them) and hence only occurs when the necessary probes have bound to the analyte, thereby lending improved specificity to the detection system.

In PEA, nucleic acid moieties linked to the analyte-binding domains of a probe pair hybridise to one another when the probes are in close proximity (i.e. when bound to a target), and are then extended using a nucleic acid polymerase. The extension product forms a reporter nucleic acid, detection of which demonstrates the presence in a sample of interest of a particular analyte (the analyte bound by the relevant probe pair). In PLA, nucleic acid moieties linked to the analyte-binding domains of a probe pair come into proximity when the probes of the probe pair bind their target, and may be ligated together, or alternatively they may together template the ligation of separately added oligonucleotides which are able to hybridise to the nucleic acid domains when they are in proximity. The ligation product is then amplified, acting as a reporter nucleic acid. Multiplex analyte detection using PEA or PLA may be achieved by including a unique barcode sequence in the nucleic acid moiety of each probe. A reporter nucleic acid molecule corresponding to a particular analyte may be identified by the barcode sequences it contains. The methods of the present invention find particular utility in multiplex PEA and PLA methods.

The methods of the invention may be of utility in at least any field in which proteomics is used, in particular in diagnostics in the context of biomarker identification and quantification. Modern personalised medicine requires the ability to assess large panels of biomarkers, e.g. in the field of oncology. As personalised medicine becomes ever more widespread, the ability to accurately identify and quantify a large number of biomarkers in a sample (across a range of concentrations) is increasing in importance. This need is addressed by the present invention.

Summary

To this end, in one aspect the present disclosure provides a method of detecting multiple non-nucleic acid analytes in a sample of a sample type, said analytes having varying levels of abundance in the sample, said method comprising: (i) providing multiple aliquots from the said sample; and (ii) performing a separate block of detection assays on each of said multiple aliquots, wherein each detection assay block detects a subset of analytes different to the subset which is detected by another detection assay block, and wherein each analyte in each subset of analytes is assigned to the respective detection assay block based on predicted abundance of the analyte,

wherein the predicted abundance of any analyte is predicted based on a normal concentration of that analyte in the same sample type or on measurements obtainable from the used detection assay;

wherein detection assays for analytes which are expected to be present in the sample at a similar concentration are included in the same detection assay block, and wherein detection assays for analytes which are expected to be present in the sample at different concentrations are included in different detection assay blocks;

wherein one or more aliquots are diluted prior to performing the detection assays, by an appropriate dilution factor in view of the predicted abundance of the analytes; and wherein the analytes are detected by detecting analyte-specific reporter nucleic acid molecules generated by the detection assays in each aliquot.

In one example, the disclosure provides a method of detecting an analyte in a sample, wherein the analyte is detected by detecting a reporter nucleic acid molecule specific for the analyte, said method comprising performing a PCR reaction to generate a PCR product of the reporter nucleic acid molecule and detecting said PCR product;

Claims

1. A method of detecting multiple non-nucleic acid analytes in a sample of a sample type, said analytes having varying levels of abundance in the sample, said method comprising:

(i) providing multiple aliquots from the said sample; and

(ii) performing a separate block of detection assays on each of said multiple aliquots, wherein each detection assay block detects a subset of analytes different to the subset which is detected by another detection assay block, and wherein each analyte in each subset of analytes is assigned to the respective detection assay block based on predicted abundance of the analyte,

wherein the predicted abundance of any analyte is predicted based on a normal concentration of that analyte in the same sample type or on measurements obtainable from the used detection assay;

wherein detection assays for analytes which are expected to be present in the sample at a similar concentration are included in the same detection assay block, and wherein detection assays for analytes which are expected to be present in the sample at different concentrations are included in different detection assay blocks;

wherein one or more aliquots are diluted prior to performing the detection assays, by an appropriate dilution factor in view of the predicted abundance of the analytes; and

wherein the analytes are detected by detecting analyte-specific reporter nucleic acid molecules generated by the detection assays in each aliquot.

2. The method of claim 1, wherein:

(a) an aliquot in which analytes are expected to be present at the lowest concentrations is undiluted or has the lowest dilution factor;

(b) an aliquot in which analytes are expected to be present at the highest concentrations has the highest dilution factor; and

(c) an aliquot in which analytes are expected to be present at concentrations in between the lowest and the highest concentrations has a dilution factor in between the lowest and the highest dilution factors.

3. The method of claim 1 or 2, wherein the analyte is or comprises a protein.

4. The method of any one of claims 1 to 3, wherein an analyte is included in more than one subset if it has an expected concentration in between the expected concentrations of two subsets or if it is known to occur in the sample in a wide range of concentrations.

5. The method of any one of claims 1 to 4, wherein the detection assays performed on each aliquot provide a relative or absolute quantitation of each analyte in the subset of analytes assigned to that aliquot.

6. The method of any one of claims 1 to 5, wherein the reporter nucleic acid molecules are amplified by PCR, and preferably are detected by nucleic acid sequencing.

7. The method of claim 6, wherein one or more adaptors for sequencing are added to the reporter nucleic acid molecules in one or more amplification and/or ligation steps.

8. The method of claim 6 or 7, wherein the reporter nucleic acid molecules are subjected to at least a first PCR reaction to add at least a first adaptor for nucleic acid sequencing.

9. The method of claim 8, wherein the PCR products from the first PCR reaction are subjected to a second PCR reaction to add a second adaptor for nucleic acid sequencing.

10. The method of any one of claims 6 to 9, wherein at least one PCR reaction is run to saturation.

11. The method of any one of claims 1 to 10, wherein the reporter nucleic acid molecules, or the amplification products thereof, are pooled to create a first pool, and are amplified in the first pool.

12. The method of claim 11, wherein the method comprises:

amplifying the reporter nucleic acid molecules in first PCR reactions performed separately on each individual aliquot to generate first PCR products, pooling the first PCR products from individual aliquots to create a first pool, and performing a second PCR reaction on the first pool.

13. The method of claim 11 or 12, wherein different amounts of the reporter nucleic acid molecules or amplification products thereof are added to the first pool.

14. The method of any one of claims 11 to 13, wherein the method is performed in parallel for multiple different samples separately to generate reporter nucleic acid molecules , or amplification products thereof, for each sample, and wherein for each sample

a separate first pool is created and a sample index is added to the reporter nucleic acid molecules in the first pool by an amplification and/or ligation reaction.

15. The method of claim 14, wherein the separate first pool created for each sample comprises first PCR products, and wherein a sample index is added to the first PCR products in the second PCR reaction which is performed on the first pool for each sample.

16. The method of claim 14 or 15, wherein the indexed first pools generated for each sample are pooled together to create a second pool for performing nucleic acid sequencing.

17. The method of any one of claims 6 to 16, wherein the PCR reaction comprises an internal control for each aliquot.

18. The method of any one of the preceding claims, wherein the reporter nucleic acid molecule is generated in a proximity probe detection assay, in particular a proximity extension assay (PEA).

19. The method of any one of the preceding claims, wherein the reporter nucleic acid molecule comprises at least one barcode sequence, and detection of the reporter nucleic acid molecule comprises detecting the at least one barcode sequence, optionally in conjunction with a sample index,

preferably wherein the reporter nucleic acid molecule comprises a combination of barcode sequences from the nucleic acid domains of a pair of proximity probes, and detection of the reporter nucleic acid molecule comprises detection of the combination of barcode sequences.

20. The method of any one of the preceding claims, wherein the sample is a plasma or serum sample.

21. The method of any one of claims 18 to 20, wherein the analytes are detected using pairs of proximity probes, each proximity probe comprising:

(i) an analyte-binding domain specific for an analyte; and

(ii) a nucleic acid domain,

wherein both probes within each pair comprise analyte-binding domains specific for the same analyte, and each probe pair is specific for a different analyte, and wherein each probe pair is designed such that on proximal binding of the pair of proximity probes to their

respective analyte the nucleic acid domains of the proximity probes interact to generate a reporter nucleic acid molecule;

wherein at least 2 panels of proximity probe pairs are used, each panel being for the detection of a different group of analytes, and for each panel separate aliquots of the sample are provided for the detection of a different subset of the analytes in the group; and

wherein (a) within each panel, every probe pair comprises a different pair of nucleic acid domains; and (b) in different panels the probe pairs comprise the same pairs of nucleic acid domains.

22. The method of claim 21, for detecting analytes from different samples, wherein the PCR products generated by amplification of the reporter nucleic acid molecules generated for each sample are provided with a sample index;

and wherein the PCR products generated from each different sample using the same panel of proximity probe pairs are pooled into a panel pool for nucleic acid sequencing, the PCR products generated using each panel being pooled into separate panel pools;

and wherein each panel pool is sequenced separately.

23. The method of any one of claims 7 to 22, wherein said nucleic acid sequencing is massively parallel DNA sequencing.