CLAIMS

WHAT IS CLAIMED IS:

1. A sequencing method, comprising:

contacting a methylation control with a plurality of genomic fragments, thereby generating a sample, wherein the methylation control comprises a target methylation level;

converting unmethylated cytosines in the sample to uracil, thereby generating converted fragments;

contacting the converted fragments with a probe library comprising a plurality of polynucleotide probes configured to hybridize to a genomic region comprising a CpG site;

enriching a converted fragment that binds to a polynucleotide probe of the plurality of polynucleotide probes to generate an enriched target polynucleotide; and

sequencing the enriched target polynucleotide.

2. The method of claim 1, wherein the target methylation level is produced by mixing a first population of a polynucleotide comprising CpG sites wherein all CpG sites are methylated, with a second population of the polynucleotide wherein all CpG sites are unmethylated, wherein the first population and the second population are present at a ratio corresponding to the target methylation level.

3. The method of claim 1, further comprising adding the methylation control at a plurality of target methylation levels, and generating a calibration curve by plotting measured methylation levels of the methylation control against the plurality of target methylation levels.

4. The method of claim 1, further comprising quantitating methylation levels in the plurality of genomic fragments by normalizing sequencing data against a measured methylation level of the methylation control at the target methylation level.

5. The method of claim 1, wherein the methylation control comprises polynucleotides having at least 8 different sequences, and wherein the polynucleotides do not have sequence identity to a reference genome.

6. The method of claim 1, further comprising identifying a false positive rate based on a deviation between a measured methylation level of the methylation control and the target methylation level.

7. The method of claim 1, further comprising:

contacting the converted fragments with a control panel, wherein the control panel comprises a plurality of control probes that are complementary to the methylation control; and

enriching converted fragments of the methylation control that bind to the control panel.

8. The method of claim 1, further comprising:

measuring a methylation level of the methylation control based on data obtained during sequencing of the enriched target polynucleotide; and

comparing the measured methylation level to the target methylation level to determine a conversion efficiency.

9. The method of claim 1, further comprising contacting the converted fragments with a blocking library, wherein:

the blocking library comprises a plurality of polynucleotides comprising sequences derived from a reference genome,

at least 80% of cytosine bases in the plurality of polynucleotides of the blocking library are replaced with thymine, and

each polynucleotide of the plurality of polynucleotides in the blocking library have a C_0t value less than 2.

10. The method of claim 1, wherein the plurality of genomic fragments comprise cell-free DNA.

11. A methylation detection system comprising:

a probe library comprising a plurality of polynucleotide probes configured to hybridize to genomic fragments comprising CpG sites;

a blocking library; and

a methylation control comprising:

a first polynucleotide comprising one or more CpG sites, wherein all of the one or more CpG sites of the first polynucleotide are methylated,

a second polynucleotide comprising one or more CpG sites and a nucleotide sequence that is identical to a nucleotide sequence of the first polynucleotide, wherein the one or more CpG sites of the second polynucleotide are unmethylated, and

wherein the first polynucleotide is mixed with the second polynucleotide at a defined ratio to produce a target methylation level.

12. The system of claim 11, wherein the methylation control comprises at least 8 different target methylation levels.

13. The system of claim 11, wherein the methylation control comprises polynucleotides having sequences that are not homologous to a reference genome.

14. The system of claim 11, wherein the methylation control comprises polynucleotides having a length of 150 to 175 bases.

15. The system of claim 11, wherein the methylation control comprises polynucleotides having at least 8 different sequences.

16. The system of claim 11, further comprising a control panel comprising a plurality of polynucleotide probes configured to hybridize to polynucleotides of the methylation control.

17. The system of claim 11, wherein the first polynucleotide and the second polynucleotide do not comprise homopolymers.

18. The system of claim 11, further comprising one or more enzymes configured to convert unmethylated cytosines to uracil.

19. The system of claim 11, wherein the blocking library comprises a plurality of polynucleotides comprising sequences derived from a reference genome, wherein at least 80% of cytosine bases in the plurality of polynucleotides of the blocking library are replaced with thymine.

20. The system of claim 11, wherein the blocking library comprises a plurality of polynucleotides having a Cot value less than 2.