Claims

Claim 1. A method of detecting single strand break (SSB) sites in double stranded (ds) DNA comprising;

(i) contacting a sample of dsDNA with an oligonucleotide probe comprising a first sequencing adaptor and a tag,

(ii) contacting the sample of dsDNA with DNA ligase, such that the DNA ligase covalently links the oligonucleotide probe to a strand of the dsDNA at the site of a single strand break in the dsDNA,

(iii) contacting the sample of dsDNA with a transposase loaded with a second sequencing adaptor, such that the transposase cleaves the dsDNA to generate a population of fragments comprising a terminal second sequencing adaptor,

(iv) isolating from said population, DNA strands that comprise the first and second sequencing adaptors and;

(v) determining the sequence of the isolated DNA strands.

Claim 2. A method according to claim 1 wherein the sequences of the isolated DNA strands are indicative of single strand breaks (SSBs) sites within the dsDNA.

Claim 3. A method according to any one of the preceding claims wherein

(i) the dsDNA is genomic DNA,

(ii) the dsDNA is in a cell nucleus or extract thereof,

(iii) the dsDNA is from a diseased cell; an embryonic cell; an age-associated cell; or a treated cell,

optionally wherein the treated cell has undergone a treatment selected from;

exposure to one or more compounds,

exposure to light or irradiation,

exposure to cell culture conditions,

exposure to a nuclease, optionally Cas9 or Cpf1, or

exposure to reprogramming factors, optionally Oct3/4, Sox2, Klf4 and c-Myc.

Claim 4. A method according to any one of the preceding claims wherein

(a) the sample of dsDNA is produced by a method comprising extracting the nucleus from a eukaryotic cell, optionally wherein the eukaryotic cell is fixed and permeabilised.

(b) the sample of dsDNA is subjected to one or more of the following steps prior to contacting the sample with the oligonucleotide probe:

(i) samples (e.g. cells) comprising dsDNA are subjected to wash and lysis,

(ii) said samples comprising dsDNA are subjected to buffer exchange e.g. by centrifugation of the sample or by dilution.

(c) the sample of dsDNA is purified by proteinase digestion after contacting the dsDNA with the ligase

(d) the isolation of the DNA strands that comprise the first and second sequencing adaptors is performed using magnetic beads, and/or

(e) the tag is biotin.

Claim 5. A method according to any one of the preceding claims wherein the probe further comprises a hybridisation sequence that anneals to the dsDNA at SSB sites,
optionally wherein

(i) the hybridisation sequence comprises comprise 5-10 nucleotides.

(ii) the hybridisation sequence is diverse, preferably random and/or

(iii) the hybridisation sequence is complementary to a target sequence in the dsDNA, optionally wherein the target sequence is a promoter.

Claim 6. A method according to any one of the preceding claims wherein

(i) the oligonucleotide probe is ligated to the 3' end of the broken strand of the dsDNA at the SSB site, optionally wherein the tag is located at the 3' end of the oligonucleotide probe and the oligonucleotide probe further comprises a 5' phosphate group, or

(ii) the oligonucleotide probe is ligated to the 5' end of the broken strand of the dsDNA at the SSB site, optionally wherein the tag is located at the 5' end of the oligonucleotide probe and the oligonucleotide probe further comprises a 3' OH group.

Claim 7. A method according to any one of the preceding claims wherein

(i) the transposase is Tn5 transposase.

(ii) fragments comprising the first and second sequencing adaptors are isolated by contacting the tag with an immobilised binding member, optionally wherein the tag is biotin and the binding member is streptavidin and/or the binding member is immobilised on a bead,

(iii) the method comprises treating the isolated DNA strands with bisulfite, optionally wherein the sequences of the bisulfite treated strands are compared to a reference genome to identify methylated cytosine residues in the isolated DNA strands,

(iv) the method comprises amplifying the isolated DNA strands using sequencing primers to produce amplification products, and/or

(v) the method comprises sequencing the isolated DNA strands or amplification products,

Claim 8. A method according to any one of the preceding claims further comprising generating a set of sequence reads of the isolated DNA strands or amplification products,

Claim 9. A method according to claim 8 comprising mapping the sequence reads in the set to one or more sites in a reference genome.

Claim 10. A method according to claim 9 comprising determining the frequency of SSBs at a site in the reference genome from the numbers of sequence reads mapped to the site.

Claim 11. A method according to claim 10 comprising mapping the frequency of SSBs at sites within a first dsDNA and a second dsDNA and identifying sites with increased frequency of SSB occurrence in the first dsDNA relative to the second dsDNA or in the second dsDNA relative to the first dsDNA.

Claim 12. A method according to claim 11 wherein first dsDNA is a test sample, and the second dsDNA is a reference or control sample.

Claim 13. A method according to claim 12 wherein the first dsDNA is from a disease cell; an embryonic cell; an age-associated cell; or a treated cell, optionally wherein the treated cell has undergone a treatment selected from;

exposure to one or more compounds,

exposure to light or irradiation,

exposure to cell culture conditions,

exposure to a nuclease, optionally Cas9 or Cpf1, or

exposure to Oct3/4, Sox2, Klf4 and c-Myc.

Claim 14. A kit for detecting sites of single strand breaks (SSBs) in double stranded (ds) DNA; the kit comprising;

an oligonucleotide probe comprising a first sequencing adaptor and a tag,

a DNA ligase,

a transposase and

a second sequencing adaptor loaded or loadable onto the transposase.

optionally, wherein the kit further comprises instructions for use in a method according to any one of claims 1 to 13.

Claim 15. A method of screening for an agent, such as a chemical and/or biological agent (e.g. a small molecule and/or a peptide and/or protein therapeutic) that can enable repair of SSBs in dsDNA, wherein said screening method comprises the following steps:

(a) detecting single strand break (SSB) sites in a control sample of double stranded (ds) DNA that has not been exposed to said agent and also detecting single strand break (SSB) sites in a test version of the same sample of the dsDNA that has been exposed to said agent by the steps of;

(i) contacting a sample of the test and control dsDNA with an oligonucleotide probe comprising a first sequencing adaptor and a tag,

(ii) contacting the sample of test and control dsDNA with DNA ligase, such that the DNA ligase covalently links the oligonucleotide probe to a strand of the dsDNA at the site of a single strand break in the dsDNA,

(iii) contacting the sample of test and control dsDNA with a transposase loaded with a second sequencing adaptor, such that the transposase cleaves the dsDNA to generate a population of fragments comprising a terminal second sequencing adaptor, and then

(b) isolating from said population, DNA strands that comprise the first and second sequencing adaptors,

(c) determining the sequence of the isolated DNA strands from the test and control samples,

(d) comparing the profile of SSBs in both the test and control samples,

(e) isolating the agent e.g. chemical and/or biological agent, that is associated with a reduction in SSBs in test sample compared to control samples, and

(f) formulating said isolated agent obtained