opposite adenine, thymine opposite guanine,
ethenocytosine opposite guanine, adenine opposite 8-
20 ~~oxo-7,8-dihydroguanine, or 2-hydroxyladenine
opposite guanine.~~~~~~~~

25 ~~30. The method according to Claim 22, wherein the
substrate base of the linking nucleotide is uracil
or hypoxanthine.~~

~~31. The method according to Claim 22, wherein the substrate base is uracil thereby forming deoxyuridine.~~

~~32. The method according to Claim 22, wherein the substrate base is hypoxanthine thereby forming deoxyinosine.~~

~~33. The method according to Claim 22, wherein the initiator is in a template-independent form or a template-dependent form.~~

~~34. The method according to Claim 22, wherein the polymerase is originated from family-A DNA polymerases, family-B DNA polymerases, family-C DNA polymerases, family-D DNA polymerases, family-X DNA polymerases, family-Y DNA polymerases, or reverse transcriptases.~~

~~35~~18. A kit for synthesizing a nucleic acid, the kit comprising:

a polymerase;

an initiator attached to a solid support;

a mono-functional DNA glycosylase;

an abasic site endonuclease; and

a 3' phosphatase activity-possessing enzyme~~7~~L

wherein the kit is used according to the method of Claim ~~22~~3.

~~36. A method of regenerating an initiator for nucleic acid synthesis, the method comprising the following steps of:~~

5 ~~a) providing an initiator attached to a solid support, wherein the initiator is coupled with a linking nucleotide and a newly synthesized nucleic acid; and the linking nucleotide has a substrate base and a substrate sugar;~~

10 ~~b) subjecting the substrate base to an excision treatment with a mono functional DNA glycosylase, so that the substrate base is excised by the mono functional DNA glycosylase to generate an abasic site;~~

15 ~~c) subjecting the abasic site to a cleavage treatment with an abasic site endonuclease, so that the substrate sugar and a sugar phosphate backbone at the abasic site are both cleaved to release the newly synthesized nucleic acid from the initiator, thereby forming a 3' phosphate group at a 3' terminal nucleotide of the initiator; and~~

20 ~~d) subjecting the 3' terminal nucleotide having the 3' phosphate group to a dephosphorylation treatment with a 3' phosphatase activity possessing enzyme, so that an hydroxyl group is formed at the~~
25 ~~3' terminal nucleotide to regenerate the initiator for being reused for further nucleic acid synthesis.~~

37. The method according to Claim 36, wherein the mono-functional DNA glycosylase is uracil-DNA glycosylase, alkyladenine DNA glycosylase, single-strand-selective monofunctional uracil DNA glycosylase 1, methyl-binding domain glycosylase 4, thymine DNA glycosylase, mutY homolog DNA glycosylase, alkylpurine glycosylase C, alkylpurine glycosylase D, 8-oxo-guanine glycosylase 1 without abasic site lyase activity, endonuclease III-like 1 without abasic site lyase activity, endonuclease VIII-like glycosylase 1 without abasic site lyase activity, endonuclease VIII-like glycosylase 2 without abasic site lyase activity, or endonuclease VIII-like glycosylase 3 without abasic site lyase activity.

38. The method according to Claim 36, wherein the mono-functional DNA glycosylase is uracil-DNA glycosylase or alkyladenine DNA glycosylase.

39. The method according to Claim 34, wherein the abasic site endonuclease is endonuclease VIII, endonuclease III, enzymatically active fragment of endonuclease VIII, or enzymatically active fragment of endonuclease III.

40. The method according to Claim 36, wherein the abasic site endonuclease is endonuclease VIII.

~~41. The method according to Claim 36, wherein the 3' phosphatase activity-possessing enzyme is a polynucleotide kinase 3'-phosphatase or a 3'-phosphoesterase.~~

~~42. The method according to Claim 36, wherein the 3' phosphatase activity-possessing enzyme is T4 polynucleotide kinase with 3' phosphatase activity or zinc finger DNA 3'-phosphoesterase.~~

~~43. The method according to Claim 36, wherein the substrate base of the linking nucleotide is uracil, hypoxanthine, thymine, cytosine, guanine, 5-fluorouracil, 5-hydroxymethyluracil, 5-formylcytosine, 5-carboxylecytosine, 3-methyladenine, 3-methylguanine, 7-methyladenine, 7-methylguanine, N6-methyladenine, 8-oxo-7,8-dihydroguanine, 5-hydroxyl cytosine, 5-hydroxyl uracil, dihydroxyuracil, ethenocytosine, ethenoadenine, thymine glycol, cytosine glycol, 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine, a formamidopyrimidine derivative of adenine, a formamidopyrimidine derivative of guanine, adenine opposite guanine, uracil opposite guanine, uracil opposite adenine, thymine opposite guanine, ethenocytosine opposite guanine, adenine opposite 8-~~

~~exo-7,8-dihydroguanine, or 2-hydroxyladenine
opposite guanine.~~

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~~44. The method according to Claim 36, wherein the
substrate base of the linking nucleotide is uracil
or hypoxanthine.~~

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~~45. The method according to Claim 36, wherein the
substrate base is uracil thereby forming deoxyuridine.~~

~~46. The method according to Claim 36, wherein the
substrate base is hypoxanthine thereby forming
deoxyinosine.~~

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~~47. The method according to Claim 36, wherein the
initiator is in a template-independent form or a
template-dependent form.~~

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~~48~~19. A kit for regenerating an initiator for nucleic
acid synthesis, the kit comprising:

an initiator attached to a solid support;

a mono-functional DNA glycosylase;

an abasic site endonuclease; and

a 3' phosphatase activity-possessing enzyme~~7~~1

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wherein the kit is used according to the method
of Claim ~~36~~4.