CLAIMS

1. A method for providing an array, comprising:

(a) irradiating a substrate comprising an unmasked first region and a masked second region, whereby a photoresist in the first region is degraded to render oligonucleotide molecules in the first region available for hybridization and/or ligation, whereas oligonucleotide molecules in the second region are protected by a photoresist in the second region from hybridization and/or ligation; and

(b) attaching an oligonucleotide comprising a barcode sequence to oligonucleotide molecules in the first region via hybridization and/or ligation, wherein oligonucleotide molecules in the second region do not receive the barcode sequence,

thereby providing on the substrate an array comprising different oligonucleotide molecules in the first and second regions.

2. The method of claim 1, wherein oligonucleotide molecules on the substrate comprise one or more common sequences, optionally wherein the one or more common sequences comprise a common primer sequence, optionally wherein the common sequence is between about 10 and about 35 nucleotides in length.

~~3. The method of claim 1 or 2, wherein oligonucleotide molecules in the first region and oligonucleotide molecules in the second region are identical in sequence.~~

~~4. The method of any of claims 1-3, wherein oligonucleotide molecules on the substrate prior to the irradiating step are identical in sequence.~~

~~5. The method of claim 1 or 2, wherein oligonucleotide molecules in the first region and oligonucleotide molecules in the second region are different in sequences, optionally wherein oligonucleotide molecules in the first region and oligonucleotide molecules in the second region comprise different barcode sequences.~~

~~6. The method of any of claims 1-3 and 5, wherein oligonucleotide molecules on the substrate comprise two or more different sequences.~~

~~7. The method of any of claims 1-6, wherein oligonucleotide molecules on the substrate are immobilized in a plurality of features.~~

~~8~~3. The method of claim ~~7~~1, wherein oligonucleotide molecules on the substrate are immobilized in a plurality of features, wherein the 3' terminal nucleotides of immobilized oligonucleotide molecules are distal to the substrate.

~~9. The method of claim 7, wherein the 5' terminal nucleotides of immobilized oligonucleotide molecules are distal to the substrate.~~

~~10~~4. The method of claim 1~~any of claims 1-9~~, wherein oligonucleotide molecules on the substrate prior to the irradiating step are between about 4 and about 50 nucleotides in length.

~~11~~5. The method of claim 1~~any of claims 1-10~~, wherein oligonucleotide molecules on the substrate comprise functional groups, optionally wherein the functional groups are amino or hydroxyl groups.

~~12~~6. The method of claim ~~11~~5, wherein the functional groups are not protected prior to the irradiating step, optionally wherein the functional groups are not protected by a photo-sensitive group, moiety, or molecule prior to the irradiating step.

~~13. The method of claim 11 or 12, wherein the functional groups are 3' hydroxyl groups of nucleotides.~~

~~14. The method of any of claims 1-13, further comprising a step of providing the substrate, wherein the first and second regions have the same photoresist.~~

7~~15~~. The method of claim ~~14~~1, further comprising a step of providing the substrate, wherein the first and second regions have the same photoresist, wherein the providing step comprises applying the photoresist to the substrate, thereby forming a photoresist layer on the substrate, optionally wherein the photoresist is applied via spin coating.

8~~16~~. The method of claim 7~~14 or 15~~, wherein oligonucleotide molecules on the substrate are embedded in the photoresist.

9~~17~~. The method of claim ~~14 or 15~~7, wherein oligonucleotide molecules on the substrate are embedded in an underlayer, and the photoresist forms a photoresist layer on top of the underlayer, optionally wherein the underlayer is a soluble polymer.

10~~18~~. The method of any of claims 1-9~~17~~, further comprising forming a pattern of oligonucleotide molecules on the substrate prior to applying the photoresist to the substrate~~.~~,

~~19. The method of claim 18,~~ wherein the forming step comprises:

irradiating a substrate comprising a plurality of functional groups and a photoresist through a patterned mask, whereby the photoresist in a first region of the substrate is degraded, rendering functional groups in the first region available for reacting with functional groups in functionalized oligonucleotide molecules, whereas functional groups in a second region of the substrate are protected by the photoresist from reacting with functional groups in the oligonucleotide molecules; and

contacting the substrate with the functionalized oligonucleotide molecules, wherein the functionalized oligonucleotide molecules are coupled to functional groups in the first region but not to functional groups in the second region,

thereby forming a pattern of oligonucleotide molecules on the substrate.

~~20. The method of claim 19, wherein the plurality of functional groups of the substrate are not protected prior to the irradiating step, optionally wherein the functional groups of the substrate are not protected by a photo-sensitive group, moiety, or molecule.~~

~~21. The method of claim 19 or 20, wherein the plurality of functional groups of the substrate are aldehyde groups or click chemistry groups, optionally wherein the click chemistry groups are capable of a nucleophilic addition reaction, a cyclopropane-tetrazine reaction, a strain-promoted azide-alkyne cycloaddition (SPAAC) reaction, an alkyne hydrothiolation reaction, an alkene hydrothiolation reaction, a strain-promoted alkyne-nitrone cycloaddition (SPANC) reaction, an inverse electron-demand Diels-Alder (IED-DA) reaction, a cyanobenzothiazole condensation reaction, an aldehyde/ketone condensation reaction, or a Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction.~~

~~22. The method of any of claims 19-21, wherein the functional groups in the functionalized oligonucleotide molecules are amino groups.~~

~~23. The method of claim 22, wherein the functionalized oligonucleotide molecules are 5' amine-terminated.~~

~~24. The method of any of claims 19-23, further comprising heating the substrate to dryness during or after the contacting step.~~

~~25. The method of any of claims 19-24, further comprising blocking unreacted functional groups of the substrate.~~

11~~26~~. The method of ~~any of claims 19-25~~claim 10, further comprising rendering the reaction between the plurality of functional groups of the substrate and the functionalized oligonucleotide molecules irreversible.

~~27. The method of any of claims 19-26, wherein aldehyde groups of the substrate are reacted with 5' amino groups of the functionalized oligonucleotide molecules, and the substrate is contacted with a reagent to block unreacted aldehyde groups and render the reaction irreversible, optionally wherein the reagent is sodium borohydride.~~

12~~28~~. The method of claim 10~~any of claims 19-27~~, wherein the irradiating and contacting steps are repeated in one or more cycles.

~~29. The method of claim 28, wherein the photoresist is not removed prior to, during, or between the one or more cycles, optionally wherein the method does not comprise re-applying a photoresist to the substrate prior to, during, or between the one or more cycles.~~

~~30. The method of claim 28, wherein the photoresist is removed in a cycle and re-applied in the next cycle, optionally wherein the removed photoresist and the re-applied photoresist are the same or different.~~

~~31. The method of any of claims 1-30, wherein the photoresist in the first and/or second regions comprises a photoacid generator.~~

~~32. The method of claim 31, wherein the photoresist further comprises an acid scavenger.~~

~~33. The method of claim 31 or 32, wherein the photoresist comprises a base quencher and/or a photosensitizer.~~

~~34. The method of any of claims 31-33, wherein the photoresist further comprises a surfactant and/or a casting solvent.~~

~~35. The method of any of claims 1-34, wherein the substrate is irradiated with a UV light.~~

13~~36~~. The method of any of claims 1-12~~35~~, wherein the substrate is irradiated through a patterned mask.

14~~37~~. The method of claim 13~~36~~, comprising removing the patterned mask after the irradiating step,

optionally wherein the same patterned mask is re-used in a subsequent cycle of the irradiating and contacting steps, wherein the patterned mask is moved relative to the substrate; or

optionally wherein a different patterned mask is used in a subsequent cycle of the irradiating and contacting steps.

15~~38~~. The method of any of claims 1-14~~37~~, wherein the photoresist in the first region of the substrate is dissolved by a developer and removed.

16~~39~~. The method of any of claims 1-15~~38~~, wherein the barcode sequence is between about 4 and about 25 nucleotides in length.

~~40. The method of any of claims 1-39, wherein the oligonucleotide comprising the barcode sequence is between about 10 and about 50 nucleotides in length.~~

17~~41~~. The method of any of claims 1-16~~40~~, wherein the oligonucleotide comprising the barcode sequence is hybridized to an oligonucleotide molecule in the first region.

1842. The method of any of claims 1-1740, wherein the oligonucleotide comprising the barcode sequence is ligated to an oligonucleotide molecule in the first region.

1943. The method of any of claims 1-1840, wherein the oligonucleotide comprising the barcode sequence is hybridized to a splint which is in turn hybridized to an oligonucleotide molecule in the first region.

2044. The method of claim 1943, further comprising ligating the oligonucleotide comprising the barcode sequence to the oligonucleotide molecule to generate a barcoded oligonucleotide molecule in the first region.

~~45. The method of claim 44, wherein the oligonucleotide and the oligonucleotide molecule are ligated using the splint as template, without or without gap filling prior to the ligation.~~

~~46. The method of claim 44 or 45, further comprising removing the splint after the ligation.~~

~~47. The method of any of claims 44-46, further comprising blocking the 3' or 5' termini of barcoded oligonucleotide molecules and/or unligated oligonucleotide molecules in the first region from ligation.~~

~~48. The method of claim 47, wherein the blocking comprises adding a 3' dideoxy, a non-ligating 3' phosphoramidate, or a triphenylmethyl (trityl) group to the barcoded oligonucleotide molecules and/or unligated oligonucleotide molecules, optionally wherein the blocking by the trityl group is removed with a mild acid after ligation is completed.~~

~~49. The method of claim 48, wherein the addition is catalyzed by a terminal transferase, optionally wherein the terminal transferase is a terminal deoxynucleotidyl transferase (TdT).~~

~~50. The method of any of claims 44-49, wherein the blocking is removed using an internal digestion of the barcoded oligonucleotide molecules after ligation is completed.~~

2151. The method of any of claims 1-2050, wherein the method comprises ***N*** cycles, ***N*** is an integer of 2 or greater, and one of the ***N*** cycles comprises the irradiating and the attaching steps.

2252. The method of claim 2151, wherein the barcode sequences received by oligonucleotide molecules in feature(s) on the substrate in cycle ***I*** and in feature(s) on in cycle ***J*** are different, wherein ***I*** and ***J*** are integers and $1 \leq I < J \leq N$.

2353. The method of claim ~~51~~ 21 or 2252, wherein the photoresist is not removed prior to, during, or between one or more of the ***N*** cycles.

~~54. The method of any of claims 51-53, wherein the method does not comprise re-applying a photoresist to the substrate prior to, during, or between one or more of the *N* cycles.~~

2455. The method of any of claims 21-2351-54, wherein the method comprises ***M*** rounds, ***M*** is an integer of 2 or greater, and each of the ***M*** rounds comprises one or more cycles.

~~56. The method of claim 55, comprising removing photoresist from the substrate after each round or after one or more or all of a plurality of rounds and re-applying photoresist to the substrate prior to a new round.~~

~~57. The method of claim 55 or 56, wherein each of the *M* rounds comprises *N* cycles, wherein *N* is 3 or greater.~~

2558. The method of claim 2457, wherein oligonucleotide molecules in a feature of the substrate receive a first barcode sequence in one of the cycles in round ***K***, wherein ***K*** is an integer and $1 \leq K < M$, and oligonucleotide molecules in the feature comprising the first barcode sequence receive a second barcode sequence in one of the cycles in round (K+1), thereby forming oligonucleotide molecules comprising the first and second barcode sequences.

2659. The method of claim 2558, wherein the diversity of barcode sequences in the oligonucleotides in a plurality of features on the substrate is N^M.

2760. The method of claim 2558 or 2659, wherein the feature(s) is/are no more than 0.5 micron, no more than 1 micron, no more than 5 microns, no more than 10 microns, or no more than 15 microns, no more than 20 microns, no more than 25 microns, no more than 30 microns, or no more than 35 microns, no more than 40 microns, no more than 45 microns, or no more than 50 microns in diameter.

2861. A method for providing an array, comprising:

(a) irradiating a substrate comprising an unmasked first region and a masked second region, whereby a photoresist in the first region is degraded to render oligonucleotide molecules in the first region available for hybridization and/or ligation, whereas oligonucleotide molecules in the second region are protected by ~~the~~ a photoresist in the second region from hybridization and/or ligation; and

(b) contacting oligonucleotide molecules in the first region with a first splint and a first oligonucleotide comprising a first barcode sequence, wherein the first splint hybridizes to the first oligonucleotide and the oligonucleotide molecules in the first region,

wherein the first oligonucleotide is ligated to the oligonucleotide molecules in the first region, and the first oligonucleotide is not ligated to oligonucleotide molecules in the second region,

thereby providing on the substrate an array comprising different oligonucleotide molecules in the first and second regions.

~~62. The method of claim 61, wherein the hybridization region between the first splint and the oligonucleotide molecules is at least 3, 4, 5, 6, 7, 8, 9, 10 bp or more than 10 bp.~~

~~63. The method of claim 61 or 62, wherein the hybridization region between the first splint and the first oligonucleotide is at least 3, 4, 5, 6, 7, 8, 9, 10 bp or more than 10 bp.~~

~~64. The method of any of claims 61-63, wherein hybridization to the first splint brings the terminal nucleotides of the first oligonucleotide and the oligonucleotide molecules immediately next to each other, and the ligation does not require gap-filling.~~

~~65. The method of any of claims 61-63, wherein hybridization to the first splint brings the terminal nucleotides of the first oligonucleotide and the oligonucleotide molecules in proximity to each other and separated by one or more nucleotides, and the ligation is preceded by gap-filling.~~

~~66. The method of any of claims 61-65, wherein the photoresist is a first photoresist, and the first oligonucleotide is ligated to the oligonucleotide molecules in the first region to generate first extended oligonucleotide molecules, and the method further comprises:~~

~~(c) applying a second photoresist to the substrate, optionally wherein the second photoresist is applied after the first photoresist is removed from the substrate;~~

~~(d) irradiating the substrate while the first region is masked and the second region is unmasked, whereby the first or second photoresist in the second region is degraded to render oligonucleotide molecules in the second region available for hybridization and/or ligation, whereas the first extended oligonucleotide molecules in the first region are protected by the second photoresist in the first region from hybridization and/or ligation; and~~

~~(e) contacting oligonucleotide molecules in the second region with a second splint and a second oligonucleotide comprising a second barcode sequence, wherein the second splint hybridizes to the second oligonucleotide and the oligonucleotide molecules in the second region,~~

~~wherein the second oligonucleotide is ligated to the oligonucleotide molecules in the second region to generate second extended oligonucleotide molecules, and the second oligonucleotide is not ligated to the first extended oligonucleotide molecules in the first region.~~

67. The method of claim 66, wherein steps (a)-(b) are part of a first cycle, steps (d)-(e) are part of a second cycle, and steps (a)-(e) are part of a first round, and wherein the method comprises one or more additional rounds.

68. The method of claim 66 or 67, wherein steps (a)-(e) are part of a first round, the first and second oligonucleotides are Round 1 oligonucleotides, the first and second barcode sequences are Round 1 barcode sequences, and the method further comprises:

(a') irradiating the substrate while the first region is unmasked and the second region is masked, whereby a photoresist in the first region is degraded to render the first extended oligonucleotide molecules in the first region available for hybridization and/or ligation, whereas the second extended oligonucleotide molecules in the second region are protected by the photoresist in the second region from hybridization and/or ligation; and

(b') attaching a first Round 2 oligonucleotide comprising a first Round 2 barcode sequence to the first extended oligonucleotide molecules in the first region via hybridization and/or ligation, wherein the second extended oligonucleotide molecules in the second region do not receive the first Round 2 barcode sequence.

69. The method of claim 68, wherein the photoresist is a first photoresist, and the first Round 2 oligonucleotide is ligated to the first extended oligonucleotide molecules in the first region to generate first further extended oligonucleotide molecules, and the method further comprises:

(c') applying a second photoresist to the substrate, optionally wherein the second photoresist is applied after the first photoresist is removed from the substrate;

(d') irradiating the substrate while the first region is masked and the second region is unmasked, whereby the first or second photoresist in the second region is degraded to render the second extended oligonucleotide molecules in the second region available for hybridization and/or ligation, whereas the first further extended oligonucleotide molecules in the first region are protected by the second photoresist in the first region from hybridization and/or ligation; and

(e') attaching a second Round 2 oligonucleotide comprising a second Round 2 barcode sequence to the second extended oligonucleotide molecules in the second region via hybridization and/or ligation, wherein the first further extended oligonucleotide molecules in the first region do not receive the second Round 2 barcode sequence.

70. The method of claim 68 or 69, wherein the Round 1 barcode sequences are different from each other.

~~71. The method of claim any of claims 68-70, wherein the Round 1 barcode sequences are different from the Round 2 barcode sequences.~~

29~~72~~. A composition, comprising:

(i) a substrate comprising a first region and a second region,

(ii) hybridization complexes in the first region, wherein at least one of the hybridization complexes comprise an oligonucleotide molecule immobilized in the first region hybridized to a first splint, which comprises a sequence complementary ~~is in turn hybridized~~ to a first oligonucleotide comprising a first barcode sequence, and

(iii) oligonucleotide molecules immobilized in the second region and protected by a photoresist from hybridization and/or ligation.

~~73. The composition of claim 72, further comprising the photoresist.~~

~~74. The composition of claim 72 or 73, wherein the photoresist forms a photoresist layer and oligonucleotide molecules immobilized in the second region are embedded in the photoresist layer.~~

~~75. The composition of any of claims 72-74, further comprising a ligase capable of ligating the first oligonucleotide and the oligonucleotide molecule immobilized in the first region using the first splint as template, and optionally a polymerase capable of gap filling using the first splint as template prior to the ligation.~~

~~76. The composition of any of claims 72-75, wherein the oligonucleotide molecules on the substrate comprise functional groups, optionally wherein the functional groups are amino or hydroxyl groups.~~

~~77. The composition of claim 76, wherein the functional groups are not protected by a chemical group, optionally wherein the functional groups are not protected by a photo-sensitive group.~~

~~78. The composition of claim 76 or 77, wherein the functional groups are 3' hydroxyl groups of nucleotides.~~

30~~79~~. A composition, comprising:

(i) a substrate comprising a first region and a second region,

(ii) hybridization complexes in the first region, wherein at least one of the hybridization complexes comprise an oligonucleotide molecule immobilized in the first region hybridized to a first splint, which comprises a sequence complementary ~~is in turn hybridized~~ to a first

oligonucleotide comprising a first barcode sequence, wherein the hybridization complexes are protected by a first photoresist from hybridization and/or ligation, and

(iii) oligonucleotide molecules immobilized in the second region and protected by a second photoresist from hybridization and/or ligation.

~~80. The method of claim 79, wherein the first photoresist and the second photoresist are the same.~~

~~81. The method of claim 79, wherein the first photoresist and the second photoresist are different.~~

~~82. The composition of any of claims 79-81, wherein the oligonucleotide molecules on the substrate comprise functional groups, optionally wherein the functional groups are amino or hydroxyl groups.~~

~~83. The composition of claim 82, wherein the functional groups are not protected by a chemical group, optionally wherein the functional groups are not protected by a photo-sensitive group.~~

~~84. The composition of claim 82 or 83, wherein the functional groups are 3' hydroxyl groups of nucleotides.~~

~~85. A composition, comprising a substrate comprising a plurality of universal oligonucleotide molecules immobilized thereon, wherein the universal oligonucleotide molecules in a first region of the substrate are available for hybridization and/or ligation, and the universal oligonucleotide molecules in a second region of the substrate are embedded in a photoresist and protected from hybridization and/or ligation.~~

~~86. The composition of claim 85, further comprising a photomask masking the second region while exposing the first region to light.~~

~~87. The composition of claim 85 or 86, further comprising hybridization complexes in the first region, wherein at least one of the hybridization complexes comprise a universal oligonucleotide molecule immobilized in the first region hybridized to a first splint, which is in turn hybridized to a first oligonucleotide comprising a first barcode sequence.~~

~~88. The composition of any of claims 85-87, wherein the universal oligonucleotide molecules on the substrate comprise functional groups, optionally wherein the functional groups are amino or hydroxyl groups.~~

~~89. The composition of claim 88, wherein the functional groups are not protected by a chemical group, optionally wherein the functional groups are not protected by a photo-sensitive group.~~

~~90. The composition of claim 88 or 89, wherein the functional groups are 3' hydroxyl groups of nucleotides.~~