Claims

1. A method of synthesizing a polynucleotide having a predetermined sequence, the method comprising the steps of:

a) providing an initiator having a free 3'-hydroxyl; and

b) repeating until the polynucleotide is synthesized cycles of (i) contacting under elongation conditions the initiator or elongated fragments having free 3'-O-hydroxyls with a 3'-O-blocked nucleoside triphosphate and a template-independent DNA polymerase so that the initiator or elongated fragments are elongated by incorporation of a 3'-O-blocked, base protected nucleoside triphosphate to form 3'-O-blocked elongated fragments, and (ii) deblocking the elongated fragments to form elongated fragments having free 3'-hydroxyls, until the polynucleotide is formed, wherein the elongation conditions are selected to prevent hydrogen bonding or base stacking.

2. The method of claim 1, wherein said elongation conditions provide that at least one 3'-O-blocked nucleoside triphosphate has a base protecting moiety attached to its base to prevent hydrogen bonding.

3. The method of claim 2, wherein said 3'-O-blocked nucleoside triphosphate has a base protecting moiety attached to a nitrogen or to an oxygen of its base.

4. The method of claim 3, wherein said 3'-O-blocked nucleoside triphosphate has said base protecting moiety attached to a nitrogen.

5. The method of claim 4, wherein said nitrogen of said base of said 3'-O-blocked nucleoside triphosphate is an exocyclic nitrogen.

6. The method of claim 5, wherein said base protecting moiety is attached to 6-nitrogen of deoxyadenosine triphosphate, 2-nitrogen of deoxyguanosine triphosphate, or 4-nitrogen of deoxycytidine triphosphate.

7. The method of claim 6, wherein said base protecting moiety is an acyl protecting group.

8. The method of claim 6 or 7, wherein said base protecting moiety attached to said 6-nitrogen of deoxyadenosine triphosphate is selected from the group consisting of benzoyl, phthaloyl, phenoxyacetyl, and methoxyacetyl; wherein said base protecting moiety attached to said 2-nitrogen of deoxyguanosine triphosphate is selected from the group consisting of isobutyryl, isobutyryloxyethylene, acetyl, 4-isopropyl-phenoxyacetyl, phenoxyacetyl, and methoxyacetyl; and wherein said base protecting moiety attached to said 4-nitrogen of deoxycytidine triphosphate is selected from the group consisting of benzoyl, phthaloyl, acetyl, and isobutyryl.

9. The method of claim 6, wherein said base protecting moiety attached to said 6-nitrogen of deoxyadenosine triphosphate is benzoyl or dimethylformamidine; wherein said base protecting moiety attached to said 2-nitrogen of deoxyguanosine triphosphate is acetyl or dimethylformamidine; and wherein said base protecting moiety attached to said 4-nitrogen of deoxycytidine triphosphate is acetyl.

10. The method of claim 6, wherein said base protecting moiety attached to said 6-nitrogen of deoxyadenosine triphosphate is dimethylformamidine; wherein said base protecting moiety attached to said 2-nitrogen of deoxyguanosine triphosphate is dimethylformamidine; and wherein said base protecting moiety attached to said 4-nitrogen of deoxycytidine triphosphate is acetyl.

11. The method of any of claims 2 to 10, wherein said base protecting moiety is base labile.

12. The method of any of claims 2 to 11, wherein said base protecting moiety is an amidine.

13. The method of any of claims 2 to 12, wherein said method includes removing said base protecting moieties from nucleotides of the polynucleotide.

14. The method of any of claims 1 to 13, wherein said initiator is attached to a solid support.

15. The method of any of claims 2 to 14, wherein said initiator comprises a base-cleavable nucleoside and said base protecting moieties are base labile and wherein said step of removing comprises treating said polynucleotide with base so that base protecting moieties and the base-cleavable nucleoside are cleaved in the same reaction.

16. The method of any of claims 1 to 15, wherein said elongation conditions include a denaturation agent.

17. The method of claim 16, wherein said denaturation agent is selected from the group consisting of water miscible solvents having a dielectic constant less than that of water and chaotropic agents.

18. The method of claim 16 or 17 wherein said denaturation agent is selected from the group consisting of formamide, guanidine, sodium salicylate, dimethyl sulfoxide (DMSO), propylene glycol, and urea.

19. The method of any of claims 2 to 18 wherein said 3'-O-protecting group is selected from the group consisting of 3'-O-methyl, 3'-O-(2-nitrobenzyl), 3'-O-allyl, 3'-O-amine, 3'-O-azidomethyl, 3'-O-tert-butoxy ethoxy, 3'-O-(2-cyanoethyl), and 3'-O-propargyl.

20. The method of claim 19 wherein said 3'-O-protecting group is azidomethyl.

21. The method of claim 19 wherein said 3'-O-protecting group is amine.

22. A method of synthesizing a polynucleotide having a predetermined sequence, the method comprising the steps of:

a) providing an initiator having a free 3'-hydroxyl;

b) repeating until the polynucleotide is synthesized cycles of (i) contacting under elongation conditions the initiator or elongated fragments having free 3'-O-hydroxyls with a 3'-O-blocked nucleoside triphosphate and a template-independent DNA polymerase so that the initiator or elongated fragments are elongated by incorporation of a 3'-O-blocked, base protected nucleoside triphosphate to form 3'-O-blocked elongated fragments, and (ii) deblocking the elongated fragments to form elongated fragments having free 3'-hydroxyls, until the polynucleotide is formed, wherein the elongation conditions are selected to prevent hydrogen bonding or base stacking; wherein a final cycle comprises only step (i) and wherein the 3'-O-blocked, base protected nucleoside triphosphate comprises a base protecting moiety comprising a capture moiety; and

c) capturing the polynucleotide with a complement of the capture moiety.

23. The method of claim 22 further comprising a step of deblocking said captured polynucleotide.