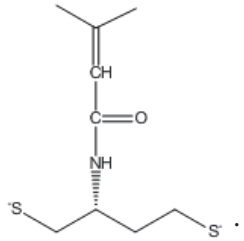
In some cases, after subjecting the reducing agent to the activation condition, the method further comprises cleaving the reducible cross-linkage.

[0016] In some cases, the reducible linkage comprises a disulfide linkage linking the nucleic acid molecule to the polymer matrix. In some cases, the nucleic acid molecule comprises a barcode sequence. In some cases, the reaction volume is provided in the aqueous interior of a droplet in a water-in-oil emulsion. In some cases, the reaction volume comprises an enzyme which can be, for example, a polymerase.

[0017] In another aspect, the disclosure provides a reducing agent comprising:



[0017a] In another aspect, the disclosure provides a method for processing a nucleic acid molecule, comprising:

(a) providing a reaction volume comprising a reagent that comprises a first component coupled to a second component via a linker, wherein the linker comprises a cleavable moiety; the nucleic acid molecule; and an agent, wherein the agent comprises a sterically hindered -SH group;

(b) subjecting the reaction volume to conditions sufficient for the agent to cleave the cleavable moiety, thereby separating the first component from the second component; and

using the first component to subject the nucleic acid molecule to an extension reaction to yield an extension product comprising a sequence that is complementary to a sequence of the nucleic acid molecule.

[0017b] In another aspect, the disclosure provides a method for processing a nucleic acid molecule, comprising:

(a) providing a reaction volume comprising a reagent that comprises a first component coupled to a second component via a linker, wherein the linker comprises a first cleavable moiety; the nucleic acid molecule; and an agent that is in an inactive state and comprises one or more -SH groups and a second cleavable moiety;

(b) subjecting the reaction volume to conditions sufficient to cleave the second cleavable moiety of the agent to provide an activated agent that cleaves the first cleavable moiety of the linker, thereby separating the first component from the second component; and

using the first component to subject the nucleic acid molecule to an extension reaction to yield an extension product comprising a sequence that is complementary to a sequence of the nucleic acid molecule.

[0018] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0019] 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. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative

Claims

1. A method for processing a nucleic acid molecule, comprising:
   (a) providing a reaction volume comprising a reagent that comprises a first component coupled to a second component via a linker, wherein the linker comprises a cleavable moiety; the nucleic acid molecule; and an agent, wherein the agent comprises a sterically hindered -SH group;
   (b) subjecting the reaction volume to conditions sufficient for the agent to cleave the cleavable moiety, thereby separating the first component from the second component; and
   (c) using the first component to subject the nucleic acid molecule to an extension reaction to yield an extension product comprising a sequence that is complementary to a sequence of the nucleic acid molecule.
2. The method of Claim 1, wherein the subjecting in (b) comprises increasing a temperature of the reaction volume.
3. The method of Claim 1, wherein the second component comprises a bead.
4. The method of Claim 1, wherein the first component comprises a nucleic acid barcode molecule.
5. The method of Claim 4, wherein the nucleic acid barcode molecule comprises a barcode sequence, a primer sequence, or a combination thereof.
6. The method of Claim 1, wherein the second component comprises a polymer matrix.
7. The method of Claim 6, wherein the polymer matrix comprises a hydrogel.
8. The method of Claim 1, wherein the cleavable moiety is a disulfide linkage.
9. The method of Claim 5, wherein the extension product comprises the barcode sequence.
10. The method of Claim 1, wherein the reaction volume is within an aqueous droplet.
11. The method of Claim 1, wherein the -SH group of the agent is bound to a carbon atom comprising two or more alkyl groups.
12. A method for processing a nucleic acid molecule, comprising:
   (a) providing a reaction volume comprising a reagent that comprises a first component coupled to a second component via a linker, wherein the linker comprises a first

cleavable moiety; the nucleic acid molecule; and an agent that is in an inactive state and comprises one or more -SH groups and a second cleavable moiety;

(b) subjecting the reaction volume to conditions sufficient to cleave the second cleavable moiety of the agent to provide an activated agent that cleaves the first cleavable moiety of the linker, thereby separating the first component from the second component; and

(c) using the first component to subject the nucleic acid molecule to an extension reaction to yield an extension product comprising a sequence that is complementary to a sequence of the nucleic acid molecule.

13. The method of Claim 12, wherein cleaving the second cleavable moiety of the agent comprises cleaving a covalent bond of the agent.

14. The method of Claim 12, wherein the second cleavable moiety is an amide bond.

15. The method of Claim 12, wherein the subjecting in (b) comprises increasing a temperature of the reaction volume.

16. The method of Claim 12, wherein the first component comprises a nucleic acid barcode molecule.

17. The method of Claim 16, wherein the nucleic acid barcode molecule comprises a barcode sequence, a primer sequence, or a combination thereof.

18. The method of Claim 17, wherein the extension product comprises the barcode sequence.

19. The method of Claim 12, wherein the second component comprises a bead.

20. The method of Claim 12, wherein the second component comprises a polymer matrix.

21. The method of Claim 20, wherein the polymer matrix comprises a hydrogel.

22. The method of Claim 12, wherein the reaction volume is within an aqueous droplet.