

particular embodiment the functional sequence is sequestered and releasable in a releasing step including a stimulus selected from the list consisting of thermal increase and chemical cleavage. In a different embodiment the releasing step includes degrading at least a portion the beads of the bead population including oligonucleotide barcode sequence segments. In a specific embodiment degrading the beads includes cleaving a chemical linkage including a disulfide bridge linkage between the barcode sequence segments and the bead, and the releasing step includes exposing the beads to a reducing agent. In a particular embodiment the reducing agent includes a reducing agent selected from the group consisting of DTT and TCEP.

[0024a] In general, in another aspect there is provided a method of preparing a barcoded nucleic acid molecule, comprising: (a) providing a template nucleic acid molecule, deoxynucleotide triphosphates (dNTPs), deoxyuridine triphosphates (dUTPs), a random primer, a polymerase, an enzyme capable of excising uracil, and a bead comprising a plurality of oligonucleotide barcode molecules releasably attached thereto; (b) subjecting the template nucleic acid molecule, the polymerase, dNTPs, dUTPs and the random primer to conditions sufficient to synthesize a double stranded nucleic acid molecule comprising a first strand, wherein the first strand comprises a uracil; (c) excising the uracil from the double stranded nucleic acid molecule with the enzyme to provide a nick in the double stranded nucleic acid molecule; (d) subjecting the double stranded nucleic acid molecule to a nucleic acid extension reaction under conditions sufficient to yield a plurality of single stranded nucleic acid fragments; and (e) using an oligonucleotide barcode molecule of the plurality of oligonucleotide barcode molecules and a nucleic acid fragment of the plurality of single stranded nucleic acid fragments to generate a barcoded nucleic acid molecule.

INCORPORATION BY REFERENCE

[0025] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] Figure 1 is a diagram illustrating the process of priming free amplification of templates.

[0027] Figure 2A is a diagram illustrating barcoding of templates using an extension barcoding approach.

[0028] Figure 2B is a diagram illustrating barcoding of single or double stranded templates using a ligation approach.

[0029] Figure 2C is a diagram illustrating barcoding of single stranded library molecules using and APP DNA/RNA ligase approach.

[0030] Figure 3 shows results of testing for T base bias based on whole genome sequencing data.

[0031] Figure 4A is a plot of a primed amplification showing coverage evenness over 1000 base pairs binned GC content of the human genome.

[0032] Figure 4B is a plot of a primer free amplification showing coverage evenness over 1000 base pairs binned GC content of the human genome.

[0033] Figure 5A is a GC coverage plot for a reaction with no dUTP added.

[0034] Figure 5B is a GC coverage plot for a reaction with 0.5% dUTP added.

[0035] Figure 5C is a GC coverage plot for a reaction with 1% dUTP added.

[0036] Figure 5D is a GC coverage plot for a reaction with 2% dUTP added.

[0037] Figure 5E is a GC coverage plot for a reaction with 3% dUTP added.

[0038] Figure 6 shows the results of titration of dUTP and the effect on chimera rate, Depth Positional CV (DPCV).

CLAIMS

WHAT IS CLAIMED IS:

1. A method of preparing a barcoded nucleic acid molecule, comprising:
 - (a) providing a template nucleic acid molecule, deoxynucleotide triphosphates (dNTPs), deoxyuridine triphosphates (dUTPs), a random primer, a polymerase, an enzyme capable of excising uracil, and a bead comprising a plurality of oligonucleotide barcode molecules releasably attached thereto;
 - (b) subjecting the template nucleic acid molecule, the polymerase, dNTPs, dUTPs and the random primer to conditions sufficient to synthesize a double stranded nucleic acid molecule comprising a first strand, wherein the first strand comprises a uracil;
 - (c) excising the uracil from the double stranded nucleic acid molecule with the enzyme to provide a nick in the double stranded nucleic acid molecule;
 - (d) subjecting the double stranded nucleic acid molecule to a nucleic acid extension reaction under conditions sufficient to yield a plurality of single stranded nucleic acid fragments; and
 - (e) using an oligonucleotide barcode molecule of the plurality of oligonucleotide barcode molecules and a nucleic acid fragment of the plurality of single stranded nucleic acid fragments to generate a barcoded nucleic acid molecule.
2. The method of claim 1, wherein (a)-(e) are performed in a single reaction volume.
3. The method of claim 1, wherein the bead, the template nucleic acid molecule, the dNTPs, the dUTPs, the random primer, the polymerase and the dUTP excising enzyme are provided in a partition.
4. The method of claim 3, wherein the partition is a droplet in an emulsion.
5. The method of claim 1, wherein the bead is a degradable bead selected from the group consisting of a chemically degradable bead, a photodegradable bead and a thermally degradable bead.
6. The method of claim 5, wherein the bead is a chemically degradable bead, and wherein the chemically degradable bead comprises chemically reducible cross-linkers.
7. The method of claim 6, wherein the chemically reducible cross-linkers comprise disulfide linkages.

8. The method of any of claims 1, wherein, in (d), the nucleic acid extension reaction is isothermal.
9. The method of claim 1, wherein, in (e), the barcoded nucleic acid molecule is generated through use of a ligating enzyme, a polymerase enzyme, or a topoisomerase.
10. The method of claim 9 wherein the ligating enzyme is an adenosine triphosphate (ATP) independent enzyme.
11. The method of claim 10, wherein each of the plurality of oligonucleotide barcode molecules is adenylated and wherein the ATP independent enzyme is a thermostable 5' App DNA/RNA ligase.
12. The method of claim 9, wherein the topoisomerase is topoisomerase I.
13. The method of claim 9, wherein the ligating enzyme comprises a T4 DNA ligase.
14. The method of claim 1, wherein each of the plurality of oligonucleotide barcode molecules comprise a barcode sequence that is at least 4 nucleotides in length.
15. The method of claim 1, wherein the bead comprises at least 1,000,000 oligonucleotide molecules releasably attached thereto.
16. The method of claim 1, wherein each of the plurality of oligonucleotide barcode molecules comprises one or more functional sequences.
17. The method of claim 16, wherein the one or more functional sequences are selected from an adapter sequence, a primer sequence, a primer annealing sequence, an attachment sequence, and a sequencing primer sequence.
18. The method of claim 16, wherein the one or more functional sequences comprise a blocking group.
19. The method of claim 16, wherein the one or more functional sequences comprise a random N-mer sequence 5 to 500 nucleotides in length.
20. The method of claim 1, further comprising releasing oligonucleotide barcode molecules of the plurality of oligonucleotide barcode molecules from the bead.
21. The method of claim 20, wherein the releasing comprises degrading the bead.
22. The method of claim 20, wherein the releasing comprises using a reducing agent to cleave a disulfide linkage between the plurality of oligonucleotide barcode molecules and the bead.
23. The method of claim 22, wherein the reducing agent is dithiothreitol (DTT) or tris(2-carboxyethyl) phosphine (TCEP).
24. The method of claim 1, wherein, in (a), the dNTPs are provided at a concentration that is greater than a concentration of the dUTPs.

25. The method of claim 1, further comprising sequencing the barcoded nucleic acid molecule or a derivative thereof.
26. The method of claim 1, wherein (b) comprises (i) coupling the random primer to the template nucleic acid molecule and (ii) extending the random primer to synthesize the double stranded nucleic acid molecule comprising the uracil.
27. The method of claim 17, wherein each of the plurality of oligonucleotide barcode molecules comprise a primer sequence and wherein, in (e), the barcoded nucleic acid molecule is generated by nucleic acid extension of the primer sequence.
28. The method of claim 1, wherein, in (d), the polymerase engages the double stranded nucleic acid molecule at the nick, wherein the nucleic acid extension reaction comprises a primer independent extension process, and wherein the polymerase comprises strand displacement activity.
29. The method of claim 1, wherein the random primer is a random N-mer sequence 5 to 500 nucleotides in length.
30. The method of claim 29, wherein the random N-mer sequence is 5 to 25 nucleotides in length.
31. The method of claim 19, wherein the random N-mer sequence is 5 to 25 nucleotides in length.
32. The method of claim 18, wherein the blocking group is releasable in a releasing step comprising applying a stimulus selected from the group consisting of a thermal stimulus and a chemical stimulus.
33. The method of claim 27, wherein in (e), the nucleic acid extension is isothermal.
34. The method of claim 1, wherein each of the plurality of barcode oligonucleotide molecules is releasably attached to the bead through a labile bond.
35. The method of claim 34, wherein the labile bond is selected from the group consisting of a thermally cleavable bond, a chemically labile bond, and a photo-sensitive bond.
36. The method of claim 34, wherein the labile bond comprises a linkage selected from the group consisting of an ester linkage, a vicinal diol linkage, a Diels-Alder linkage, a sulfone linkage, a silyl ester linkage, a glycosidic linkage, a peptide linkage, or a phosphodiester linkage.
37. The method of claim 3, wherein the partition is a well.