

CLAIMS

1. A method for detecting cMET alterations, the assay comprising

contacting a portion of a nucleic acid sample with a set of primers which detect alterations in cMET gene copy number variation;

- 5 in which the set of primers comprises subsets of primer pairs that amplify at least one gDNA-specific sequence of cMET, the subset comprising at least one primer pair, in which a primer pair consists of :

a first primer selected from the group consisting of: SEQ ID NOs: 10, 11 and 12 and a second primer selected from the group consisting of SEQ ID NOs: 28, 29 and 30; or

a first primer selected from the group consisting of: SEQ ID NOs: 89, 90 and 91 and a second primer selected from the group consisting of: SEQ ID NOs: 107, 108 and 109;

at least one gDNA-specific reference gene sequence of each of:

- 15 (i) KDEL2 which is located on chromosome 7, the subset comprising at least one primer pair consisting of a first primer selected from the group consisting of: SEQ ID NOs: 16 and 113 and a second primer selected from the group consisting of: SEQ ID NOs: 34 and 95; and
- 20 (ii) SOD1 or SPG21, which are not located on chromosome 7, in which a primer pair for amplifying SOD1 consists of a first primer selected from the group consisting of: SEQ ID NOs: 17 and 114 and a second primer selected from the group consisting of: SEQ ID NOs: 35 and 96 and in which a primer pair for amplifying SPG21 consists of a first primer selected from the group consisting of: SEQ ID NOs: 18 and 115 and a second primer selected from
- 25 the group consisting of: SEQ ID NOs: 36 and 97;

to detect cMET gene copy number variation;

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 set of primers;

detecting the level of the cMET amplicons relative to the reference gene amplicons, thereby detecting the presence or absence of:

- (a) a chromosome 7 amplification comprising a cMET alteration; and
- (b) cMET gene amplification alteration.

5 2. A method according to Claim 1, in which the set of primers further comprises a subset of primer pairs that amplify at least one gDNA-specific sequence of EGFR, the subset comprising at least one primer pair consisting of a first primer selected from the group consisting of: SEQ ID NOs: 13, 14, 15, 110, 111 and 112 and a second primer selected from the group consisting of: SEQ ID NOs: 31, 32, 33, 92, 93 and 94; and

10 the assay further comprises comparing the normalized level of EGFR amplicons to a gDNA EGFR reference level; in which a higher level of a gDNA-specific EGFR amplicon as compared to the gDNA EGFR reference level indicates the presence of a gene amplification alteration of EGFR in the sample.

15 3. A method according to Claim 1 or 2, in which the method further comprises comparing the normalized level of KDEL2 amplicons to a gDNA KDEL2 reference level;

in which a higher level of a gDNA-specific KDEL2 amplicon as compared to the gDNA KDEL2 reference level indicates the presence of a gene amplification alteration of KDEL2 in the sample.

20 4. A method according to Claim 2 or 3, in which the presence of a gene amplification alteration of cMET, EGFR and KDEL2 indicates the presence of chromosome 7 amplification.

5. A method according to Claim 2, 3 or 4, in which the primer set comprises subsets of primer pairs that amplify one gDNA-specific sequence of KDEL2 and three gDNA-specific sequences of each of cMET and EGFR.

25 6. A method according to any preceding claim, in which the primer set comprises subsets of primer pairs that amplify at least one gDNA-specific sequence of SOD1 and SPG21.

7. A method according to any preceding claim, further comprising contacting the portion of a nucleic acid sample with a second set of primers, in which the second set of primers detects changes in cMET gene expression level;

in which the second set of primers comprises subsets of primer pairs that amplify mRNA-specific sequences of cMET and at least mRNA specific sequences of at least two reference genes, in which a primer pair consists of:

a first primer selected from the group consisting of: SEQ ID NOs: 1, 2, 3, 19, 20 and 21
and a second primer selected from the group consisting of: SEQ ID NOs: 37, 38 and 39
or

a first primer selected from the group consisting of: SEQ ID NOs: 1, 2, 3, 116, 117 and 118 and a second primer selected from the group consisting of: SEQ ID NOs: 98, 99 and 100; and in which an altered level of a mRNA-specific cMET amplicon as compared to a mRNA cMET reference level indicates the presence of a gene expression level alteration of cMET in the sample.

8. A method according to Claim 7, in which a primer set comprises primer pair subsets that amplify at least one amplicon of each gene.

9. A method according to Claim 7, in which the at least two reference genes amplified by the subsets of primers that amplify mRNA-specific sequences are SOD1 and SGP21,

in which a primer pair for amplifying SOD1 consists of:

a first primer selected from the group consisting of: SEQ ID NOs: 6, 7, 24 and 25
and a second primer selected from the group consisting of: SEQ ID NOs: 42 and 43, or

a first primer selected from the group consisting of: SEQ ID NOs: 6, 7, 121 and 122 and a second primer selected from the group consisting of: SEQ ID NOs: 103 and 104

and in which a primer pair for amplifying SGP21 consists of:

a first primer selected from the group consisting of: SEQ ID NOs: 8, 9, 26 and 27
and a second primer selected from the group consisting of: SEQ ID NOs: 44 and 45, or

a first primer selected from the group consisting of: SEQ ID NOs: 8, 9, 123 and 124 and a second primer selected from the group consisting of: SEQ ID NOs: 105 and 106.10. A method according to Claim 9, in which the primer sets comprise primer pair subsets that

amplify at least two gDNA-specific amplicons of each of cMET, EGFR, and KDELR-2 and at least two mRNA-specific amplicons of each of cMET, SOD1 and SGP21.

11. A method according to any preceding claim, further comprising:

contacting a second portion of the sample with a third set of primer pairs in which the third set of primers comprises subsets of primer pairs that amplify cMET sequences comprising sequence variations;

performing a PCR amplification regimen comprising cycles of strand separation, primer annealing, and primer extension on a reaction mixture comprising the second portion of the sample and the third set of primers;

detecting the level of the amplicon for each primer pair, in which the presence of an amplicon indicates the presence of the sequence variation for which that primer pair is specific.

12. A method according to Claim 11, in which one or more sequence variations of cMET are SNPs.

13. A method according to Claim 12, in which the cMET SNP is selected from the group consisting of:

S1058P; V1101I; H1112Y; H1124D; G1137V; M1149T; V1206L; L1213V; K1262R; M1268T; V1238I; Y1248C; and D1246N (SEQ ID NO: 131).

14. A method according to Claim 11, 12 or 13, in which the same PCR thermocycling regimens are used for both reactions.

15. A method according to any preceding claim, in which the nucleic acid sample is prepared from a FFPE tumor sample.

16. A method 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.

17. A method according to any preceding claim, in which one or more primers are dual domain primers.
18. A method according to any preceding claim, in which the amplified products from two or more primer pairs of a primer subset can be distinguished.
- 5 19. A method according to any preceding claim, in which the amplified products from two or more primer pairs of a primer subset are distinguished by being of distinct sizes or by being labeled with different detectable labels.
20. A method according to any preceding claim, in which the amplified products from the first set of primers and the second set of primers are distinguished by being labeled with
10 different detectable labels.
21. A method according to any preceding claim, in which the primers are present in the reaction mixture at the concentrations of Table 2.