Claims

1. A method of determining epigenetic modification status, e.g. methylation status of at least one nucleotide of interest (NOI) in a non-modified target nucleic acid sequence of interest, wherein said target nucleic acid sequence comprises a target anchor sequence comprising said NOI, said method comprising the steps of

   a. Providing an oligonucleotide comprising an anchor sequence (An), wherein the anchor sequence is a sequence at least 50% complementary to said target anchor sequence, wherein the anchor sequence comprises at least one hydrophobic nucleotide (H) positioned between the nucleotide complementary to said NOI and the nucleotide immediately 5' thereof;

   b. Incubating said oligonucleotide with said target nucleic acid of interest at a temperature which is higher than the melting temperature between said oligonucleotide and the target nucleic acid sequence of interest when said NOI is unmodified, such as unmethylated,

   c. Detecting whether said oligonucleotide anneals to said target nucleic acid of interest, thereby determining the epigenetic modification status, e.g. the methylation status, wherein

      i. said hydrophobic nucleotide (H) has the structure

         X-Y-Q,

         wherein

         X is a nucleotide or nucleotide analogue or a backbone monomer unit capable of being incorporated into the backbone of a nucleic acid or nucleic acid analogue;

         Q is an intercalator which is not taking part in Watson-Crick hydrogen bonding; and

         Y is a linker moiety linking said nucleotide or nucleotide analogue or backbone monomer unit and said intercalator; and

      ii. said oligonucleotide has the structure 5'-An-Lp-St-3', wherein
         An is the anchor sequence;

         Lp is a loop sequence, which is not complementary to the target nucleic acid sequence of interest, wherein the loop sequence consists of a single nucleic acid sequence capable of forming a protruding structure, such as a loop structure or a stem structure, or consists of two or more nucleic acid sequences capable of hybridising at least partly to one another to form a complex which is capable of forming a protruding structure, such as a loop structure or a stem structure;

St is a starter sequence, capable of hybridizing to a target starter sequence, wherein the target starter sequence is a sequence of the target nucleic acid sequence positioned 5' to the target anchor sequence.

2. The method according to claim 1, wherein the epigenetic modification is methylation.

3. The method according to any one of the preceding claims, wherein the target nucleic acid of interest is DNA or RNA.

4. The method according to any one of the preceding claims, wherein the non-modified nucleic acid sequence of interest has not been subjected to treatment comprising bisulfite conversion, restriction enzyme digestion or TET enzymatic conversion prior to performing the method.

5. The method according to any one of the preceding claims, wherein the NOI is a cytosine.

6. The method according to any one of the preceding claims, wherein the difference between

   A. the absolute difference between the melting temperatures of
      i. the oligonucleotide comprising said hydrophobic nucleotide(s) when hybridized to said target nucleic acid sequence of interest, wherein said nucleic acid sequence of interest comprises at least one methylated NOI, such as at least one methylated cytosine; and
      ii. the oligonucleotide comprising said hydrophobic nucleotide(s) when hybridized to said target nucleic acid sequence of interest, wherein said nucleic acid sequence of interest comprises no methylated NOIs, such as no methylated cytosines; and
   B. the absolute difference between the melting temperatures of
      i. an oligonucleotide identical to the oligonucleotide of A., however which does not comprise said hydrophobic nucleotide(s), when hybridized to said target nucleic acid sequence of interest, wherein said nucleic acid sequence of interest comprises the same number of methylated NOIs as in A. i., such as the same number of methylated cytosines as in A. i.; and
      ii. an oligonucleotide identical to the oligonucleotide of A., however which does not comprise said hydrophobic nucleotide(s), when hybridized to said target

nucleic acid sequence of interest, wherein said nucleic acid sequence of interest comprises no methylated NOIs, such as no methylated cytosines,

is of at least 0.50 °C, such as at least 0.75 °C, preferably at least 1.0 °C per NOI, such as per cytosine, that is methylated instead of unmethylated in said target nucleic acid sequence of interest, wherein the difference is A-B and wherein the difference is a positive difference, and wherein the melting temperature is measured in TM buffer comprising 0.02 M Na_2HPO_4, 0.02 M NaCl, and 2 mM EDTA.

7. The method according to any one of the preceding claims, wherein the anchor sequence comprises at least one -N-H-G- sequence, wherein each -G- is complementary to a cytosine of interest and each -N- individually is selected from the group consisting of C, G, A and T and is complementary to the neighbouring nucleotide of said cytosine of interest.

8. The method according to any one of the preceding claims, wherein steps b) and c) together comprises performing a PCR, wherein said temperature is used as the annealing temperature in one or more cycles of said PCR.

9. The method according to any one of the preceding claims, wherein said oligonucleotide consists of a first and a second nucleic acid sequences, wherein the first nucleic acid sequence comprises the anchor sequence (An) complementary to the target DNA and a first part of a loop sequence not complementary to the target nucleic sequence of interest, and the second nucleic acid sequence comprises a second part of the loop sequence, capable of hybridising at least partly to the first part of the loop sequence, said second nucleic acid sequence further comprising the starter sequence (St), and wherein the first and the second nucleic acids once hybridized are capable of forming the protruding structure.

10. The method according to any one of the preceding claims, wherein the target anchor sequence and the target starter sequence are positioned within 10, for example within 8, such as within 5 nucleotides from each other.

11. The method according to any one of the preceding claims, wherein Lp and St are selected such that an oligonucleotide consisting of Lp-St has a melting temperature with its complementary sequence of at least 50°C, for example at least 55°C, such as at least

65°C.

12. The method according to any one of the preceding claims, wherein Lp consists of two or more nucleic acid sequences, and wherein the nucleic acid sequence(s) not covalently linked to the St sequence comprise(s) one or more hydrophobic nucleotides.

13. The method according to any one of the preceding claims, wherein St does not comprise a hydrophobic nucleotide.

14. The method according to any one of the preceding claims, wherein the difference in melting temperature between said oligonucleotide and the methylated target nucleic acid sequence of interest is at least 5°C higher, for example at least 6°C higher, such as in the range of 6 to 15°C higher, for example in the range of 6 to 12°C higher than the difference in melting temperature between said oligonucleotide and the unmethylated target nucleic acid sequence of interest.

15. The method according to any one of the preceding claims, wherein the difference in melting temperature between said oligonucleotide and the methylated target nucleic acid sequence compared to an unmethylated target nucleic acid sequence of interest is at least 1°C higher than the difference in melting temperature between an oligonucleotide of identical sequence except lacking the hydrophobic nucleotides and the methylated target nucleic acid sequence compared to an unmethylated target nucleic acid sequence.

16. The method according to claim 8, wherein said PCR comprises one or more methyl-specific amplification cycles and one or more general amplification cycles, wherein the methyl specific amplification cycle(s) comprise the steps of
    a. Melting nucleic acids
    b. Annealing and extension under methyl specific conditions

    and the general amplification cycles comprise the steps of
    a. Melting nucleic acids
    b. Annealing and extension under general conditions

    wherein the melting comprises incubation at a temperature of at least 90 °C, and wherein the annealing and extension under methyl specific conditions is performed at a higher temperature than annealing and extension under general conditions.

17. The method according to any one of claims 8 to 15, wherein said PCR is performed using said oligonucleotide as forward primer, wherein said PCR further comprises use of a reverse primer at least 90% identical, for example 100% identical to a sequence downstream of said target nucleic acid sequence of interest.

18. The method according to any of the preceding claims, wherein target nucleic acid sequence of interest is comprised in DNA purified from a sample obtained from an individual suffering from or at risk of having a clinical condition associated with methylation in the nucleic acid of interest.

19. A method for determining the risk of whether an individual suffers from a clinical condition, or the risk of an individual to contract a clinical condition, or determining the likelihood of effect of a treatment of a clinical condition in an individual, wherein the clinical condition is associated with the methylation status of a NOI in a nucleic acid of interest, said method comprising
    a. Providing a sample obtained from said individual;
    b. Determining the methylation status of said NOI in said sample by performing the method according to any one of the preceding claims, wherein said methylation status is indicative of the presence of or risk of contracting said clinical condition or of the effects of different treatment regimens on said clinical condition.

20. An oligonucleotide comprising or consisting of the following general structure:

    5'$(N)_n$-HG-$(N)_m$-HG-$(N)_p$-HG-$(N)_q$-3',
    wherein N is any nucleotide or nucleotide analogue; and
    G is the nucleotide guanine; and
    n is an integer ≥0; and
    m is an integer ≥1; and
    p is an integer ≥1; and
    q is an integer ≥0; and
    H is a hydrophobic nucleotide
    or
    5'$(N)_n$-CGH-$(N)_m$-CGH-$(N)_p$-3',
    wherein N is any nucleotide or nucleotide analogue; and
    C is the nucleotide cytosine; and
    G is the nucleotide guanine; and

n is an integer ≥0; and

m is an integer ≥1; and

p is an integer ≥0; and

H is a hydrophobic nucleotide

wherein said hydrophobic nucleotide has the structure

X-Y-Q

wherein

X is a nucleotide or nucleotide analogue or a backbone monomer unit capable of being incorporated into the backbone of a nucleic acid or nucleic acid analogue,

Q is an intercalator which is not taking part in Watson-Crick hydrogen bonding; and

Y is a linker moiety linking said nucleotide or nucleotide analogue or backbone monomer unit and said intercalator.

21. The oligonucleotide according to claim 19, wherein the oligonucleotide comprises or consists of the following general structure:

$5'(N)_n$-HG-$(N)_m$-HG-$(N)_p$-HG-$(N)_q$-HG-$(N)_u$-3', wherein p is an integer ≥1

or

$5'(N)_n$-CGH-$(N)_m$-CGH-$(N)_p$-CGH-$(N)_q$-CGH-$(N)_u$-3', wherein p is an integer ≥0;

wherein

q is an integer ≥1; and

u is an integer ≥0.

22. The oligonucleotide according to any of claims 19 to 20, wherein the nucleotide located immediately 5' to at least one H is a cytosine (C).