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 ~~of Invention~~

To this end, in ~~a first~~ one aspect the present ~~invention~~disclosure provides a method of detecting multiple analytes in a sample, wherein said analytes have varying levels of abundance in the sample, said method comprising:

(i) providing multiple aliquots from the sample; and

(ii) in each aliquot, detecting a different subset of the analytes by performing a separate multiplex assay for each aliquot, wherein the analytes in each subset are selected based on their predicted abundance in the sample.

In ~~a second aspect~~one example, the ~~invention~~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;

wherein an internal control is provided for the PCR reaction, and said internal control is:

(i) a separate component which is present in a pre-determined amount, and which is, or comprises, or leads to the generation of, a control nucleic acid molecule which is amplified by the same primers as the reporter nucleic acid molecules; and/or

(ii) a unique molecular identifier (UMI) sequence present in each reporter nucleic acid molecule and/or in each control nucleic acid molecule, which is unique to each molecule.

In another example~~a third aspect~~, the ~~invention~~disclosure provides a method of detecting an analyte in a sample, wherein the analyte is detected by detecting a reporter nucleic acid molecule 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, wherein an internal control is included in the PCR reaction and said internal control is present in a pre-determined amount and is, or comprises, or leads to the generation of, a control nucleic acid molecule wherein the control nucleic acid molecule comprises a sequence which is the reverse sequence of the reporter nucleic acid molecule.

Detailed Description

As detailed above, the first aspect of the present invention provides a method for detecting multiple analytes in a sample, wherein the analytes have varying levels of abundance in the sample. The method relies on performing separate sets of assays grouped according to the abundance of the analytes to be assayed.

Accordingly, alternatively viewed, the method as disclosed herein may be defined as a method of detecting multiple analytes in a sample, wherein said analytes have varying levels of abundance in the sample, said method comprising:

performing a separate block of assays on each of separate multiple aliquots from said sample, to detect in each separate aliquot a subset of the analytes, wherein the analytes in each subset are selected based on their predicted abundance in the sample.

Each block of assays performed on an individual aliquot is accordingly a multiplex assay. The multiplex assay to detect multiple analytes in the analyte subset (i.e. the analyte subset designated to be detected in any one particular aliquot) may thus be viewed as an "abundance block". The term "abundance block" as used herein thus refers to a block of assays (or set of assays) performed to detect a particular group, or subset, of the analytes to be detected (i.e. assayed for) in the sample, wherein the analytes are assigned to each block (or set) of assays based on their abundance in the sample, namely their expected or predicted abundance, or relative abundance in the sample. In other words, the assays are grouped, or "blocked" based on abundance. Thus, different aliquots, or different abundance blocks, may be designated for the detection of a particular subset of analytes, based on, for