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 is sequestered by a first photo-cleavable polymer that inhibits or blocks hybridization and/or ligation to the first polynucleotide, and the second polynucleotide is sequestered by a second photo-cleavable polymer that inhibits or blocks hybridization and/or ligation to the second polynucleotide,

thereby cleaving the first photo-cleavable polymer such that it is released from the substrate and 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 remains inhibited or blocked by the second photo-cleavable polymer,

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 polymer such that it is released from the substrate and the inhibition or blocking of hybridization and/or ligation to the second polynucleotide is reduced or eliminated, wherein the second polynucleotide is irradiated with the second light while the first polynucleotide is not irradiated with the second light.

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 is a DNA oligo, optionally wherein the first polynucleotide and/or the second polynucleotide are between about 6 and about 30 nucleotides in length, e.g., about 10 and about 20 nucleotides in length.

5. The method of any of claims 1-4, wherein the first barcode and/or the second barcode are DNA oligonucleotides, and 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.

6. The method of any of claims 1-5, wherein the substrate comprises a plurality of differentially barcoded polynucleotides immobilized thereon.

7. The method of any of claims 1-6, wherein the irradiation comprises using a photomask to selectively irradiate the first polynucleotide or the second polynucleotide.

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

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

10. The method of claim 9, 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.

11. The method of claim 10, wherein the first/second barcode is directly ligated to the first/second polynucleotide, respectively, without gap filling.

12. The method of claim 10, wherein ligating the first/second barcode to the first/second polynucleotide, respectively, is preceded by gap filling.

13. The method of any of claims 9-12, wherein the first splint and/or the second splint are DNA oligos, 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.

14. The method of any of claims 1-13, wherein the first photo-cleavable polymer and/or the second photo-cleavable polymer bind to the first and second polynucleotides, respectively, in a non-sequence-specific manner.

15. The method of any of claims 1-14, wherein the first photo-cleavable polymer and/or the second photo-cleavable polymer are UV degradable.

16. The method of any of claims 1-15, wherein the first photo-cleavable polymer and/or the second photo-cleavable polymer are synthetic, semi-synthetic, or naturally derived.

17. The method of any of claims 1-16, wherein the first photo-cleavable polymer and/or the second photo-cleavable polymer comprise a material selected from the group consisting of a PEG (polyethylene glycol), a PDMS (polydimethylsiloxane), a polyethylenimine (PEI), a polyacrylate, a lipid, a nanoparticle, a DNA, an RNA, a synthetic oligodeoxynucleotide (ODN), a xeno nucleic acid (XNA), a peptide nucleic acid (PNA), a locked nucleic acid (LNA), a 1,5-anhydrohexitol nucleic acid (HNA), a cyclohexene nucleic acid (CeNA), a threose nucleic acid (TNA), a glycol nucleic acid (GNA), a fluoro arabino nucleic acid (FANA), and a polypeptide.

18. The method of claim 17, wherein the polyacrylate and/or the lipid is cationic, optionally wherein the cationic lipid is Lipofectamine.

19. The method of any of claims 1-18, wherein the first photo-cleavable polymer and/or the second photo-cleavable polymer comprise $(dNTP)_6$-PC-$(dNTP)_6$-PC-$(dNTP)_6$-PC-$(dNTP)_6$, wherein PC is a photo-cleavable moiety.

20. The method of any of claims 1-19, wherein the first photo-cleavable polymer and/or the second photo-cleavable polymer comprise a DNA-binding protein.

21. The method of any of claims 1-20, wherein the first photo-cleavable polymer and/or the second photo-cleavable polymer comprise a polyethylenimine (PEI).

22. The method of any of claims 1-21, wherein the first photo-cleavable polymer and/or the second photo-cleavable polymer comprise a UV-degradable group.

23. The method of claim 22, wherein the UV-degradable group is in the backbone or at each subunit of the first photo-cleavable polymer and/or the second photo-cleavable polymer.

24. The method of claim 22 or 23, wherein the UV-degradable group is a nitrobenzyl group.

25. 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 are sequestered by a photo-cleavable polymer that inhibits or blocks hybridization and/or ligation to P1 and P2, respectively,

thereby cleaving the photo-cleavable polymer to release it from the substrate and allow hybridization and/or ligation to P1, whereas hybridization and/or ligation to P2 remain inhibited or blocked by the photo-cleavable polymer; and

(b1) attaching barcode 1A to P1 via hybridization and/or ligation to form a barcoded polynucleotide 1A-P1,

thereby providing on the substrate an array comprising polynucleotides 1A-P1 and P2.

26. The method of claim 25, further comprising:

(c1) irradiating P2 with light, thereby cleaving the photo-cleavable polymer to release it from the substrate and allow hybridization and/or ligation to P2; and

(d1) attaching barcode 1B to P2 via hybridization and/or ligation to form a barcoded polynucleotide 1B-P2,

thereby providing on the substrate an array comprising barcoded polynucleotides 1A-P1 and 1B-P2.

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 composition, comprising:

a first polynucleotide immobilized on a substrate;

a first splint hybridized to one end of the first polynucleotide, wherein the first splint comprises a sequence complementary to one end of a first barcode; and

a second polynucleotide immobilized on a substrate wherein the second polynucleotide is sequestered by a photo-cleavable polymer that inhibits or blocks hybridization and/or ligation to the second polynucleotide.