

Docket No.: CGLC-034WO
International Application No. PCT/IB2018/050137

CLAIMS

1. A method for normalizing a sample, comprising:
 - (a) obtaining a sample comprising nucleic acid;
 - (b) tethering at least some of the nucleic acid molecules in the sample to a support;
 - (c) reacting the tethered nucleic acid molecules of (b) with a limiting amount of a first Cas9-RNA complex that recognizes a first target sequence in the sample, thereby cleaving a portion of the tethered nucleic acid molecules that comprise the target sequence and releasing a normalized amount of a first cleavage product into solution, and
 - (d) isolating the first cleavage product released in step (c);wherein the amount of the first cleavage product isolated in step (d) is determined by the limiting amount of the Cas9-RNA complex used in step (c).
2. The method of claim 1, wherein the nucleic acid in the sample is an amplification product.
3. The method of claim 1 or 2, wherein the amplification product comprises PCR products.
4. The method of claim 1, 2 or 3, wherein step (c) of the method comprises:
 - (c) reacting the tethered nucleic acid molecules of (b) with:
 - (i) a limiting amount of a first Cas9-RNA complex that recognizes a first target sequence in the sample, thereby cleaving a portion of the tethered nucleic acid molecules that comprise the target sequence and releasing a normalized amount of a first cleavage product into solution; and

Docket No.: CGLC-034WO

International Application No. PCT/IB2018/050137

- (ii) a limiting amount of a second Cas9-RNA complex that recognizes a second target sequence in the sample, thereby cleaving a portion of the tethered nucleic acid molecules that comprise the second target sequence and releasing a normalized amount of second cleavage product into solution; and

step (d) comprises:

- (d) isolating the first and second cleavage products released in step (c);

wherein the amounts of the first and cleavage products isolated in step (d) are determined by the limiting amounts of the first and second Cas9-RNA complexes used in step (c).

5. The method of claim 4, wherein the first Cas9-RNA complex cleaves a first amplification product and the second Cas9-RNA complex cleaves a sequence in a second amplification product.

6. The method of claim 4 or 5, wherein the first and second amplification products are made in separate reactions and then pooled together.

7. The method of claim 4 or 5, wherein the first and second amplification products are made in the same reaction.

8. The method of claim 1, 2 or 3, wherein the method further comprising:
 (e) obtaining a second sample comprising nucleic acid;
 (f) tethering at least some of the nucleic acid molecules in the second sample to a support;

(g) reacting the tethered nucleic acid molecules of (f) with the same amount of the first Cas9-RNA complex as used in step (c), thereby cleaving a portion of the tethered nucleic acid molecules that comprise the target

Docket No.: CGLC-034WO

International Application No. PCT/IB2018/050137

sequence and releasing a normalized amount of a second cleavage product into solution; and

(h) isolating the second cleavage product released in step (g);

wherein the amount of the second cleavage product released in step (g) is determined by the amount of the first Cas9-RNA complex used in step (g), and wherein the amounts of the first and second cleavage products isolated in steps (d) and (h) are approximately the same.

9. The method of any preceding claim, further comprising preparing a sequencing library from the released cleavage product.

10. The method of any preceding claim, further comprising ligating an adaptor to the released cleavage product.

11. The method of claim 10, wherein the ligating is done by ligating an adaptor to the ends of the released cleavage product or by tagmenting the cleavage product.

12. The method of any preceding claim, further comprising sequencing the released cleavage product.

13. The method of any preceding claim, further comprising amplifying the released cleavage product.

14. The method of any preceding claim, wherein the nucleic acid in the sample has been enriched from an initial sample.

15. The method of any preceding claim, wherein the nucleic acid molecules are tethered to the support by a covalent bond.

Docket No.: CGLC-034WO
International Application No. PCT/IB2018/050137

16. The method of claim 15, wherein the tethering is done using click chemistry.

17. The method of any preceding claim, wherein the nucleic acid molecules are tethered to the support by a non-covalent bond.

18. The method of claim 17, wherein the tethering is done using a biotin/streptavidin interaction.

19. A kit comprising:

a pair of primers for amplifying a product by PCR, wherein one of the primers comprises an affinity moiety; and

a Cas9-RNA complex that targets a sequence in the primer, in double-stranded form.

20. The kit of claim 19, further comprising a support that binds to or reacts with the affinity moiety.