

Claims

1. A method of detecting multiple analytes in a sample, wherein said analytes have varying levels of abundance in the sample, said method comprising:
 - 5 (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.
- 10 2. The method of claim 1, wherein the analyte is a non-nucleic acid analyte.
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 in each aliquot the analytes are
15 detected by detecting a reporter nucleic acid molecule specific for each analyte.
5. The method of claim 4, wherein the reporter nucleic acid molecules are generated in the multiplex detection assay performed for each aliquot.
- 20 6. The method of claim 4 or 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.
- 25 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.
- 30 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.
- 35 11. The method of any one of claims 1 to 10, wherein the reaction products of the separate multiplex assays or, where said reaction products are nucleic acid molecules,

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 reaction products of the multiplex assays are reporter nucleic acid molecules, and 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.

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13. The method of claim 11 or 12, wherein different amounts of the reaction products 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 reaction products, 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 products 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 claims 4 to 17, 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 claims 4 to 16, 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.

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20. The method of any one of claims 1 to 19, 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
10 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
15 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
20 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
30 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.