

WHAT IS CLAIMED IS:

1. A method of producing two or more linked nucleic acid molecules from a single cell, comprising:
 - (i) isolating a single cell in a first container, and lysing the single cell to release mRNA molecules;
 - (ii) reverse transcribing the mRNA molecules to produce cDNA molecules in the first container; and
 - (iii) linking the cDNA molecules derived from the single cell in step (ii) in a second container,thereby producing linked nucleic acid molecules.
2. The method of claim 1, wherein the first container comprises one or more solid supports attached to an oligonucleotide comprising a sequence complementary to a portion of the mRNA molecules.
3. The method of claim 2, wherein the mRNA molecules are attached to the oligonucleotide via binding to the complementary sequence.
4. The method of claim 3, wherein the reverse transcribing comprises extending the oligonucleotide with a reverse transcriptase to produce the cDNA molecules.
5. The method of claim 4, wherein the cDNA molecules from step (ii) are covalently linked to the solid supports.
6. The method of claim 5, wherein each of the one or more solid supports is isolated in a different second container prior to step (iii).
7. The method of claim 2, wherein the oligonucleotide is attached to the solid support by a linker.
8. The method of claim 7, wherein the linker is located between a surface of the solid support and the sequence complementary to a portion of the mRNA molecules.
9. The method of claim 7, wherein the linker is a photocleavable linker.
10. The method of claim 9, wherein the cDNA molecules are released from the solid support by exposing the photocleavable linker to light in the second container.

11. The method of claim 2, wherein 1 to 20 solid supports are present in the first container.
12. The method of claim 2, wherein an average of 3 to 5 solid supports are present in the first container.
13. The method of claim 2, wherein an average of 15 solid supports are present in the first container.
14. The method of claim 2, wherein the solid support is a bead or particle.
15. The method of claim 2, wherein the solid support is a spherical particle having a diameter of 1 to 20 micrometers.
16. The method of claim 2, wherein the solid support has an average diameter between 5 and 10 micrometers.
17. The method of claim 2, wherein linking the cDNA molecules in step (iii) comprises amplifying and linking the cDNA molecules by overlap extension PCR.
18. The method of claim 17, wherein the cDNA molecules are released from the solid support in the second container prior to step (iii).
19. The method of claim 17, wherein the overlap extension PCR comprises amplifying the cDNA molecules using one or more internal primers comprising a biotin tag.
20. The method of claim 19, wherein cDNA molecules comprising the biotin tag are removed after step (iii).
21. The method of claim 17, wherein the overlap extension PCR comprises amplifying the cDNA molecules using one or more external primers chemically modified to resist nuclease degradation.
22. The method of claim 21, wherein the one or more external primers are chemically modified to include phosphorothioate bonds.
23. The method of claim 22, wherein the cDNA molecules are contacted with a 5'-exonuclease after step (iii).

24. The method of claim 1, wherein the single cell is an immune system cell.
25. The method of claim 1, wherein the single cell is a B cell, a memory B cell, an activated B cell, a blasting B cell, a plasma cell, or a plasmablast.
26. The method of claim 25, wherein the mRNA molecules encode a heavy chain variable region and a light chain variable region.
27. The method of claim 25, wherein the cDNA molecules encode a cognate pair of heavy and light chain variable regions.
28. The method of claim 1, wherein the single cell is a T cell.
29. The method of claim 1, wherein the single cell is a natural killer T (NKT) cell.
30. The method of claim 27, wherein the cDNA molecules encode a cognate pair of T cell receptor alpha and beta chains.
31. The method of claim 1, wherein the first or second container comprises a partition, an aqueous droplet in an emulsion, a microvesicle, a tube, or a multiwell plate.
32. The method of claim 31, wherein the droplet is 2 to 500 micrometers in diameter.
33. The method of claim 1, further comprising digesting the mRNA following step (ii).
34. The method of claim 33, wherein the mRNA is digested in the first container, or between steps (ii) and (iii).
35. A method for producing a library of linked nucleic acid molecules, comprising:
- a) isolating a plurality of single cells in a plurality of first containers, where the first containers comprise a single cell;
 - b) lysing the single cells to release mRNA molecules in the first container;

c) reverse transcribing the mRNA molecules to produce cDNA molecules derived from single cells in the first container;
d) linking the cDNA molecules from step (c) in a second container;
e) combining the linked cDNA molecules from step (d) to produce a library of linked nucleic acid molecules.

36. The method of claim 35, wherein the single cells are B cells, and the percentage of heavy chain variable regions that are correctly paired with the cognate light chain variable regions in the library is increased compared to a method where steps (c) and (d) are performed in the same container.

37. The method of claim 35, wherein the single cells are T cells, and the percentage of T cell receptor alpha chains that are correctly paired with the cognate T cell receptor beta chains in the library is increased compared to a method where steps (c) and (d) are performed in the same container.

38. The method of claim 35, wherein the single cells are NKT cells, and the percentage of T cell receptor alpha chains that are correctly paired with the cognate T cell receptor beta chains in the library is increased compared to a method where steps (c) and (d) are performed in the same container.

39. The method of claim 35, wherein step (d) comprises amplifying and linking the cDNA molecules by overlap extension PCR.

40. The method of claim 39, wherein the overlap extension PCR comprises amplifying the cDNA molecules using one or more internal primers comprising a biotin tag.

41. The method of claim 40, wherein cDNA molecules comprising the biotin tag are removed after step (d).

42. The method of claim 39, wherein the overlap extension PCR comprises amplifying the cDNA molecules using one or more external primers chemically modified to resist nuclease degradation.

43. The method of claim 42, wherein the one or more external primers are chemically modified to include phosphorothioate bonds.

44. The method of claim 43, wherein the cDNA molecules are contacted with a 5'-exonuclease after step (d).

45. A method for producing two or more linked nucleic acid molecules from a single cell, comprising:

- (i) isolating a single cell in a first container, and lysing the single cell to release mRNA molecules;
- (ii) hybridizing the mRNA molecules to a capture oligonucleotide attached to a solid support, wherein the capture oligonucleotide comprises a sequence complementary to a portion of the mRNA sequence;
- (iii) reverse transcribing the mRNA molecules to produce cDNA molecules attached to the solid support in the first container;
- (iv) linking the cDNA molecules derived from step (iii) in a second container, thereby producing linked nucleic acid molecules.

46. The method of claim 45, wherein the capture oligonucleotide further comprises a linker positioned between the solid support and the sequence complementary to a portion of the mRNA sequence.

47. The method of claim 46, wherein the linker is cleaved, releasing the cDNA molecules from the solid support prior to step (iv).

48. The method of claim 45, wherein step (iv) comprises amplifying and linking the cDNA molecules by overlap extension PCR.

49. The method of claim 48, wherein the overlap extension PCR comprises amplifying the cDNA molecules using one or more internal primers comprising a biotin tag.

50. The method of claim 48, wherein cDNA molecules comprising the biotin tag are removed after step (iv).

51. The method of claim 48, wherein the overlap extension PCR comprises amplifying the cDNA molecules using one or more external primers chemically modified to resist nuclease degradation.

52. The method of claim 51, wherein the one or more external primers are chemically modified to include phosphorothioate bonds.

53. The method of claim 52, wherein the cDNA molecules are contacted with a 5'-exonuclease after step (iv).