

CLAIMS

1. An *in vitro* method for analysing cell free nucleosomes of tumor origin from a biological sample by affinity purification wherein said method comprises the steps of:
 - (i) contacting the sample with a histone H1 binding agent;
 - (ii) isolating nucleosomes from the sample which are not bound to the histone H1 binding agent; and
 - (iii) analysing the isolated nucleosomes by immunoassay or mass spectroscopy.
2. The *in vitro* method of claim 1, wherein the histone H1 binding agent is selected from: a binding agent that binds to histone H1 protein, variant, isoform or modification thereof.
3. The *in vitro* method of claim 1 or claim 2, wherein the histone H1 binding agent is selected from: a binding agent that binds to histone H1 protein or a histone H1 variant selected from H1.0, H1.10 or H1.X
4. The *in vitro* method of any one of claims 1 to 3, wherein the isolated nucleosomes are analysed by immunoassay or mass spectroscopy for inclusion of an epigenetic feature selected from: a post-translational histone modification, a histone isoform, a modified nucleotide or an adducted non-histone protein.
5. An *in vitro* method for isolating purified tumor DNA from a biological sample, wherein said method comprises the steps of:
 - (i) contacting the sample with a histone H1 binding agent;
 - (ii) isolating nucleosomes which are not bound to the histone H1 binding agent;
 - (iii) extracting DNA from the nucleosome sample isolated in step (ii); and
 - (iv) analysing the extracted DNA.
6. The *in vitro* method according to claim 5, wherein the step of analysing the extracted DNA comprises: DNA sequencing, methylated DNA sequencing analysis, PCR, BEAMing, NGS (targeted or whole genome), digital PCR, cold PCR (co-amplification at lower denaturation temperature-PCR), MAP (MIDI-Activated Pyrophosphorolysis), PARE (personalized analysis of rearranged ends) or Mass Spectrometry.
7. The *in vitro* method of any one of claims 1 to 6, which additionally comprises contacting the isolated nucleosomes with a histone H3.1 and/or H3.2 and/or H3t binding agent.

8. An *in vitro* immunoassay method for detecting an epigenetic epitope of tumor derived nucleosomes in a biological sample, wherein said method comprises the steps of:

- (i) contacting the sample with a first binding agent comprising a histone H1 binding agent;
- (ii) isolating nucleosomes which are not bound to the first binding agent;
- (iii) contacting the nucleosomes from step (ii) with a second binding agent which binds to said epitope;
- (iv) detecting and/or quantifying the binding of said second binding agent to said epitope; and
- (v) using the presence or degree of such binding as a measure of the presence of the particular epitope of tumor derived nucleosomes in the sample.

9. The *in vitro* method according to claim 8, wherein the epitope comprises: a histone modification (such as H3K27Ac); a modified nucleotide (such as 5-methylcytosine); a histone H2A, H2B, H3 or H4 variant or isoform; or a nucleosome adduct or variant thereof.

10. The *in vitro* method according to any one of claims 1 to 9, wherein the biological sample comprises blood, serum, plasma, menstrual blood, cerebrospinal fluid (CSF), endometrial fluid, urine, saliva, or other bodily fluid (stool, tear fluid, synovial fluid, sputum), breath, e.g. as condensed breath, or an extract or purification therefrom, or dilution thereof.

11. The *in vitro* method of claim 10, wherein the biological sample comprises a blood, serum or plasma sample.

12. A method for detecting cancer in an animal or a human subject which comprises the steps of:

- (i) analyzing a biological sample obtained from the subject for cell free nucleosomes that contain an epigenetic feature and also contain histone H1 by means of an immunoassay using two binding agents, one of which is directed to bind to the particular epigenetic feature and the other of which is directed to bind to histone H1;
- (ii) analyzing the sample for circulating cell free nucleosomes that contain the particular epigenetic feature, both with and without histone H1, by means of an immunoassay using two binding agents, one of which is directed to bind to the epigenetic feature and the other of which is directed to bind to a common core nucleosome epitope including any histone H2, H3 or H4 epitope, any

DNA epitope or any other epitope present in chromatin fragments other than a histone H1 epitope; and

- (iii) using a combination of the immunoassay results obtained in steps (i) and (ii) as a combination biomarker indicative of the presence of said cancer in the subject.

13. The method of claim 12, wherein the cancer is selected from: breast, bladder, colorectal, skin (such as melanoma), ovarian, prostate, lung, pancreatic, bowel, liver, endometrial, lymphoma, oral, head and neck cancer, leukaemia and osteosarcoma.

14. Use of a kit comprising a histone H1 binding agent in a method described in any one of claims 1 to 13.