Claims

1. A method of detecting DNA sequences from multiple pools, wherein each pool comprises multiple species of DNA molecule, the method comprising:

(i) combining the pools;

(ii) generating multiple linear DNA concatemers of a pre-defined length, wherein each concatemer is generated by joining together one random DNA molecule from each pool in a pre-determined order such that the position of each DNA molecule within the concatemer indicates the pool from which it is derived and each concatemer comprises a pre-determined number of DNA molecules; and

(iii) sequencing the concatemers, thereby detecting a DNA sequence from each pool in each concatemer, wherein the DNA sequence from each pool is assigned to that pool based upon its position within its concatemer.

2. The method of claim 1, wherein the method comprises, prior to step (i), a step of preparing multiple pools of DNA molecules for concatenation, wherein said preparing comprises providing the DNA molecules within each pool with defined end sequences which may be joined in a concatenation step, the DNA molecules in the same pool having the same end sequences and the different pools having different end sequences, such that a DNA molecule from one pool may only be joined to a DNA molecule from one or two pre-determined different pools.

~~3. The method of claim 1 or claim 2, wherein the DNA molecule is an amplicon generated in a DNA amplification reaction.~~

~~4. The method of claim 3, wherein the DNA amplification reaction is a PCR.~~

3~~5~~. The method of any one of claims 1 or 2~~to 4~~, wherein each DNA molecule is a reporter DNA molecule specific for an analyte, and sequencing of each reporter DNA molecule results in detection of the corresponding analyte.

~~6. The method of claim 5, wherein the analyte is or comprises a protein.~~

4~~7~~. The method of claim ~~5 or 6~~3, wherein the reporter DNA molecules are generated by a multiplex detection assay performed on a sample; and

the method comprises performing multiple multiplex detection assays on one or more samples, in order to detect multiple analytes in each sample, and each multiplex detection assay yields a pool of reporter DNA molecules.

~~8. The method of claim 7, wherein the multiplex detection assays comprise a first PCR which generates a first PCR product;~~

~~and wherein the first PCR products are modified by a second PCR, in order to prepare the first PCR products for concatenation, wherein the second PCR generates the pools of DNA molecules.~~

~~9. The method of claim 7 or 8, wherein the detection assay is a proximity extension assay, comprising an extension step that generates the reporter DNA molecules, and an amplification step in which the reporter DNA molecules are amplified, and the extension and amplification steps take place within a single PCR.~~

~~10. The method of claim 9, wherein the multiple multiplex proximity extension assays are performed on the same sample; and~~

~~wherein each proximity extension assay comprises detecting analytes 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 DNA 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 each multiplex proximity extension assay uses one panel of proximity probe pairs;~~

~~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; and~~

~~wherein the product of each panel of proximity probe pairs forms a pool.~~

~~11. The method of any one of claims 7 to 10, wherein the sample is a plasma or serum sample.~~

5~~12~~. The method of any one of claims 1 to 4~~11~~, wherein concatenation is performed by USER assembly or Gibson assembly.

6~~13~~. The method of claim 5~~12~~, wherein the method comprises performing a PCR on each pool using assembly primers, wherein all the DNA molecules in each pool are amplified using the same primer pair, and a different primer pair is used for amplification in each pool, and each species of assembly primer comprises a unique assembly site, such that all the PCR products in each pool comprise a unique pre-defined assembly site at one or both ends; and

wherein in step (ii), the PCR products of each pool are joined to the PCR products of different pools having complementary assembly sites, thereby generating the concatemers.

7~~14~~. The method of claim 6~~13~~, wherein concatenation is performed by USER assembly, and each assembly site comprises multiple uracil residues, preferably at least 3 uracil residues.

8~~15~~. The method of claim 7~~13 or 14~~, wherein the method comprises:

(a) performing multiple multiplex proximity extension assays, thereby generating multiple pools of reporter DNA molecules, wherein the reporter DNA molecules in each pool comprise universal primer binding sites at their 3' and 5' termini;

(b) performing a PCR on each pool using assembly primers comprising assembly sites for USER assembly;

(c) assembling the PCR products from the pools into linear concatemers by USER assembly, the assembly step comprising:

(i) processing the PCR products in each pool to generate 3' overhangs comprising the assembly sites;

(ii) combining the pools; and

(iii) generating the multiple linear DNA concatemers, the PCR products of each pool being joined to the PCR products of different pools having complementary 3' overhangs; and

(d) sequencing the concatemers, thereby identifying the analytes detected in each proximity extension assay; wherein the analytes detected in each proximity extension assay are identified based on the combination of the sequence of each reporter DNA molecule and its position within its concatemer.

9~~16~~. The method of any one of claims 1 to 8~~15~~, wherein the linear DNA concatemers are subjected to a PCR to add at least a first sequencing adaptor and/or at least a first sequencing primer binding site to the concatemers.

10~~17~~. The method of claim 9~~16~~, wherein in the PCR a first sequencing adaptor and/or at least a first sequencing primer binding site is added to one end of the concatemers, and a second sequencing adaptor and/or at least a second sequencing primer binding site is added to the other end of the concatemers.

~~18. The method of any one of claims 1 to 17, wherein the linear DNA concatemers are subjected to a PCR to add at least a first sequencing primer binding site to the concatemers.~~

~~19. The method of claim 18, wherein in the PCR a first sequencing primer binding site is added at one end of the concatemers, and a second sequencing primer binding site is added at the other end of the concatemers.~~

11~~20~~. The method of any one of claims 1 to 10~~19~~, wherein:

(I) multiple sets of pools are individually combined and a separate concatenation reaction performed for each set of pools, yielding multiple concatenation reaction products;

(II) a unique index sequence is added to each concatenation reaction product by PCR;

(III) the concatenation reaction products are combined; and

(IV) the concatemers are sequenced, and the index sequence identifies the set of pools from which each concatemer originates.

12~~21~~. The method of claim 11~~20~~, wherein in the PCR a first index sequence is added at one end of the concatemers, and a second index sequence is added at the other end of the concatemers.

13~~22~~. The method of claim 12~~21~~, wherein the concatemers are subjected to a single PCR, in which a sequencing adaptor, a sequencing primer binding site and an index sequence are added to both ends of each concatemer.

14~~23~~. The method of claim 13~~22~~, wherein the PCR to which the concatemers are subjected yields products comprising, at each end, from 5' to 3', a sequencing adaptor, a sequencing primer binding site and an index sequence.

~~24. The method of any one of claims 7 to 9, wherein the multiple analytes have a range of levels of abundance in the sample, and the method comprises:~~

~~(i) providing multiple aliquots from the sample;~~

~~(ii) in each aliquot, detecting a different subset of the analytes by performing a separate multiplex detection assay for each aliquot, and generating a first PCR product from each aliquot, wherein the analytes in each subset are selected based on their predicted abundance in the sample;~~

~~(iii) combining the first PCR products into multiple pools; and~~

~~(iv) performing the second PCR to modify the first PCR products, to prepare the first PCR products for concatenation.~~

~~25. The method of claim 24, wherein the method is performed as defined in claim 10, and multiple aliquots are provided from the sample for each panel of proximity probe pairs, the first PCR products from each panel are combined and the second PCR performed, thereby yielding a pool from each panel of the sample.~~

~~26. The method of any one of claims 1 to 25, wherein all the steps prior to sequencing are performed in the same buffer.~~

~~27. The method of any one of claims 1 to 26, wherein the concatemers are sequenced by massively parallel DNA sequencing.~~

15~~28~~. A kit comprising:

(i) multiple proximity probe pairs, wherein each proximity probe ~~and proximity probe pair are as defined in claim 10~~ comprises:

(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 DNA molecule, and wherein in each pair one proximity probe comprises a nucleic acid domain comprising a first universal primer binding site and a barcode sequence 3' thereof, and the other proximity probe comprises a nucleic acid domain comprising a second universal primer binding site and a barcode sequence 3' thereof;

(ii) a first primer pair, wherein the primers are designed to bind the first and second universal primer binding sites;

(iii) a set of assembly primer pairs suitable for preparing DNA molecules for directed assembly by USER assembly or Gibson assembly into a linear concatemer, wherein each primer comprises, from 5' to 3', an assembly site and a hybridisation site, and in each primer

pair the hybridisation sites are designed to bind the first and second universal primer binding sites;

(iv) enzymes suitable for assembling DNA fragments by USER assembly or Gibson assembly, wherein the enzymes are suitable for use in the same means of DNA assembly as the assembly primer pairs; and

(v) a second primer pair, wherein each primer comprises a sequencing adaptor, a sequencing primer binding site, an index sequence and a hybridisation site, wherein the hybridisation sites are designed to bind the assembly sites of the assembly primers designed to form the ends of the linear concatemer;

and wherein the first primer in the pair comprises a first sequencing adaptor, a first sequencing primer binding site and a first index sequence, and the second primer in the pair comprises a second sequencing adaptor, a second sequencing primer binding site and a second index sequence.

1629. The kit of claim 1528, wherein each primer in the second primer pair comprises, from 5' to 3', the sequencing adaptor, the sequencing primer binding site, the index sequence and the hybridisation site.

1730. The kit of claim 1528 or 1629, wherein the assembly primers and the enzymes are suitable for preparing DNA molecules for assembly and assembling DNA molecules by USER assembly, and wherein the each assembly primer comprises an assembly site comprising multiple uracil residues, preferably at least 3 uracil residues.

1831. The kit of any one of claims 1528 to 1730, wherein the kit further comprises a reaction buffer suitable for use as defined in claim 26.

1932. The kit of any one of claims 1528 to 1831, further comprising a DNA polymerase and a dNTP mix.