

challenges relating to the sheer number of different genomes in a given sample and the degree of homology between members, presenting a complex problem for already laborious procedures.

[0005] The existing techniques for detecting sequence variations are particularly ineffective in detecting fusion gene variations and chromosome rearrangements. Often the 'partner' gene fused with the rearranged gene is not known, which makes the detection challenging. Fusions genes may also be difficult to detect if the junction site is not observed.

SUMMARY

[0006] In view of the foregoing, there is a need for alternative and/or robust methods and compositions of detecting rare sequence variations, particularly rare sequence changes and gene fusion events. The compositions and methods of the present disclosure address this need, and provide additional advantages as well. In particular, the various examples of the disclosure provide amplicons containing multiple copies of a target polynucleotide that can be used for massively parallel sequencing methods. Using amplicons containing multiple copies of a target polynucleotide, a target polynucleotide can be sequenced more than once, decreasing the error in sequencing rare sequence variants and fusion genes.

[0006a] In one aspect, provided here is a method for enriching amplicons comprising a concatemer of at least two or more copies of a target polynucleotide, the method comprising: (a) generating a concatemer comprising a single-stranded polynucleotide from a circular target polynucleotide by multiple cycles of amplification with a first primer and polymerase with strand displacement activity, wherein each cycle of the multiple cycles of amplification comprises denaturation at a denaturing temperature, primer annealing at an annealing temperature, and primer elongation at an elongation temperature, and wherein the first primer comprises a first 3' end that specifically hybridizes to the target polynucleotide via sequence complementarity and a first 5' end comprising a first common sequence that does not specifically hybridize to the target polynucleotide via sequence complementarity; (b) generating a plurality of extension products containing one or more copies of the target polynucleotide by extension of a second primer comprising a second 3' end that specifically hybridizes to the concatemer via sequence complementarity and a second 5' end comprising a second common sequence that does not specifically hybridize to the concatemer via sequence complementarity, wherein the first common sequence and the second common sequence each comprise at least 10 contiguous nucleotides at a 5' end and are at least 90% identical when optimally aligned; and

CLAIMS

WHAT IS CLAIMED IS:

1. A method for enriching amplicons comprising a concatemer of at least two or more copies of a target polynucleotide, the method comprising:

(a) generating a concatemer comprising a single-stranded polynucleotide from a circular target polynucleotide by multiple cycles of amplification with a first primer and polymerase with strand displacement activity, wherein each cycle of the multiple cycles of amplification comprises denaturation at a denaturing temperature, primer annealing at an annealing temperature, and primer elongation at an elongation temperature, and wherein the first primer comprises a first 3' end that specifically hybridizes to the target polynucleotide via sequence complementarity and a first 5' end comprising a first common sequence that does not specifically hybridize to the target polynucleotide via sequence complementarity;

(b) generating a plurality of extension products containing one or more copies of the target polynucleotide by extension of a second primer comprising a second 3' end that specifically hybridizes to the concatemer via sequence complementarity and a second 5' end comprising a second common sequence that does not specifically hybridize to the concatemer via sequence complementarity, wherein the first common sequence and the second common sequence each comprise at least 10 contiguous nucleotides at a 5' end and are at least 90% identical when optimally aligned; and

(c) amplifying the plurality of extension products of step (b) under conditions to generate a plurality of amplicons, wherein amplicons comprising at least 2 or more copies of the target polynucleotide are enriched.

2. The method of claim 1, wherein the first common sequence and the second common sequence are identical.

3. The method of claim 1, wherein in the amplifying of step (c) comprises primer extension of a third primer, wherein the third primer comprises a sequence that specifically hybridizes to the first common sequence or the second common sequence via sequence complementarity.

4. The method of claim 1, wherein the amplifying step of (c) yields a percentage of amplicons having two or more copies of the target polynucleotide that is greater than a percentage of amplicons having fewer than two copies of the target polynucleotide.

5. The method of claim 4, wherein the percentage of amplicons having two or more copies of the target polynucleotide is at least 60%.

6. The method of claim 1, wherein the extension products form stem loop structures comprising intramolecular hybridization between (i) the first common sequence and a complement of the second common sequence, or (ii) the second common sequence and a complement of the first common sequence.
7. The method of claim 1, wherein the circular target polynucleotide is a circularized cell free DNA.
8. The method of claim 1, wherein the circular target polynucleotide is a circularized fragment of genomic DNA.
9. The method of claim 1, wherein the circular target polynucleotide is single-stranded.
10. The method of claim 1, further comprising sequencing the plurality of amplicons produced in step (c).
11. The method of claim 1, wherein a plurality of different target polynucleotides are amplified in the same reaction mixture.
12. The method of claim 9, wherein sequencing is performed after selectively purifying amplicons comprising more than one copy of the target polynucleotide from amplicons comprising only one copy of the target polynucleotide.