

Claims

1. An apparatus for synthesizing with a template-free polymerase a plurality of polynucleotides each with a predetermined sequence, the apparatus comprising:

(a) a plurality of reaction chambers, each reaction chamber having a synthesis support with initiators attached, wherein each initiator has a free 3'-hydroxyl, and wherein each reaction chamber has an inlet and an outlet and a filter that retains the synthesis support and that is operationally associated with the outlet so that reaction solutions exiting the reaction chamber pass through the filter;

(b) a waste manifold operationally associated with the outlets of the reaction chambers such that reaction solutions are removed from the reaction chambers and enter the waste manifold whenever a positive pressure differential is established between the reaction chambers and the waste manifold;

(c) a fluid delivery system for delivering reaction solutions to the reaction chambers, the reaction solutions comprising 3'-O-protected nucleoside triphosphates, a deprotection solution; and a template-free polymerase;

(d) a user interface for accepting nucleotide sequences of polynucleotides to be synthesized; and

(e) a control system operationally associated with the user interface, the reaction chambers, the fluid delivery system and the waste manifold, wherein the control system assigns the predetermined sequence of each polynucleotide to a reaction chamber for synthesis, and wherein for each reaction chamber, the control system directs repeated steps of: (i) delivering under coupling conditions to the initiators or deprotected elongated fragments a 3'-O-protected nucleoside triphosphate and a template-free polymerase, wherein the coupling conditions include a predetermined coupling incubation time and incubation temperature to allow initiator oligonucleotides or deprotected elongated fragments to be elongated by the 3'-O-protected nucleoside triphosphate to form 3'-O-protected elongated fragments, (ii) delivering the deprotection solution to the reaction chambers so that the 3'-O-protected elongated fragments are deprotected, and (iii) producing a pressure differential between the reaction chambers and the waste manifold to remove deprotection solution from the reaction chambers at a predetermined rate.

2. The apparatus of claim 1 further comprising one or more liquid level sensors for measuring a liquid level in each of the reaction chambers, and wherein said control system directs repeated steps of: (i) delivering under coupling conditions to the initiators or deprotected elongated fragments a 3'-O-protected nucleoside triphosphate and a template-free polymerase, wherein the coupling conditions include a predetermined coupling incubation time and incubation temperature to allow initiator oligonucleotides or deprotected elongated fragments to be elongated by the 3'-O-protected nucleoside triphosphate to form 3'-O-protected elongated fragments, (ii) delivering the deprotection solution to the reaction chambers so that the 3'-O-protected elongated fragments are deprotected, (iii) producing a pressure differential between the reaction chambers and the waste manifold to remove deprotection solution from the reaction chambers at a predetermined rate, and (iv) measuring with the one or more liquid level sensors a liquid level in each of said reaction chambers and whenever a reaction chamber is identified whose liquid level is outside of predetermined bounds, bypassing the identified reaction chamber in subsequent reagent delivery steps.

3. The apparatus of claim 1 or 2 wherein said plurality of reaction chambers are uniformly spaced in a planar synthesis plate positioned at a first location in said apparatus and wherein said apparatus further comprises (i) a polynucleotide isolation plate positioned at a second location in said apparatus, wherein the polynucleotide isolation plate comprises a plurality of chambers in a planar array spaced to align with said outlets of said plurality of reaction chambers of the synthesis plate, each chamber of the polynucleotide isolation plate having an inlet, an outlet and a separation material for isolating polynucleotides, and (ii) a plate mover operationally associated with said control system, the synthesis plate and the polynucleotide isolation plate, such that, upon instructions from said control system, the plate mover moves the synthesis plate and the polynucleotide isolation plate so that at the first or second location the polynucleotide isolation plate is operationally mounted on a waste manifold and the synthesis plate is operationally mounted on the polynucleotide isolation plate so that whenever a positive pressure differential is establish between said reaction chambers of the synthesis plate and the waste manifold isolation reagents in each reaction chamber flow through a chamber of the isolation plate to said waste manifold.

4. The apparatus of claim 1 or 2 wherein said plurality of reaction chambers are uniformly spaced in a planar synthesis plate positioned at a first location in said apparatus and wherein said apparatus further comprises (i) a polynucleotide isolation plate positioned at a second location in said apparatus, wherein the polynucleotide isolation plate comprises a plurality of chambers in a planar array spaced to align with said plurality of reaction chambers of the synthesis plate, each chamber of the polynucleotide isolation plate having an inlet, an outlet and a separation material for isolating polynucleotides, and (ii) a pipettor operationally associated with said control system, the synthesis plate and the polynucleotide isolation plate, such that, upon instructions from said control system, the pipettor moves contents of each reaction chamber to a separate chamber of the polynucleotide isolation plate.

5. The apparatus of any one of claims 1 to 4, wherein said control system directs repeated steps of: (i) delivering under coupling conditions to said initiators or deprotected elongated fragments a 3'-O-protected nucleoside triphosphate and a template-free polymerase, wherein the coupling conditions include a predetermined coupling incubation time and incubation temperature to allow initiator oligonucleotides or deprotected elongated fragments to be elongated by said 3'-O-protected nucleoside triphosphate to form 3'-O-protected elongated fragments, (ii) delivering said deprotection solution to said reaction chambers so that said 3'-O-protected elongated fragments are deprotected, (iii) producing a pressure differential between said reaction chambers and said waste manifold to remove deprotection solution from said reaction chambers at a predetermined rate, (iv) delivering a wash solution to said reaction chambers, and (v) producing a pressure differential between said reaction chambers and said waste manifold to remove wash solution from said reaction chambers at a predetermined rate.

6. An apparatus for synthesizing with a template-free polymerase a plurality of polynucleotides each with a predetermined sequence, the apparatus comprising:

a) a plurality of reaction chambers, each reaction chamber having disposed therein a synthesis support with initiators attached, wherein each initiator has a free 3'-hydroxyl, and wherein each reaction chamber has an inlet and an outlet and a filter that retains the synthesis support and that is operationally associated with the outlet so that reaction solutions exiting the reaction chamber pass through the filter;

(b) a waste manifold operationally associated with the outlets of the reaction chambers such that reaction solutions are removed from the reaction chambers and enter the waste manifold whenever a positive pressure differential is established between the reaction chambers and the waste manifold;

(c) a fluid delivery system for delivering reaction solutions to the reaction chambers, the reaction solutions comprising 3'-O-protected nucleoside triphosphates, a deprotection solution, a template-free polymerase and a protease solution;

(d) one or more liquid level sensors for measuring rates of change of liquid levels in individual reaction chambers;

(e) a user interface for accepting nucleotide sequences of polynucleotides to be synthesized; and

(f) a control system operationally associated with the user interface, the reaction chambers, the fluid delivery system and the waste manifold, wherein the control system assigns the predetermined sequence of each polynucleotide to a reaction chamber for synthesis, and wherein for each reaction chamber, the control system directs repeated steps of: (i) delivering under coupling conditions to the initiators or deprotected elongated fragments a 3'-O-protected nucleoside triphosphate and a template-free polymerase, wherein the coupling conditions include a predetermined coupling incubation time and incubation temperature to allow initiator oligonucleotides or deprotected elongated fragments to be elongated by the 3'-O-protected nucleoside triphosphate to form 3'-O-protected elongated fragments, (ii) delivering the deprotection solution to the reaction chambers so that the 3'-O-protected elongated fragments are deprotected, (iii) producing a predetermined pressure differential between the reaction chambers and the waste manifold to remove deprotection solution from the reaction chambers, (iv) delivering a wash solution to the reaction chambers, (v) producing a predetermined pressure differential between the reaction chambers and the waste manifold to remove wash solution from the reaction chambers, and (vi) measuring with the one or more liquid level sensors a rate of change of liquid level in each of a portion of the reaction chambers and whenever a reaction chamber is detected whose rate of liquid removal is below the predetermined rate a corrective action is actuated; wherein the kind of 3'-protected nucleoside triphosphate contacted in step (i) in a reaction chamber is determined by the predetermined sequence assigned to the reaction chamber.

7. The apparatus of claim 6 wherein said control system directs said (iv) measurement of said rates of change of liquid levels during removal of said deprotection solution or during removal of said wash solution.
8. The apparatus of claim 6 or 7 wherein said corrective action comprises a further step of delivering said protease solution to said reaction chamber whose rate of liquid removal is below said predetermined rate.
9. The apparatus of claim 6 or 7 wherein said corrective action comprises a further step of bypassing in subsequence reagent delivery steps to said reaction chamber whose rate of liquid removal is below said predetermined rate.
10. The apparatus of any one of claims 6 to 9, wherein said plurality of said reaction chambers is in the range of from 24 to 100 and said one or more liquid level sensors is in the range of from 2 to 32 liquid level sensors.
11. The apparatus of any one of claims 6 to 9 wherein said plurality of said reaction chambers is in the range of from 200 to 1600 and said one or more liquid level sensors is in the range of from 32 to 50 liquid level sensors.
12. The apparatus of any one of claims 6 to 11, wherein said control system directs repeated steps of: (i) delivering under coupling conditions to said initiators or deprotected elongated fragments a 3'-O-protected nucleoside triphosphate and a template-free polymerase, wherein the coupling conditions include a predetermined coupling incubation time and incubation temperature to allow initiator oligonucleotides or deprotected elongated fragments to be elongated by said 3'-O-protected nucleoside triphosphate to form 3'-O-protected elongated fragments, (ii) delivering said deprotection solution to said reaction chambers so that said 3'-O-protected elongated fragments are deprotected, (iii) producing a pressure differential between said reaction chambers and said waste manifold to remove deprotection solution from said reaction chambers at a predetermined rate, (iv) delivering a wash solution to said reaction

chambers, and (v) producing a pressure differential between said reaction chambers and said waste manifold to remove wash solution from said reaction chambers at a predetermined rate.

13. The apparatus of any one of claims 6 to 12, wherein said plurality of reaction chambers are uniformly spaced in a planar reaction plate positioned at a first location in said apparatus and wherein said apparatus further comprises (i) a polynucleotide isolation plate positioned at a second location in said apparatus, wherein the isolation plate comprises a plurality of chambers in a planar array spaced to align with said outlets of said plurality of reaction chambers of the reaction plate, each chamber of the isolation plate having an inlet, an outlet and a separation material for isolating polynucleotides, and (ii) a plate mover operationally associated with said control system, the reaction plate and the isolation plate, such that, upon instructions from said control system, the plate mover moves the reaction plate and the isolation plate so that at the first or second location the isolation plate is operationally mounted on a waste manifold and the reaction plate is operationally mounted on the isolation plate so that whenever a positive pressure differential is establish between said reaction chambers of the reaction plate and the waste manifold isolation reagents in each reaction chamber flow through a chamber of the isolation plate to said waste manifold.

14. A kit for implementing enzymatic synthesis of a plurality of polynucleotides on an automated synthesizer, the kit comprising a synthesis plate having a plurality of reaction wells wherein each reaction well contains a predetermined amount of synthesis support material having initiators attached in a viscous humectant solution or in a dissolvable gel.

15. The kit of claim 14 wherein said synthesis plate is enclosed in an air-tight packaging material.

16. The kit of claim 15 wherein said synthesis plate is vacuum packed in a packaging material comprising plastic or foil.

17. The kit of any one of claims 14 to 16, wherein said synthesis plate comprises 96 wells or 384 wells.

18. The kit of any one of claims 14 to 17, wherein said viscous humectant solution has a viscosity equivalent to a glycerol:water solution in the range 40-60% (v/v).

19. The kit of claim 18 wherein said viscous humectant solution comprises glycerol, alcohol sugars, ethylhexylglycerin, panthenol, sorbitol, xylitol, maltitol, propylene glycol, hexylene glycol, butylene glycol, sodium lactate, hyaluronic acid, or polydextrose.

20. The kit of any one of claims 14 to 19, further including a container containing a solution comprising 3'-O-protected-dATP, a container containing a solution comprising 3'-O-protected-dCTP, a container containing a solution comprising 3'-O-protected-dGTP, a container containing a solution comprising 3'-O-protected dTTP, a container containing a solution comprising a template-free polymerase and a container containing a deprotection solution.

21. The kit of claim 20 wherein said template-free polymerase is a TdT variant, said 3'-O-protected-dATP is 3'-O-amino-dATP, said 3'-O-protected-dCTP is 3'-O-amino-dCTP, said 3'-O-protected-dGTP is 3'-O-amino-dGTP, and said 3'-O-protected-dTTP is 3'-O-amino-dTTP.