

CLAIMS

1. A method for separating circulating cell free nucleosomes comprising linker DNA from a biological fluid sample, wherein said method comprises the steps of:
- 5 (i) contacting the sample with a binding agent which binds to:
- (a) nucleosome associated linker DNA;
 - (b) a chromatin binding protein which binds to linker DNA; or
 - (c) a core nucleosome feature associated with linker DNA; and
- 10 (ii) isolating nucleosomes from the sample which are bound to the binding agent in step (i).
2. A method for separating circulating cell free nucleosomes which do not comprise linker DNA from a biological fluid sample, wherein said method comprises the steps of:
- 15 (i) contacting the sample with a binding agent which binds to:
- (a) nucleosome associated linker DNA;
 - (b) a chromatin binding protein which binds to linker DNA; or
 - (c) a core nucleosome feature associated with linker DNA; and
- 20 (ii) isolating nucleosomes from the sample which are not bound to the binding agent in step (i).
3. The method as defined in claim 1 or claim 2, wherein the binding agent which binds to nucleosome associated linker DNA is a histone protein selected from histone H1, macroH2A or a fragment or an engineered analogue thereof.
- 25 4. The method as defined in claim 1 or claim 2, wherein the binding agent which binds to nucleosome associated linker DNA is a chromatin binding protein or a fragment or an engineered analogue thereof.
- 30 5. The method as defined in claim 4, wherein the chromatin binding protein which binds to linker DNA is selected from:
- (a) a Chromodomain Helicase DNA Binding (CHD) protein;
 - (b) a DNA (cytosine-5)-methyltransferase (DNMT) protein;
 - (c) a High mobility group box protein (HMGB) protein;

35 (d) a Poly [ADP-ribose] polymerase (PARP) protein; or

(e) a Methyl-CpG-binding domain (MBD) protein, such as MBD1, MBD2, MBD3, MBD4 or Methyl CpG binding protein 2 (MECP2)).

6. The method as defined in claim 1 or claim 2, wherein the binding agent that
5 binds to a chromatin binding protein which binds to linker DNA is selected from an antibody, an affimer or an aptamer.

7. The method as defined in claim 6, wherein the chromatin binding protein which
binds to linker DNA is selected from:
10 a) a Chromodomain Helicase DNA Binding (CHD) protein;
(b) a DNA (cytosine-5)-methyltransferase (DNMT) protein;
(c) a High mobility group box protein (HMGB) protein;
(d) a Poly [ADP-ribose] polymerase (PARP) protein; or
(e) a Methyl-CpG-binding domain (MBD) protein, such as MBD1, MBD2,
15 MBD3, MBD4 or Methyl CpG binding protein 2 (MECP2)).

8. The method as defined in claim 1 or claim 2, wherein the core nucleosome
feature associated with linker DNA is histone variant macroH2A.

20 9. The method as defined in any one of claims 1 to 8, which additionally
comprises:
(iii) analysing the isolated cell free nucleosomes of step (ii) by
immunoassay, mass spectroscopy and/or DNA analysis.

25 10. The method as defined in claim 9, wherein step (iii) comprises analysing the
isolated cell free nucleosome for epigenetic nucleosome features that are selected
from: histone type (for example, H2A, H2B, H3, H4 histone), post-translational
modifications, histone isoforms, particular nucleotides or modified nucleotides (for
example methylated, hydroxyl-methylated or other nucleotide modifications), proteins
30 adducted to the nucleosome or combinations thereof.

11. The method as defined in claim 9, wherein the DNA analysis comprises DNA
sequencing including Next Generation Sequencing (targeted or whole genome) and
methylated DNA sequencing analysis, BEAMing, PCR including digital PCR and cold
35 PCR (co-amplification at lower denaturation temperature-PCR), isothermal

amplification, MIDI-Activated Pyrophosphorolysis (MAP) or Personalized Analysis of Re-arranged Ends (PARE).

12. The method as defined in any one of claims 1 to 11, which additionally
5 comprises contacting the sample bound in step (i) with a second binding agent which binds to nucleosomes or a component thereof.

13. The method as defined in any one of claims 1 to 12, wherein the biological fluid
10 sample is selected from a blood, serum or plasma sample.

14. The method as defined in any one of claims 1 to 13, wherein the sample is contacted with more than one type of binding agent which binds to nucleosomes comprising linker DNA.

15. A method for separating circulating cell free nucleosomes which do not comprise linker DNA from a biological fluid sample, wherein said method comprises the steps of:

- (i) contacting the sample with a binding agent which binds to a clipped histone molecule from which all or part of the histone tail is absent:
- 20 (ii) isolating nucleosomes from the sample which are bound to the binding agent in step (i).

16. The method as defined in claim 15, which additionally comprises:
25 (iii) analysing the isolated cell free nucleosomes of step (ii) by immunoassay, mass spectrometry and/or DNA analysis.

17. The method as defined in claim 16, wherein step (iii) comprises analysing the isolated cell free nucleosome for epigenetic nucleosome features that are selected from: histone type (for example, H2A, H2B, H3, H4 histone), post-translational
30 modifications, histone isoforms, particular nucleotides or modified nucleotides (for example methylated, hydroxyl-methylated or other nucleotide modifications), proteins adducted to the nucleosome or combinations thereof.

18. The method as defined in claim 16, wherein the DNA analysis comprises DNA
35 sequencing including Next Generation Sequencing (targeted or whole genome) and methylated DNA sequencing analysis, BEAMing, PCR including digital PCR and cold

PCR (co-amplification at lower denaturation temperature-PCR), isothermal amplification, hybridization, MIDI-Activated Pyrophosphorolysis (MAP) or Personalized Analysis of Re-arranged Ends (PARE).

- 5 19. A method for isolating purified circulating tumour DNA (ctDNA) from a biological fluid sample, wherein said method comprises the steps of:
- (i) performing the method as defined in any one of claims 2 to 18; and
 - (ii) extracting the DNA from the isolated nucleosomes.
- 10 20. A method of diagnosing cancer comprising:
- (i) performing the method as defined in any one of claims 2 to 18 to isolate circulating tumour nucleosomes from a biological sample obtained from a patient; and
 - (ii) analysing the isolated circulating tumour nucleosomes and/or associated DNA.
- 15 21. The method as defined in claim 20, wherein step (ii) comprises analysing the isolated circulating tumour nucleosomes for epigenetic nucleosome features that are selected from: histone type (for example, H2A, H2B, H3, H4 histone), histone post-translational modifications, histone isoforms, particular nucleotides or modified
- 20 nucleotides (for example methylated, hydroxyl-methylated or other nucleotide modifications), proteins adducted to the nucleosome or combinations thereof.
- 22 The method as defined in claim 20, wherein the associated DNA is analysed using DNA sequencing, for example a sequencing method selected from Next
- 25 Generation Sequencing (targeted or whole genome) and methylated DNA sequencing analysis, BEAMing, PCR including digital PCR and cold PCR (co-amplification at lower denaturation temperature-PCR), isothermal amplification, hybridization, MIDI-Activated Pyrophosphorolysis (MAP) or Personalized Analysis of Re-arranged Ends (PARE).
- 30 23. A method for obtaining an antibody or other binding agent that preferentially binds to cell free nucleosomes of disease origin, wherein said method comprises the steps of:
- (i) using a nucleosome containing approximately 150 base pairs or less of
- 35 DNA as an antigen against which to produce an antibody or other binding agent;

(ii) presenting the antibody or other binding agent obtained in step (i) to a nucleosome containing approximately 165 base pairs or more of DNA; and

(iii) selecting the antibody or other binding agent that binds to the nucleosome of step (i), but not to the nucleosome of step (ii).

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24. A method for obtaining an antibody or other binding agent that preferentially binds to cell free nucleosomes of non-disease origin, wherein said method comprises the steps of:

(i) using a nucleosome containing approximately 165 base pairs or more of DNA as an antigen against which to produce an antibody or other binding agent;

(ii) presenting the antibody or other binding agent obtained in step (i) to a nucleosome containing approximately 150 base pairs or less of DNA; and

(iii) selecting the antibody or other binding agent that binds to the nucleosome of step (i), but not to the nucleosome of step (ii).

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25. The method as defined in claim 23 or claim 24, wherein the method comprises phage display library antibody selection.