

What is claimed is:

1. A method for sequencing nucleic acid templates, comprising
 - 5 (a) providing an array comprising a plurality of sites, wherein each site comprises a first nucleic acid template and a second nucleic acid template,
wherein the first nucleic acid template has a sequence that is different from the sequence of the second nucleic acid template,
wherein a first primer is bound to the first nucleic acid template, a reversible
10 blocking moiety being attached to the first primer,
wherein a second primer is bound to the second nucleic acid template, a reversible blocking moiety being attached to the second primer, and
wherein the reversible blocking moiety that is attached to the first primer is different from the reversible blocking moiety that is attached to the second primer;
 - 15 (b) selectively removing the reversible blocking moiety that is attached to the first primer while retaining the reversible blocking moiety that is attached to the second primer;
 - (c) extending the first primer by addition of a first nucleotide analog comprising a reversible blocking moiety;
 - (d) selectively removing the reversible blocking moiety that is attached to the second
20 primer while retaining the reversible blocking moiety that is attached to the nucleotide analog that is added to the first primer;
 - (e) extending the second primer by addition of a second nucleotide analog comprising a reversible blocking moiety, wherein the reversible blocking moiety of the first nucleotide analog is different from the reversible blocking moiety of the second nucleotide analog; and
25 (f) detecting the nucleotide analog that is added to the first primer and the nucleotide analog that is added to the second primer, at each of the sites, thereby determining the different sequences of the first template and the second template at each of the sites in an orthogonal manner by using a selective deblocking of nucleotides.
- 30 2. The method of claim 1, further comprising

- (g) selectively removing the reversible blocking moiety of the first nucleotide analog that is added to the first primer while retaining the reversible blocking moiety of the second nucleotide analog that is added to the second primer;
- (h) extending the first primer, after (g), by addition of a third nucleotide analog
5 comprising a reversible blocking moiety;
- (i) selectively removing the reversible blocking moiety of the second nucleotide analog that is added to the second primer while retaining the reversible blocking moiety of the third nucleotide analog that is added to the first primer;
- (j) extending the second primer, after (i), by addition of a fourth nucleotide analog
10 comprising a reversible blocking moiety, wherein the reversible blocking moiety of the third nucleotide analog is different from the reversible blocking moiety of the fourth nucleotide analog; and
- (k) detecting the nucleotide analog that is added to the first primer in (h) and the nucleotide analog that is added to the second primer in (j), at each of the sites, thereby
15 determining the different sequences of the first template and the second template at each of the sites in an orthogonal manner by using a selective deblocking of nucleotides.
3. The method of claim 2, further comprising repeating steps (g) through (k).
- 20 4. The method of claim 1 or 2, wherein the detecting uses a detector having a spatial resolution that is too low to resolve points at distance equivalent to the spacing between the first primer and the second primer at each of the sites.
5. The method of any one of claims 1-4, wherein the detector is an optical
25 detector.
6. The method of any one of claims 1-5, wherein the nucleotide analogs comprise optical labels.
- 30 7. The method of claim 6, wherein the first nucleotide analog is from a first set of nucleotide analogs, wherein the second nucleotide analog is from a second set of

nucleotide analogs, and wherein the optical labels of the first set of nucleotide analogs are different from the optical labels of the second set of nucleotide analogs.

8. The method of any one of claims 1-7, wherein the first nucleotide analog is
5 from a first set of nucleotide analogs, wherein the second nucleotide analog is from a second set of nucleotide analogs, and wherein a subset of the nucleotide analogs in the first set of nucleotide analogs comprises optical labels.

9. The method of claim 8, wherein a subset of the nucleotide analogs in the
10 second set of nucleotide analogs comprises optical labels.

10. The method of claim 9, wherein the subset of nucleotide analogs in the first set of nucleotide analogs comprises optical labels that are different from the optical labels of the subset of nucleotide analogs in the second set of nucleotide analogs.

15

11. The method of any one of claims 1-7, wherein the first nucleotide analog is from a first set of nucleotide analogs, wherein the second nucleotide analog is from a second set of nucleotide analogs, wherein the first set of nucleotide analogs comprises only one type of optical label that is detected in step (f) and the second set of nucleotide analogs
20 comprises only one type of optical label that is detected in step (f).

12. The method of claim 11, wherein the one type of optical label is attached to substantially all of the nucleotide analogs of a first species in the first set,

the one type of optical label is attached to a subset of the nucleotide analogs of a
25 second species in the first set,

substantially all of the nucleotide analogs of a third species in the first set are attached to a ligand, and

substantially all of the nucleotide analogs of a fourth species in the first set are not attached to the one type of optical label or to the ligand.

30

13. The method of any one of claims 1-7, wherein the first nucleotide analog is from a first set of nucleotide analogs, wherein the second nucleotide analog is from a second set of nucleotide analogs, wherein the first set of nucleotide analogs comprises only two types of optical labels that are detected in step (f) and the second set of nucleotide
5 analogs comprises only two types of optical labels that are detected in step (f).

14. The method of claim 13, wherein only one of the two types of optical labels is attached to substantially all of the nucleotide analogs of a first species in the first set,
only a second of the two types of optical labels is attached to substantially all of the
10 nucleotide analogs of a second species in the first set,
the one of the two types of optical labels and the second of the two types of optical labels are attached to nucleotide analogs of a third species in the first set, and
substantially all of the nucleotide analogs of a fourth species in the first set are not attached to the one of the two types of optical labels or the second of the two types of
15 optical labels.

15. The method of any one of claims 1-14, wherein a pixel of the detector simultaneously acquires signals from both the first primer and the second primer.

20 16. The method of any one of claims 1-15, wherein the first nucleic acid template comprises at least one base moiety that is the same species as a base moiety in the second nucleic acid template.

17. The method of any one of claims 1-16, wherein steps (b) and (d) are carried
25 out simultaneously.

18. The method of any one of claims 1-17, wherein steps (c) and (e) are carried out sequentially.

30 19. The method of any one of claims 1-18, wherein a single nucleic acid molecule contains the first nucleic acid template and the second nucleic acid template.

20. The method of any one of claims 1-18, wherein the first nucleic acid template and the second nucleic acid template are on different nucleic acid molecules.
- 5 21. The method of any one of claims 1-20, wherein the sites have an area that is $100\text{ }\mu\text{m}^2$ or less.
- 10 22. The method of any one of claims 1-21, wherein the sites comprise multiple amplicons of the first nucleic acid template and multiple amplicons of the second nucleic acid template.
23. The method of claim 22, wherein the multiple amplicons comprise a nucleic acid cluster.
- 15 24. The method of any one of claims 1-23, wherein the plurality of sites has a pitch of $10\text{ }\mu\text{m}$ or less.
25. The method of any one of claims 1-24, wherein the plurality of sites comprises at least 1×10^6 sites.
- 20 26. The method of any one of claims 1-25, wherein each of the sites comprises a nucleic acid sequence that is unique compared to the nucleic acid sequences at the other sites in the plurality.
- 25 27. The method of any one of claims 1-26, wherein the first primer comprises a first universal primer sequence and the first nucleic acid template at each site in the plurality of sites comprises a first universal primer binding sequence that is complementary to the first universal primer sequence.
- 30 28. The method of claim 27, wherein the second primer comprises a second universal primer sequence and the second nucleic acid template at each site in the plurality

of sites comprises a second universal primer binding sequence that is complementary to the second universal primer sequence, wherein the first universal primer binding sequence is different from the second universal primer binding sequence.

5 29. The method of any one of claims 1-28, wherein steps (c) and (e) are carried out by the same polymerase or the same species of polymerases.

 30. The method of any one of claims 1-29, wherein azidomethyl and tert-butoxy-ethoxy are used as reversible blocking moieties.

10

 31. The method of claim 30, wherein the azidomethyl is selectively removed by phosphine treatment and the tert-butoxy-ethoxy is selectively removed by treatment with acid.

15 32. The method of any one of claims 1-29, wherein the selective removal of the reversible blocking moieties comprise chemical treatment, heat treatment, irradiation or electrical treatment.

 33. The method of any one of claims 1-32, wherein the reversible blocking
20 moiety that is attached to the first primer is the same species as the reversible blocking moiety that is attached to the first nucleotide analog.

 34. The method of claim 33, wherein the reversible blocking moiety that is
25 attached to the second primer is the same species as the reversible blocking moiety that is attached to the second nucleotide analog.

 35. A method for sequencing nucleic acid templates, comprising
(a) providing an array of sites, wherein each site comprises a mixture of at least two
different nucleic acid templates;

- (b) extending primers hybridized to the different nucleic acid templates at each of the sites with different nucleotide analogs comprising different reversible blocking moieties, respectively, thereby producing different primer extension products at each site;
- (c) detecting the different primer extension products to distinguish the different nucleotide analogs at each site;
- (d) removing the different reversible blocking moieties from the primer extension products at each of the sites using a first treatment that is selective for a first of the different reversible blocking moieties and a second treatment that is selective for a second of the different reversible blocking moieties; and
- (e) repeating (b) through (d) to determine the sequence of different nucleotide analogs added to each of the different extension products at each of the sites in an orthogonal manner.

36. The method of claim 35, wherein the primers that are hybridized to the different nucleic acid templates are simultaneously present during the extending in (b).

37. The method of any one of claims 35 through 36, wherein the different primer extension products are simultaneously present during the detecting in (c).

38. The method of any one of claims 35 through 37, wherein the first of the different reversible blocking moieties and the second of the different reversible blocking moieties are simultaneously present on the primer extension products during the removing in (d).

39. The method of claim 35, wherein a first of the primers that is hybridized to a first of the different nucleic acid templates is removed from the array of sites prior to the extending of a second of the primers that is hybridized to a second of the different nucleic acid templates.

40. The method of claim 35, wherein a first of the different primer extension products is removed from the array of sites prior to the detecting of a second of the different primer extension products.
- 5 41. The method of claim 35, wherein a first of the different primer extension products is removed from the array of sites prior to the second treatment.
42. The method of any one of claims 35-41, wherein (c) is carried out after (d).
- 10 43. The method of any one of claims 35-42, wherein the detecting uses a detector having a spatial resolution that is too low to resolve points at distance equivalent to the spacing between the different primer extension products at each of the sites.
44. The method of any one of claims 35-43, wherein the detector is an optical
15 detector.
45. The method of any one of claims 35-44, wherein the nucleotide analogs comprise optical labels.
- 20 46. The method of any one of claims 35-45, wherein a first set of nucleotide analogs is added to a first of the different primer extension products and a second set of nucleotide analogs is added to a second of the different primer extension products, wherein the first set of nucleotide analogs comprise optical labels that are different from the optical labels of the second set of nucleotide analogs.
- 25 47. The method of any one of claims 35-46, wherein a pixel of a detector simultaneously acquires signals from both the first primer extension product and the second primer extension product.

48. The method of any one of claims 35-47, wherein the first nucleic acid template comprises at least one base moiety that is the same species as a base moiety in the second nucleic acid template.
- 5 49. The method of any one of claims 35-48, wherein a single nucleic acid molecule contains the at least two different nucleic acid templates.
50. The method of any one of claims 35-48, wherein a first nucleic acid template and a second nucleic acid template of the at least two different nucleic acid
10 templates are on different nucleic acid molecules.
51. The method of any one of claims 35-50, wherein the sites have an area that is $100\ \mu\text{m}^2$ or less.
- 15 52. The method of any one of claims 35-51, wherein the sites comprise multiple amplicons of each of the at least two different nucleic acid templates.
53. The method of claim 52, wherein the multiple amplicons comprise a nucleic acid cluster.
20
54. The method of any one of claims 35-53, wherein the plurality of sites has a pitch of $10\ \mu\text{m}$ or less.
55. The method of any one of claims 35-54, wherein the plurality of sites
25 comprises at least 1×10^6 sites.
56. The method of any one of claims 35-55, wherein each of the sites comprises a nucleic acid sequence that is unique compared to the nucleic acid sequences at the other sites in the plurality.
30

57. The method of any one of claims 35-56, wherein the same polymerase or the same species of polymerase performs the extending of the primers hybridized to the different nucleic acid templates at each of the sites.
- 5 58. The method of any one of claims 35-57, wherein the selective treatments comprise selectively removing a reversible blocking moiety that comprises azidomethyl using phosphine treatment and selectively removing a reversible blocking moiety that comprises tert-butoxy-ethoxy using acid treatment.
- 10 59. The method of any one of claims 35-57, wherein the selective removal of the reversible blocking moieties comprise chemical treatment, heat treatment, irradiation or electrical treatment.
60. A nucleic acid array, comprising
- 15 a plurality of sites on a solid support, wherein each site comprises a first nucleic acid template and a second nucleic acid template,
- wherein the first nucleic acid template has a sequence that is different from the sequence of the second nucleic acid template,
- wherein a first primer is bound to the first nucleic acid template, a first reversible
- 20 blocking moiety being attached to the first primer,
- wherein a second primer is bound to the second nucleic acid template, a second reversible blocking moiety being attached to the second primer, and
- wherein the first reversible blocking moiety is different from the second reversible blocking moiety,
- 25 wherein the different sequences of the first template and the second template at each of the sites can be determined in an orthogonal manner by using a selective deblocking of nucleotides.
61. The nucleic acid array of claim 60, wherein each site occupies an area on the solid support that is $100\text{ }\mu\text{m}^2$ or less.

30

62. The nucleic acid array of claim 60 or 61, wherein the plurality of sites has a pitch of 10 μm or less.
63. The nucleic acid array of any one of claims 60-62, wherein the plurality of
5 sites comprises at least 1×10^6 sites.
64. The nucleic acid array of claim 63, wherein each of the sites comprises a nucleic acid sequence that is unique compared to the nucleic acid sequences at the other sites in the plurality.
- 10 65. The nucleic acid array of any one of claims 60-64, wherein the first reversible blocking moiety is attached to the 3' nucleotide of the first primer.
66. The nucleic acid array of claim 65, wherein the 3' nucleotide of the first
15 primer is attached to a first optical label.
67. The nucleic acid array of any one of claims 65-66, wherein the second reversible blocking moiety is attached to the 3' nucleotide of the second primer.
- 20 68. The nucleic acid array of claim 67, wherein the 3' nucleotide of the second primer is attached to a second optical label, wherein the second optical label is optically distinguishable from the first optical label.
69. The nucleic acid array of any one of claims 65-68, wherein the first and
25 second optical labels comprise fluorophores.
70. The nucleic acid array of any one of claims 60-69, wherein the first nucleic acid template and the second nucleic acid template comprise DNA.
- 30 71. The nucleic acid array of any one of claims 60-70, wherein a single nucleic acid molecule contains the first nucleic acid template and the second nucleic acid template.

72. The nucleic acid array of any one of claims 60-71, wherein the first nucleic acid template and the second nucleic acid template are on different nucleic acid molecules.
- 5 73. The nucleic acid array of any one of claims 60-72, wherein the sites comprise multiple amplicons of the first nucleic acid template and multiple amplicons of the second nucleic acid template.
74. The nucleic acid array of claim 73, wherein the multiple amplicons
10 comprise a nucleic acid cluster.
75. The nucleic acid array of any one of claims 60-74, wherein the first primer comprises a first universal primer sequence and the first nucleic acid template at each site in the plurality of sites comprises a first universal primer binding sequence that is
15 complementary to the first universal primer sequence.
76. The nucleic acid array of claim 75, wherein the second primer comprises a second universal primer sequence and the second nucleic acid template at each site in the plurality of sites comprises a second universal primer binding sequence that is
20 complementary to the second universal primer sequence, wherein the first universal primer binding sequence is different from the second universal primer binding sequence.
77. The nucleic acid array of any one of claims 60-76, wherein a first polymerase is bound to the first primer and the first nucleic acid template.
25
78. The nucleic acid array of claim 77, wherein a second polymerase is bound to the second primer and the second nucleic acid template, and wherein the first polymerase and second polymerase are the same species of polymerase.

79. The nucleic acid array of any one of claims 60-78, wherein the first reversible blocking moiety comprises azidomethyl and wherein the second reversible blocking moiety comprises tert-butoxy-ethoxy.
- 5 80. A detection apparatus, comprising
the nucleic acid array of any one of claims 60-79, and
a detector positioned to detect the plurality of sites, wherein said detector is capable of detecting a label moiety attached to a nucleotide or nucleotide analog.
- 10 81. The detection apparatus of claim 80, wherein the detector has a spatial resolution that is too low to resolve points at distance equivalent to the spacing between the first primer and the second primer at each of the sites.
- 15 82. The detection apparatus of claim 80 or 81, wherein the detector is an optical detector.
- 20 83. The detection apparatus of any one of claims 80-81, wherein a pixel of the detector is configured to simultaneously acquire signals from the first primer and the second primer.