

CLAIMS

1. A process for treating chromatin in a population of mammalian cells, the process comprising the steps:

- 5 (i) cross-linking the chromatin within the cells in a population of mammalian cells;
(ii) permeabilising the outer and nuclear cell membranes of the mammalian cells;
and
(iii) fragmenting the cross-linked chromatin within the permeabilised cells using
micrococcal nuclease to produce mono-nucleosomes wherein the inter-
10 nucleosomal linkers are at least partially intact.

2. A process for treating nucleic acids in a population of ~~eukaryotic~~ mammalian cells, the process comprising the steps:

- 15 (i) immobilising the nucleic acids within the cells in a population of ~~eukaryotic~~ mammalian cells;
(ii) permeabilising or removing the cell membranes of the ~~eukaryotic~~ mammalian cells; and
(iii) fragmenting the immobilised nucleic acids within the cells with a micrococcal nuclease to produce nucleic acid fragments,
20 ~~preferably wherein the eukaryotic cells are mammalian cells.~~

3. A process as claimed in claim 2, wherein the nucleic acids are chromatin comprising nucleosomes which are linked by inter-nucleosomal linkers.

4. A process as claimed in claim 2 or claim 3, wherein in Step (i), the nucleic acids are immobilised by cross-linking the nucleic acids.

5. A process as claimed in any one of claims 2-4, wherein in Step (ii), the outer cell membranes and nuclear membranes are permeabilised.

6. A process as claimed any one of claims 2-4, wherein in Step (ii), the outer cell membranes are removed and the nuclear membranes are permeabilised.

~~6. A process as claimed in any one of claims 2-5, wherein in Step (iii), the immobilised nucleic acids are fragmented using an endo-exonuclease, preferably using micrococcal nuclease.~~

7. A process as claimed in any one of claims 2-6, wherein in Step (iii), the immobilised nucleic acids are fragmented such that mono-nucleosomes are produced, wherein the inter-nucleosomal linkers are at least partially intact.

8. A process as claimed in any one of claims 2-7, wherein the number of cells in the population of mammalian cells is 1-10,000, 10,000-1 million, or 1 million to 100 million.

9. A process for producing a 3C library, the process comprising the steps:
(a) treating nucleic acids by a process as defined in any one of claims 2-8;
(b) ligating the nucleic acid fragments to produce ligated nucleic acid fragments;
and
(c) de-immobilising (preferably de-crosslinking) the ligated nucleic acid fragments.

10. 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 process as defined in claim 9;

- (d) fragmenting the 3C library to produce nucleic acid fragments;
(e) optionally, adding sequencing adaptors to the ends of the nucleic acid fragments and/or amplifying the nucleic acid fragments;
(f) 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;

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- (g) isolating the subgroup of nucleic acid fragments which have been bound by the targeting nucleic acid using the second half of the binding pair;
- (h) amplifying the isolated subgroup of nucleic acid fragments;
- (j) optionally repeating Steps (f), (g) and (h) one or more times; and
- 5 (k) 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.

11. A method as claimed in claim 10, wherein the targeting nucleic acid is a DNA
10 oligonucleotide.

12. A method as claimed in claim 10 or claim 11, wherein the concentration of the
targeting nucleic acid (preferably a DNA oligonucleotide) is ~~5 μ M to 1 μ M, preferably,~~
~~2.9 μ M to 29 μ M, and more preferably 1 μ M to 30 μ M or 300nM to 30pM.~~

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13. A method as claimed in claim 11 or claim 12, 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
20 nucleic acids.

14. A method as claimed in claim 13, 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
25 interest, or of a regulatory element (preferably an enhancer, repressor or CTCF binding
site) in the nucleic acids.

15. A method as claimed in any one of claims 10-14, wherein Step (j) is repeated 1, 2,
3, 4 or 5 times.

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16. 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 10-15 including sequencing the amplified isolated subgroup of nucleic acid fragments in order to identify allele-specific interaction profiles in SNP-containing regions.

17. 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 10-15 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.

~~18. A kit for identifying nucleic acid regions within a nucleic acid sample which interact with one another, the kit comprising buffers and reagents for performing a method as defined in any one of claims 1-17.~~

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