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. The method of claim 1, 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.

3. The method of 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.

4. The method of any of claims 1-3, 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.

5. The method of any of claims 1-4, 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. The method of any of claims 1-5, 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.

7. The method of any of claims 1-6, 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. The method of any of claims 1-7, 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. The method of any of claims 1-8, 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. The method of any of claims 1-9, 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.

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

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

13. The method of any of claims 1-12, 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.

14. The method of any of claims 1-13, 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.

15. The method of claim 14, 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.

16. The method of 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.

17. 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. The method of claim 17, 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.

19. The method of 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. The method of claim 19, 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,

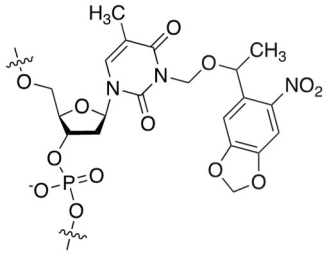
thereby providing on the substrate an array comprising barcoded polynucleotides each comprising the photo-cleavable moiety that inhibits or blocks hybridization and/or ligation.

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

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

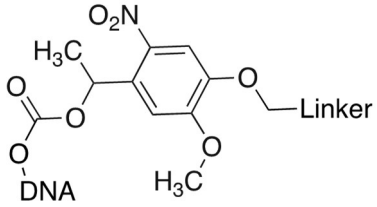
23. The method of any of claims 20-22, wherein steps a1-d1 form round 1 and steps a2-d2 form round 2, the method further comprising steps a*i*-d*i* in round *i*, wherein barcodes *i*A and *i*B are attached to provide barcoded polynucleotides on the substrate, and wherein *i* is an integer greater than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

24. The method of any of claims 17-23, 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:



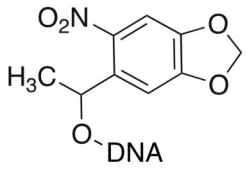
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25. The method of any of claims 17-24, wherein the photo-cleavable moiety comprises a photo-cleavable hairpin, and optionally wherein the photo-cleavable moiety comprises the following structure:



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26. The method of any of claims 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:



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27. The method of any of claims 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.

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. A composition, comprising the hybridization complex of claim 28 and the substrate.

30. The composition of claim 29, 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. The composition of claim 29, 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.