

Claims

1. A method of performing a non-isothermal nucleic acid amplification reaction,
 5 the method comprising the steps of:
 - (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
 10 near opposite ends of the duplex; and performing an amplification process by;
 - (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 recreates 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), and wherein the temperature of the reaction does not return
 20 to a predetermined temperature.
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,
 25 such that the 3' ends of the forward and reverse primers are oriented 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
 30 least 15 °C during the amplification reaction, and wherein the average rate of temperature reduction during the amplification reaction is in the range -0.10 to -6.0°C min⁻¹, preferably -0.20 to -3.5°C min⁻¹, more preferably -0.30 to -3.5°C min⁻¹, and most preferably -0.40 to -3.5°C min⁻¹.

4. 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.
- 5 5. The method according to any one of the preceding claims, wherein step (a) is performed at a temperature in the range 55-62°C, preferably 57-61°C, more preferably 58-60°C.
6. The method according to any one of the preceding claims, further comprising
10 the step of detecting, directly or indirectly, the newly synthesised nucleic acid.
7. The method according to claim 6, 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.
- 15 8. The method according to any one of the preceding claims, wherein step (b) comprises the use of a nicking enzyme.
9. The method according to any one of the preceding claims, comprising the use
20 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, and wherein the second polymerase is
25 preferably Bsu polymerase or Klenow fragment of DNA polymerase.
10. The method according to claim 9, 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
30 reaction to a temperature below the optimum temperature of the first nicking enzyme, and preferably wherein the temperature of the amplification reaction is reduced to, or below, the optimum temperature of the second polymerase and/or second nicking enzyme.

11. 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, and wherein the enzyme is preferably cod uracil-DNA Glycosylase (UDG), or Antarctic thermolabile UDG.
12. 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, optionally further comprising the step of making double stranded DNA from the DNA transcript.
13. The method according to any one of the preceding claims, further comprising a pre-amplification or enrichment step.
14. The method according to any one of the preceding claims, wherein one or more of the primers comprises a modified nucleotide, preferably wherein the modified nucleotide is in a target-complementary portion of the primer.
15. The method according to claim 14, wherein the one or more primers comprise a 2'-modified nucleotide, preferably a 2' O-methyl modified nucleotide, more preferably a plurality of 2' O-methyl modified nucleotides, and most preferably up to seven 2' O-methyl modified nucleotides.
16. 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.
17. The method according to any one of the preceding claims, wherein one or more of the primers comprises a self-complementary portion, which self-

complementary portion typically forms a hairpin structure, and wherein said hairpin structure preferably comprises 5 to 10 base pairs.

18. The method according to any one of the preceding claims, wherein the
5 magnitude of the temperature reduction during steps (b)-(d) is in the range 8-20°C.

19. Apparatus for performing the method of any one of the preceding claims,
the apparatus comprising temperature regulation means and programmable control
means, the programmable control means being programed to operate the
10 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, and wherein the temperature regulation means is
programmed not to return to a predetermined temperature during the amplification
process.

15

20