

Claims

1. A method for identifying DNA double-strand breaks (DSBs) in a nucleic acid sample comprising

5 i) exposing a sample of nucleic acid suspected of containing DSBs, under ligation conditions, to a first pair of oligonucleotides a first one of which comprises a 5' binding feature that enables ligation of said oligonucleotide to a first strand of said DSB, a hybridization site (RD1 SP) to which a first sequencing primer can bind and a binding sequence for separating said
10 DSB from a pool of DSBs; and a second oligonucleotide that is complementary to said first oligonucleotide of the first pair and comprises a 3' binding feature that enables ligation of said oligonucleotide to a second strand of said DSBs; wherein either or both of said oligonucleotides comprise, a 3' and/or 5' protective feature;

15 ii) fragmenting the nucleic acid of said sample into fragments;

iii) exposing said fragments, under ligation conditions, to a second pair of oligonucleotides a first one of which comprises a 5' binding feature, that enables ligation of said oligonucleotide to a first strand of said fragmented nucleic acid and a hybridization site (RD2 SP) to which a second sequencing primer can bind; and a second longer oligonucleotide that is in
20 part complementary to said first oligonucleotide of the second pair and comprises a 3' binding feature for binding to a second strand of said fragmented nucleic acid, a sequence complimentary to said hybridization site, and a further sequence that is, optionally, a binding sequence for enabling bridge amplification; and wherein either or both of said
25 oligonucleotides comprise a 3' and/or 5' protective feature;

iv) denaturing the fragments to provide single strand nucleic acids;

v) separating the strands of part iv) into two groups: group A those fragments that have ligated at a first end the first hybridization site and binding
30 sequence provided by the oligonucleotide of part i) and at another end the second hybridization site and further sequence provided by the oligonucleotide of part iii) and group B those fragments that do not have ligated at a first end the hybridization site and binding sequence provided

- by the oligonucleotide of part i) and at another end the second hybridization site and further sequence provided by the oligonucleotide of part iii); and
- vi) sequencing the strands of group A using primers that bind to the first and/or second hybridization sites where each sequence is equivalent to a DSB break and further wherein the number and nature of base pair deletions can be determined by comparing each sequence with a genome representative of said species from which the sample was taken.
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2. The method of claim 1, wherein the oligonucleotide of the second pair that comprises a 5' binding feature does not comprise a binding sequence for separating said fragmented nucleic acid.
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3. The method of claim 1 or claim 2 wherein the oligonucleotides of part i) and part iii) are interchanged whereby, after fragmenting in step ii), the nucleic acid is first exposed to the oligonucleotides of part iii) and the nucleic acid is then exposed to the oligonucleotides of part i).
- 15
4. The method of any one of claims 1 to 3 wherein said nucleic acid sample is gDNA.
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5. The method according to any one of the preceding claims wherein said 5' and/or 3' binding feature comprises one of the following: a phosphate group; a triphosphate 'T-tail', preferably a deoxythymidine triphosphate 'T-tail'; a triphosphate 'A-tail', preferably a deoxyadenosine triphosphate 'A-tail'; at least one random N nucleotide, and a plurality of N nucleotides.
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6. The method according to any one of the preceding claims wherein said 5' and/or 3' protective feature comprises a feature that provides resistance to any one or more of the following: phosphorylation activity, phosphatase activity, terminal transferase activity, nucleic acid hybridization, endonuclease activity, exonuclease activity, ligase activity, polymerase activity, and protein binding.
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7. The method according to claim 6 wherein said protective features comprises a phosphorothioate linkage, a dideoxynucleotide or a covalent block, a phosphoramidite, a C3 Spacer phosphoramidite (3SpC3).

5 8. The method according to any one of the preceding claims wherein said 5' binding feature of said first oligonucleotide of part i) is a phosphate group and said 3' binding feature of said second oligonucleotide of part i) is a triphosphate tail.

10 9. The method according to any one of the preceding claims wherein said first and second oligonucleotides of part i) also comprise an index feature that is a particular sequence of nucleotides that enables the origin of pooled samples to be determined.

15 10. The method according to any one of the preceding claims wherein said first oligonucleotide of part i), reading 5' to 3', comprises a 5' binding feature and then, optionally, a protective feature, a hybridization site (RD1 SP) to which a sequencing primer can bind, an index sequence, a binding sequence for separating said DSB from a pool of DSBs, and a 3' binding and/or protective
20 feature.

11. The method according to claim 10 wherein said binding feature is a phosphate group.

25 12. The method according to any one of the preceding claims wherein said second oligonucleotide of part i), reading 3' to 5', comprises a 3' binding feature and then, optionally, a protective feature, a hybridization sequence (RD1 SP) to which a sequencing primer can bind, an index sequence, a binding sequence for separating said DSB from a pool of DSBs and a 5' binding and/or protective
30 feature.

13. The method according to claim 12 wherein the 3' binding feature comprises a 3' deoxythymidine triphosphate 'T-tail' and also a phosphorothioate linkage.

14. The method according to any one of the preceding claims wherein either the first and/or second oligonucleotide of the first oligonucleotide pair of part i) comprises two different terminal protective features.

15. The method according to any one of the preceding claims wherein said 5' binding feature of said first oligonucleotide of the second oligonucleotide pair of part iii) is a phosphate group and said 3' binding feature of said second oligonucleotide of part iii) is a triphosphate tail.

16. The method according to any one of the preceding claims wherein said first and second oligonucleotides of part iii) also comprise an index feature that is a particular sequence of nucleotides that enables the origin of pooled samples to be determined.

17. The method according to any one of the preceding claims wherein said second oligonucleotide of part iii), reading 5' to 3' comprises a 5' binding feature and then, optionally, a protective feature, a further sequence for, optionally, enabling bridge amplification, an index sequence, a hybridization site (RD2 SP) to which a sequencing primer can bind, and a 3' binding and/or protective feature.

18. The method according to any one of the preceding claims wherein either the first or second oligonucleotide of this second oligonucleotide pair of part iii) comprises two different terminal protective features.

19. The method according to any one of the preceding claims wherein said oligonucleotides of part i) comprises a first oligonucleotide having SEQ ID NO. 1 and a second oligonucleotide having SEQ ID NO. 2; or an oligonucleotide that shares at least 80% identity or homology with SEQ ID NO. 1 or 2.

20. The method according to any one of the preceding claims wherein said second pair of oligonucleotides of part iii) comprises a first oligonucleotide having SEQ ID NO. 3 and a second oligonucleotide having SEQ ID NO. 4; or an oligonucleotide that shares at least 80% identity or homology with SEQ ID NO. 3 or 4.

21. The method according to any one of the preceding claims wherein said second oligonucleotide of said second pair of oligonucleotides of part iii) comprises any one of the following sequences; SEQ ID NOs. 4 – 28; or an oligonucleotide that shares at least 80% identity or homology with one of SEQ ID NO. 4-28.

22. The method according to any one of the preceding claims wherein said sample is mammalian or human.

23. The method according to any one of the preceding claims wherein said ligation in part i) occurs *in situ* or *in vitro* using a cell or tissue sample.

24. The method according to any preceding claim wherein said sample is exposed to a permeabilizing agent before step i) is undertaken.

25. The method according to any one of the preceding claims wherein said sample is exposed to at least one agent for performing arginine tail repair before step i) is undertaken.

26. The method according to any one of the preceding claims wherein part i) also includes extracting gDNA from said sample prior to performing the subsequent steps.

27. The method according to any one of the preceding claims wherein said method further comprises after part ii) and/or part iv), removing fragments whose size is less than about 100bp, or less than about 150bp, and/or retaining fragments whose size is greater than about 150bp.

28. The method according to any one of the preceding claims wherein said separating of part v) involves using said binding sequence provided by the oligonucleotide of part i) to bind a partner and so separate the Group A strands of part iv) from any other strands.

29. The method of claim 28 wherein a complementary binding strand to said binding sequence provided by the oligonucleotide of part i) is anchored to a substrate and said single strands of nucleic acids flow by, or over, the anchored complementary binding strand.

30. The method according to any one of the preceding claims wherein part vi) involves bridge amplification where the single strands separated under part v) are clonally amplified on a substrate that has anchored thereon oligonucleotides/binding sites for the binding sequence of the first oligonucleotide of part i) and the further sequence of the second oligonucleotide of part iii).

31. The method according to any one of the preceding claims wherein prior to performing the claimed method, a sample containing or suspected of containing, a single strand break is ligated or broken to ensure the single strand break is converted into a double strand break.

32. A kit of parts for identifying DNA double-strand breaks (DSBs) in a gDNA sample comprising

- i) a first pair of oligonucleotides a first one of which comprises a 5' binding feature that enables ligation of said oligonucleotide to a first strand of said DSB, a hybridization site (RD1 SP) to which a first sequencing primer can bind and a binding sequence for separating said DSB from a pool of DSBs; and a second oligonucleotide that is complementary to said first oligonucleotide of this first pair and comprises a 3' binding feature for binding to a second strand of said DSBs; and wherein either or both of said oligonucleotides comprise a 3' and/or 5' protective feature; and

- 5 ii) a second pair of oligonucleotides a first one of which comprises a 5' binding feature, that enables ligation of said oligonucleotide to a first strand of said DSB, and a hybridization site (RD2 SP) to which a second sequencing primer can bind; and a second longer oligonucleotide that is in part complementary to said first oligonucleotide of this second pair and comprises a 3' binding feature for binding to a second strand of said DSBs, a sequence complimentary to said hybridization site, and a further sequence which is, optionally, a binding sequence for enabling bridge amplification; and wherein either or both of said oligonucleotides comprise a 3' and/or 5' protective feature.
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33. A kit for sample preparation for identifying DSBs in a gDNA sample, comprising

- 15 i) a first pair of oligonucleotides, a first one of which comprises a 5' binding feature that enables ligation of said oligonucleotide to a strand of a double-stranded nucleic acid, and comprises a sequence according to TCGGTGGTCGCGTATCATT (SEQ ID NO: 31); and a second oligonucleotide that is complementary to said first oligonucleotide of the first pair; and wherein either or both of said oligonucleotides comprise, respectively, a 3' and/or 5' protective feature; and
- 20 ii) a second pair of oligonucleotides, a first one of which does not comprise a sequence of more than 5, 10, 15, or 20 bases, or does not comprise all 24 bases, of the sequence
- 25 ATCTCGTATGCCGTCTTCTGCTTG (SEQ ID NO: 30); and a second oligonucleotide that comprises a 3' binding feature that enables ligation of said oligonucleotide to a strand of a double-stranded nucleic acid and comprises a sequence according to
- 30 CAAGCAGAAGACGGCATAACGAGAT (SEQ ID NO: 32); and wherein either or both of said oligonucleotides comprise, respectively, a 3' and/or 5' protective feature.

34. A kit for sample preparation for identifying DSBs in a gDNA sample, comprising

- i) a first pair of oligonucleotides, a first one of which comprises a 5' binding feature that enables ligation of said oligonucleotide to a strand of a double-stranded nucleic acid, and comprises a sequence according to ATCTCGTATGCCGTCTTCTGCTTG (SEQ ID NO: 30);
5 and a second oligonucleotide that is complementary to said first oligonucleotide of the first pair; and wherein either or both of said oligonucleotides comprise, respectively, a 3' and/or 5' protective feature; and
- ii) a second pair of oligonucleotides, a first one of which does not
10 comprise a sequence of more than 5, 10, or 15 bases, or does not comprise all 20 bases, of the sequence TCGGTGGTCGCCGTATCATT (SEQ ID NO: 31); and a second oligonucleotide that comprises a 3' binding feature that enables
15 ligation of said oligonucleotide to a strand of a double-stranded nucleic acid and comprises a sequence according to AATGATACGGCGACCACCGA (SEQ ID NO: 34); and wherein either or both of said oligonucleotides comprise, respectively, a 3' and/or 5' protective feature.

20 35. The kit according to claim 33 or 34, wherein the first oligonucleotide of the first pair and/or the second pair of oligonucleotides comprises a hybridization site to which a first sequencing primer can bind.

25 36. The kit according to any one of claims 32 or 35 wherein said first and second oligonucleotides of part i) and/or part ii) also comprise an index feature that is a particular sequence of nucleotides that enables the origin of pooled samples to be determined.

30 37. The kit according to any one of claims 32, 35, or 36, wherein said kit further comprises at least one primer that bind to the first and/or second hybridization sites for the purpose of sequencing.

38. The kit according to claims 32 to 37, wherein said kit further comprises fragmenting agents and/or denaturing agents for fragmenting and/or denaturing the nucleic acid into fragments and/or single strands, respectively.

5 39. A double strand adaptor for identifying DNA double-strand breaks (DSBs) in a nucleic acid sample, such as a gDNA sample, comprising:

a first oligonucleotide strand comprising a 5' binding feature that enables ligation of said oligonucleotide to a first strand of said DSB, a hybridization site (RD1 SP) to which a sequencing primer can bind and a binding sequence for separating said DSB from a pool of DSBs; and a second
10 oligonucleotide strand that is complementary to said first oligonucleotide and comprises a 3' binding feature for binding to a second strand of said DSBs; and wherein either or both of said oligonucleotides comprise a 3' and/or 5' protective feature.

15 40. A double strand adaptor for identifying DNA double-strand breaks (DSBs) in a nucleic acid sample, such as a gDNA sample, comprising:

a first oligonucleotide strand comprising a 5' binding feature, that enables ligation of said oligonucleotide to a first strand of said DSB, and a
20 hybridization site (RD2 SP) to which a sequencing primer can bind; and a second longer oligonucleotide strand that is in part complementary to said first oligonucleotide and comprises a 3' binding feature for binding to a second strand of said DSBs, a sequence complimentary to said hybridization site, and a further sequence, which is, optionally, a binding
25 sequence for enabling bridge amplification; and wherein either or both of said oligonucleotides comprise a 3' and/or 5' protective feature.

30 41. The adaptor of claim 39 or 40 wherein said first and second oligonucleotides also comprise an index feature that is a particular sequence of nucleotides that enables the origin of pooled samples to be determined.

42. A double strand adaptor for identifying DNA double-strand breaks (DSBs) in a nucleic acid sample, comprising:

a first oligonucleotide strand which does not comprise a sequence of more than 5, 10, 15, or 20 bases, or does not comprise all 24 bases, of the sequence ATCTCGTATGCCGTCTTCTGCTTG (SEQ ID NO: 30); and a second oligonucleotide that comprises a 3' binding feature that enables ligation of said oligonucleotide to a strand of a double-stranded nucleic acid and comprises a sequence according to CAAGCAGAAGACGGCATACGAGAT (SEQ ID NO: 32); and wherein either or both of said oligonucleotides comprise, respectively, a 3' and/or 5' protective feature.

43. A double strand adaptor for identifying DNA double-strand breaks (DSBs) in a nucleic acid sample, comprising:

a first oligonucleotide strand which does not comprise a sequence of more than 5, 10, or 15 bases, or does not comprise all 20 bases, of the sequence TCGGTGGTCGCCGTATCATT (SEQ ID NO: 31); and a second oligonucleotide that comprises a 3' binding feature that enables ligation of said oligonucleotide to a strand of a double-stranded nucleic acid and comprises a sequence according to AATGATACGGCGACCACCGA (SEQ ID NO: 34); and wherein either or both of said oligonucleotides comprise, respectively, a 3' and/or 5' protective feature.

44. A method of sample preparation for identifying DNA double-strand breaks (DSBs) in a nucleic acid sample, wherein the preparation comprises modifying DSB-associated nucleic acids to be suitable for binding to a substrate comprising immobilised primers, the method comprising:

- a) providing a sample comprising a plurality of nucleic acids;
- b) exposing the plurality of nucleic acids to a first adaptor under conditions conducive to ligation, wherein the first adaptor comprises an oligonucleotide capable of being ligated to a 3' terminus of a strand of a DSB and which comprises a sequence that is capable of binding to a primer immobilised to the substrate by hybridisation;
- c) fragmenting the plurality of nucleic acids; and

d) exposing the plurality of nucleic acids to a second adaptor under conditions conducive to ligation, wherein the second adaptor comprises an oligonucleotide capable of being ligated to a 5' terminus of a strand at a break induced by fragmentation, but is not capable of being ligated to the first adaptor, and which does not comprise a sequence that is capable of binding to a primer immobilised to the substrate by hybridisation.

45. The method of claim 44, wherein the oligonucleotide of step d) comprises a sequence identical to a region of a second primer.

46. The method of claim 44 or 45, wherein
the substrate comprises a first and a second immobilised primer;
the oligonucleotide of step b) comprises a sequence that is capable of binding to the first immobilised primer by hybridisation; and
the oligonucleotide of step d) comprises a sequence identical to a region of the second immobilised primer.

47. The method of any one of claims 44 to 46, wherein step b) is:

exposing the plurality of nucleic acids to a first adaptor pair under conditions conducive to ligation, wherein the first adaptor pair is capable of being ligated to at least a 3' terminus of a strand of a DSB, and wherein the first adaptor pair comprises first and second oligonucleotides that are at least partially complementary, and the first oligonucleotide is ligatable to a 3' terminus and comprises a sequence that is capable of binding to a primer immobilised to the substrate by hybridisation; and

wherein step d) is:

exposing the plurality of nucleic acids to a second adaptor pair under conditions conducive to ligation, wherein the second adaptor pair is capable of being ligated to at least a 5' terminus of a strand at a break induced by fragmentation but is not capable of being ligated to the first oligonucleotide of the first adaptor pair, wherein the second adaptor

comprises first and second partially complementary oligonucleotides, and the first oligonucleotide is ligatable to a 5' terminus and comprises a sequence identical to a region of a second primer, and the second oligonucleotide does not comprise a sequence that is complementary to said sequence identical to a region of the second primer.

48. The method of any one of claims 44 to 47, wherein the second adaptor pair comprises:

a first oligonucleotide comprising a sequence according to CAAGCAGAAGACGGCATACGAGAT (SEQ ID NO: 32), and a second oligonucleotide comprising a sequence that does not comprise a sequence of more than 5, 10, 15, or 20 bases, or does not comprise all 24 bases, of the sequence ATCTCGTATGCCGTCTTCTGCTTG (SEQ ID NO: 30); or

a first oligonucleotide comprising a sequence according to AATGATACGGCGACCACCGA (SEQ ID NO: 34), and a second oligonucleotide comprising a sequence that does not comprise a sequence of more than 5, 10, or 15 bases, or does not comprise all 20 bases, of the sequence TCGGTGGTCGCCGTATCATT (SEQ ID NO: 31).

49. The method of claim 47 or claim 48, wherein the first and/or the second oligonucleotide of the first adaptor pair comprises a 3' and/or 5' protective feature; and/or wherein the first and/or the second oligonucleotide of the second adaptor pair comprises a 3' and/or 5' protective feature.

50. The method of any one of claims 44 to 49, wherein the second adaptor is not capable of being ligated to the first adaptor due to the presence of a 3' modification of the first adaptor.

51. The method of any one of claims 44 to 50, wherein the oligonucleotide of the second adaptor that is ligatable to a 5' terminus comprises a sequence identical to 5, 10, 15, 20, 21, 24, or more bases of an immobilised primer.

52. The method of any one of claims 44 to 51, further comprising denaturing the plurality of nucleic acids to form a plurality of single-stranded nucleic acids.

53. The method of any one of claims 44 to 52, further comprising contacting the plurality of nucleic acids with the substrate comprising immobilised primers under conditions suitable for hybridisation of the immobilised primers to complementary nucleic acids.

54. The method of claim 53, further comprising obtaining sequence information for any nucleic acids hybridised to the substrate.

55. The method of any one of claims 44 to 54, wherein said sample comprising a plurality of nucleic acids is gDNA.

56. The method of any one of claims 44 to 55, wherein:
the steps are performed in the order a), b), c), and then d); or
the steps are performed in the order a), c), d), and then b); wherein the sample is exposed to conditions capable of causing or suspected of being capable of causing a DSB between steps d) and b).

57. The method of claim 56, wherein the sample is exposed to conditions capable of causing a DSB at a feature of interest in the nucleic acid sample.

58. A method of sample preparation for identifying DNA double-strand breaks (DSBs) in a nucleic acid sample, wherein the preparation comprises modifying DSB-associated nucleic acids to be suitable for binding to a substrate comprising an immobilised first primer, the method comprising:

- 1) providing a sample comprising a plurality of nucleic acids;
- 2) exposing the plurality of nucleic acids to a first adaptor under conditions conducive to ligation, wherein the first adaptor comprises an oligonucleotide capable of being ligated to a 3' terminus of a strand of a DSB and which comprises a sequence that is capable of hybridising to a second primer;
- 3) fragmenting the plurality of nucleic acids;
- 4) exposing the plurality of nucleic acids to a second adaptor under conditions conducive to ligation, wherein the second adaptor comprises

an oligonucleotide capable of being ligated to a 5' terminus of a strand at a break induced by fragmentation, but is not capable of being ligated to the first adaptor, and which comprises a sequence identical to a region of the immobilised first primer; and

5) contacting the plurality of nucleic acids with the second primer under conditions suitable for extension of the primer.

59. The method of claim 58,
wherein step 2) is:

exposing the plurality of nucleic acids to a first adaptor pair under conditions conducive to ligation, wherein the first adaptor pair is capable of being ligated to at least a 3' terminus of a strand of a DSB, and wherein the first adaptor pair comprises first and second oligonucleotides that are at least partially complementary, and the first oligonucleotide is ligatable to a 3' terminus and comprises a sequence that is capable of hybridising to a second primer; and

wherein step 4) is:

exposing the plurality of nucleic acids to a second adaptor pair under conditions conducive to ligation, wherein the second adaptor pair is capable of being ligated to at least a 5' terminus of a strand at a break induced by fragmentation but is not capable of being ligated to the first oligonucleotide of the first adaptor pair, wherein the second adaptor comprises first and second partially complementary oligonucleotides, and the first oligonucleotide is ligatable to a 5' terminus and comprises a sequence identical to a region of the immobilised first primer, and the second oligonucleotide does not comprise a sequence that is complementary to said sequence identical to a region of the immobilised first primer.

60. The method of claim 58 or claim 59, wherein the second adaptor pair comprises:

a first oligonucleotide comprising a sequence according to AACCCACTACGCCTCCGCTTTCC (SEQ ID NO: 40); and
a second oligonucleotide that does not comprise a sequence of more

than 5, 10, 15, 20 bases, or does not comprise all 22 bases, of the sequence GGAAAGCGGAGGCGTAGTGGTT (SEQ ID NO: 36).

61. The method of claim 59 or claim 60, wherein the first and/or the second oligonucleotide of the first adaptor pair comprises a 3' and/or 5' protective feature; and/or wherein the first and/or the second oligonucleotide of the second adaptor pair comprises a 3' and/or 5' protective feature.
62. The method of any one of claims 58 to 61, wherein the second adaptor is not capable of being ligated to the first adaptor due to the presence of a 3' modification of the first adaptor.
63. The method of any one of claims 58 to 62, wherein the oligonucleotide of the second adaptor that is ligatable to a 5' terminus comprises a sequence identical to 5, 10, 15, 20, 21, 24, or more bases of an immobilised primer.
64. The method of any one of claims 58 to 63, further comprising denaturing the plurality of nucleic acids to form a plurality of single-stranded nucleic acids.
65. The method of any one of claims 58 to 64, further comprising contacting the plurality of nucleic acids with the substrate comprising the immobilised first primer under conditions suitable for hybridisation of the immobilised first primer to complementary nucleic acids.
66. The method of claim 65, further comprising obtaining sequence information for any nucleic acids hybridised to the substrate.
67. The method of any one of claims 58 to 66, wherein said sample comprising a plurality of nucleic acids is gDNA.
68. The method of any one of claims 58 to 67, wherein:
the steps are performed in the order 1), 2), 3), 4), and then 5); or
the steps are performed in the order 1), 3), 4), 2), and then 5); wherein
the sample is exposed to conditions capable of causing or suspected of being

capable of causing a DSB between steps 4) and 2).

69. The method of claim 68, wherein the sample is exposed to conditions capable of causing a DSB at a feature of interest in the nucleic acid sample.

70. A method of sample preparation for identifying a feature of interest in a nucleic acid sample, wherein the preparation comprises modifying nucleic acids associated with a feature of interest to be suitable for binding to a substrate comprising immobilised primers, the method comprising:

- a) providing a sample comprising a plurality of nucleic acids, exposing the plurality of nucleic acids to conditions capable of cleaving at least one strand of a nucleic acid at a feature of interest, and denaturing the plurality of nucleic acids into single-stranded nucleic acids;
- b) exposing the plurality of nucleic acids to a first adaptor under conditions conducive to ligation, wherein the first adaptor comprises an oligonucleotide capable of being ligated to a 3' terminus of a strand of a cleavage site and which comprises a sequence that is capable of binding to a primer immobilised to the substrate by hybridisation;
- c) fragmenting the plurality of nucleic acids; and
- d) exposing the plurality of nucleic acids to a second adaptor under conditions conducive to ligation, wherein the second adaptor comprises an oligonucleotide capable of being ligated to a 5' terminus of a strand at a break induced by fragmentation, but is not capable of being ligated to the first adaptor, and which does not comprise a sequence that is capable of binding to a primer immobilised to the substrate by hybridisation.

71. The method of claim 70, wherein the feature of interest is any feature capable of being specifically cleaved.

72. The method of claim 70 or claim 71, wherein the feature of interest is a cyclobutane pyrimidine dimer (CPD), 8-oxoguanine, or an abasic site.

73. The method of any one of claims 70 to 72, wherein the oligonucleotide of step d) comprises a sequence identical to a region of a second primer.

74. The method of any one of claims 70 to 73, wherein
the substrate comprises a first and a second immobilised primer;
the oligonucleotide of step b) comprises a sequence that is capable of
binding to the first immobilised primer by hybridisation; and
the oligonucleotide of step d) comprises a sequence identical to a
region of the second immobilised primer.

75. The method of any one of claims 70 to 74, wherein
step b) is:

exposing the plurality of nucleic acids to a first adaptor pair under
conditions conducive to ligation, wherein the first adaptor pair is
capable of being ligated to at least a 3' terminus of a strand of a
cleavage site, and wherein the first adaptor pair comprises first and
second oligonucleotides that are at least partially complementary, and
the first oligonucleotide is ligatable to a 3' terminus and comprises a
sequence that is capable of binding to a primer immobilised to the
substrate by hybridisation; and

wherein step d) is:

exposing the plurality of nucleic acids to a second adaptor pair under
conditions conducive to ligation, wherein the second adaptor pair is
capable of being ligated to at least a 5' terminus of a strand at a break
induced by fragmentation but is not capable of being ligated to the first
oligonucleotide of the first adaptor pair, wherein the second adaptor
comprises first and second partially complementary oligonucleotides,
and the first oligonucleotide is ligatable to a 5' terminus and comprises
a sequence identical to a region of a second primer, and the second
oligonucleotide does not comprise a sequence that is complementary
to said sequence identical to a region of the second primer.

76. The method of any one of claims 70 to 75, wherein the second adaptor pair
comprises:

a first oligonucleotide comprising a sequence according to CAAGCAGAAGACGGCATACGAGAT (SEQ ID NO: 32), and a second oligonucleotide comprising a sequence that does not comprise a sequence of more than 5, 10, 15, or 20 bases, or does not comprise all 24 bases, of the sequence ATCTCGTATGCCGTCTTCTGCTTG (SEQ ID NO: 30); or

a first oligonucleotide comprising a sequence according to AATGATACGGCGACCACCGA (SEQ ID NO: 34), and a second oligonucleotide comprising a sequence that does not comprise a sequence of more than 5, 10, or 15 bases, or does not comprise all 20 bases, of the sequence TCGGTGGTCGCCGTATCATT (SEQ ID NO: 31).

77. The method of claim 75 or claim 76, wherein the first and/or the second oligonucleotide of the first adaptor pair comprises a 3' and/or 5' protective feature; and/or wherein the first and/or the second oligonucleotide of the second adaptor pair comprises a 3' and/or 5' protective feature.

78. The method of any one of claims 70 to 77, wherein the second adaptor is not capable of being ligated to the first adaptor due to the presence of a 3' modification of the first adaptor.

79. The method of any one of claims 70 to 78, wherein the oligonucleotide of the second adaptor that is ligatable to a 5' terminus comprises a sequence identical to 5, 10, 15, 20, 21, 24, or more bases of an immobilised primer.

80. The method of any one of claims 70 to 79, further comprising denaturing the plurality of nucleic acids to form a plurality of single-stranded nucleic acids.

81. The method of any one of claims 70 to 80, further comprising contacting the plurality of nucleic acids with the substrate comprising immobilised primers under conditions suitable for hybridisation of the immobilised primers to complementary nucleic acids.

82. The method of claim 81, further comprising obtaining sequence information for any nucleic acids hybridised to the substrate.

83. The method of any one of claims 70 to 82, wherein said sample comprising a plurality of nucleic acids is gDNA.

5 84. The method of any one of claims 70 to 83, wherein:
the steps are performed in the order a), b), c), and then d); or
the steps are performed in the order c), d), a), and then b).

10 85. A double strand adaptor for identifying DNA double-strand breaks (DSBs) in a nucleic acid sample, such as a gDNA sample, comprising:
a first oligonucleotide strand that does not comprise a sequence of more than 5, 10, 15, 20 bases, or does not comprise all 22 bases, of the sequence GGAAAGCGGAGGCGTAGTGGTT (SEQ ID NO: 36); and a
15 second oligonucleotide that comprises a 3' binding feature that enables ligation of said oligonucleotide to a strand of a double-stranded nucleic acid and comprises a sequence according to AACCCTACTACGCCTCCGCTTTCC (SEQ ID NO: 40); and wherein
either or both of said oligonucleotides comprise, respectively, a 3' and/or
20 5' protective feature.

CLAIMS

1. A method of sample preparation for identifying DNA double-strand breaks (DSBs) in a nucleic acid sample, comprising:

(i) exposing a sample of nucleic acid suspected of containing DSBs, under ligation conditions, to a first pair of oligonucleotides a first one of which comprises a 5' binding feature that enables ligation of the oligonucleotide to a first strand of the DSB, a hybridization site (RD1 SP) to which a first sequencing primer can bind and a binding sequence for separating the DSB from a pool of DSBs; and a second oligonucleotide that is complementary to the first oligonucleotide of the first pair and comprises a 3' binding feature that enables ligation of the oligonucleotide to a second strand of the DSBs; in which either or both of the oligonucleotides comprise, a 3' and/or 5' protective feature;

(ii) fragmenting the nucleic acid of the sample into fragments; and

(iii) exposing the fragments, under ligation conditions, to a second pair of oligonucleotides a first one of which comprises a 5' binding feature, that enables ligation of the oligonucleotide to a first strand of the fragmented nucleic acid and a hybridization site (RD2 SP) to which a second sequencing primer can bind; and a second longer oligonucleotide that is in part complementary to the first oligonucleotide of the second pair and comprises a 3' binding feature for binding to a second strand of the fragmented nucleic acid, a sequence complimentary to the hybridization site, and a further sequence that is, optionally, a binding sequence for enabling bridge amplification; and in which either or both of the oligonucleotides comprise a 3' and/or 5' protective feature.

2. A method according to Claim 1, in which the method is a method for identifying DNA DSBs in a nucleic acid sample, and further comprises:

(iv) denaturing the fragments to provide single strand nucleic acids;

(v) separating the strands of part (iv) into two groups: group A those fragments that have ligated at a first end the first hybridization site and binding sequence provided by the oligonucleotide of part (i) and at another end the second hybridization site and further sequence provided by the oligonucleotide of part (iii) and group B those fragments that do not have ligated at a first end the hybridization site and binding

sequence provided by the oligonucleotide of part (i) and at another end the second hybridization site and further sequence provided by the oligonucleotide of part (iii); and
 (vi) sequencing the strands of group A using primers that bind to the first and/or second hybridization sites where each sequence is equivalent to a DSB break and further
 5 in which the number and nature of base pair deletions can be determined by comparing each sequence with a genome representative of the species from which the sample was taken.

3. A method according to Claim 1 or 2, in which the oligonucleotide of the second pair that comprises a 5' binding feature does not comprise a binding sequence for separating the
 10 fragmented nucleic acid.

4. A method according to Claims 1, 2 or 3 in which the oligonucleotides of part (i) and part (iii) are interchanged whereby, after fragmenting in step (ii), the nucleic acid is first exposed to the oligonucleotides of part (iii) and the nucleic acid is then exposed to the oligonucleotides of part (i).

15 5. A method according to any preceding claim, in which:

(a) the 5' and/or 3' binding feature comprises one of the following: a phosphate group; a triphosphate 'T-tail', preferably a deoxythymidine triphosphate 'T-tail'; a triphosphate 'A-tail', preferably a deoxyadenosine triphosphate 'A-tail'; at least one random N nucleotide, and a plurality of N nucleotides; and/or

20 (b) the 5' and/or 3' protective feature comprises a feature that provides resistance to any one or more of the following: phosphorylation activity, phosphatase activity, terminal transferase activity, nucleic acid hybridization, endonuclease activity, exonuclease activity, ligase activity, polymerase activity, and protein binding; optionally in which the protective features comprises a phosphorothioate linkage, a
 25 dideoxynucleotide or a covalent block, a phosphoramidite, a C3 Spacer phosphoramidite (3SpC3).

6. A method according to any preceding claim, in which:

either the first and/or second oligonucleotide of the first oligonucleotide pair of part (i) comprises two different terminal protective features; and/or

either the first or second oligonucleotide of this second oligonucleotide pair of part (iii) comprises two different terminal protective features.

7. A method according to any preceding claim, in which:

the oligonucleotides of part (i) comprises a first oligonucleotide having SEQ ID NO. 1 and a second oligonucleotide having SEQ ID NO. 2; or an oligonucleotide that shares at least 80% identity or homology with SEQ ID NO. 1 or 2; and/or

the second pair of oligonucleotides of part (iii) comprises a first oligonucleotide having SEQ ID NO. 3 and a second oligonucleotide having SEQ ID NO. 4; or an oligonucleotide that shares at least 80% identity or homology with SEQ ID NO. 3 or 4; and/or

the second oligonucleotide of the second pair of oligonucleotides of part (iii) comprises any one of the following sequences: SEQ ID Nos. 4 – 28; or an oligonucleotide that shares at least 80% identity or homology with one of SEQ ID NO. 4-28.

8. A method according to any preceding claim, in which:

the nucleic acid sample is gDNA; and/or

the sample is mammalian or human.

9. A method according to any preceding claim, in which:

(a) the ligation in part (i) occurs *in situ* or *in vitro* using a cell or tissue sample; and/or

(b) the sample is exposed to a permeabilizing agent before step (i) is undertaken; and/or

(c) the sample is exposed to at least one agent for performing arginine tail repair before step (i) is undertaken; and/or

(d) part (i) also includes extracting gDNA from the sample prior to performing the subsequent steps.

10. A method according to any preceding claim, in which the method further comprises after part (ii) and/or part (iv), removing fragments whose size is less than about 100bp, or less than about 150bp, and/or retaining fragments whose size is greater than about 150bp.

11. A method according to any preceding claim, in which the separating of part (v) involves using the binding sequence provided by the oligonucleotide of part (i) to bind a partner and so separate the Group A strands of part (iv) from any other strands; optionally in which a complementary binding strand to the binding sequence provided by the oligonucleotide of part (i) is anchored to a substrate and the single strands of nucleic acids flow by, or over, the anchored complementary binding strand.
12. A method according to any preceding claim, in which part (vi) involves bridge amplification where the single strands separated under part (v) are clonally amplified on a substrate that has anchored thereon oligonucleotides/binding sites for the binding sequence of the first oligonucleotide of part (i) and the further sequence of the second oligonucleotide of part (iii).
13. A method according to any preceding claim, in which prior to performing the claimed method, a sample containing or suspected of containing, a single strand break is ligated or broken to ensure the single strand break is converted into a double strand break.
14. A kit of parts for identifying DNA double-strand breaks (DSBs) in a gDNA sample comprising:
- (i) a first pair of oligonucleotides a first one of which comprises a 5' binding feature that enables ligation of the oligonucleotide to a first strand of the DSB, a hybridization site (RD1 SP) to which a first sequencing primer can bind and a binding sequence for separating the DSB from a pool of DSBs; and a second oligonucleotide that is complementary to the first oligonucleotide of this first pair and comprises a 3' binding feature for binding to a second strand of the DSBs; and in which either or both of the oligonucleotides comprise a 3' and/or 5' protective feature; and
 - (ii) a second pair of oligonucleotides a first one of which comprises a 5' binding feature, that enables ligation of the oligonucleotide to a first strand of the DSB, and a hybridization site (RD2 SP) to which a second sequencing primer can bind; and a second longer oligonucleotide that is in part complementary to the first oligonucleotide of this second pair and comprises a 3' binding feature for binding to a second strand of the DSBs, a sequence complimentary to the hybridization site, and a further sequence which is, optionally, a binding sequence for enabling bridge amplification; and in which either or both of the oligonucleotides comprise a 3' and/or 5' protective feature.

15. A kit for sample preparation for identifying DSBs in a gDNA sample, comprising:

(i) a first pair of oligonucleotides, a first one of which comprises a 5' binding feature that enables ligation of the oligonucleotide to a strand of a double-stranded nucleic acid, and comprises a sequence according to TCGGTGGTCGCCGTATCATT (SEQ ID NO: 31); and a second oligonucleotide that is complementary to the first oligonucleotide of the first pair; and in which either or both of the oligonucleotides comprise, respectively, a 3' and/or 5' protective feature; and

(ii) a second pair of oligonucleotides, a first one of which does not comprise a sequence of more than 5, 10, 15, or 20 bases, or does not comprise all 24 bases, of the sequence ATCTCGTATGCCGTCTTCTGCTTG (SEQ ID NO: 30); and a second oligonucleotide that comprises a 3' binding feature that enables ligation of the oligonucleotide to a strand of a double-stranded nucleic acid and comprises a sequence according to CAAGCAGAAGACGGCATAACGAGAT (SEQ ID NO: 32); and in which either or both of the oligonucleotides comprise, respectively, a 3' and/or 5' protective feature;

or

(i) a first pair of oligonucleotides, a first one of which comprises a 5' binding feature that enables ligation of the oligonucleotide to a strand of a double-stranded nucleic acid, and comprises a sequence according to ATCTCGTATGCCGTCTTCTGCTTG (SEQ ID NO: 30); and a second oligonucleotide that is complementary to the first oligonucleotide of the first pair; and in which either or both of the oligonucleotides comprise, respectively, a 3' and/or 5' protective feature; and

(ii) a second pair of oligonucleotides, a first one of which does not comprise a sequence of more than 5, 10, or 15 bases, or does not comprise all 20 bases, of the sequence TCGGTGGTCGCCGTATCATT (SEQ ID NO: 31); and a second oligonucleotide that comprises a 3' binding feature that enables ligation of the oligonucleotide to a strand of a double-stranded nucleic acid and comprises a sequence according to AATGATACGGCGACCACCGA (SEQ ID NO: 34); and in which either or both of the oligonucleotides comprise, respectively, a 3' and/or 5' protective feature.

16. A kit according to Claim 15, in which:

(a) the first oligonucleotide of the first pair and/or the second pair of oligonucleotides comprises a hybridization site to which a first sequencing primer can bind; and/or

(b) the first and second oligonucleotides of part (i) and/or part (ii) also comprise an index feature that is a particular sequence of nucleotides that enables the origin of pooled samples to be determined.

17. A kit according to Claim 14 or 16, in which the kit further comprises at least one primer that bind to the first and/or second hybridization sites for the purpose of sequencing.

18. A kit according to any of Claims 14 to 17, in which the kit further comprises fragmenting agents and/or denaturing agents for fragmenting and/or denaturing the nucleic acid into fragments and/or single strands, respectively.

19. A double strand adaptor for identifying DNA double-strand breaks (DSBs) in a nucleic acid sample, such as a gDNA sample, comprising:

a first oligonucleotide strand comprising a 5' binding feature that enables ligation of the oligonucleotide to a first strand of the DSB, a hybridization site (RD1 SP) to which a sequencing primer can bind and a binding sequence for separating the DSB from a pool of DSBs; and a second oligonucleotide strand that is complementary to the first oligonucleotide and comprises a 3' binding feature for binding to a second strand of the DSBs; and in which either or both of the oligonucleotides comprise a 3' and/or 5' protective feature.

20. A double strand adaptor for identifying DNA double-strand breaks (DSBs) in a nucleic acid sample, such as a gDNA sample, comprising:

a first oligonucleotide strand comprising a 5' binding feature, that enables ligation of the oligonucleotide to a first strand of the DSB, and a hybridization site (RD2 SP) to which a sequencing primer can bind; and a second longer oligonucleotide strand that is in part complementary to the first oligonucleotide and comprises a 3' binding feature for binding to a second strand of the DSBs, a sequence complimentary to the hybridization site, and a further sequence, which is, optionally, a binding sequence for enabling bridge amplification; and in which either or both of the oligonucleotides comprise a 3' and/or 5' protective feature.

21. A double strand adaptor for identifying DNA double-strand breaks (DSBs) in a nucleic acid sample, comprising:

(a) a first oligonucleotide strand which does not comprise a sequence of more than 5, 10, 15, or 20 bases, or does not comprise all 24 bases, of the sequence

5 ATCTCGTATGCCGTCTTCTGCTTG (SEQ ID NO: 30); and a second oligonucleotide that comprises a 3' binding feature that enables ligation of the oligonucleotide to a strand of a double-stranded nucleic acid and comprises a sequence according to CAAGCAGAAGACGGCATAACGAGAT (SEQ ID NO: 32); and in which either or both of the oligonucleotides comprise, respectively, a 3' and/or 5' protective feature; or

10 (b) a first oligonucleotide strand which does not comprise a sequence of more than 5, 10, or 15 bases, or does not comprise all 20 bases, of the sequence

TCGGTGGTCGCCGTATCATT (SEQ ID NO: 31); and a second oligonucleotide that comprises a 3' binding feature that enables ligation of the oligonucleotide to a strand of a double-stranded nucleic acid and comprises a sequence according to
15 AATGATACGGCGACCACCGA (SEQ ID NO: 34); and in which either or both of the oligonucleotides comprise, respectively, a 3' and/or 5' protective feature.

22. A method of sample preparation for identifying DNA double-strand breaks (DSBs) in a nucleic acid sample, in which the preparation comprises modifying DSB-associated nucleic acids to be suitable for binding to a substrate comprising immobilised primers, the method
20 comprising:

(a) providing a sample comprising a plurality of nucleic acids;

(b) exposing the plurality of nucleic acids to a first adaptor under conditions conducive to ligation, in which the first adaptor comprises an oligonucleotide capable of being ligated to a 3' terminus of a strand of a DSB and which comprises a sequence that
25 is capable of binding to a primer immobilised to the substrate by hybridisation;

(c) fragmenting the plurality of nucleic acids; and

(d) exposing the plurality of nucleic acids to a second adaptor under conditions conducive to ligation, in which the second adaptor comprises an oligonucleotide capable of being ligated to a 5' terminus of a strand at a break induced by fragmentation, but is
30 not capable of being ligated to the first adaptor, and which does not comprise a sequence that is capable of binding to a primer immobilised to the substrate by hybridisation.

23. A method according to Claim 22, in which the oligonucleotide of step (d) comprises a sequence identical to a region of a second primer.

24. A method according to Claim 22 or 23, in which:

the substrate comprises a first and a second immobilised primer;

5 the oligonucleotide of step (b) comprises a sequence that is capable of binding to the first immobilised primer by hybridisation; and

the oligonucleotide of step (d) comprises a sequence identical to a region of the second immobilised primer.

25. A method according to Claim 22, 23 or 24, in which:

10 step (b) is:

exposing the plurality of nucleic acids to a first adaptor pair under conditions conducive to ligation, in which the first adaptor pair is capable of being ligated to at least a 3' terminus of a strand of a DSB, and in which the first adaptor pair comprises first and second oligonucleotides that are at least partially

15 complementary, and the first oligonucleotide is ligatable to a 3' terminus and comprises a sequence that is capable of binding to a primer immobilised to the substrate by hybridisation; and

in which step (d) is:

20 exposing the plurality of nucleic acids to a second adaptor pair under conditions conducive to ligation, in which the second adaptor pair is capable of being ligated to at least a 5' terminus of a strand at a break induced by fragmentation but is not capable of being ligated to the first oligonucleotide of the first adaptor pair, in which the second adaptor comprises first and second partially complementary oligonucleotides, and the first oligonucleotide is ligatable to a 5' terminus and

25 comprises a sequence identical to a region of a second primer, and the second oligonucleotide does not comprise a sequence that is complementary to the sequence identical to a region of the second primer.

26. A method according to any of Claims 22 to 25, in which the second adaptor pair comprises:

a first oligonucleotide comprising a sequence according to
 CAAGCAGAAGACGGCATAACGAGAT (SEQ ID NO: 32), and a second
 oligonucleotide comprising a sequence that does not comprise a sequence of more than
 5, 10, 15, or 20 bases, or does not comprise all 24 bases, of the sequence
 5 ATCTCGTATGCCGTCTTCTGCTTG (SEQ ID NO: 30); or

a first oligonucleotide comprising a sequence according to
 AATGATACGGCGACCACCGA (SEQ ID NO: 34), and a second oligonucleotide
 comprising a sequence that does not comprise a sequence of more than 5, 10, or 15
 bases, or does not comprise all 20 bases, of the sequence
 10 TCGGTGGTCGCCGTATCATT (SEQ ID NO: 31).

27. A method according to any of Claims 22 to 26, further comprising contacting the
 plurality of nucleic acids with the substrate comprising immobilised primers under conditions
 suitable for hybridisation of the immobilised primers to complementary nucleic acids.

28. A method according to any of Claims 22 to 27, in which:

15 the steps are performed in the order (a), (b), (c), and then (d); or
 the steps are performed in the order (a), (c), (d), and then (b); in which the sample is
 exposed to conditions capable of causing or suspected of being capable of causing a
 DSB between steps (d) and (b); optionally in which the sample is exposed to conditions
 capable of causing a DSB at a feature of interest in the nucleic acid sample.

20 29. A method of sample preparation for identifying DNA double-strand breaks (DSBs) in a
 nucleic acid sample, in which the preparation comprises modifying DSB-associated nucleic
 acids to be suitable for binding to a substrate comprising an immobilised first primer, the
 method comprising:

(1) providing a sample comprising a plurality of nucleic acids;

25 (2) exposing the plurality of nucleic acids to a first adaptor under conditions
 conducive to ligation, in which the first adaptor comprises an oligonucleotide capable of
 being ligated to a 3' terminus of a strand of a DSB and which comprises a sequence that
 is capable of hybridising to a second primer;

(3) fragmenting the plurality of nucleic acids;

- (4) exposing the plurality of nucleic acids to a second adaptor under conditions conducive to ligation, in which the second adaptor comprises an oligonucleotide capable of being ligated to a 5' terminus of a strand at a break induced by fragmentation, but is not capable of being ligated to the first adaptor, and which comprises a sequence identical to a region of the immobilised first primer; and
- (5) contacting the plurality of nucleic acids with the second primer under conditions suitable for extension of the primer.

30. A method according to Claim 29, in which
step (2) is:

- exposing the plurality of nucleic acids to a first adaptor pair under conditions conducive to ligation, in which the first adaptor pair is capable of being ligated to at least a 3' terminus of a strand of a DSB, and in which the first adaptor pair comprises first and second oligonucleotides that are at least partially complementary, and the first oligonucleotide is ligatable to a 3' terminus and comprises a sequence that is capable of hybridising to a second primer; and
- in which step (4) is:

- exposing the plurality of nucleic acids to a second adaptor pair under conditions conducive to ligation, in which the second adaptor pair is capable of being ligated to at least a 5' terminus of a strand at a break induced by fragmentation but is not capable of being ligated to the first oligonucleotide of the first adaptor pair, in which the second adaptor comprises first and second partially complementary oligonucleotides, and the first oligonucleotide is ligatable to a 5' terminus and comprises a sequence identical to a region of the immobilised first primer, and the second oligonucleotide does not comprise a sequence that is complementary to the sequence identical to a region of the immobilised first primer.

31. A method according to Claim 29 or 30, in which the second adaptor pair comprises:
a first oligonucleotide comprising a sequence according to
AACCCACTACGCCTCCGCTTTCC (SEQ ID NO: 40); and

a second oligonucleotide that does not comprise a sequence of more than 5, 10, 15, 20 bases, or does not comprise all 22 bases, of the sequence GGAAAGCGGAGGCGTAGTGGTT (SEQ ID NO: 36).

32. A method according to Claim 29, 30 or 31, in which:

5 the steps are performed in the order (1), (2), (3), (4), and then (5); or

the steps are performed in the order (1), (3), (4), (2), and then (5); in which the sample is exposed to conditions capable of causing or suspected of being capable of causing a DSB between steps (4) and (2); optionally

10 in which the sample is exposed to conditions capable of causing a DSB at a feature of interest in the nucleic acid sample.

33. A method according to any of Claims 29 to 32, further comprising contacting the plurality of nucleic acids with the substrate comprising the immobilised first primer under conditions suitable for hybridisation of the immobilised first primer to complementary nucleic acids.

15 34. A method according to Claim 27 or 33, further comprising obtaining sequence information for any nucleic acids hybridised to the substrate.

35. A method according to any of Claims 22 to 34, in which the sample comprising a plurality of nucleic acids is gDNA.

20 36. A method according to any of Claims 25, 26, 30 or 31, in which the first and/or the second oligonucleotide of the first adaptor pair comprises a 3' and/or 5' protective feature; and/or in which the first and/or the second oligonucleotide of the second adaptor pair comprises a 3' and/or 5' protective feature.

25 37. A method according to any of Claims 22 to 36, in which the second adaptor is not capable of being ligated to the first adaptor due to the presence of a 3' modification of the first adaptor.

38. A method according to any of Claims 22 to 37, in which the oligonucleotide of the second adaptor that is ligatable to a 5' terminus comprises a sequence identical to 5, 10, 15, 20, 21, 24, or more bases of an immobilised primer.

39. A double strand adaptor for identifying DNA double-strand breaks (DSBs) in a nucleic acid sample, such as a gDNA sample, comprising:

a first oligonucleotide strand that does not comprise a sequence of more than 5, 10, 15, 20 bases, or does not comprise all 22 bases, of the sequence

5 GGAAAGCGGAGGCGTAGTGGTT (SEQ ID NO: 36); and a second oligonucleotide that comprises a 3' binding feature that enables ligation of the oligonucleotide to a strand of a double-stranded nucleic acid and comprises a sequence according to AACCCACTACGCCTCCGCTTTCC (SEQ ID NO: 40); and in which either or both of the oligonucleotides comprise, respectively, a 3' and/or 5' protective feature.