CLAIMS

WHAT IS CLAIMED IS:

1. A method for construction of a directional cDNA library comprising:
    a. reverse transcribing a RNA sample to generate a first strand cDNA;
    b. generating a second strand cDNA from the first strand cDNA, wherein at least one of the four dNTPs dATP, dCTP, dGTP or dTTP is replaced by a modified dNTP during second strand synthesis and incorporated into the second strand, thereby generating a double-stranded cDNA;
    c. performing end repair on the double-stranded cDNA;
    d. ligating adaptors to the double-stranded cDNA, wherein only one of the adaptors has the modified dNTP incorporated into a ligation strand of the adaptor;
    e. performing gap repair; and
    f. selectively cleaving the second strand and the adaptor that has the modified dNTP by a suitable cleavage agent, thereby generating a directional cDNA library comprising the first strand cDNA.
2. The method of claim 1, wherein the modified dNTP comprises dUTP.
3. The method of claim 1, wherein the method~~, optionally,~~ further comprises fragmenting the double-stranded cDNA prior to step (c).
4. The method of claim 1, wherein step (f) comprises cleaving a base portion of the modified nucleotide, thereby forming an abasic site.
5. The method of claim 4, further comprising cleaving a phosphodiester backbone at the abasic site.
6. The method of claim 4, wherein the cleaving the base portion of the modified nucleotide comprises use of an enzyme.
7. The method of claim 5, wherein the cleaving the phosphodiester backbone at the abasic site comprises use of an enzyme, chemical agent, and/or heat.
8. The method of claim 7, wherein the enzyme is an endonuclease.
9. The method of claim 8, wherein the endonuclease is an apurinic/apyrimidinic endonuclease (APE).
10. The method of claim 6, wherein the enzyme comprises a glycosylase.
11. The method of claim 10, wherein the glycosylase is uracil-N-glycosylase (UNG) or uracil DNA glycosylase (UDG).
12. The method of claim 7, wherein the chemical agent comprises a primary amine.
13. The method of claim 7, wherein the chemical agent comprises a polyamine.
14. The method of claim 13, wherein the polyamine comprises N,N-dimethylethylenediamine (DMED).

51. The method of claim 50, wherein the RNase is RNase H.

52. The method of claim 49, wherein cleaving the RNA sample comprises exposing the RNA sample to heat or chemical treatment or a combination thereof.

53. The method of claim 49 further comprising reducing or depleting non-desired nucleic acid sequences from the directional cDNA library.

54. The method of claim 53, wherein the non-desired nucleic acid is ribosomal RNA (rRNA).

55. A kit comprising:
    a. One or more primers;
    b. a reverse transcription enzyme;
    c. a glycosylase;
    d. one or more adaptors wherein one of the adaptors comprises at least one modified nucleotide in a ligation strand of the adaptor.

~~56. The kit of claim 55, further comprising written instructions for use of the kit.~~

~~57.~~56. The kit of claim 55, further comprising at least one modified nucleotide or dNTP.

~~58.~~57. The kit of claim 55, further comprising reagents for amplification.

~~59.~~58. The kit of claim 55, further comprising reagents for sequencing.

~~60.~~59. The kit of claim 55, further comprising a polyamine, an APE, or a combination thereof.

~~61.~~60. The kit of claim 55, wherein the modified nucleotide comprises dUTP, and wherein the glycosylase comprises UNG.

~~62.~~61. The kit of claim 60, wherein the modified nucleotide comprises dUTP.

~~63.~~62. A method for construction of a directional cDNA library comprising:
    a. synthesizing first and second strand cDNA from template RNA, wherein either first strand cDNA or second strand cDNA synthesis comprises incorporating a modified dNTP, to generate double stranded cDNA;
    b. performing end repair on the double-stranded cDNA;
    c. ligating adaptors to the double-stranded cDNA, wherein only one of the adaptors has the modified dNTP incorporated into a ligation strand of the adaptor;
    d. performing gap repair; and
    e. selectively cleaving the first or the second strand cDNA and the adaptor that has the modified dNTP by a suitable cleavage agent, thereby generating a directional cDNA library comprising the first or the second strand cDNA.