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(54) **Title:** EXTREME PCR

(57) **Abstract:** Methods, devices, and kits are provided for performing PCR in <20 seconds cycle, with improved efficiency and yield.



AMENDED CLAIMS

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1. A method for amplifying a target nucleic acid sequence in a biological sample during amplification comprising the steps of:
 - 5 adding a thermostable polymerase and primers configured for amplification of the target nucleic acid sequence to the biological sample, wherein the polymerase is provided at a concentration of at least 0.5 μM and primers are each provided at a concentration of at least 2 μM ; and
 - 10 amplifying the target nucleic acid sequence by polymerase chain reaction by thermally cycling the biological sample between at least a denaturation temperature and an elongation temperature through a plurality of amplification cycles using an extreme temperature cycling profile wherein each cycle is completed in a cycle time less than 20 seconds per cycle.
2. The method of claim 1, wherein each cycle is completed in less than 2
15 seconds per cycle.
3. The method of claim 1, wherein the ramp rate is at least 200°C/s.
4. The method of claim 3, wherein the ramp rate is at least 300°C/s.
5. The method of claim 4, wherein the ramp rate is at least 400°C/s.
6. The method of claim 1, wherein the plateau is at least 20 cycles after Cp.
- 20 7. The method of claim 1, wherein the concentration for each primer is greater than 2.5 μM .
8. The method of any of claim 1,
further comprising an annealing temperature that is different from the elongation temperature,
25 wherein the amplifying step generates an amplicon greater than 100 bp, and
wherein the extreme temperature cycling profile includes a hold at the elongation temperature of a time about equal to extension length (amplicon length – primer length) in base pairs divided by an extension rate of the thermostable polymerase in bases/second.
9. The method of claim 1, wherein the amplifying step further includes an
30 annealing temperature which is the same or lower than the elongation temperature, and the amplifying step includes a hold of 0.05 to 0.9 seconds at the annealing temperature.
10. The method of claim 1, wherein the Mg^{++} concentration is at least 4 mM.
11. The method of claim 1, wherein the polymerase and primer concentrations

are increased by a factor and the cycle time is divided by the factor.

12. The method of claim 1, wherein the extreme temperature profile includes an annealing time defined by
annealing time = $k_1/[primer]$

5 wherein k_1 is a constant and
[primer] is the concentration of each primer.

13. The method of claim 12, wherein k_1 is experimentally determined.

14. The method of claim 1, wherein the temperature cycling profile includes a time at the elongation temperature defined by
10 elongation time = $k_2(\text{extension length})/([polymerase]*(polymerase\ speed))$
wherein k_2 is a proportionality constant,
[polymerase] is the concentration of the polymerase, and
polymerase speed is a rate of polymerase incorporation of bases in nucleotides/s.

15. The method of claim 14, wherein k_2 is experimentally determined.

15 16. The method of claim 1, wherein the target nucleic acid is amplified with an efficiency of at least 70%.

17. A method for amplifying a target nucleic acid sequence in a biological sample during amplification comprising the steps of:

20 adding a thermostable polymerase and primers configured for amplification of the target nucleic acid sequence to the biological sample, wherein the polymerase to primer ratio is (about 0.03 to about 0.4 polymerase):(total primer concentration), and the polymerase concentration is at least 0.5 μM ; and

25 amplifying the target nucleic acid sequence by polymerase chain reaction by thermally cycling the biological sample between at least a denaturation temperature and an elongation temperature through a plurality of amplification cycles using an extreme temperature cycling profile wherein each cycle is completed in less than 20 seconds.

18. The method of claim 17, wherein each cycle is completed in less than 10 seconds.

30 19. The method of claim 18, wherein each cycle is completed in less than 5 seconds.

20. The method of claim 19, wherein each cycle is completed in less than 2 seconds.

21. The method of claim 17, wherein the target nucleic acid is genomic DNA.
22. The method of claim 17, wherein the biological sample contains eukaryotic genomic DNA.
23. The method of claim 17, wherein the polymerase is KlenTaq provided at a concentration of at least 1.0 μM .
24. The method of claim 17, wherein non-specific amplification is below detectible levels.
25. The method of claim 17, wherein total primer concentration is at least 5 μM .
26. A device for performing PCR comprising;
a sample container for holding a PCR sample;
means for heating the sample;
means for cooling the sample; and
control means for repeatedly subjecting the sample container to the means for heating the sample and the means for cooling the sample to thermal cycle the sample at a ramp rate of at least 200°C/s.
27. The device of claim 26, further comprising means for optically exciting the sample in the monitoring position to cause the sample to fluoresce; and means for detecting the fluorescence of the excited sample during amplification.
28. The device of claim 27, wherein the ramp rate is at least 300°C/s.
29. The device of claim 28, wherein the ramp rate is at least 400°C/s.
30. The device of claim 26, wherein the means for heating is a hot water bath and the means for cooling is a cool water bath.
31. The device of claim 26, wherein the device is a microfluidics system.
32. The device of claim 26, wherein the sample container comprises a target nucleic acid, dNTPs, a polymerase provided at a concentration of at least 0.5 μM , and a pair of primers configured for amplifying the target nucleic acid, wherein the primers are each provided at a concentration of at least 2 μM .
33. A kit for performing PCR on a target nucleic acid, the kit comprising: dNTPs,

a polymerase provided at a concentration of at least 0.5 μM , and
a pair of primers configured for amplifying the target nucleic acid, wherein the
primers are each provided at a concentration of at least 2 μM .

34. The kit of claim 33, further comprising instructions for performing PCR
5 using a temperature cycling profile wherein each cycle is completed in a cycle time less
than 20 seconds per cycle.