Claims

1. A method for detecting the presence of a variant sequence in a target nucleic acid sequence consisting of two strands and comprising nucleotide(s) of interest (NOI), wherein the NOI is a microsatellite sequence of *M* tandem repeats having a total length of *n* nucleotides, and wherein the variant sequence has *M'* tandem repeats having a total length of *n'* nucleotides, wherein *M* and *M'* are different integers, wherein *n* is 15 or more, such as 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50 or more, and wherein said target nucleic acid sequence consists of a variant sequence or of a reference sequence, said method comprising the steps of:

a) Providing a first sample comprising nucleic acids suspected of comprising said variant sequence;

b) Providing a second sample comprising nucleic acids comprising said reference sequence, wherein the second sample is a reference sample;

c) Providing a reporter oligonucleotide;

d) Providing a set of primers consisting of a first primer and a second primer, wherein the set of primers together are capable of amplifying the target nucleic acid sequence comprising the NOI;

e) Amplifying the target nucleic acid sequence in the presence of said first sample, said first primer and said second primer, thereby obtaining a first amplicon comprising nucleic acids suspected of comprising a variant sequence; and amplifying the target nucleic acid sequence in the presence of said second sample, said first primer and said second primer, thereby obtaining a second amplicon comprising the reference sequence, wherein the second amplicon is a reference amplicon;

f) Performing melting analysis, such as high-resolution melt (HRM) analysis, of the first amplicon, thereby obtaining a first profile characterised by a first melt curve, and performing melting analysis, such as HRM analysis, of the second amplicon, thereby obtaining a second profile, characterised by a second melt curve wherein the second profile is a reference profile characterised by a reference melt curve; wherein each amplicon comprises a first strand and a second strand, wherein the melting analysis involves hybridisation of the reporter oligonucleotide to one strand of each amplicon,

detection of a signal emitted by the fluorophore, and obtaining the first and the second melt curves;

wherein the reporter oligonucleotide is a sequence of in the range of 10 to 50, preferably in the range of 15 to 50 nucleotides, into which in the range of 2 to 10 hydrophobic nucleotides have been inserted,

wherein the reporter oligonucleotide comprises a first fluorophore, preferably in its 5'-end or within 4 nucleotides from the 5'-end, and a first quencher, preferably in its 3'-end or within 4 nucleotides from the 3'-end, and

wherein the reporter oligonucleotide comprises a hybridization sequence H, wherein the hybridisation sequence is identical to a consecutive stretch of the sequence of a first strand of the target nucleic acid sequence, and wherein the hybridisation sequence is complementary to a consecutive stretch of the sequence of the second strand of the target nucleic acid sequence, and

wherein the length of the hybridisation sequence H of the reporter oligonucleotide is *n''*, wherein $n'' \geq n + 1$, preferably $n'' \geq n + 1$ or $n'' \geq n + 2$, and wherein the hybridisation sequence of the reporter oligonucleotide comprises or consists of a repetitive sequence and at least one helper sequence in its 5'-end and/or in its 3'-end, wherein said helper sequence does not comprise repeats, and can hybridise to the first and second amplicons when the hybridisation sequence is hybridized thereto; and

g) Comparing the first profile to the reference profile, wherein a difference between the first profile and the reference profile indicates that the first sample contains a variant sequence.

2. The method according to claim 1, wherein step g) comprises or consists of the steps of:

i) aligning the first and the second melt curves at a given fluorescent intensity along the temperature axis, thereby nullifying differences in melting temperatures between the first and the second melt curves;

ii) determining the difference in the signal emitted by the fluorophore between the first and the second melt curves, preferably wherein the difference is a numerical difference; and

iii) comparing the difference determined in ii) to a threshold value, wherein a difference greater than the threshold indicates that the first sample comprises a

variant sequence and a difference smaller than the threshold indicates that the first sample comprises the reference sequence.

3. The method according to any one of the preceding claims, wherein the reference sequence has a length of 15 nucleotides or more, such as 16, 17, 18, 19 , 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides or more.

4. The method according to any one of the preceding claims, wherein the reporter oligonucleotide consists of a repetitive sequence and only one helper sequence in its 5'-end or its 3'-end or wherein the reporter oligonucleotide consists of a repetitive sequence and two helper sequences, such as one helper sequence in both the 5'-end and the 3'-end of the reporter oligonucleotide.

5. The method according to any one of the preceding claims, wherein
at least one of said hydrophobic nucleotides is positioned at the 5'-end or within 10 nucleotides from the 5'-end of the reporter oligonucleotide; and/or
at least one hydrophobic nucleotide is positioned at the 3'-end or within 10 nucleotides from the 3'-end of the reporter oligonucleotide; and
wherein the 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.

6. The method according to any one of the preceding claims, wherein the helper sequence comprises or consists of 1 to 20 nucleotides, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 ,15, 16 ,17, 18, 19 or 20 nucleotides.

7. The method according to any one of the preceding claims, wherein the helper sequence comprises at least one hydrophobic oligonucleotide as defined in claim 5.

8. The method according to any one of the preceding claims, wherein at least one hydrophobic nucleotide as defined in claim 5, such as 1, 2, or 3 hydrophobic nucleotides, is inserted within 1 to 10 nucleotides from the 3'-end of the reporter oligonucleotide, such as within 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotides from the 3'-end.

9. The method according to any one of the preceding claims, wherein at least one hydrophobic nucleotide as defined in claim 5, such as 1, 2, or 3 hydrophobic nucleotides, is inserted within 1 to 10 nucleotides from the 5'-end of the reporter oligonucleotide, such as within 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotides from the 5'-end.

10. The method according to any one of the preceding claims, wherein amplifying in step e) is performed by polymerase chain reaction (PCR), preferably by asymmetric PCR wherein the first and second primers are provided in different amounts, thereby directing the PCR towards amplifying more of one strand of each amplicon than of the other strand of each amplicon.

11. The method according to any one of the preceding claims, wherein the first sample has been isolated from an individual suffering from or suspected of suffering from a disease, such as a cancer, preferably the cancer is hereditary non-polyposis colorectal cancer, or from a disorder, preferably a disorder associated with microsatellite instability.

12. The method according to any one of the preceding claims, wherein the first sample is a sample of a tissue comprising or suspected of comprising cells with mutations characteristic of said disease or disorder.

13. A kit of parts for detecting the presence of a variant sequence in a target nucleic acid sequence consisting of two strands and comprising nucleotide(s) of interest (NOI), wherein the NOI is a microsatellite sequence of M tandem repeats having a total length of n nucleotides, and wherein the variant sequence has M' tandem repeats having a total length of n' nucleotides, wherein M and M' are different integers, wherein n is 15 or more, such as 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50 or more, and wherein said target nucleic acid sequence consists of a variant sequence or of a reference sequence, said kit of parts comprising:

a) a reporter oligonucleotide comprising a first fluorophore, preferably in its 5'-end or within 4 nucleotides from the 5'-end, and a first quencher, preferably in its 3'-end or within 4 nucleotides from the 3'-end,
wherein the reporter oligonucleotide is a sequence of in the range of 10 to 50 nucleotides, preferably in the range of 15 to 50 nucleotides, into which in the range of 2 to 10 hydrophobic nucleotides have been inserted and wherein the reporter oligonucleotide comprises a hybridization sequence H, and

wherein the hybridisation sequence is identical to a consecutive stretch of the sequence of a first strand of the target nucleic acid, and wherein the hybridisation sequence is complementary to a consecutive stretch of the sequence of a second strand of the target nucleic acid, and wherein the length of the hybridisation sequence H of the reporter oligonucleotide is n'', wherein $n'' \geq n + 1$, preferably $n'' \geq n + 1$ or $n'' \geq n + 2$; such as n'' is 16 or more, such as 17, 18, 19, 20 nucleotides or more, such as 25, 30, 35, 40, 45, 50 or more,
and wherein the hybridisation sequence of the reporter oligonucleotide comprises or consists of a repetitive sequence and at least one helper sequence in its 5'-end and/or in its 3'-end, wherein said helper sequence does not comprise repeats, and can hybridise to the first and second amplicons when the hybridisation sequence is hybridized thereto; and

b) a set of primers consisting of a first primer and a second primer, wherein the set of primers together are capable of amplifying the target nucleic acid sequence.

14. The kit according to claim 13, preferably wherein the reporter oligonucleotide, the first primer and the second primer are as defined in any one of claims 1 to 12.

15. The kit according to any one of claims 13 to 14, wherein the reporter oligonucleotide consists of a repetitive sequence and only one helper sequence in its 5'-end or its 3'-end or wherein the reporter oligonucleotide consists of a repetitive sequence and two helper sequences, such as one helper sequence in both the 5'-end and the 3'-end of the reporter oligonucleotide.

16. The kit according to any one of claims 13 to 15, wherein
at least one of said hydrophobic nucleotides is positioned at the 5'-end or within 10 nucleotides from the 5'-end of the reporter oligonucleotide; and/or
at least one hydrophobic nucleotide is positioned at the 3'-end or within 10 nucleotides from the 3'-end of the reporter oligonucleotide; and
wherein the 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;

17. A reporter oligonucleotide which can hybridise to one strand of a target nucleic acid consisting of two strands and comprising nucleotide(s) of interest (NOI), wherein the NOI is a microsatellite sequence of M tandem repeats having a total length of n nucleotides, and wherein the variant sequence has M' tandem repeats having a total length of n' nucleotides, wherein M and M' are different integers, wherein n is 15 or more, such as 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50 or more, said reporter oligonucleotide comprising a first fluorophore, preferably in its 5'-end or within 4 nucleotides from the 5'-end, and a first quencher, preferably in its 3'-end or within 4 nucleotides from the 3'-end, wherein the reporter oligonucleotide is a sequence of in the range of 10 to 50, preferably in the range of 15 to 50 nucleotides, into which in the range of 2 to 10 hydrophobic nucleotides have been inserted and wherein the reporter oligonucleotide comprises a hybridization sequence H,

wherein the hybridisation sequence is identical to a consecutive stretch of the sequence of a first strand of the target nucleic acid, and wherein the hybridisation sequence is complementary to a consecutive stretch of the sequence of a second strand of the target nucleic acid, and wherein the length of the hybridisation sequence H of the reporter oligonucleotide is n'', wherein $n'' \geq n + 1$, preferably $n'' \geq n + 1$ or $n'' \geq n + 2$; such as n'' is 16 or more, such as 17, 18, 19, 20 nucleotides or more, such as 25, 30, 35, 40, 45, 50 or more, and wherein the hybridisation sequence of the reporter oligonucleotide comprises or consists of a repetitive sequence and at least one helper sequence in its 5'-end and/or in its 3'-end, wherein said helper sequence does not comprise repeats, and can hybridise to the second strand of the first and second amplicons when the hybridisation sequence is hybridized thereto.

18. The reporter oligonucleotide according to claim 17, wherein the reporter oligonucleotide consists of a repetitive sequence and only one helper sequence in its 5'-end or its 3'-end or wherein the reporter oligonucleotide consists of a repetitive sequence and two helper sequences, such as one helper sequence in both the 5'-end and the 3'-end of the reporter oligonucleotide.

19. The reporter oligonucleotide according to any one of claims 17 to 18, wherein

at least one of said hydrophobic nucleotides is positioned at the 5'-end or within 10 nucleotides from the 5'-end of the reporter oligonucleotide; and/or
at least one of said hydrophobic nucleotides is positioned at the 3'-end or within 10 nucleotides from the 3'-end of the reporter oligonucleotide; and
wherein the 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.

20. The reporter oligonucleotide according to any one of claims 17 to 19, wherein the helper sequence comprises or consists of 1 to 20 nucleotides, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 ,15, 16 ,17, 18, 19 or 20 nucleotides, preferably wherein the helper sequence comprises at least one hydrophobic oligonucleotide as defined in claim 3.

21. A method of predicting the efficacy of treatment of a clinical condition in an individual in need thereof with a predetermined drug, wherein the efficacy of treatment of said clinical condition with said drug is associated with the presence of a variant sequence, said method comprising the steps of

a. providing a sample from said individual
b. performing the method according to any one of claims 1 to 12 to determine the presence of said variant sequence;

wherein the presence of said variant sequence is indicative of whether said drug is efficient in treating said clinical condition in said individual.

22. A method of predicting the presence of a clinical condition in an individual in need thereof, wherein said clinical condition is associated with the presence of a target nucleic acid sequence comprising a variant sequence, said method comprising the steps of

a. providing a sample from said individual
b. performing the method according to any one of claims 1 to 12 to determine the presence of a variant sequence;

wherein the presence of a variant sequence is indicative of said individual suffering from said clinical condition.