

Claims

1. A method of performing a non-isothermal nucleic acid amplification reaction, the method comprising the steps of:
 - 5 (a) mixing a target sequence with one or more complementary single stranded primers in conditions which permit a hybridisation event in which the primers hybridise to the target, which hybridisation event, directly or indirectly, leads to the formation of a duplex structure comprising two nicking sites disposed at or near opposite ends of the duplex; and performing an amplification process by;
 - 10 (b) causing a nick at each of said nicking sites in the strands of the duplex;
 - (c) using a polymerase to extend the nicked strands so as to form newly synthesised nucleic acid, which extension with the polymerase creates nicking sites;
 - 15 (d) repeating steps (b) and (c) as desired so as to cause the production of multiple copies of the newly synthesised nucleic acid;characterised in that the temperature at which the method is performed is non-isothermal, and subject to a reduction of at least 2°C during the amplification process of steps (b)-(d).
- 20 2. A method according to claim 1, wherein in step (a) the target comprises two complementary strands of nucleic acid, and the primer comprises forward and reverse primers which are each complementary to a respective strand of the target, such that the 3' ends of the forward and reverse primers are oriented
25 towards each other.
3. The method according to claim 1 or 2, wherein the temperature is subject to a controlled reduction of at least 5 °C, preferably at least 10°C, more preferably at least 15 °C during the amplification reaction.
- 30 4. The method according to any one of the preceding claims, wherein the average rate of temperature reduction during the amplification reaction is in the range --

-0.10 to $-6.0^{\circ}\text{C min}^{-1}$, preferably -0.20 to $-3.5^{\circ}\text{C min}^{-1}$, more preferably -0.30 to $-3.5^{\circ}\text{C min}^{-1}$, and most preferably -0.40 to $-3.5^{\circ}\text{C min}^{-1}$.

- 5 5. The method according to any one of the preceding claims, wherein steps (b)-(d) are performed substantially immediately after step (a), and wherein steps (a)-(d) are preferably performed in the same reaction vessel or on the same solid support.
- 10 6. The method according to any one of the preceding claims, wherein step (a) is performed at a temperature in the range $55-62^{\circ}\text{C}$, preferably $57-61^{\circ}\text{C}$, more preferably $58-60^{\circ}\text{C}$.
- 15 7. The method according to any one of the preceding claims, further comprising the step of detecting, directly or indirectly, the newly synthesised nucleic acid.
8. The method according to claim 7, wherein said detecting step comprises the use of a molecular beacon or a fluorescent dye, a lateral flow labelled probe, or an enzyme which catalyses an electrochemical reaction.
- 20 9. The method according to any one of the preceding claims, wherein step (b) comprises the use of a nicking enzyme.
- 25 10. The method according to any one of the preceding claims, comprising the use of a first polymerase and/or a first nicking enzyme having an optimum temperature, and a second polymerase and/or a second nicking enzyme having an optimum temperature, wherein the optimum temperature of the second polymerase and/or second nicking enzyme is lower than the optimum temperature of the respective first polymerase and/or first nicking enzyme.
- 30 11. The method according to claim 10, wherein the second polymerase is Bsu polymerase or Klenow fragment of DNA polymerase.

12. The method according to claim 10, wherein the initial temperature of the amplification reaction is at or above the optimum temperature of the first nicking enzyme, and the temperature is reduced during the course of the amplification reaction to a temperature below the optimum temperature of the first nicking enzyme.
13. The method according to claim 10, 11 or 12, wherein the temperature of the amplification reaction is reduced to, or below, the optimum temperature of the second polymerase and/or second nicking enzyme.
14. The method according to any one of the preceding claims, further comprising the step of contacting the mixture obtained by performance of the method with a thermolabile enzyme which degrades nucleic acid, the mixture being contacted with the thermolabile enzyme at a temperature at which the thermolabile enzyme is substantially active.
15. The method according to claim 14, wherein the enzyme is cod uracil-DNA Glycosylase (UDG), or Antarctic thermolabile UDG.
16. The method according to any one of the preceding claims, wherein step (a) is preceded by performing a reverse transcription step, comprising contacting an RNA analyte of interest with a reverse transcriptase so as to form a DNA transcript of the RNA analyte of interest.
17. The method according to claim 16, further comprising the step of making double stranded DNA from the DNA transcript.
18. The method according to any one of the preceding claims, further comprising a pre-amplification or enrichment step.
19. The method according to any one of the preceding claims, wherein one or more of the primers comprises a modified nucleotide.

20. The method according to claim 19, wherein the modified nucleotide is in a target-complementary portion of the primer.
- 5 21. The method according to claim 19 or 20, wherein the one or more primers comprise a 2'-modified nucleotide.
22. The method according to any one of claims 19-21, wherein the one or more primers comprise a 2' O-methyl modified nucleotide.
- 10 23. The method according to claim 22, wherein the one or more primers comprise a plurality of 2' O-methyl modified nucleotides.
24. The method according to claim 23, wherein the one or more primers comprise
15 up to seven 2' O-methyl modified nucleotides.
25. The method according to any one of the preceding claims, wherein the temperature of the reaction during steps (b)-(d) does not return to the temperature at which step (a) is performed.
- 20 26. The method according to any one of the preceding claims, in which the temperature of the reaction does not return to a predetermined temperature.
27. The method according to any one of the preceding claims, wherein one or
25 more of the primers comprises a self-complementary portion.
28. The method according to claim 27, wherein the self-complementary portion forms a hairpin structure.
- 30 29. The method according to claim 28, wherein the hairpin comprises 5 to 10 base pairs.

30. The method according to any one of the preceding claims and substantially as hereinbefore described and with reference to the accompanying drawings.

31. Apparatus for performing the method of any one of the preceding claims, the
5 apparatus comprising temperature regulation means, the programmable control means being programmed to operate the temperature regulation means to perform a temperature reduction of at least 2°C, preferably at least 5°C, during the amplification process of a reaction mixture used to perform the method.