CLAIMS

1. A method for providing an array of polynucleotides, comprising:

irradiating a first polynucleotide immobilized on a substrate with a first light while a second polynucleotide immobilized on the substrate is not irradiated with the first light, wherein the first polynucleotide comprises a first photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the first polynucleotide, and the second polynucleotide comprises a second photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the second polynucleotide,

thereby cleaving the first photo-cleavable moiety such that the inhibition or blocking of hybridization and/or ligation to the first polynucleotide is reduced or eliminated, whereas hybridization and/or ligation to the second polynucleotide remain inhibited or blocked by the second photo-cleavable moiety,

wherein a first barcode is attached to the first polynucleotide via hybridization and/or ligation,

thereby providing on the substrate an array comprising the first and second polynucleotides, wherein the first polynucleotide is barcoded with the first barcode and the second polynucleotide is not barcoded with the first barcode.

~~2. A method for providing an array of polynucleotides, wherein a first polynucleotide immobilized on a substrate is irradiated with a first light while a second polynucleotide immobilized on the substrate is not irradiated with the first light, wherein the first polynucleotide comprises a first photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the first polynucleotide, and the second polynucleotide comprises a second photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the second polynucleotide, and the first photo-cleavable moiety is cleaved such that the inhibition or blocking of hybridization and/or ligation to the first polynucleotide is reduced or eliminated, whereas hybridization and/or ligation to the second polynucleotide remain inhibited or blocked by the second photo-cleavable moiety,~~

~~the method comprising attaching a first barcode to the first polynucleotide via hybridization and/or ligation,~~

~~thereby providing on the substrate an array comprising the first and second polynucleotides, wherein the first polynucleotide is barcoded with the first barcode and the second polynucleotide is not barcoded with the first barcode.~~

~~3. The method of claim 1 or 2, wherein the second polynucleotide is irradiated with a second light, and wherein the second photo-cleavable moiety is cleaved such that the inhibition or blocking of hybridization and/or ligation to the second polynucleotide is reduced or eliminated.~~

~~4~~2. The method of claim 1~~ or 2~~, further comprising irradiating the second polynucleotide with a second light, thereby cleaving the second photo-cleavable moiety such that the inhibition or blocking of hybridization and/or ligation to the second polynucleotide is reduced or eliminated.

~~5. The method of claim 3 or 4, wherein the second polynucleotide is irradiated with the second light while the first polynucleotide is not irradiated with the second light.~~

~~6. The method of any of claims 3-5, wherein a second barcode is attached to the second polynucleotide via hybridization and/or ligation, wherein an array comprising the first and second polynucleotides is provided on the substrate, and wherein the first polynucleotide is barcoded with the first barcode and the second polynucleotide is barcoded with the second barcode.~~

3~~7~~. The method of ~~any of claims 3-5~~claim 2, further comprising attaching a second barcode to the second polynucleotide via hybridization and/or ligation, thereby providing on the substrate an array comprising the first polynucleotide barcoded with the first barcode and the second polynucleotide barcoded with the second barcode.

~~8. A method for providing an array of polynucleotides, comprising:~~

~~(a) irradiating a first polynucleotide immobilized on a substrate with a first light while a second polynucleotide immobilized on the substrate is not irradiated with the first light, wherein the first polynucleotide comprises a first photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the first polynucleotide, and the second polynucleotide comprises a second photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the second polynucleotide,~~

~~thereby cleaving the first photo-cleavable moiety such that the inhibition or blocking of hybridization and/or ligation to the first polynucleotide is reduced or eliminated, whereas~~

~~hybridization and/or ligation to the second polynucleotide remain inhibited or blocked by the second photo-cleavable moiety, and~~

~~(b) attaching a first barcode to the first polynucleotide via hybridization and/or ligation,~~

~~thereby providing on the substrate an array comprising the first and second polynucleotides, wherein the first polynucleotide is barcoded with the first barcode and the second polynucleotide is not barcoded with the first barcode.~~

~~9. The method of claim 8, further comprising:~~

~~(c) irradiating the second polynucleotide with a second light, thereby cleaving the second photo-cleavable moiety such that the inhibition or blocking of hybridization and/or ligation to the second polynucleotide is reduced or eliminated.~~

~~10. The method of claim 9, wherein the second polynucleotide is irradiated with the second light while the first polynucleotide is not irradiated with the second light.~~

~~11. The method of claim 9 or 10, further comprising:~~

~~(d) attaching a second barcode to the second polynucleotide via hybridization and/or ligation,~~

~~thereby providing on the substrate an array comprising the first polynucleotide barcoded with the first barcode and the second polynucleotide barcoded with the second barcode.~~

~~12. The method of any of claims 1-11, wherein the first polynucleotide and the second polynucleotide are of the same nucleic acid sequence.~~

~~13. The method of any of claims 1-11, wherein the first polynucleotide and the second polynucleotide are of different nucleic acid sequences.~~

~~14. The method of any of claims 1-13, wherein the first polynucleotide and/or the second polynucleotide are single stranded.~~

~~15. The method of any of claims 1-13, wherein the first polynucleotide and/or the second polynucleotide are double stranded.~~

4~~16~~. The method of any of claims 1-~~15~~3, wherein the first polynucleotide and/or the second polynucleotide are DNA oligonucleotides, optionally wherein the first polynucleotide and/or the second polynucleotide are between about 6 and about 30 nucleotides in length, optionally wherein the first polynucleotide and or the second polynucleotide are between about 10 and about 20 nucleotides in length.

~~17. The method of any of claims 3-7 and 9-16, wherein the first light and the second light are the same.~~

~~18. The method of any of claims 3-7 and 9-16, wherein the first light and the second light are different, optionally wherein the first light and the second light are of different wavelengths, intensities, or durations of irradiation, or any combination thereof.~~

5~~19~~. The method of any of claims 1-4~~18~~, wherein hybridization and/or ligation to the first polynucleotide barcoded with the first barcode is inhibited or blocked, and/or

wherein hybridization and/or ligation to the second polynucleotide barcoded with the second barcode is inhibited or blocked.

6~~20~~. The method of any of claims 1-5~~19~~, wherein the first barcode comprises a third photo-cleavable moiety that that inhibits or blocks hybridization and/or ligation, thereby inhibiting or blocking hybridization and/or ligation to the first polynucleotide barcoded with the first barcode, and/or

wherein the second barcode comprises a fourth photo-cleavable moiety that that inhibits or blocks hybridization and/or ligation, thereby inhibiting or blocking hybridization and/or ligation to the second polynucleotide barcoded with the second barcode.

~~21. The method of any of claims 1-20, wherein the first photo-cleavable moiety, the second photo-cleavable moiety, the third photo-cleavable moiety, and/or the fourth photo-cleavable moiety are the same.~~

~~22. The method of any of claims 1-20, wherein the first photo-cleavable moiety, the second photo-cleavable moiety, the third photo-cleavable moiety, and/or the fourth photo-cleavable moiety are different.~~

~~23. The method of any of claims 1-22, wherein the first photo-cleavable moiety, the second photo-cleavable moiety, the third photo-cleavable moiety, and/or the fourth photo-cleavable moiety inhibit or block hybridization.~~

~~24. The method of any of claims 1-23, wherein the first photo-cleavable moiety, the second photo-cleavable moiety, the third photo-cleavable moiety, and/or the fourth photo-cleavable moiety inhibit or block ligation.~~

7~~25~~. The method of any of claims 1-6~~24~~, wherein the first photo-cleavable moiety, the second photo-cleavable moiety, the third photo-cleavable moiety, and/or the fourth photo-cleavable moiety comprise a photo-caged nucleobase.

8~~26~~. The method of any of claims 1-7~~25~~, wherein the first photo-cleavable moiety, the second photo-cleavable moiety, the third photo-cleavable moiety, and/or the fourth photo-cleavable moiety comprise a photo-cleavable hairpin.

9~~27~~. The method of any of claims 1-8~~26~~, wherein the first photo-cleavable moiety, the second photo-cleavable moiety, the third photo-cleavable moiety, and/or the fourth photo-cleavable moiety comprise a photo-caged 3'-hydroxyl group.

10~~28~~. The method of any of claims 1-9~~27~~, wherein the first photo-cleavable moiety, the second photo-cleavable moiety, the third photo-cleavable moiety, and/or the fourth photo-cleavable moiety comprise a photo-cleavable polymer.

~~29. The method of any of claims 3-7 and 9-28, wherein the first barcode and the second barcode are of the same nucleic acid sequence.~~

~~30. The method of any of claims 3-7 and 9-28, wherein the first barcode and the second barcode are of different nucleic acid sequences.~~

~~31. The method of any of claims 1-30, wherein the first barcode and/or the second barcode are single stranded.~~

~~32. The method of any of claims 1-30, wherein the first barcode and/or the second barcode are double stranded.~~

~~33. The method of any of claims 1-32, wherein the first barcode and/or the second barcode are DNA oligonucleotides.~~

~~34. The method of any of claims 1-33, wherein the first barcode and/or the second barcode are between about 5 and about 20 nucleotides in length, optionally wherein the first barcode and/or the second barcode are between about 6 and about 16 nucleotides in length.~~

11~~35~~. The method of any of claims 1-10~~34~~, wherein the substrate comprises a plurality of differentially barcoded polynucleotides immobilized thereon.

~~36. The method of any of claims 1-35, wherein the substrate is transparent, translucent, or opaque.~~

12~~37~~. The method of any of claims 1-11~~36~~, wherein the irradiation comprises using a photomask to selectively irradiate the first polynucleotide or the second polynucleotide.

13~~38~~. The method of any of claims 1-12~~37~~, wherein the attachment of the first barcode and/or the second barcode comprises ligating one end of the first/second barcode to one end of the first/second polynucleotide, respectively.

~~39. The method of claim 38, wherein the 5' end nucleotide of the first/second barcode is ligated to the 3' end nucleotide of the first/second polynucleotide, respectively.~~

~~40. The method of claim 39, wherein the 3' end nucleotide of the first/second barcode is ligated to the 5' end nucleotide of the first/second polynucleotide, respectively.~~

1441. The method of any of claims 1-1340, wherein the attachment of the first barcode comprises hybridizing one end of the first barcode and one end of the first polynucleotide to a first splint, and/or

wherein the attachment of the second barcode comprises hybridizing one end of the second barcode and one end of the second polynucleotide to a second splint.

1542. The method of claim 1441, further comprising ligating the first barcode to the first polynucleotide hybridized to the first splint, and/or ligating the second barcode to the second polynucleotide hybridized to the second splint.

~~43. The method of claim 42, wherein the first/second barcode is directly ligated to the first/second polynucleotide, respectively, without gap filling.~~

~~44. The method of claim 42, wherein ligating the first/second barcode to the first/second polynucleotide, respectively, is preceded by gap filling.~~

~~45. The method of any of claims 41-44, wherein the first splint and the second splint are of the same nucleic acid sequence.~~

~~46. The method of any of claims 41-44, wherein the first splint and the second splint are of different nucleic acid sequences.~~

~~47. The method of any of claims 41-46, wherein the first splint and/or the second splint are single stranded.~~

1648. The method of ~~any of claims 41-47~~claim 14 or 15, wherein the first splint and/or the second splint are DNA oligonucleotides, optionally wherein the first splint and/or the second splint are at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 nucleotides in length.

~~49. The method of any of claims 1-48, further comprising providing the first polynucleotide and the second polynucleotide immobilized on the substrate.~~

1750. A method for providing an array of polynucleotides, comprising:

(a1) irradiating polynucleotide P1 immobilized on a substrate with light while polynucleotide P2 immobilized on the substrate is photomasked, wherein polynucleotides P1 and P2 comprise a photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to P1 and P2, respectively,

thereby cleaving the photo-cleavable moiety to allow hybridization and/or ligation to P1, whereas hybridization and/or ligation to P2 remain inhibited or blocked by the photo-cleavable moiety; and

(b1) attaching barcode 1A to P1 via hybridization and/or ligation to form a barcoded polynucleotide 1A-P1, wherein barcode 1A comprises the photo-cleavable moiety which inhibits or blocks hybridization and/or ligation to 1A-P1,

thereby providing on the substrate an array comprising polynucleotides 1A-P1 and P2 each comprising the photo-cleavable moiety that inhibits or blocks hybridization and/or ligation.

18~~51~~. The method of claim 17~~50~~, further comprising:

(c1) irradiating P2 with light while 1A-P1 is photomasked, thereby cleaving the photo-cleavable moiety to allow hybridization and/or ligation to P2, whereas hybridization and/or ligation to 1A-P1 remain inhibited or blocked by the photo-cleavable moiety; and

(d1) attaching barcode 1B to P2 via hybridization and/or ligation to form a barcoded polynucleotide 1B-P2, wherein barcode 1B comprises the photo-cleavable moiety which inhibits or blocks hybridization and/or ligation to 1B-P2,

thereby providing on the substrate an array comprising barcoded polynucleotides 1A-P1 and 1B-P2 each comprising the photo-cleavable moiety that inhibits or blocks hybridization and/or ligation.

~~52. The method of claim 51, wherein barcodes 1A and 1B comprise the same nucleic acid sequence.~~

~~53. The method of claim 51, wherein barcodes 1A and 1B comprise different nucleic acid sequences.~~

19~~54~~. The method of ~~any of claims 50-53~~claim 17 or 18, further comprising:

(a2) irradiating one of 1A-P1 and 1B-P2 with light while the other is photomasked,

thereby cleaving the photo-cleavable moiety to allow hybridization and/or ligation to the irradiated polynucleotide, whereas hybridization and/or ligation to the photomasked polynucleotide remains inhibited or blocked by the photo-cleavable moiety; and

(b2) attaching barcode 2A to the irradiated polynucleotide via hybridization and/or ligation to form a 2A-barcoded polynucleotide, wherein barcode 2A comprises the photo-cleavable moiety which inhibits or blocks hybridization and/or ligation,

thereby providing on the substrate an array comprising barcoded polynucleotides each comprising the photo-cleavable moiety that inhibits or blocks hybridization and/or ligation.

20~~55~~. The method of claim 19~~54~~, further comprising:

(c2) irradiating the photomasked polynucleotide in step a2 with light while the 2A-barcoded polynucleotide is photomasked, thereby cleaving the photo-cleavable moiety to allow

hybridization and/or ligation, whereas hybridization and/or ligation to the 2A-barcoded polynucleotide remain inhibited or blocked by the photo-cleavable moiety; and

(d2) attaching barcode 2B to the irradiated polynucleotide in step c2 via hybridization and/or ligation to form a 2B-barcoded polynucleotide, wherein barcode 2B comprises the photo-cleavable moiety which inhibits or blocks hybridization and/or ligation,

thereby providing on the substrate an array comprising barcoded polynucleotides each comprising the photo-cleavable moiety that inhibits or blocks hybridization and/or ligation.

21~~56~~. The method of claim 20~~55~~, wherein the barcoded polynucleotides on the array comprise polynucleotides 2A-1A-P1 and 2B-1B-P2.

22~~57~~. The method of claim 20~~55~~, wherein the barcoded polynucleotides on the array comprise polynucleotides 2B-1A-P1 and 2A-1B-P2.

23~~58~~. The method of any of claims 20~~55~~-22~~57~~, wherein steps a1-d1 form round 1 and steps a2-d2 form round 2, the method further comprising steps a*i*-d*i* in round *i*, wherein barcodes *i*A and *i*B are attached to provide barcoded polynucleotides on the substrate, and wherein *i* is an integer greater than 2.

~~59. The method of claim 58, wherein barcodes *i*A and *i*B comprise the same nucleic acid sequence.~~

~~60. The method of claim 58, wherein barcodes *i*A and *i*B comprise different nucleic acid sequences.~~

~~61. A method for providing an array of polynucleotides, comprising:~~

~~(a) irradiating polynucleotide P1 and polynucleotide P3 immobilized on a substrate with light while polynucleotide P2 and polynucleotide P4 immobilized on the substrate are photomasked, wherein polynucleotides P1-P4 comprise a photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to P1-P4, respectively,~~

~~thereby cleaving the photo-cleavable moiety to allow hybridization and/or ligation to P1 and P3, whereas hybridization and/or ligation to P2 and P4 remain inhibited or blocked by the photo-cleavable moiety;~~

~~(b) attaching barcode 1A to P1 and P3 via hybridization and/or ligation to form barcoded polynucleotides 1A-P1 and 1A-P3, wherein barcode 1A comprises the photo-cleavable moiety which inhibits or blocks hybridization and/or ligation;~~

~~(c) irradiating P2 and P4 with light while 1A-P1 and 1A-P3 are photomasked, thereby cleaving the photo-cleavable moiety to allow hybridization and/or ligation to P2 and P4,~~

whereas hybridization and/or ligation to 1A-P1 and 1A-P3 remain inhibited or blocked by the photo-cleavable moiety; and

(d) attaching barcode 1B to P2 and P4 via hybridization and/or ligation to form barcoded polynucleotides 1B-P2 and 1B-P4, wherein barcode 1B comprises the photo-cleavable moiety which inhibits or blocks hybridization and/or ligation,

thereby providing on the substrate an array comprising barcoded polynucleotides 1A-P1, 1B-P2, 1A-P3, and 1B-P4, each comprising the photo-cleavable moiety that inhibits or blocks hybridization and/or ligation.

62. The method of claim 61, further comprising:

(a') irradiating polynucleotides 1A-P1 and 1B-P2 with light while polynucleotides 1A-P3 and 1B-P4 are photomasked,

thereby cleaving the photo-cleavable moiety to allow hybridization and/or ligation to 1A-P1 and 1B-P2, whereas hybridization and/or ligation to 1A-P3 and 1B-P4 remain inhibited or blocked by the photo-cleavable moiety,

(b') attaching barcode 2A to 1A-P1 and 1B-P2 via hybridization and/or ligation to form barcoded polynucleotides 2A-1A-P1 and 2A-1B-P2, wherein barcode 2A comprises the photo-cleavable moiety which inhibits or blocks hybridization and/or ligation;

(c') irradiating 1A-P3 and 1B-P4 with light while 2A-1A-P1 and 2A-1B-P2 are photomasked, thereby cleaving the photo-cleavable moiety to allow hybridization and/or ligation to 1A-P3 and 1B-P4, whereas hybridization and/or ligation to 2A-1A-P1 and 2A-1B-P2 remain inhibited or blocked by the photo-cleavable moiety; and

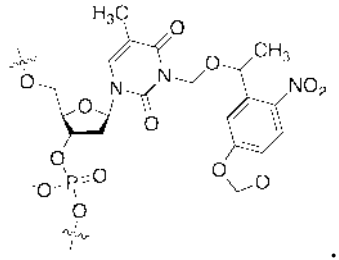
(d') attaching barcode 2B to 1A-P3 and 1B-P4 via hybridization and/or ligation to form barcoded polynucleotides 2B-1A-P3 and 2B-1B-P4, wherein barcode 2B comprises the photo-cleavable moiety which inhibits or blocks hybridization and/or ligation,

thereby providing on the substrate an array comprising barcoded polynucleotides 2A-1A-P1, 2A-1B-P2, 2B-1A-P3, and 2B-1B-P4, each comprising the photo-cleavable moiety that inhibits or blocks hybridization and/or ligation.

63. The method of claim 61 or 62, wherein barcodes 1A, 1B, 2A, and/or 2B comprise the same nucleic acid sequence.

64. The method of claim 61 or 62, wherein barcodes 1A, 1B, 2A, and/or 2B comprise different nucleic acid sequences.

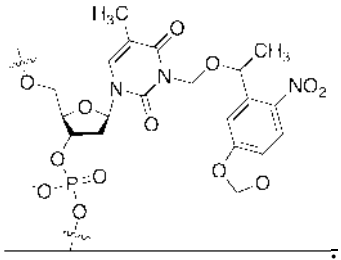
2465. The method of any of claims 17-2350-64, wherein the photo-cleavable moiety comprises a photo-caged nucleobase, optionally wherein the photo-caged nucleobase is a photo-caged deoxythymidine (dT), and optionally wherein the photo-cleavable moiety comprises the following structure:



.

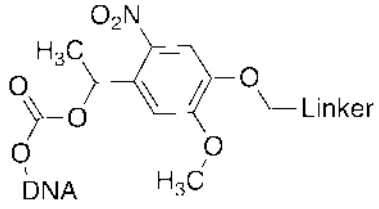
~~66. The method of claim 65, wherein the photo-caged nucleobase is a photo-caged deoxythymidine (dT).~~

~~67. The method of claim 65 or 66, wherein the photo-cleavable moiety comprises the following structure:~~



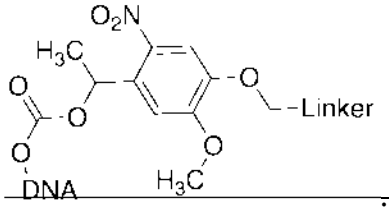
.

2568. The method of any of claims 17-2450-67, wherein the photo-cleavable moiety comprises a photo-cleavable hairpin, and optionally wherein the photo-cleavable moiety comprises the following structure:



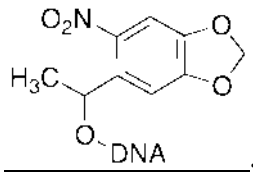
.

~~69. The method of claim 68, wherein the photo-cleavable moiety comprises the following structure:~~

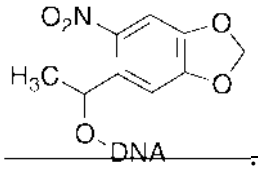


.

26~~70~~. The method of any of claims ~~50-69~~17-25, wherein the photo-cleavable moiety comprises a photo-caged 3'-hydroxyl group, and optionally wherein the photo-cleavable moiety comprises the following structure:



~~71. The method of claim 70, wherein the photo-cleavable moiety comprises the following structure:~~



~~72. The method of any of claims 50-71, wherein the photo-cleavable moiety comprises a photo-cleavable polymer.~~

~~73. A method for providing an array of polynucleotides, comprising:~~

~~(a) irradiating a plurality of first polynucleotides immobilized on a substrate with a first light while a plurality of second polynucleotides immobilized on the substrate are not irradiated with the first light, wherein each first polynucleotide comprises a first photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the first polynucleotide, and each second polynucleotide comprises a second photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the second polynucleotide,~~

~~thereby cleaving the first photo-cleavable moieties such that the inhibition or blocking of hybridization and/or ligation to the plurality of first polynucleotides is reduced or eliminated, whereas hybridization and/or ligation to the second plurality of polynucleotides remain inhibited or blocked by the second photo-cleavable moieties, and~~

~~(b) attaching first barcodes to the plurality of first polynucleotide via hybridization and/or ligation,~~

~~thereby providing on the substrate an array comprising the first polynucleotides barcoded with the first barcodes and the second polynucleotides not barcoded with the first barcodes.~~

~~74. The method of claim 73, wherein at least about 90%, at least about 95%, at least about 99%, or 100% of the first barcodes comprise a third photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the first barcodes.~~

75. The method of claim 74, wherein at least about 90%, at least about 95%, at least about 99%, or 100% of the first polynucleotides barcoded with the first barcodes comprise the third photo-cleavable moiety.

76. The method of any of claims 73-75, further comprising:

(c) irradiating the plurality of second polynucleotides with a second light, thereby cleaving the second photo-cleavable moieties such that the inhibition or blocking of hybridization and/or ligation to the plurality of second polynucleotides is reduced or eliminated.

77. The method of claim 76, wherein the plurality of second polynucleotides are irradiated with the second light while the plurality of first polynucleotides are not irradiated with the second light.

78. The method of claim 76 or 77, further comprising:

(d) attaching second barcodes to the plurality of second polynucleotides via hybridization and/or ligation, wherein hybridization and/or ligation to the first polynucleotide barcoded with the first barcodes is inhibited or blocked,

thereby providing on the substrate an array comprising the first polynucleotides barcoded with the first barcodes and the second polynucleotides barcoded with the second barcodes.

79. The method of claim 78, wherein at least about 90%, at least about 95%, at least about 99%, or 100% of the second barcodes comprise a fourth photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the second barcodes.

80. The method of claim 79, wherein at least about 90%, at least about 95%, at least about 99%, or 100% of the second polynucleotides barcoded with the second barcodes comprise the fourth photo-cleavable moiety.

81. The method of any of claims 73-80, wherein at least about 90%, at least about 95%, at least about 99%, or 100% of the plurality of first polynucleotides have the same nucleic acid sequences.

82. The method of any of claims 73-81, wherein at least about 90%, at least about 95%, at least about 99%, or 100% of the plurality of second polynucleotides have the same nucleic acid sequences.

83. The method of any of claims 73-82, wherein at least about 90%, at least about 95%, at least about 99%, or 100% of the plurality of first polynucleotides have the same nucleic acid sequences as at least about 90%, at least about 95%, at least about 99%, or 100% of the plurality of second polynucleotides.

~~84. The method of any of claims 73-83, wherein the plurality of first polynucleotides and the plurality of second polynucleotides have the same nucleic acid sequences.~~

~~85. The method of any of claims 73-84, wherein polynucleotides of a universal nucleic acid sequence are immobilized on the substrate prior to the irradiation.~~

27~~86~~. The method of any of claims ~~73-85~~1-26, wherein polynucleotides of different nucleic acid sequences are immobilized on the substrate in a pattern prior to the irradiation, wherein the pattern comprises rows and/or columns.

~~87. The method of claim 86, wherein the pattern comprises rows and/or columns.~~

~~88. The method of any of claims 73-87, wherein at least about 90%, at least about 95%, at least about 99%, or 100% of the plurality of first polynucleotides are barcoded with the first barcodes, and/or~~

~~wherein at least about 90%, at least about 95%, at least about 99%, or 100% of the plurality of second polynucleotides are barcoded with the second barcodes.~~

~~89. A method for providing an array of polynucleotides, comprising:~~

~~(i) irradiating a first polynucleotide immobilized on a substrate with light while a second polynucleotide immobilized on the substrate is not irradiated with light, wherein the first and second polynucleotides each comprises a photo-cleavable moiety that inhibits or blocks hybridization,~~

~~thereby cleaving the photo-cleavable moiety to allow hybridization to the first polynucleotide, whereas hybridization to the second polynucleotide remains inhibited or blocked by the photo-cleavable moiety;~~

~~(ii) attaching a first barcode to the first polynucleotide via hybridization to a first splint followed by ligation, wherein the first splint hybridizes to one end of the first polynucleotide and one end of the first barcode, and wherein the first barcode comprises the photo-cleavable moiety;~~

~~(iii) irradiating the second polynucleotide with light, thereby cleaving the photo-cleavable moiety to allow hybridization to the second polynucleotide; and~~

~~(iv) attaching a second barcode to the second polynucleotide via hybridization to a second splint followed by ligation, wherein the second splint hybridizes to one end of the second polynucleotide and one end of the second barcode, and wherein the second barcode comprises the photo-cleavable moiety,~~

thereby providing on the substrate an array comprising the first polynucleotide barcoded with the first barcode and the second polynucleotide barcoded with the second barcode.

90. The method of claim 89, wherein the photo-cleavable moiety comprises a photo-caged nucleobase.

91. The method of claim 89 or 90, wherein the photo-cleavable moiety comprises a photo-cleavable hairpin.

92. The method of any of claims 89-91, wherein the photo-cleavable moiety comprises a photo-cleavable polymer.

93. A method for providing an array of polynucleotides, comprising:

(i) irradiating a first polynucleotide immobilized on a substrate with light while a second polynucleotide immobilized on the substrate is not irradiated with light, wherein the first and second polynucleotides each comprises a photo-cleavable moiety that inhibits or blocks ligation to the '3 end of the first and second polynucleotides, respectively,

thereby cleaving the photo-cleavable moiety to allow ligation to the '3 end of the first polynucleotide, whereas ligation to the '3 end of the second polynucleotide remains inhibited or blocked by the photo-cleavable moiety;

(ii) attaching a first barcode to the first polynucleotide via hybridization to a first splint followed by ligation, wherein the first splint hybridizes to a '3 end portion of the first polynucleotide and a '5 end portion of the first barcode, and wherein the first barcode comprises the photo-cleavable moiety which blocks ligation to the '3 end of the first barcode;

(iii) irradiating the second polynucleotide with light, thereby cleaving the photo-cleavable moiety to allow hybridization to the '3 end of the second polynucleotide; and

(iv) attaching a second barcode to the second polynucleotide via hybridization to a second splint followed by ligation, wherein the second splint hybridizes to a '3 end portion of the second polynucleotide and a '5 end portion of the second barcode, and wherein the second barcode comprises the photo-cleavable moiety which blocks ligation to the '3 end of the second barcode,

thereby providing on the substrate an array comprising the first polynucleotide barcoded with the first barcode and the second polynucleotide barcoded with the second barcode.

94. The method of claim 93, wherein the photo-cleavable moiety comprises a photo-caged 3'-hydroxyl group.

95. The method of claim 93 or 94, wherein the photo-cleavable moiety comprises a photo-cleavable polymer.

96. The method of any of claims 89-95, wherein the first polynucleotide and the second polynucleotide are of the same nucleic acid sequence.

97. The method of any of claims 89-95, wherein the first polynucleotide and the second polynucleotide are of different nucleic acid sequences.

98. The method of any of claims 89-97, wherein the first barcode and the second barcode are of the same nucleic acid sequence.

99. The method of any of claims 89-97, wherein the first barcode and the second barcode are of different nucleic acid sequences.

100. The method of any of claims 89-99, wherein the first splint and the second splint are of the same nucleic acid sequence.

101. The method of any of claims 89-99, wherein the first splint and the second splint are of different nucleic acid sequences.

102. A method for providing an array of polynucleotides, comprising:

attaching a first barcode to a first polynucleotide immobilized on a substrate, wherein the first barcode comprises a first photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the first barcode, and wherein the substrate has immobilized thereon (i) the first polynucleotide, (ii) a second polynucleotide comprising a second photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the second polynucleotide, and (iii) a third polynucleotide available for hybridization and/or ligation,

wherein after the attaching step, (i) the first polynucleotide is attached to the first barcode comprising the first photo-cleavable moiety, (ii) the second polynucleotide retains the second photo-cleavable moiety, and (iii) the third polynucleotide remains available for hybridization and/or ligation, and

wherein the third polynucleotide is rendered unavailable for hybridization and/or ligation.

103. The method of claim 102, wherein the second polynucleotide is irradiated with light while the first polynucleotide is photomasked, thereby rendering the second polynucleotide available for hybridization and/or ligation whereas the first polynucleotide remains unavailable for hybridization and/or ligation.

~~104. The method of claim 103, wherein a second barcode is attached to the second polynucleotide via hybridization and/or ligation, wherein the second barcode is not attached to the first polynucleotide or the third polynucleotide, thereby providing on the substrate an array comprising the first polynucleotide barcoded with the first barcode and the second polynucleotide barcoded with the second barcode.~~

~~105. A method for providing an array of polynucleotides, comprising:~~

~~irradiating a substrate with light, wherein the substrate has immobilized thereon (i) a first polynucleotide comprising a first barcode comprising a first photo-cleavable moiety, (ii) a second polynucleotide comprising a second photo-cleavable moiety, and (iii) a third polynucleotide available for hybridization and/or ligation, wherein the first photo-cleavable moiety and the second photo-cleavable moiety inhibit or block hybridization and/or ligation to the first polynucleotide and the second polynucleotide, respectively,~~

~~wherein the third polynucleotide is rendered unavailable for hybridization and/or ligation,~~

~~wherein the irradiating step comprises irradiating the second polynucleotide with light while the first polynucleotide is photomasked, thereby rendering the second polynucleotide available for hybridization and/or ligation whereas the first polynucleotide remains unavailable for hybridization and/or ligation, and~~

~~wherein a second barcode is attached to the second polynucleotide via hybridization and/or ligation, wherein the second barcode is not attached to the first polynucleotide or the third polynucleotide,~~

~~thereby providing on the substrate an array comprising the first polynucleotide barcoded with the first barcode and the second polynucleotide barcoded with the second barcode.~~

~~106. The method of claim 105, further comprising rendering the third polynucleotide unavailable for hybridization and/or ligation.~~

~~107. The method of any of claims 102-106, wherein the third polynucleotide is rendered unavailable for hybridization and/or ligation by enzymatic digestion of the third polynucleotide.~~

~~108. The method of any of claims 102-107, wherein the third polynucleotide has a 3'-hydroxyl group that is not protected and is susceptible to exonuclease digestion.~~

~~109. The method of any of claims 102-108, wherein the first and second photo-cleavable moieties protect the first and second polynucleotides, respectively, from exonuclease digestion.~~

~~110. The method of any of claims 102-106, wherein the third polynucleotide is capped at the 3' end, rendering it unavailable for ligation.~~

~~111. A method for providing an array of polynucleotides, comprising:~~

~~attaching a first barcode which is nuclease resistant to a first polynucleotide immobilized on a substrate, wherein the substrate has immobilized thereon (i) the first polynucleotide, (ii) a second polynucleotide comprising a first photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the second polynucleotide, and (iii) a third polynucleotide available for hybridization and/or ligation,~~

~~wherein after the attaching step, (i) the first polynucleotide is attached to the first barcode, (ii) the second polynucleotide retains the first photo-cleavable moiety, and (iii) the third polynucleotide remains available for hybridization and/or ligation, and~~

~~wherein the third polynucleotide is rendered unavailable for hybridization and/or ligation.~~

~~112. The method of claim 111, wherein the second polynucleotide is rendered unavailable for hybridization and/or ligation.~~

~~113. The method of claim 111 or 112, wherein the second and third polynucleotides are rendered unavailable for hybridization and/or ligation by nuclease digestion, whereas the first polynucleotide is rendered nuclease resistant due to its attachment to the first barcode.~~

~~114. The method of any of claims 111-113, further comprising attaching an adaptor to the first barcode which is in turn attached to the first polynucleotide, wherein the adaptor comprises a second photo-cleavable moiety that inhibits or blocks hybridization and/or ligation.~~

~~115. The method of claim 114, wherein the adaptor is a universal adaptor.~~

~~116. The method of claim 114 or 115, further comprising irradiating the first polynucleotide attached to the first barcode and the adaptor with light.~~

~~117. The method of claim 116, further comprising attaching a second barcode to the adaptor via hybridization and/or ligation.~~

~~118. An array of polynucleotides produced by the method of any of claims 1-117.~~

~~119~~28. A hybridization complex, comprising:

a first polynucleotide immobilized on a substrate;

a first splint hybridized to one end of the first polynucleotide and one end of a first barcode; and

the first barcode which comprise a first photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the other end of the first barcode.

29~~120~~. A composition, comprising the hybridization complex of claim 28~~119~~ and the substrate.

30~~121~~. The composition of claim 29~~120~~, further comprising a second polynucleotide immobilized on the substrate, wherein the second polynucleotide comprises a second photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to second polynucleotide.

31~~122~~. The composition of claim 29~~120~~, further comprising a second hybridization complex comprising:

a second polynucleotide immobilized on the substrate;

a second splint hybridized to one end of the second polynucleotide and one end of a second barcode; and

the second barcode which comprise a second photo-cleavable moiety that inhibits or blocks hybridization and/or ligation to the other end of the second barcode.

~~123. The composition of claim 122, wherein the first polynucleotide and the second polynucleotide are of the same nucleic acid sequence.~~

~~124. The composition of claim 122, wherein the first polynucleotide and the second polynucleotide are of different nucleic acid sequences.~~

~~125. The composition of any of claims 122-124, wherein the first barcode and the second barcode are of the same nucleic acid sequence.~~

~~126. The composition of any of claims 122-124, wherein the first barcode and the second barcode are of different nucleic acid sequences.~~

~~127. The composition of any of claims 122-126, wherein the first splint and the second splint are of the same nucleic acid sequence.~~

~~128. The composition of any of claims 122-126, wherein the first splint and the second splint are of different nucleic acid sequences.~~

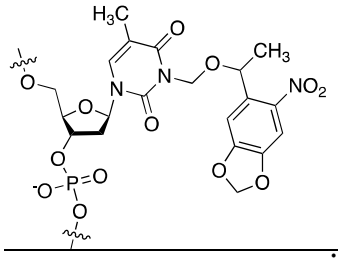
~~129. The composition of any of claims 120-128, wherein the first polynucleotide and/or the second polynucleotide are single stranded.~~

~~130. The composition of any of claims 120-129, wherein the first polynucleotide and/or the second polynucleotide are DNA oligonucleotides.~~

131. The composition of any of claims 120-130, wherein the first photo-cleavable moiety and/or the second photo-cleavable moiety comprise a photo-caged nucleobase.

132. The composition of claim 131, wherein the photo-caged nucleobase is a photo-caged deoxythymidine (dT).

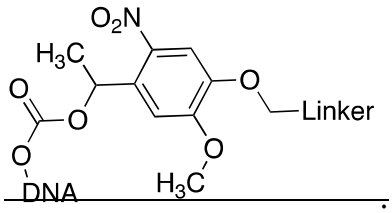
133. The composition of claim 131 or 132, wherein the photo-cleavable moiety comprises the following structure:



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134. The composition of any of claims 120-133, wherein the first photo-cleavable moiety and/or the second photo-cleavable moiety comprise a photo-cleavable hairpin.

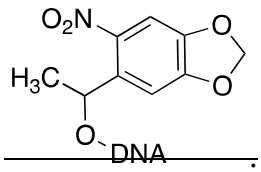
135. The composition of claim 134, wherein the photo-cleavable moiety comprises the following structure:



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136. The composition of any of claims 120-135, wherein the first photo-cleavable moiety and/or the second photo-cleavable moiety comprise a photo-caged 3'-hydroxyl group.

137. The composition of claim 136, wherein the photo-cleavable moiety comprises the following structure:



.

138. The composition of any of claims 120-137, wherein the first photo-cleavable moiety and/or the second photo-cleavable moiety comprise a photo-cleavable polymer.