Annex D

1. A method of sequencing at least two nucleotides of a template nucleic acid by successive cycles of sequencing-by-synthesis comprising repeating the steps of:
    a) incorporating one or more fluorescently labelled nucleotides into a strand of nucleic acid complementary to said template nucleic acid; and
    b) determining the identity of one or more of the incorporated nucleotide(s), wherein the steps of determining the identity of the incorporated nucleotide(s) is carried out in a buffer which comprises ascorbic acid, or a salt thereof.

2. The method according to claim 1, wherein said substrate for incorporation of fluorescently labelled nucleotide is a nucleoside triphosphate.

3. The method according to claim 1 or claim 2, wherein the ascorbic acid or salt thereof is present in the buffer at a concentration of at least 10 mM.

4. The method according to claim 1 or claim 2, wherein the ascorbic acid or salt thereof is present in the buffer at a concentration of at least 20 mM.

5. The method according to claim 1 or claim 2, wherein the ascorbic acid or salt thereof is present in the buffer at a concentration of up to 100 mM.

6. The method according to claim 1, wherein the salt of ascorbic acid is sodium ascorbate.

7. The method according to any one preceding claim wherein the buffer further comprises additional antioxidants.

8. The method according to any one preceding claim wherein the buffer has a pH of about 5.5 to about 8.6.

9. The method according to any one preceding claim wherein the buffer has a pH of about 7.

10. The method according to any one of the preceding claims wherein the template nucleic acid is present in an array.

11. The method according to claim 10 wherein the array is a clustered array.

12. The method according to claim 10 wherein the array is a single molecule array:

13. The method according claim 1, wherein at least 10 nucleotides are successively incorporated and the identity of the base present in each of the incorporated nucleotides is determined.

14. The method according to claim 1, wherein at least 16 nucleotides are successively incorporated and the identity of the base present in each of the incorporated nucleotides is determined.

15. A kit for use in a method according to any one of claims 1 to 14 comprising:

one or more fluorescently labelled nucleotides, wherein the fluorescent label is linked to the nucleotides via a cleavable linker;

an enzyme capable of catalysing incorporation of said nucleotides into a nucleic acid strand complementary to a nucleic acid template to be sequenced; and

a buffer comprising ascorbic acid or a salt thereof, or a supply of ascorbic acid or a salt thereof.