

Claims

1. A method for determining the sequence of a nucleic acid comprising:
 - providing at least one nucleic acid bound to a support;
 - contacting the nucleic acid with an enzyme in the presence of (i) at least two primers able to hybridise to the same strand of said at least one nucleic acid at different positions and (ii) four labelled moieties each comprising at least one nucleotide 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;
 - hybridising the at least two primers to the same strand of the nucleic acid;
 - performing primer extensions on said same strand of the nucleic acid at each of the at least two primers to give a plurality of extension reads, each extension read extending from one of the at least two primers, wherein the same set of four labelled moieties are used for each extension read;
 - obtaining signal data corresponding to at least one nucleotide base incorporated at each of the primer extensions; and
 - determining from said signal data the identity of the nucleotide bases and allocating said bases to an extension read; wherein the at least two primers comprise differing levels of locked and unblocked primers such that the plurality of extension reads are chemically differentiated by making one of the reads brighter than the other.
2. The method of claim 1, wherein the method comprises a step of removing unbound labelled moieties.
3. The method of claim 1 or 2, wherein the plurality of extension reads comprise simultaneous extension reads.
4. The method of any of claims 1 to 3, wherein the four labelled moieties each comprise a single reversibly terminating nucleotide or reversible terminator selected from dGTP, dCTP, dTTP, dUTP, and dATP.
5. The method of any of claims 1 to 4, wherein the enzyme comprises a polymerase or ligase.
6. The method of any one of claims 1 to 5, wherein the support comprises a chip or bead.

7. The method of any of claims 1 to 6, wherein the unique label comprises a dye, a fluorophore, a chromophore, a combinatorial fluorescence energy transfer tag, a mass tag, or an electrophore.
8. The method of any of claims 1 to 7, wherein the least two primers have overlapping sequences and/or differ by a single base addition.
9. The method of any of claims 1 to 8, wherein bioinformatics information is used to allocate the bases to the extension read.
10. The method of any of claims 1 to 9, wherein the signal data corresponding to each of the at least two primer extensions is obtained simultaneously.
11. The method of any of claims 1 to 10, 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.
12. The method of any of claims 1 to 11, wherein the signal data comprises one or more images, optionally wherein the signal data is detected as colour signals corresponding to a plurality of nucleotide analogues incorporated in the primer extensions and wherein each colour signal corresponds to a different nucleotide analogue or combination of nucleotide analogues.
13. The method of any of claims 1 to 12, wherein the method comprises a step of signal processing comprising one or more of signal deconvolution, signal refinement and signal selection.
14. The method of any of claims 1 to 13, wherein multiple base calls are made at each hybridisation cycle.
15. The method of any of claims 1 to 14, wherein the step of allocating the bases to an extension read comprises determining the position of the nucleotide bases and/or providing a preliminary base call and a final base call for each extension read, wherein optionally the final base call is provided by comparison of the preliminary base call data with a reference genome.