

Claims

1. A method for evaluating levels of microsatellite instability in a sample comprising:
 - 5 (a) providing primers for amplifying a plurality of the selected group of microsatellite loci of human genomic DNA identified in Table A
 - (b) amplifying from the sample the plurality of selected microsatellite mono-nucleotide repeat loci to give microsatellite amplicons;
 - (c) sequencing the microsatellite amplicons; and
 - 10 (d) comparing the sequences from the microsatellite amplicons to predetermined sequences and determining any deviation, indicative of instability, from the predetermined sequences.
2. A method as in Claim 1 wherein, in step (a) primers are provided for
15 amplifying at least 6 or at least 7 of the selected group of microsatellite loci identified in Table A, optionally wherein the at least 6 or at least 7 microsatellite loci are selected group consisting of GM07, LR11, LR36, LR44, LR48, IM49 and GM14.
- 20 3. A method as in any of the previous Claims wherein, in step (a) primers are also provided for amplifying the microsatellite loci DEPDC2 and AP003532_2.
- 25 4. A method as in any of the previous Claims wherein, in step (a) primers are provided for amplifying at least 10 of the selected group of microsatellite loci of human genomic DNA of Table A and/or Table B or at least 17 of the selected group of microsatellite loci of human genomic DNA of Table A and/or Table B or at least 18 of the selected group of microsatellite loci of human genomic DNA of Table A and/or Table B.
- 30 5. A method as in any of the previous Claims wherein step (a) is providing primers for amplifying at least 10 of the selected group of microsatellite loci of human genomic DNA comprising;
DEPDC2, LR46, AL359238, AL954650, AP003532_2, TTK, AL355154,

AVIL, GM29, LR32, ASTE1, GM07, GM14, LR11, LR48, IM49, LR36, LR44,
LR49, IM66, LR20, GM11, LR24, IM16, GM17, GM9.

- 5 6. A method as in any of the previous Claims wherein step (a) is providing
primers for amplifying at least 10 of the selected group of microsatellite
loci of human genomic DNA comprising;
DEPDC2, LR46, AL359238, AL954650, AP003532_2, TTK, AL355154,
AVIL, GM29, LR32, ASTE1, GM07, GM14, LR11, LR48, IM49, LR36, LR44.
- 10 7. A method as in any of the previous Claims wherein step (a) is providing
primers for amplifying the selected group of microsatellite loci of human
genomic DNA comprising;
DEPDC2, LR46, AL359238, AL954650, AP003532_2, TTK, AL355154,
AVIL, GM29, LR32, ASTE1, GM07, GM14, LR11, LR48, IM49, LR36, LR44.
- 15 8. A method as in any of the previous Claims wherein step (a) is providing
primers for amplifying at least 10 of the selected group of microsatellite
loci of human genomic DNA comprising;
LR49, IM66, LR20, GM11, LR24, IM16, GM17, GM9, GM07, LR36, LR44,
20 LR48, LR11, AP003532_2, DEPDC2, GM14, IM49.
- 25 9. A method as in any of the previous Claims wherein step (a) is providing
primers for amplifying the selected group of microsatellite loci of human
genomic DNA comprising;
LR49, IM66, LR20, GM11, LR24, IM16, GM17, GM9, GM07, LR36, LR44,
LR48, LR11, AP003532_2, DEPDC2, GM14, IM49.
- 30 10. A method as in any of the previous Claims wherein the step of (b)
amplifying from the sample the plurality of selected microsatellite mono-
nucleotide repeat loci to give microsatellite amplicons, comprises co-
amplifying the set of selected loci in a multiplex amplification reaction.

11. A method as in any of the previous Claims wherein a number of separate microsatellite mono-nucleotide repeat loci are amplified and sequenced as separate reactions.
- 5 12. A method as in any of the previous Claims wherein amplification is by polymerase chain reaction and uses using primer pairs, each primer pair comprising a forward primer which is complimentary to a portion upstream from a selected microsatellite mono-nucleotide repeat loci, and a reverse primer which is complimentary to a portion downstream from
10 said selected microsatellite mono-nucleotide repeat loci.
13. A method as in any of the previous Claims wherein the selected group of microsatellite loci also include a single nucleotide polymorphism (SNP) within a short distance of the microsatellite loci, preferably wherein the
15 selected group of microsatellite loci also include a single nucleotide polymorphism (SNP) within 100 base pairs of the microsatellite loci.
14. A method as in any of the previous Claims wherein the single nucleotide polymorphism (SNP) has a minor allele frequency between 0.05 – 0.95,
20 and/or wherein the single nucleotide polymorphism (SNP) has a high frequency.
15. A method as in any of the previous Claims wherein the method includes the step of determining allelic imbalance, and/or wherein the method
25 includes the step of diagnosing MSI induced CRC or Lynch syndrome.
16. A method as in any of the previous Claims wherein deviation from the predetermined sequences for two or more microsatellite mono-nucleotide repeat loci is indicative of a mismatch repair defect, and/or
30 wherein deviation from the predetermined sequences for two or more microsatellite mono-nucleotide repeat loci is indicative of the sample having high levels of microsatellite instability (MSI-H).

17. A method as in any of the previous Claims wherein the sample is a tumor sample or a body tissue or fluid suitable for detecting tumor cells.
- 5 18. A kit for evaluating levels of microsatellite instability in a sample, comprising:
oligonucleotide primers for co-amplifying a plurality of microsatellite loci of human genomic DNA selected from the set of microsatellite loci identified in Table A.
- 10 19. A kit as in Claim 18 wherein the primers are for amplifying at least 6 or at least 7 of the selected group of microsatellite loci identified in Table A, optionally wherein the at least 6 or at least 7 microsatellite loci are selected group consisting of GM07, LR11, LR36, LR44, LR48, IM49 and GM14.
- 15 20. A kit as in claim 18 or 19 further comprising primers that are for amplifying the microsatellite loci DEPDC2 and AP003532_2.
- 20 21. A kit as in any of Claims 18 to 20, wherein the primers are for amplifying at least 10 of the selected group of microsatellite loci of human genomic DNA of Table A and/or Table B or at least 17 of the selected group of microsatellite loci of human genomic DNA of Table A and/or Table B or at least 18 of the selected group of microsatellite loci of human genomic DNA of Table A and/or Table B.
- 25 22. A kit as in any of Claims 18 to 21 wherein the primers are for amplifying at least 10 of the selected group of microsatellite loci of human genomic DNA comprising;
DEPDC2, LR46, AL359238, AL954650, AP003532_2, TTK, AL355154,
30 AVIL, GM29, LR32, ASTE1, GM07, GM14, LR11, LR48, IM49, LR36, LR44, LR49, IM66, LR20, GM11, LR24, IM16, GM17, GM9.

23. A kit as in any of Claims 18 to 22 wherein the primers are for amplifying at least 10 of the selected group of microsatellite loci of human genomic DNA comprising;
DEPDC2, LR46, AL359238, AL954650, AP003532_2, TTK, AL355154,
5 AVIL, GM29, LR32, ASTE1, GM07, GM14, LR11, LR48, IM49, LR36, LR44.
24. A kit as in any of Claims 18 to 23 wherein the primers are for amplifying the selected group of microsatellite loci of human genomic DNA comprising;
10 DEPDC2, LR46, AL359238, AL954650, AP003532_2, TTK, AL355154, AVIL, GM29, LR32, ASTE1, GM07, GM14, LR11, LR48, IM49, LR36, LR44.
25. A kit as in any of Claims 18 to 24 wherein the primers are for amplifying at least 10 of the selected group of microsatellite loci of human genomic DNA comprising;
15 LR49, IM66, LR20, GM11, LR24, IM16, GM17, GM9, GM07, LR36, LR44, LR48, LR11, AP003532_2, DEPDC2, GM14, IM49.
26. A kit as in any of 18 to 25 wherein the primers are for amplifying the selected group of microsatellite loci of human genomic DNA comprising;
20 LR49, IM66, LR20, GM11, LR24, IM16, GM17, GM9, GM07, LR36, LR44, LR48, LR11, AP003532_2, DEPDC2, GM14, IM49.
27. A method for evaluating the biological significance of mutations identified during sequencing;
25 (a) providing primers for amplifying a plurality of a selected group of microsatellite mono-nucleotide repeat loci of human genomic DNA, said microsatellite loci having a single nucleotide polymorphisms loci within a short distance of the microsatellite loci and said primers selected to amplify both the microsatellite
30 loci and the SNP in a single amplicon;
(b) amplifying from the sample the plurality of selected microsatellite mono-nucleotide repeat loci to give microsatellite amplicons;

- (c) sequencing the microsatellite amplicons; and
- (d) comparing the sequences from the microsatellite amplicons to predetermined sequences (wild type sequences) and determining any deviation, indicative of instability, from the predetermined sequences; and
- (e) for heterozygous SNPs, determining whether there is a bias between indel frequencies for the two alleles.
28. A method as in Claim 27 wherein the selected group of microsatellite loci include a single nucleotide polymorphism (SNP) within 100 base pairs of the microsatellite loci.
29. A method as in any of Claims 27 or 28 wherein the selected group of microsatellite loci include a single nucleotide polymorphism (SNP) within 80 base pairs of the microsatellite loci.
30. A method as in any of Claims 27 to 29 wherein the selected group of microsatellite loci include a single nucleotide polymorphism (SNP) within 50 base pairs of the microsatellite loci.
31. A method as in any of Claims 27 to 30 wherein the selected group of microsatellite loci include a single nucleotide polymorphism (SNP) within 30 base pairs of the microsatellite loci.
32. A method as in any of Claims 27 to 31 wherein the single nucleotide polymorphism (SNP) has a minor allele frequency between 0.05 – 0.95.
33. A method as in any of Claims 27 to 32 wherein the single nucleotide polymorphism (SNP) has a high frequency.