METHODS FOR TAGGING DNA-ENCODED LIBRARIES

Cross-reference to Related Applications

This application claims benefit of U.S. Provisional Application Nos. 61/531,820, filed September 7, 2011, and 61/536,929, filed September 20, 2011, each of which is hereby incorporated by reference.

Background of the Invention

In general, this invention relates to DNA-encoded libraries of compounds and methods of using and creating such libraries. The invention also relates to compositions for use in such libraries.

DNA-encoded combinatorial libraries afford many benefits for drug discovery. These libraries can provide a large number of diverse compounds that can be rapidly screened and interrogated. To further increase complexity, various steps of the discovery process can be programmed and automated. These steps include the use of multi-step, split-and-pool synthesis to add building blocks to atomic or polyatomic scaffolds and the use of enzymatic and/or chemical ligation to add DNA tags that encode both the synthetic steps and the building blocks.

Despite these benefits, numerous issues can arise when very large or complex libraries must be synthesized and deconvoluted. As the size of the library increases, improved methods may be needed to provide high yields of tag ligation. To create libraries under diverse reaction conditions, stable ligated nucleotide constructs would be beneficial, such as constructs that are stable under conditions of high pH and elevated temperature. To simplify deconvolution of tags, the sequence of the tags could be recognized by DNA- or RNA-dependent polymerases, such that tag population demographics can be determined by template-dependent polymerization and sequence determination. Difficulties may arise when creating a library having all of these beneficial attributes. Accordingly, there exists a need for improved, more robust methods of screening and identifying small compounds in DNA-encoded libraries.

Summary ~~of the Invention~~

The present disclosure~~invention~~ features methods of creating libraries, where the method includes one or more conditions that improve single-stranded ligation of tags, and compositions for use in creating libraries. Exemplary conditions include the use of one or more 2'-substituted bases within the tags, such as 2'-O-methyl or 2'-fluoro; the use of tags of particular length; the use of one or more enzymes; optionally, the inclusion of error-recognition capabilities in the tag design; and/or the use of one or more agents during ligation.

Accordingly, the ~~invention~~ disclosure features a method of tagging a first library including an oligonucleotide-encoded chemical entity, the method including: (i) providing a headpiece having a first functional group and a second functional group, where the headpiece includes at least one 2'-substituted nucleotide; (ii) binding the first functional group of the headpiece to a first component of the chemical

entity, where the headpiece is directly connected to the first component or the headpiece is indirectly connected to the first component by a bifunctional linker (e.g., a poly ethylene glycol linker or $-(CH_2CH_2O)_nCH_2CH_2-$, where n is an integer from 1 to 50); and (iii) binding the second functional group of the headpiece to a first building block tag to form a complex, where steps (ii) and (iii) can be performed in any order and where the first building block tag encodes for the binding reaction of step (ii), thereby providing a tagged library.

In one aspect, there is provided a method of tagging a first library comprising an oligonucleotide-encoded chemical entity, said method comprising:

(i) providing a headpiece having a first functional group and a second functional group, wherein said headpiece comprises at least one 2'-substituted nucleotide;

(ii) binding said first functional group of said headpiece to a first component of said chemical entity, wherein said headpiece is directly connected to said first component or said headpiece is indirectly connected to said first component by a bifunctional linker; and

(iii) binding said second functional group of said headpiece to a first building block tag to form a complex, wherein said steps (ii) and (iii) can be performed in any order and wherein said first building block tag encodes for the binding reaction of said step (ii), thereby providing a tagged library.

In some embodiments, the headpiece includes a 2'-substituted nucleotide at one or more of the 5'-terminus, the 3'-terminus, or the internal position of the headpiece. In particular embodiments, the headpiece includes the 2'-substituted nucleotide and the second functional group at the 5'-terminus or at the 3'-terminus.

In other embodiments, the first building block tag includes at least one (e.g., at least two, three, four, five, or more) 2'-substituted nucleotides. In particular embodiments, the first building block tag includes a 2'-substituted nucleotide at one or more of the 5'-terminus, the 3'-terminus, or the internal position of the first building block tag (e.g., a 2'-O-methyl nucleotide or a 2'-fluoro nucleotide at both of the 5'- and 3'-termini). In some embodiments, the first building block tag includes a protecting group at the 3'-terminus or at the 5'-terminus.

In any of the embodiments described herein, the 2'-substituted nucleotide is a 2'-O-methyl nucleotide (e.g., 2'-O-methyl guanine or 2'-O-methyl uracil) or a 2'-fluoro nucleotide (e.g., 2'-fluoro guanine, or 2'-fluoro uracil).

In any of the above embodiments, step (ii) may include joining, binding, or operatively associating the headpiece directly to the first component (e.g., a scaffold or a first building block). In yet other embodiments, step (ii) includes binding the headpiece indirectly to the first component (e.g., a scaffold or a first building block) via a bifunctional linker (e.g., the method includes binding the headpiece with the first functional group of the linker and binding the first component with the second functional group of the linker).

In any of the above embodiments, the method may further include (iv) binding a second building block tag to the 5'-terminus or 3'-terminus of the complex; and (v) binding a second component (e.g., a first building block or a second building block) of the chemical library to the first component, where steps (iv) and (v) can be performed in any order. In some embodiments, the second building block tag encodes for the binding reaction of step (v). In other embodiments, step (iv) may include binding the second building block tag to the 5'-terminus of the complex; the complex includes a phosphate group at the 5'-terminus; and the second building block tag includes a hydroxyl group at both of the 3'- and 5'-termini. In other embodiments, step (iv) may further include purifying the complex and reacting the complex with a polynucleotide kinase to form a phosphate group on the 5'-terminus prior to binding the second building block tag. In other embodiments, step (iv) may include binding the second building block tag to the 3'-terminus of the complex; the complex includes a protecting group at the 3'-terminus; and the second building block tag includes a phosphate group at the 5'-terminus and a protecting group at the 3'-

from 1.5 mM to 5.0 mM, from 1.5 mM to 6.0 mM, from 1.5 mM to 7.0 mM, from 1.5 mM to 8.0 mM, from 1.5 mM to 9.0 mM, from 1.5 mM to 10.0 mM, from 1.5 mM to 10.5 mM, from 2.0 mM to 3.0 mM, from 2.0 mM to 4.0 mM, from 2.0 mM to 5.0 mM, from 2.0 mM to 6.0 mM, from 2.0 mM to 7.0 mM, from 2.0 mM to 8.0 mM, from 2.0 mM to 9.0 mM, from 2.0 mM to 10.0 mM, and from 2.0 mM to 10.5 mM). In some embodiments, one or more multivalent cations are in an amount of about 1 mM (e.g., from 0.5 mM to 1.5 mM). In a particular embodiment, the multivalent cation is in the form of hexamine cobalt (III) chloride.

In other embodiments, the method further includes separating the complex from any unreacted tag or unreacted headpiece before any one of binding steps (ii)-(v). In other embodiments, the method further includes purifying the complex before any one of binding steps (ii)-(v). In other embodiments, the method further includes binding one or more additional components (e.g., a scaffold or a first building block) and one or more additional building block tags, in any order and after any one of binding step (ii)-(v).

The ~~invention~~ disclsoure also features a method of tagging a first library including an oligonucleotide-encoded chemical entity, the method including: (i) providing a headpiece having a first functional group and a second functional group, where the headpiece includes a 2'-substituted nucleotide at the 5'-terminus, optionally one or more nucleotides at the internal position of the headpiece, and a protecting group at the 2'-position and/or the 3'-position at the 3'-terminus; (ii) binding the first functional group of the headpiece to a first component of the chemical entity, where the headpiece is directly connected to the first component or the headpiece is indirectly connected to the first component by a bifunctional linker; and (iii) binding the second functional group of the headpiece to a first building block tag, where the first building block tag includes a 2'-substituted nucleotide and a hydroxyl group at the 5'-terminus, optionally one or more nucleotides at the internal position of the tag, and a 2'-substituted nucleotide and a hydroxyl group at the 3'-terminus; where steps (ii) and (iii) can be performed in any order and where the first building block tag encodes for the binding reaction of step (ii), thereby providing a tagged library.

In some embodiments, the 2'-substituted nucleotide is a 2'-O-methyl nucleotide (e.g., 2'-O-methyl guanine) or a 2'-fluoro nucleotide (e.g., 2'-fluoro guanine). In other embodiments, one or more nucleotides at the internal position of the headpiece are 2'-deoxynucleotides. In yet other embodiments, the bifunctional linker is a poly ethylene glycol linker (e.g., $-(CH_2CH_2O)_n CH_2CH_2-$, where n is an integer from 1 to 50).

In other embodiments, one or more nucleotides (e.g., one or more 2'-deoxynucleotides) are present at the internal position of the headpiece or the tag.

In some embodiments, step (iii) may include the use of one or more soluble multivalent cations (e.g., magnesium chloride, manganese (II) chloride, or hexamine cobalt (III) chloride), poly ethylene glycol (e.g., having an average molecular weight of about 4,600 Daltons), and RNA ligase (e.g., T4 RNA ligase).

In another aspect, the ~~invention~~ disclosure features methods to identify and/or discover a chemical entity, the method including tagging a first library including an oligonucleotide-encoded chemical entity (e.g., including steps (i) to (iii) and optionally including steps (iv) to (v)) and selecting for a particular characteristic or function (e.g., selecting for binding to a protein target including exposing the oligonucleotide-encoded chemical entity or chemical entity to the protein target and selecting the one or more oligonucleotide-encoded chemical entities or chemical entities that bind to the protein target (e.g., by using size exclusion chromatography)).

The ~~invention~~ disclosure also features a complex including a headpiece and a building block tag, where the tag includes from 5 to 20 nucleotides, a 2'-substituted nucleotide at the 5'-terminus, and a 2'-substituted nucleotide at the 3'-terminus.

In one aspect, there is provided a complex comprising a headpiece and a building block tag, wherein said tag comprises from 5 to 20 nucleotides, a 2'-substituted nucleotide at the 5'-terminus, and a 2'-substituted nucleotide at the 3'-terminus.

In some embodiments, the 2'-substituted nucleotide at the 5'-terminus and/or 3'-terminus is a 2'-O-methyl nucleotide (e.g., 2'-O-methyl guanine or 2'-O-methyl uracil) or a 2'-fluoro nucleotide (e.g., 2'-fluoro guanine or 2'-fluoro uracil). In particular embodiments, the headpiece includes a hairpin structure. In some embodiments, the headpiece includes a 2'-substituted nucleotide at one or more of the 5'-terminus, the 3'-terminus, or the internal position of the headpiece. In other embodiments, the headpiece further includes a preadenylated 5'-terminus. In yet other embodiments, the headpiece includes from 5 to 20 nucleotides.

In any of the above embodiments, the headpiece, the first building block tag, the second building block tag, or the one or more additional building block tags, if present, includes a preadenylated 5'-terminus.

In any of the above embodiments, the method further includes binding one or more (e.g., one, two, three, four, five, six, seven, eight, nine, or ten) additional building block tags to the complex and binding one or more (e.g., one, two, three, four, five, six, seven, eight, nine, or ten) additional components (e.g., scaffolds or building blocks) to the complex, where the one or more additional building block tag encodes for the one or more additional components or encodes for the binding reaction of one or more additional components, thereby providing a tagged library.

In any of the above embodiments, the 2'-substituted nucleotide is a 2'-O-methyl nucleotide, such as 2'-O-methyl guanine, 2'-O-methyl uracil, 2'-O-methyl adenosine, 2'-O-methyl thymidine, 2'-O-methyl inosine, 2'-O-methyl cytidine, or 2'-O-methyl diamino purine. Alternatively, in any of the above embodiments, the 2'-substituted nucleotide is a 2'-fluoro nucleotide, such as 2'-fluoro guanine, 2'-fluoro uracil, 2'-fluoro adenosine, 2'-fluoro thymidine, 2'-fluoro inosine, 2'-fluoro cytidine, or 2'-fluoro diamino purine.

In any of the above embodiments, the RNA ligase is T4 RNA ligase and/or the DNA ligase is a ssDNA ligase.

In any of the above embodiments, the method includes a plurality of headpieces. In some embodiments of this method, each headpiece of the plurality of headpieces includes an identical sequence region and a different encoding region. In particular embodiments, the identical sequence region is a primer binding region. In other embodiments, the different encoding region is an initial building block tag that encodes for the headpiece or for an addition of an initial component.

CLAIMS

1. A method of tagging a first library comprising an oligonucleotide-encoded chemical entity, said method comprising:

(i) providing a headpiece having a first functional group and a second functional group, wherein said headpiece comprises at least one 2'-substituted nucleotide;

(ii) binding said first functional group of said headpiece to a first component of said chemical entity, wherein said headpiece is directly connected to said first component or said headpiece is indirectly connected to said first component by a bifunctional linker; and

(iii) binding said second functional group of said headpiece to a first building block tag to form a complex,

wherein said steps (ii) and (iii) can be performed in any order and wherein said first building block tag encodes for the binding reaction of said step (ii), thereby providing a tagged library.

2. The method of claim 1, wherein said first building block tag comprises at least one 2'-substituted nucleotide, such as a 2'-O-methyl nucleotide or a 2'-fluoro nucleotide.

3. The method of claim 1 or 2, wherein said 2'-substitued nucleotide is 2'-O-methyl guanine, 2'-O-methyl uracil, 2'-O-methyl adenosine, 2'-O-methyl thymidine, 2'-O-methyl inosine, 2'-O-methyl cytidine, 2'-O-methyl diamino purine, 2'-fluoro guanine, 2'-fluoro uracil, 2'-fluoro adenosine, 2'-fluoro thymidine, 2'-fluoro inosine, 2'-fluoro cytidine, or 2'-fluoro diamino purine.

4. The method of any one of claims 1-3, wherein step (ii) comprises binding said headpiece directly to said first component.

5. The method of any one of claims 1-3, wherein step (ii) comprises binding said headpiece indirectly to said first component via a bifunctional linker.

6. The method of any one of claims 1-5, further comprising

(iv) binding a second building block tag to the 5'-terminus or 3'-terminus of said complex; and

(v) binding a second component of said chemical library to said first component, wherein said steps (iv) and (v) can be performed in any order.

7. The method of claim 6, wherein said second building block tag comprises a 2'-substituted nucleotide at one or more of the 5'-terminus, the 3'-terminus, or the internal position of said second building block tag.

8. The method of any one of claims 1-7, wherein said step (iii) and/or step (iv), if present, comprises an RNA ligase and/or a DNA ligase to bind said second building block tag to said complex and/or comprises poly ethylene glycol and/or one or more soluble multivalent cations.

9. The method of any one of claims 1-8, wherein said method further comprises separating said complex from any unreacted tag or unreacted headpiece before any one of binding steps (ii)-(v) and/or purifying said complex before any one of binding steps (ii)-(v).

10. The method of any one of claims 1-9, wherein said method further comprises binding one or more additional building block tags to said complex and binding one or more additional components to said complex.

11. The method of any one of claims 1-10, wherein said headpiece comprises a hairpin structure.

12. The method of any one of claims 1-11, wherein said headpiece, said first building block tag, said second building block tag, and/or said one or more additional building block tags, if present, further comprises a first library-identifying sequence, a use sequence, and/or an origin sequence.

13. The method of any one of claims 1-12, wherein said method further comprises binding a first library-identifying tag, a use sequence, an origin sequence, and/or a tailpiece to said complex.

14. The method of any one of claims 1-13, wherein said method comprises a plurality of headpieces.

15. A complex comprising a headpiece and a building block tag, wherein said tag comprises from 5 to 20 nucleotides, a 2'-substituted nucleotide at the 5'-terminus, and a 2'-substituted nucleotide at the 3'-terminus.

Claims

1. A method of tagging a first library comprising an oligonucleotide-encoded chemical entity, said method comprising:

(i) providing a headpiece having a first functional group and a second functional group, wherein said headpiece comprises at least one 2'-substituted nucleotide;

(ii) binding said first functional group of said headpiece to a first component of said chemical entity, wherein said headpiece is directly connected to said first component or said headpiece is indirectly connected to said first component by a bifunctional linker; and

(iii) binding said second functional group of said headpiece to a first building block tag to form a complex,

wherein said steps (ii) and (iii) can be performed in any order and wherein said first building block tag encodes for the binding reaction of said step (ii), thereby providing a tagged library.

2. The method of claim 1, wherein said headpiece comprises a 2'-substituted nucleotide at one or more of the 5'-terminus, the 3'-terminus, or the internal position of said headpiece.

3. The method of claim 2, wherein said headpiece comprises said 2'-substituted nucleotide and said second functional group at the 5'-terminus or at the 3'-terminus.

4. The method of any one of claims 1-3, wherein said first building block tag comprises at least one 2'-substituted nucleotide.

5. The method of claim 4, wherein said first building block tag comprises a 2'-substituted nucleotide at one or more of the 5'-terminus, the 3'-terminus, or the internal position of said first building block tag.

6. The method of claim 5, wherein said first building block tag comprises said 2'-substituted nucleotide at both of the 5'- and 3'-termini.

7. The method of any one of claims 1-6, wherein said 2'-O-substituted nucleotide is a 2'-O-methyl nucleotide or a 2'-fluoro nucleotide.

8. The method of claim 7, wherein said 2'-O-substituted nucleotide is said 2'-O-methyl nucleotide selected from 2'-O-methyl guanine or 2'-O-methyl uracil.

9. The method of claim 7, wherein 2'-O-substituted nucleotide is said 2'-fluoro nucleotide selected from 2'-fluoro guanine or 2'-fluoro uracil.

10. The method of any one of claims 1-9, wherein said first building block tag comprises a protecting group at the 3'-terminus or at the 5'-terminus.

11. The method of any one of claims 1-10, wherein step (ii) comprises binding said headpiece directly to said first component.

12. The method of claim 11, wherein said component is a scaffold or a first building block.

13. The method of any one of claims 1-10, wherein step (ii) comprises binding said headpiece indirectly to said first component via a bifunctional linker.

14. The method of claim 13, wherein said method comprises binding said headpiece with the first functional group of said linker and binding said first component with the second functional group of said linker.

15. The method of claim 14, wherein said first component is a scaffold or a first building block.

16. The method of any one of claims 1-15, further comprising

(iv) binding a second building block tag to the 5'-terminus or 3'-terminus of said complex; and

(v) binding a second component of said chemical library to said first component, wherein said steps (iv) and (v) can be performed in any order.

17. The method of claim 16, wherein step (iv) comprises binding said second building block tag to the 5'-terminus of said complex; said complex comprises a phosphate group at the 5'-terminus; and said second building block tag comprises a hydroxyl group at both of the 3'- and 5'-termini.

18. The method of claim 17, wherein step (iv) further comprises purifying said complex and reacting said complex with a polynucleotide kinase to form a phosphate group on the 5'-terminus prior to binding said second building block tag.

19. The method of claim 16, wherein step (iv) comprises binding said second building block tag to the 3'-terminus of said complex; said complex comprises a protecting group at the 3'-terminus; and said second building block tag comprises a phosphate group at the 5'-terminus and a protecting group at the 3'-terminus.

20. The method of claim 19, wherein step (iv) further comprises reacting said complex with a hydrolyzing agent to release said protecting group from said complex prior to binding said second building block tag to said complex.

21. The method of any one of claims 16-20, wherein said second building block tag comprises a 2'-substituted nucleotide at one or more of the 5'-terminus, the 3'-terminus, or the internal position of said second building block tag.

22. The method of any one of claims 16-21, wherein said second component is a first building block or a second building block.

23. The method of any one of claims 16-22, wherein said step (iv) comprises an RNA ligase and/or a DNA ligase to bind said second building block tag to said complex.

24. The method of claim 23, wherein said step (iv) comprises said RNA ligase and said RNA ligase is T4 RNA ligase.

25. The method of claim 23, wherein said step (iv) comprises said DNA ligase and said DNA ligase is ssDNA ligase

26. The method of claim 23, wherein said step (iv) comprises said RNA ligase and said DNA ligase.

27. The method of any one of claims 1-26, wherein said step (iii) comprises an RNA ligase and/or a DNA ligase to bind said headpiece to said first building block tag.

28. The method of claim 27, wherein said step (iii) comprises said RNA ligase and said RNA ligase is T4 RNA ligase.

29. The method of claim 27, wherein said step (iii) comprises said DNA ligase and said DNA ligase is ssDNA ligase.

30. The method of claim 27, wherein said step (iii) comprises said RNA ligase and said DNA ligase.

31. The method of any one of claims 1-30, wherein said step (iii) and/or said step (iv), if present, comprises poly ethylene glycol and/or one or more soluble multivalent cations.

32. The method of claim 31, wherein said step (iii) and/or said step (iv), if present, comprises said poly ethylene in an amount from about 25% (w/v) to about 35% (w/v).

33. The method of claim 32, wherein said poly ethylene glycol has an average molecular weight from about 3,000 to about 5,500 Daltons.

34. The method of claim 33, wherein said poly ethylene glycol has an average molecular weight of about 4,600 Daltons.

35. The method of any one of claims 31-34, wherein said step (iii) and/or said step (iv), if present, comprises said one or more soluble multivalent cations in an amount of from about 0.05 mM to about 10.5 mM.

36. The method of claim 35, wherein said one or more multivalent cations are selected from the group consisting of magnesium chloride, manganese (II) chloride, and hexamine cobalt (III) chloride.

37. The method of claim 35 or 36, wherein said one or more multivalent cations are in an amount of from about 0.5 mM to about 1.5 mM.

38. The method of any one of claims 1-37, wherein said method further comprises separating said complex from any unreacted tag or unreacted headpiece before any one of binding steps (ii)-(v).

39. The method of any one of claims 1-38, wherein said method further comprises purifying said complex before any one of binding steps (ii)-(v).

40. The method of any one of claims 1-39, wherein said method further comprises binding one or more additional building block tags to said complex and binding one or more additional components to said complex.

41. The method of any one of claims 1-40, wherein said headpiece, said first building block tag, said second building block tag, or said one or more additional building block tags, if present, comprises a preadenylated 5'-terminus.

42. The method of any one of claims 1-41, wherein said headpiece comprises a hairpin structure.

43. The method of any one of claims 1-42, wherein said headpiece, said first building block tag, said second building block tag, and/or said one or more additional building block tags, if present, comprises from 5 to 20 nucleotides.

44. The method of claim 43, wherein said first building block tag, said second building block tag, and/or said one or more additional building block tags, if present, has about the same mass.

45. The method of any one of claims 1-44, wherein said headpiece, said first building block tag, said second building block tag, and/or said one or more additional building block tags, if present, further comprises a first library-identifying sequence.

46. The method of any one of claims 1-44, wherein said method further comprises binding a first library-identifying tag to said complex.

47. The method of claim 45 or 46, further comprising providing a second library and combining said first library with said second library.

48. The method of any one of claims 1-47, wherein said headpiece, said first building block tag, said second building block tag, and/or said one or more additional building block tags, if present, further comprises a use sequence and/or an origin sequence.

49. The method of any one of claims 1-47, wherein said method further comprises binding a use tag and/or an origin tag to said complex.

50. The method of any one of claims 1-49, wherein said method further comprises binding a tailpiece to said complex.

51. A method of tagging a first library comprising an oligonucleotide-encoded chemical entity, said method comprising:

(i) providing a headpiece having a first functional group and a second functional group, wherein said headpiece comprises a 2'-substituted nucleotide at the 5'-terminus, optionally one or more nucleotides at the internal position of said headpiece, and a protecting group at the 2'-position and/or the 3'-position at the 3'-terminus;

(ii) binding said first functional group of said headpiece to a first component of said chemical entity, wherein said headpiece is directly connected to said first component or said headpiece is indirectly connected to said first component by a bifunctional linker; and

(iii) binding said second functional group of said headpiece to a first building block tag, wherein said first building block tag comprises a 2'-substituted nucleotide and a hydroxyl group at the 5'-terminus, optionally one or more nucleotides at the internal position of said tag, and a 2'-substituted nucleotide and a hydroxyl group at the 3'-terminus;

wherein said steps (ii) and (iii) can be performed in any order and wherein said first building block tag encodes for the binding reaction of said step (ii), thereby providing a tagged library.

52. The method of claim 51, wherein said 2'-substituted nucleotide is a 2'-O-methyl nucleotide of 2'-O-methyl guanine.

53. The method of claim 51 or claim 52, wherein said one or more nucleotides are present at the internal position of said headpiece.

54. The method of any one of claims 51-53, wherein said one or more nucleotides are present at the internal position of said tag.

55. The method of claim 53 or claim 54, wherein said one or more nucleotides of said headpiece and/or said tag are one or more 2'-deoxynucleotides.

56. The method of any one of claims 51-55, wherein said step (iii) comprises one or more soluble multivalent cations, poly ethylene glycol, and RNA ligase.

57. The method of claim 56, wherein said poly ethylene glycol has an average molecular weight of about 4,600 Daltons and said RNA ligase is T4 RNA ligase.

58. The method of claim 56 or claim 57, wherein said one or more multivalent cations are selected from the group consisting of magnesium chloride, manganese (II) chloride, and hexamine cobalt (III) chloride.

59. The method of any one of claims 51-57, wherein said headpiece and/or said first building block tag comprises from 5 to 20 nucleotides.

60. The method of claim 59, wherein said first building block tag, said second building block tag, and/or said one or more additional building block tags, if present, has about the same mass.

61. The method of any one of claims 51-60, wherein said headpiece, said first building block tag, said second building block tag, and/or said one or more additional building block tags, if present, further comprises a first library-identifying sequence.

62. The method of any one of claims 51-60, wherein said method further comprises binding a first library-identifying tag to said complex.

63. The method of claim 61 or 62, further comprising providing a second library and combining said first library with said second library.

64. The method of any one of claims 51-63, wherein said headpiece, said first building block tag, said second building block tag, and/or said one or more additional building block tags, if present, further comprises a use sequence or an origin sequence.

65. The method of any one of claims 51-63, wherein said method further comprises binding a use tag and/or an origin tag to said complex.

66. The method of any one of claims 51-65, wherein said method further comprises binding a tailpiece to said complex.

67. The method of claim 1 or 51, wherein said 2'-substitued nucleotide is selected from the group consisting of 2'-O-methyl guanine, 2'-O-methyl uracil, 2'-O-methyl adenosine, 2'-O-methyl thymidine, 2'-O-methyl inosine, 2'-O-methyl cytidine, 2'-O-methyl diamino purine, 2'-fluoro guanine, 2'-fluoro uracil, 2'-fluoro adenosine, 2'-fluoro thymidine, 2'-fluoro inosine, 2'-fluoro cytidine, and 2'-fluoro diamino purine.

68. The method of any one of claims 1-67, wherein said method comprises a plurality of headpieces.

69. The method of claim 68, wherein each headpiece of said plurality of headpieces comprises an identical sequence region and a different encoding region.

70. The method of claim 69, wherein said identical sequence region is a primer binding region.

71. The method of claim 69 or claim 70, wherein said different encoding region is an initial building block tag that encodes for said headpiece or for an addition of an initial component.

72. The method of any one of claims 1-71, wherein said binding in at least one of steps (ii)-(iv), if present, comprises enzymatic ligation.

73. The method of claim 72, wherein said enzymatic ligation comprises use of an RNA ligase and/or a DNA ligase.

74. The method of any one of claims 1-73, wherein said binding in at least one of steps (ii)-(iv), if present, comprises chemical ligation.

75. The method of claim 74, wherein said chemical ligation comprises use of one or more chemically co-reactive pairs.

76. The method of claim 75, wherein said chemically co-reactive pair is an optionally substituted alkynyl group and an optionally substituted azido group.

77. The method of claim 75, wherein said chemically co-reactive pair is a phosphorothioate group and an iodo group.

78. The method of claim 77, wherein said phosphorothioate group is at the 5'-terminus of an oligonucleotide and said iodo group is at the 3'-terminus of an oligonucleotide.

79. The method of claim 77 or 78, wherein said chemical ligation further comprises a splint oligonucleotide in the binding reaction between said chemically co-reactive pair.

80. The method of any one of claims 75-79, wherein said chemically co-reactive pair produces a spacer having a length from about 4 to about 24 atoms.

81. The method of claim 80, wherein said spacer has a length from about 4 to about 10 atoms.

82. The method of any one of claims 74-81, wherein said chemical ligation of one or more building block tags comprises orthogonal chemically co-reactive pairs for ligating successive building block tags.

83. The method of claim 82, wherein said orthogonal chemically co-reactive pairs comprises an (i) optionally substituted alkynyl group and an optionally substituted azido group and (ii) a phosphorothioate group and an iodo group.

84. The method of any one of claims 1-83, wherein said complex, said headpiece, said first building block tag, said second building block tag, and/or said one or more additional building block tags, if present, comprises a modified phosphate group between the terminal nucleotide at the 3'-terminus and the nucleotide adjacent to said terminal nucleotide.

85. A complex comprising a headpiece and a building block tag, wherein said tag comprises from 5 to 20 nucleotides, a 2'-substituted nucleotide at the 5'-terminus, and a 2'-substituted nucleotide at the 3'-terminus.

86. The complex of claim 85, wherein said 2'-substituted nucleotide at the 5'-terminus and/or 3'-terminus is a 2'-O-methyl nucleotide or a 2'-fluoro nucleotide.

87. The complex of claim 85 or 86, wherein said headpiece comprises a hairpin structure.

88. The complex of any one of claims 85-87, wherein said headpiece comprises a 2'-substituted nucleotide at one or more of the 5'-terminus, the 3'-terminus, or the internal position of said headpiece.

89. The complex of any one of claims 85-88, wherein said headpiece further comprises a preadenylated 5'-terminus.

90. The complex of any one of claims 85-89, wherein said 2'-substituted nucleotide is a 2'-O-methyl nucleotide or a 2'-fluoro nucleotide.

91. The complex of claim 90, wherein said 2'-substitued nucleotide is selected from the group consisting of 2'-O-methyl guanine, 2'-O-methyl uracil, 2'-O-methyl adenosine, 2'-O-methyl thymidine, 2'-O-methyl inosine, 2'-O-methyl cytidine, 2'-O-methyl diamino purine, 2'-fluoro guanine, 2'-fluoro uracil, 2'-fluoro adenosine, 2'-fluoro thymidine, 2'-fluoro inosine, 2'-fluoro cytidine, and 2'-fluoro diamino purine.

92. The complex of any one of claims 85-91, further comprising a tailpiece.

93. The complex of any one of claims 85-92, wherein said complex, said headpiece, or said building block tag comprises a modified phosphate group between the terminal nucleotide at the 3'-terminus and the nucleotide adjacent to said terminal nucleotide.

94. The complex of any one of claims 85-93, wherein said complex comprises a phosphorothioate or a triazole group.