

Claims

1. A method for determining the sequence of a nucleic acid comprising
providing at least one nucleic acid bound to a support;
hybridising at least two primers to the same strand of nucleic acid;
5 performing primer extensions on the said same strand of nucleic acid at each of the at least two
primers;
obtaining signal data corresponding to at least one nucleotide base incorporated at each of the
primer extensions;
determining from said signal data the identity of the nucleotide bases and allocating said bases
10 to an extension read.
2. The method of claim 1, wherein the method comprises contacting the nucleic acid with an
enzyme in the presence of (i) the at least two primers able to hybridise to the same strand of
said nucleic acid at different positions and (ii) four labelled moieties comprising at least one
15 nucleotide analogue selected from dGTP, dCTP, dTTP, dUTP, and dATP, each of the four labelled
moieties having a unique label which is different from the unique labels of the other three
labelled moieties, under conditions permitting hybridisation to give a plurality of extensions
reads, each extension read extending from one of the at least two primers.
- 20 3. The method of claim 2, wherein the method comprises the step of removing unbound labelled
moieties.
4. The method of Claim 2 or 3, wherein the plurality of extension reads comprise substantially
simultaneous extension reads.
- 25 5. The method of any of claims 2 to 4, wherein the four labelled moieties each comprise a single
reversibly terminating nucleotide analogue or reversible terminator analogue selected from
dGTP, dCTP, dTTP, dUTP, and dATP or an oligonucleotide probe comprising at least one
nucleotide analogue selected from dGTP, dCTP, dTTP, dUTP, and dATP .
30 6. The method of any of claims 1 to 5, wherein the enzyme comprises a polymerase or ligase.

7. The method of any preceding claim, wherein the support comprises a chip or bead.
8. The method of any of claims 2 to 7, wherein the unique label comprises a dye, a fluorophore, a chromophore, a combinatorial fluorescence energy transfer tag, a mass tag, or an electrophore.
9. The method of any preceding claim wherein the at least two primers have overlapping sequences.
10. The method of any preceding claim, wherein the at least two primers differ by a single base addition.
11. The method of any preceding claim, wherein the plurality of primers comprise blocked and unblocked primers to chemically differentiate between each extension read.
12. The method of any preceding claim, wherein bioinformatics information is used to allocate the bases to the extension read.
13. The method of any preceding claim, wherein the signal data corresponding to each of the at least two primer extensions is obtained substantially simultaneously.
14. The method of any of claims 2 to 13, wherein the step of determining the identity of the nucleotide bases comprises analysing a signal intensity profile corresponding to the unique labels detected at each of the plurality of extension reads.
15. The method of any preceding claim, wherein the signal data comprises one or more images.
16. The method of claim 15, wherein the signal data is detected as colour signals corresponding to a plurality of nucleotide analogues incorporated on the primer extensions and wherein each colour signal corresponds to a different nucleotide analogue or combination of nucleotide analogues.
17. The method of any preceding claim, wherein the method comprises the step of signal processing comprising one or more of signal deconvolution, signal refinement and signal selection.
18. The method of any preceding claim, wherein multiple base calls are made at each hybridisation cycle.

19. The method of any preceding claim, wherein the step of allocating the bases to an extension read comprises determining the position of the nucleotide bases.
20. The method of any preceding claim, wherein the step of allocating the bases to an extension
5 read comprises providing a preliminary base call and a final base call for each extension read.
21. The method of claim 20, wherein the final base call is provided by comparison of the preliminary base call data with a reference genome.
22. The method of any of claims 2 to 21, wherein the same set of four labelled moieties are used
10 for each extension read.
23. A system for determining the sequence of a nucleic acid comprising sequencing apparatus having a solid support for immobilising at least one nucleic acid and means for providing at least one nucleic acid bound to a support;
hybridising at least two primers to the same strand of nucleic acid;
15 performing at least two primer extensions on the said same strand of nucleic acid at each of the at least two primers;
obtaining signal data corresponding to at least one nucleotide base incorporated at each of the at least two primer extensions;
determining from said signal data the identity of the nucleotide bases and allocating said bases
20 to an extension read.
24. The system of Claim 23, further comprising a signal processor for processing the signal data.
25. A kit for determining the sequence of a nucleic acid comprising sequencing reagents and
25 instructions for performing the method of any of Claims 1 to 22.