

CLAIMS

1. An assay for detecting mutations of NRAS, the assay comprising

contacting a portion of a nucleic acid sample with at least two primer pairs, in which each primer pair amplifies a NRAS sequence comprising a sequence variation;

- 5 performing a PCR amplification regimen comprising cycles of strand separation, primer annealing, and primer extension on a reaction mixture comprising the portion of the sample and the two or more primer pairs;

detecting the presence or absence of the amplicon for each primer pair;

- 10 in which the presence of an amplicon indicates the presence of the sequence variation for which that primer pair is specific;

and in which the at least two primer pairs are selected from the group consisting of:

- 15 SEQ ID NOs: 1 and 8; SEQ ID NOs: 2 and 8; SEQ ID NOs: 3 and 8; SEQ ID NOs: 4 and 8; SEQ ID NOs: 5 and 8; SEQ ID NOs: 6 and 8; SEQ ID NOs: 7 and 8; SEQ ID NOs: 9 and 14; SEQ ID NOs: 10 and 14; SEQ ID NOs: 11 and 14; SEQ ID NOs: 12 and 14; SEQ ID NOs: 13 and 14; SEQ ID NOs: 19 and 33; SEQ ID NOs: 20 and 33; SEQ ID NOs: 21 and 33; SEQ ID NOs: 22 and 33; SEQ ID NOs: 23 and 33; SEQ ID NOs: 24 and 33; SEQ ID NOs: 25 and 33; SEQ ID NOs: 26 and 32; SEQ ID NOs: 27 and 32; SEQ ID NOs: 28 and 32; SEQ ID NOs: 29 and 32; SEQ ID NOs: 30 and 32; and SEQ ID NOs: 31 and 32.

- 20 2. An assay according to Claim 1, in which the two or more primer pairs are selected from the group consisting of:

- 25 SEQ ID NOs: 1 and 8; SEQ ID NOs: 2 and 8; SEQ ID NOs: 3 and 8; SEQ ID NOs: 4 and 8; SEQ ID NOs: 5 and 8; SEQ ID NOs: 6 and 8; SEQ ID NOs: 7 and 8; SEQ ID NOs: 9 and 14; SEQ ID NOs: 10 and 14; SEQ ID NOs: 11 and 14; SEQ ID NOs: 12 and 14; SEQ ID NOs: 13 and 14; SEQ ID NOs: 19 and 33; SEQ ID NOs: 20 and 33; SEQ ID NOs: 21 and 33; SEQ ID NOs: 22 and 33; SEQ ID NOs: 23 and 33; SEQ ID NOs: 24 and 33; SEQ ID NOs: 25 and 33; SEQ ID NOs: 26 and 32; SEQ ID NOs: 27 and 32; SEQ ID NOs: 28 and 32; SEQ ID NOs: 29 and 32; SEQ ID NOs: 30 and 32; and SEQ ID NOs: 31 and 32.

3. An assay according to Claim 1 or 2, in which one or more sequence variations are point mutations.
4. An assay according to Claim 1, 2 or 3, in which the NRAS point mutation is selected from the group consisting of:
 - 5 G12D; G12S; G13A; G13C; G13D; G12R; G13V; Q61H1; Q61K; Q61L; Q61R1; and Q61R2.
5. An assay according to any preceding claim, in which the presence or absence of G12D; G12S; G13A; G13C; G13D; G12R; G13V; Q61H1; Q61K; Q61L; Q61R1; and Q61R2 is detected.
- 10 6. An assay according to any preceding claim, in which the set of primers further comprises one or more primer pairs selected from SEQ ID NOS: 34 and 37; SEQ ID NOS: 35 and 38; and SEQ ID NOS: 36 and 39.
7. An assay according to any preceding claim, in which the nucleic acid sample is prepared from a FFPE tumor sample.
- 15 8. An assay according to any preceding claim, in which the sample comprises tumor cells from a subject diagnosed with a condition selected from the group consisting of:

gastric cancer; renal cancer; cholanigoma; lung cancer; brain cancer; cervical cancer; colon cancer; head and neck cancer; hepatoma; non-small cell lung cancer; melanoma; mesothelioma; multiple myeloma; ovarian cancer; sarcoma; and thyroid cancer.
- 20 9. An assay according to any preceding claim, in which one or more primers are dual domain primers.
10. An assay according to any preceding claim, in which the amplified products from two or more primer pairs of a primer set can be distinguished.
11. An assay according to any preceding claim, in which the amplified products from two or
 - 25 more primer pairs of a primer set are distinguished by being of distinct sizes.
12. An assay according to any preceding claim, in which the amplified products from two or more primer pairs of a primer set are distinguished by being labeled with different detectable labels.

13. An assay according to any preceding claim, in which the primers are present in the reaction mixture at 0.3 μ M.

14. A composition comprising two or more primer pairs selected from the group consisting of molecules having the sequences of:

5 SEQ ID NOs: 1 and 8; SEQ ID NOs: 2 and 8; SEQ ID NOs: 3 and 8; SEQ ID NOs: 4 and 8; SEQ ID NOs: 5 and 8; SEQ ID NOs: 6 and 8; SEQ ID NOs: 7 and 8; SEQ ID NOs: 9 and 14; SEQ ID NOs: 10 and 14; SEQ ID NOs: 11 and 14; SEQ ID NOs: 12 and 14; SEQ ID NOs: 13 and 14; SEQ ID NOs: 19 and 33; SEQ ID NOs: 20 and 33; SEQ ID NOs: 21 and 33; SEQ ID NOs: 22 and 33; SEQ ID NOs: 23 and 33; SEQ ID NOs: 24
10 and 33; SEQ ID NOs: 25 and 33; SEQ ID NOs: 26 and 32; SEQ ID NOs: 27 and 32; SEQ ID NOs: 28 and 32; SEQ ID NOs: 29 and 32; SEQ ID NOs: 30 and 32; and SEQ ID NOs: 31 and 32.

15. A composition according to Claim 14, comprising at least two primer pairs selected from the group consisting of molecules having the sequence of:

15 SEQ ID NOs: 1 and 8; SEQ ID NOs: 2 and 8; SEQ ID NOs: 3 and 8; SEQ ID NOs: 4 and 8; SEQ ID NOs: 5 and 8; SEQ ID NOs: 6 and 8; SEQ ID NOs: 7 and 8; SEQ ID NOs: 9 and 14; SEQ ID NOs: 10 and 14; SEQ ID NOs: 11 and 14; SEQ ID NOs: 12 and 14; SEQ ID NOs: 13 and 14; SEQ ID NOs: 19 and 33; SEQ ID NOs: 20 and 33; SEQ ID NOs: 21 and 33; SEQ ID NOs: 22 and 33; SEQ ID NOs: 23 and 33; SEQ ID NOs: 24
20 and 33; SEQ ID NOs: 25 and 33; SEQ ID NOs: 26 and 32; SEQ ID NOs: 27 and 32; SEQ ID NOs: 28 and 32; SEQ ID NOs: 29 and 32; SEQ ID NOs: 30 and 32; and SEQ ID NOs: 31 and 32.

16. A composition according to Claim 14 or 15, comprising primer pairs consisting of molecules having the sequences of:

25 SEQ ID NOs: 1 and 8; SEQ ID NOs: 2 and 8; SEQ ID NOs: 3 and 8; SEQ ID NOs: 4 and 8; SEQ ID NOs: 5 and 8; SEQ ID NOs: 6 and 8; SEQ ID NOs: 7 and 8; SEQ ID NOs: 9 and 14; SEQ ID NOs: 10 and 14; SEQ ID NOs: 11 and 14; SEQ ID NOs: 12 and 14; SEQ ID NOs: 13 and 14.

17. A composition according to Claim 14, 15 or 16, comprising primer pairs consisting of
30 molecules having the sequences of:

SEQ ID NOs: 19 and 33; SEQ ID NOs: 20 and 33; SEQ ID NOs: 21 and 33; SEQ ID NOs: 22 and 33; SEQ ID NOs: 23 and 33; SEQ ID NOs: 24 and 33; SEQ ID NOs: 25 and 33; SEQ ID NOs: 26 and 32; SEQ ID NOs: 27 and 32; SEQ ID NOs: 28 and 32; SEQ ID NOs: 29 and 32; SEQ ID NOs: 30 and 32; and SEQ ID NOs: 31 and 32.

5 18. A composition according to any of Claims 14 to 17, further comprising one or more primer pairs selected from SEQ ID NOs: 34 and 37; SEQ ID NOs: 35 and 38; and SEQ ID NOs: 36 and 39.

19. A composition according to any of Claims 14 to 18, in which the primer pairs are specifically hybridized to target polynucleotides.

10 20. A composition according to any of Claims 14 to 19, in which at least one member of each primer pair is fluorescently-labelled.

21. A composition comprising fluorescently-labelled amplification products resulting from the amplification of target polynucleotides with two or more primer pairs selected from the group consisting of molecules having the sequences of:

15 SEQ ID NOs: 1 and 8; SEQ ID NOs: 2 and 8; SEQ ID NOs: 3 and 8; SEQ ID NOs: 4 and 8; SEQ ID NOs: 5 and 8; SEQ ID NOs: 6 and 8; SEQ ID NOs: 7 and 8; SEQ ID NOs: 9 and 14; SEQ ID NOs: 10 and 14; SEQ ID NOs: 11 and 14; SEQ ID NOs: 12 and 14; SEQ ID NOs: 13 and 14; SEQ ID NOs: 19 and 33; SEQ ID NOs: 20 and 33; SEQ ID NOs: 21 and 33; SEQ ID NOs: 22 and 33; SEQ ID NOs: 23 and 33; SEQ ID NOs: 24
20 and 33; SEQ ID NOs: 25 and 33; SEQ ID NOs: 26 and 32; SEQ ID NOs: 27 and 32; SEQ ID NOs: 28 and 32; SEQ ID NOs: 29 and 32; SEQ ID NOs: 30 and 32; and SEQ ID NOs: 31 and 32.

22. A composition according to Claim 21, further comprising fluorescently-labelled amplification products resulting from the amplification of target polynucleotides with one or
25 more primer pairs selected from the group consisting of molecules having the sequences of: SEQ ID NOs: 34 and 37; SEQ ID NOs: 35 and 38; and SEQ ID NOs: 36 and 39.

23. A composition according to any of Claims 14 to 22, further comprising reaction mixture components selected from the group consisting of:

buffer; dNTPs; and DNA polymerase.

24. A composition according to any of Claims 14 to 23, further comprising a nucleic acid sample prepared from a FFPE tumor sample.

25. A composition according to any of Claims 14 to 24, further comprising tumor cells from a subject diagnosed with a condition selected from the group consisting of:

- 5 gastric cancer; renal cancer; cholanigoma; lung cancer; brain cancer; cervical cancer; colon cancer; head and neck cancer; hepatoma; non-small cell lung cancer; melanoma; mesothelioma; multiple myeloma; ovarian cancer; sarcoma; and thyroid cancer.

26. A composition according to any of Claims 14 to 25, in which the primers are present at 0.3 μ M.