

CLAIMS

What is claimed is:

1. A method for performing PCR, comprising:

(a) providing a PCR mixture in a sample vessel, the PCR mixture having a volume;

5 (b) thermocycling for a first number of cycles, each of the first number of cycles having a first cycle time;

(c) reducing the volume of the PCR mixture in the sample vessel to a second volume, the second volume being smaller than the first volume; and

10 (d) thermocycling for a second number of cycles in the sample vessel, each of the second number of cycles having a second cycle time, the second cycle time being shorter than the first cycle time.

2. The method of claim 1, further comprising:

(e) reducing the volume of the PCR mixture in the sample container to a third volume, the third volume being smaller than the second volume; and

15 (f) thermocycling for a third number of cycles, each of the third number of cycles having a third cycle time, the third cycle time being shorter than the second cycle time.

3. The method of claim 1, wherein the sample vessel is compressible, and the reducing step is performed compressing the sample vessel to expel a portion of the sample from the sample vessel.

20 4. The method of claim 1, wherein the sample vessel is in contact with a first temperature zone and a second temperature zone, and the thermocycling step includes moving the sample between the first and second temperature zones.

5. The method of claim 4, further comprising moving a first portion of the PCR mixture to a second portion of the sample vessel while moving a second portion of the PCR mixture to a first portion of the sample vessel such that the first and second portions of the PCR mixture are under control of the first and second temperature zones simultaneously, wherein the first temperature zone is hotter than the second temperature zone.

25 6. The method of claim 1, wherein the sample vessel is configured to contact a first temperature zone and a second temperature zone, and the thermocycling step

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includes alternately contacting the sample vessel with the first temperature zone and then the second temperature zone.

7. The method of claim 1, wherein the sample vessel is heated by a heater that thermocycles between two or more temperatures, the heater cycling at the first cycle time in step (b) and at the second cycle time at step (d).

8. The method of claim 1, wherein the first number of cycles is 2 to 10 cycles.

9. The method of claim 1, wherein the second volume of the PCR mixture in the sample vessel is 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% or 50% smaller than the first volume.

10. The method of claim 1, wherein the second volume of the PCR mixture in the sample vessel is 50% smaller than the first volume and the second cycle time is 25 to 50% shorter than the first cycle time.

11. The method of claim 1, further comprising reducing the volume of the PCR mixture in the sample vessel 1 to 5 times and reducing the cycle time after each volume reduction.

12. The method of claim 1, wherein the sample vessel is part of a closed sample container having at least one additional fluidly connected sample vessel therein.

13. A method of thermal cycling, comprising:

(a) providing a sample container comprising a first-stage chamber fluidly connected to a second-stage reaction zone, the second-stage reaction zone comprising a plurality of second-stage reaction wells;

(b) introducing a sample into the sample container;

(c) providing an instrument comprising a temperature control element having a first temperature zone and a second temperature zone, wherein the first temperature zone is hotter than the second temperature zone;

(d) aligning the temperature control element and the first-stage chamber so that the first-stage chamber is under temperature control of at least one of the first temperature zone and the second temperature zone to effect thermal cycling of the sample in the first-stage chamber;

(e) after effecting thermal cycling of the sample in the first-stage chamber, moving at least a fraction of a product derived from the sample from the first-stage chamber into the plurality of second-stage reaction wells in the second-stage reaction zone; and

(f) aligning the temperature control element and the second-stage reaction zone to effect thermal cycling of the fraction of the sample in the second-stage reaction zone, wherein aligning the temperature control element and the second-stage reaction zone to effect thermal cycling comprises repeatedly translating the one of the temperature control element or the second-stage reaction zone so that the second-stage reaction zone is under temperature control of the first temperature zone and then the second temperature zone.

14. The method of claim 13, wherein step (f) comprises one of (i) repeatedly translating the temperature control element relative to the second-stage reaction zone or (ii) repeatedly translating the second-stage reaction zone relative to the temperature control element.

15. The method of claim 13, wherein the instrument further comprises a wiper element, and step (d) further comprises aligning the wiper element with the temperature control element and the first-stage chamber such that rotational movement of the wiper element moves a first portion of the sample from thermal control of the first temperature zone to thermal control of the second temperature zone, while simultaneously moving a second portion of the sample from thermal control of the second temperature zone to thermal control of the first temperature zone.

16. The method of claim 13, wherein the steps of the method are completed in 20 minutes or less, 15 minutes or less, or, preferably, 10 minutes or less, and wherein each thermal cycle of the first and second nucleic acid amplification zones is completed in 8 seconds or less, 6 seconds or less, or, preferably, 4 seconds or less.

17. An instrument for thermocycling a sample, the sample provided in a flexible sample container, the instrument comprising:

a first heater adjacent to a first portion of the flexible sample container for adjusting a first portion of the sample to a first temperature;

a second heater adjacent to a second portion of the flexible sample container for adjusting a second portion of the sample to a second temperature, the second temperature different from the first temperature; and

a wiper element configured to move the first portion of the sample to the second portion of the flexible sample container while moving the second portion of the sample to the first

portion of the flexible sample container such that portions of the sample are under control of each of the heaters simultaneously;

wherein the flexible sample container is provided with one or more components for a PCR reaction.

5 18. The instrument of claim 17, wherein the wiper element further comprises a blade that divides the sample into at least two discrete sections comprising at least a first section and a second section, such that the first portion is contained in the first section and the second portion is contained in the second section.

10 19. The instrument of claim 17, wherein the wiper element repeatedly moves portions of the sample to opposite portions of the sample container to thermocycle the sample.

 20. The instrument of claim 17, wherein the wiper element is movable to compress the flexible sample container to expel a portion of the sample, and the wiper element has a first speed prior to expelling the portion of the sample and a second faster speed subsequent to expelling a portion of the sample.