

CLAIMS

What is claimed is:

1. A system for determining the quality of nucleic acid in a sample comprising:
 - (a) a first set of primers comprising a plurality of first forward primers and a plurality of first reverse primers, each first forward primer and each first reverse primer comprising:
 - (i) a 3' end sequence with complementarity to a repetitive sequence in the nucleic acid and having a first melting temperature; and
 - (ii) a 5' end common sequence not present in the nucleic acid and having a second melting temperature,wherein the second melting temperature is greater than the first melting temperature; and
 - (b) a second set of primers comprising a plurality of second forward primers and a plurality of second reverse primers, each second forward primer and each second reverse primer comprising a 5' end common sequence.
2. The system of primers of claim 1, wherein the second melting temperature is greater than the first melting temperature by about 5 °C to 25 °C.
3. The system of primers of claim 1, wherein the first melting temperature is about 45 °C to 70 °C.
4. The system of primers of claim 1, wherein the second melting temperature is about 60 °C to 85°C.
5. The system of primers of claim 1, wherein the first set of primers comprises an equal number of first forward primers and first reverse primers.
6. The system of primers of claim 1, wherein the first set of primers comprises an unequal number of first forward primers and first reverse primers.
7. The system of primers of claim 1, wherein the second set of primers comprises an equal number of second forward primers and second reverse primers.
8. The system of primers of claim 1, wherein the second set of primers comprises an unequal number of second forward primers and second reverse primers.

9. The system of primers of claim 1, wherein the first set of primers comprises about 1 to 20 first forward primers and about 1 to 20 first reverse primers.
10. The system of primers of claim 1, wherein the second set of primers comprises about 1 to 20 second forward primers and about 1 to 20 second reverse primers.
11. The system of primers of claim 1, wherein the repetitive nucleic acid sequence comprises a retrotransposon.
12. The system of primers of claim 11, wherein the retrotransposon is a L1 retrotransposon.
13. The system of primers of claim 1, wherein the 5' end common sequence of each first forward primer comprises a sequence of SEQ ID NO:1.
14. The system of primers of claim 1, wherein the 5' end common sequence of each first reverse primer comprises a sequence of SEQ ID NO:2.
15. The system of primers of claim 1, wherein the 3' end sequence of each first forward primer comprises a sequence of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:8, or any combination thereof.
16. The system of primers of claim 1, wherein the 3' end sequence of each first reverse primer comprises a sequence of SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:12, or any combination thereof.
17. A method for determining quality of nucleic acid in a sample comprising:
 - (a) preparing a polymerase chain reaction (PCR) mixture comprising:
 - (i) a first set of primers comprising a plurality of first forward primers and a plurality of first reverse primers, each first forward primer and each first reverse primer comprising:
 - (i) a 3' end sequence with complementarity to a repetitive sequence in the nucleic acid and having a first melting temperature; and
 - (ii) a 5' end common sequence not present in the nucleic acid and having a second melting temperature,wherein the second melting temperature is greater than the first melting temperature; and

ii) a second set of primers comprising a plurality of second forward primers and a plurality of second reverse primers, each second forward primer and each second reverse primer comprising a 5' end common sequence;

(a) performing a first polymerase chain reaction (PCR) on the sample, wherein a first elongation step of each cycle in the first PCR is at a temperature of about the first melting temperature;

(b) performing a second polymerase chain reaction (PCR) on the sample, wherein a second elongation step of each cycle in the second PCR is at a temperature of about the second melting temperature; and

(c) determining a size range of amplicons.

18. The method of claim 17, wherein the second melting temperature is greater than the first melting temperature by about 5 °C to 25 °C.
19. The method of claim 17, wherein the first melting temperature is about 45 °C to 70 °C.
20. The method of claim 17, wherein the second melting temperature is about 60 °C to 85°C.
21. The method of claim 17, wherein the first elongation step is at a temperature that is about 5 °C below the first melting temperature to 5 °C above the first melting temperature and that is at least 5 °C below the second melting temperature.
22. The method of claim 17, wherein the second elongation step is at a temperature that is about 5 °C below the second melting temperature to 5.0 °C above the second melting temperature and that is at least 5 °C above the first melting temperature.
23. The method of claim 17, wherein the first elongation step is for about 2 minutes.
24. The method of claim 17, wherein the second elongation step is for about 1 minute.
25. The method of claim 17, wherein the first set of primers comprises an equal number of first forward primers and first reverse primers.
26. The method of claim 17, wherein the first set of primers comprises an unequal number of first forward primers and first reverse primers.
27. The method of claim 17, wherein the second set of primers comprises an equal number of second forward primers and second reverse primers.

28. The method of claim 17 wherein the second set of primers comprises an unequal number of second forward primers and second reverse primers.
29. The method of claim 17, wherein the first set of primers comprises about 1 to 20 first forward primers and about 1 to 20 first reverse primers.
30. The method of claim 17, wherein the second set of primers comprises about 1 to 20 second forward primers and about 1 to 20 second reverse primers.
31. The method of claim 17, wherein a final concentration of second forward primers and second reverse primers is greater than the final concentration of first forward primers and first reverse primers.
32. The method of claim 31, wherein the final concentration of first forward primers is the same or similar to the final concentration of first reverse primers.
33. The method of claim 31, wherein the final concentration of second forward primers is the same or similar to the final concentration of second reverse primers.
34. The method of claim 17, wherein the repetitive nucleic acid sequence comprises a retrotransposon.
35. The method of claim 34, wherein the retrotransposon is a L1 retrotransposon.
36. The method of claim 17, wherein the first PCR comprises about 3 to 5 cycles.
37. The method of claim 17, wherein the second PCR comprises about 10 to 35 cycles.
38. The method of claim 17, wherein the method further comprises determining intensity ratios of amplicons.
39. The method of claim 17, wherein a presence of predicted amplicon sizes correlates with nucleic acid quality.
40. The method of claim 17, wherein a presence of predicted amplicon sizes correlates with nucleic acid size.
41. The method of claim 17, wherein a presence of predicted amplicon intensity ratios correlates with nucleic acid quality.
42. The method of claim 17, wherein a presence of predicted amplicon intensity ratios correlates with nucleic acid size.
43. The method of claim 17, wherein the 5' end common sequence of each first forward primer comprises a sequence of SEQ ID NO:1.

44. The method of claim 17, wherein the 5' end common sequence of each first reverse primer comprises a sequence of SEQ ID NO:2.
45. The method of claim 17, wherein the 3' end sequence of each first forward primer comprises a sequence of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:8, or any combination thereof.
46. The method of claim 17, wherein the 3' end sequence of each first reverse primer comprises a sequence of SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:12, or any combination thereof.