

CLAIMS

1. A method of producing a 3C library, the method comprising the steps:
 - (a) treating chromatin in a population of eukaryotic cells by a method comprising:
 - (i) cross-linking the chromatin within the cells in a population of eukaryotic cells;
 - (ii) permeabilising or removing cell membranes of the eukaryotic cells; and
 - (iii) fragmenting the cross-linked chromatin to produce mono-nucleosomes of 180-200bp with the inter-nucleosomal linkers attached;
 - (b) ligating the mono-nucleosomes of 180-200bp with the inter-nucleosomal linkers attached to produce ligated nucleic acid fragments; and
 - (c) de-crosslinking the ligated nucleic acid fragments.
2. The method of claim 1, wherein the chromatin is digested in step (iii) to produce >70% (preferably >80% or >90%) mono nucleosomes.
3. The method of claim 1 or claim 2, wherein the fragmenting in step (iii) is carried out using micrococcal nuclease.
4. The method of any one of claims 1-3, wherein the cells are mammalian cells.
5. The method of any one of claims 1-4, wherein the number of cells in the population of eukaryotic cells is 1-10,000, 10,000-1 million, or 1 million to 100 million.
6. The method of any one of claims 1-5, wherein in step (a):
 - (a) the outer cell membrane and/or nuclear membrane is not lysed;
 - (b) the outer cell membrane or nuclear membrane is not partially or completely removed;
 - (c) the outer cell membranes and nuclear membranes of the cells are permeabilised without removing the outer cell membranes or nuclear membranes;

(d) the cell membrane is removed and the nuclear membrane is permeabilised but not removed;

(e) the cell membrane is removed and the nuclear membrane is removed; or

5 (f) the method comprises (ii) permeabilising the cell membranes of the eukaryotic cells; and (iii) fragmenting the cross-linked chromatin within the cells to produce nucleic acid fragments.

7. The method of any one of claims 1-6, wherein the length of the ligated nucleic acid fragments is greater than 200bp.

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8. A method of identifying nucleic acid regions within a nucleic acid sample which interact with one another, the method comprising the steps:

producing a 3C library by a method as defined in any one of claims 1-7;

(a) fragmenting the 3C library to produce nucleic acid fragments;

15 (b) optionally, adding sequencing adaptors to the ends of the nucleic acid fragments and/or amplifying the nucleic acid fragments;

(c) contacting the nucleic acid fragments with a targeting nucleic acid which binds to a subgroup of the nucleic acid fragments, wherein the targeting nucleic acid is labelled with the first half of a binding pair;

20 (d) isolating the subgroup of nucleic acid fragments which have been bound by the targeting nucleic acid using the second half of the binding pair;

(e) amplifying the isolated subgroup of nucleic acid fragments;

(f) optionally repeating Steps (c), (d) and (e) one or more times; and

(g) optionally sequencing the amplified isolated subgroup of nucleic acid fragments in order to identify nucleic acid regions within the nucleic acid sample which interact with one another.

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9. A method as claimed in claim 8, wherein the targeting nucleic acid is a DNA oligonucleotide.

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10. A method as claimed in claim 8 or claim 9, wherein the concentration of the targeting nucleic acid (preferably a DNA oligonucleotide) is 300nM to 30pM.

11. A method as claimed in any one of claims 8-10, wherein the targeting nucleic acid (preferably a DNA oligonucleotide) is selected such that is capable of binding within a nucleosome-depleted region of a promoter of a gene or non-coding RNA of interest, or of a regulatory element (preferably an enhancer, repressor or CTCF binding site) in the nucleic acids.

12. A method as claimed in claim 11, wherein the targeting nucleic acid (preferably a DNA oligonucleotide) is selected such that is capable of binding within the central region of the nucleosome-depleted region of a promoter of a gene or non-coding RNA of interest, or of a regulatory element (preferably an enhancer, repressor or CTCF binding site) in the nucleic acids.

13. A method as claimed in any one of claims 8-12, wherein Step (f) is repeated 1, 2, 3, 4 or 5 times.

14. A method of identifying allele-specific interaction profiles in SNP-containing regions of nucleic acids, the method comprising a method as defined in any one of claims 8-13 including sequencing the amplified isolated subgroup of nucleic acid fragments in order to identify allele-specific interaction profiles in SNP-containing regions.

15. A method of identifying one or more interacting nucleic acid regions that are indicative of a particular disease state or disorder, the method comprising:

a) carrying out a method as defined in any one of claims 8-13 on a nucleic acid sample of mammalian cells obtained from a subject with a particular disease state or disorder;

b) quantifying a frequency of interaction between a first nucleic acid region and a second nucleic acid region; and

c) comparing the frequency of interaction in the nucleic acid sample from the subject with said disease state or disorder with the frequency of interaction in a control nucleic acid sample from a healthy subject, such that a difference in the frequency of interaction in the nucleic acid samples is indicative of a particular disease state or disorder.