

CLAIMS

1. A system for determining the sequence of a nucleic acid comprising:
 - i) a sequencing apparatus having: a solid support for immobilising at least one nucleic acid to the solid support, and one or more fluidic components capable of hybridising at least two primers to the same strand of the at least one nucleic acid, wherein the fluidic system is configured to deliver amplification reagents and/or sequencing reagents to perform at least two primer extensions on the said same strand of the at least one nucleic acid at each of the at least two primers; and
 - ii) a signal processor for processing signal data wherein the signal processor is configured to:
 - obtain signal data corresponding to at least one nucleotide base incorporated at each of the at least two primer extensions; and
 - determine from said signal data the identity of the nucleotide bases and allocate the identity of the nucleotide bases to an extension read,
 wherein the at least two primers comprise differing levels of blocked and unblocked primers such that the extension reads are chemically differentiated by making one of the reads brighter than the other.
2. The system of claim 1, wherein the signal data comprises one or more images.
3. The system of claim 2, wherein the signal data is detected as color signals corresponding to a plurality of nucleotide analogues incorporated at the primer extensions and wherein each color signal corresponds to a different nucleotide analogue or combination of nucleotide analogues.
4. The system of any of claims 1-3, wherein the system further comprises means for contacting the at least one 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 each comprising a different

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 extension reads, each extension read extending from one of the at least two primers.

5. The system of claim 4, wherein the four labelled moieties each comprise a reversibly terminating nucleotide analogue.

6. The system of claim 4, wherein determining the identity of the nucleotide bases comprises analyzing a signal intensity profile corresponding to the unique labels detected at each of the extension reads.

7. The system of any of claims 1-6, wherein allocating the identity of the nucleotide bases comprises providing a preliminary base call and a final base call for each extension read.

8. The system of claim 7, wherein the final base call is provided by comparison of the preliminary base call data with a reference genome.

9. The system of any of claims 1-8, wherein allocating the identity of the nucleotide bases comprises determining the position of the nucleotide bases.

10. The system of any of claims 1-9, wherein the processor is configured to determine the distribution of the intensity measurements in the signal data by generating a histogram of intensity data.

11. The system of any of claims 1-10, wherein processing the signal data comprises one or more of signal deconvolution, signal refinement and signal selection.

12. The system of any of claims 1-11, wherein the signal data from each of the at least two primer extensions is obtained simultaneously.

13. The system of any of claims 1-12, wherein the enzyme comprises a polymerase or ligase.

14. The system of any of claims 1-13, wherein the solid support comprises a chip or bead.

15. The system of any of claims 1-14, wherein the unique label comprises a dye, a fluorophore, a chromophore, a combinatorial fluorescence energy transfer tag, a mass tag, or an electrophore.