CLAIMS

1. A method for sequencing hydroxymethylated cell-free DNA (cfDNA), comprising:

(a) adding adaptor sequences onto the ends of cfDNA, wherein the cfDNA is not pre-digested by a restriction enzyme;

(b) incubating the adaptor-ligated cfDNA with a DNA β-glucosyltransferase and UDP glucose modified with a chemoselective group, thereby covalently labeling the hydroxymethylated DNA molecules in the cfDNA with the chemoselective group;

(c) linking a biotin moiety to the chemoselectively-modified cfDNA via a cycloaddition reaction;

(d) enriching for the biotinylated DNA molecules by binding the product of step (c) to a support that binds to biotin, wherein the enriching is done without pre-amplifying the cfDNA;

(e) amplifying the enriched DNA using primers that bind to the adaptors, wherein the amplifying is done by washing the support and setting up an amplification reaction containing the support, without releasing the enriched DNA molecules from the support; and

(f) sequencing the amplified DNA to produce a plurality of sequence reads.

2. The method of claim 1, wherein the UDP glucose modified with a chemoselective group comprises UDP-6-N_3-Glu, the biotin moiety comprises dibenzocyclooctyne-modified biotin, and the support comprises avidin or streptavidin.

3. The method of claim 1, wherein the adaptor sequences comprise a molecular barcode.

4. The method of claim 3, wherein the molecular barcode comprises a sample identifier sequence and a molecule identifier sequence.

5. The method of claim 1, wherein prior to step (a), a spike-in control composition is combined with sample.

6. The method of claim 5, wherein the spike-in control composition comprises three amplicons synthesized from a cocktail of dATP, dGTP, dTTP, and (1) dCTP, (2) dmCTP, or (3) dhmCTP and dCTP.

7. A method for identifying sources of DNA in a cell-free DNA sample, comprising:

(a) providing a sample that comprises a pool of unamplified adaptor-ligated, cell-free DNA molecules that (i) have a molecular barcode to indicate their source, (ii) are hydroxymethylated, and (iii) linked to a support via a capture tag, wherein the cfDNA is not pre-digested by a restriction enzyme;

(b) amplifying the cell-free DNA molecules by washing the support and setting up an amplification reaction containing the support, without releasing the captured cell-free DNA from the support, to provide amplification products;

(c) sequencing the amplification products to produce a plurality of sequence reads; and

(d) identifying the sources of the cell-free DNA molecules from the molecular barcodes observed in the sequence reads.

8. The method of claim 7, wherein the capture tag comprises a biotin moiety.

9. The method of claim 7, wherein the sample is enriched in DNA molecules comprising one or more hydroxymethylcytosines that are modified to contain the capture tag, such that the sample comprises an enriched composition.

10. The method of claim 9, wherein at least 80% of the DNA molecules in the enriched composition comprise one or more hydroxymethylcytosines that are modified to contain the capture tag.

11. The method of claim 10, wherein at least 90% of the DNA molecules in the enriched composition comprise one or more hydroxymethylcytosines that are modified to contain the capture tag.