

CLAIMS

1. A method for detecting at least one of at least one first target DNA sequence and at least one second target DNA sequence from a library of DNA sequences, wherein the first target DNA sequence differs from the second target DNA sequence in that a single or multiple nucleotide substitution or deletion or insertion in the second target sequence generates a restriction site for a restriction endonuclease, giving rise - if cleaved by the restriction endonuclease - to a first cleaved second target sequence 3' of the generated restriction site and a second cleaved second target sequence 5' of the generated restriction site, comprising the steps of:

(a) providing the library of DNA sequences, each of the DNA sequences comprising, respectively from the 5' end to the 3' end, a first sequence segment having a length from 15 to 50 nucleotides, a second sequence segment of genomic DNA as cleaved by the restriction endonuclease, and a third sequence segment reverse complementary to the union of the first sequence segment and, if any, the 5' overhang generated by the restriction endonuclease;

(b) amplifying the library of DNA sequences by PCR using:

- at least one first reverse primer which hybridises to the 3' end region of the second sequence segment of the at least one first target sequence positive strand or at least one first cleaved second target sequence positive strand;

- at least one second forward primer which hybridises to the 3' end region of the second sequence segment of the at least one first target sequence antipositive strand;

- at least one third forward primer comprising a first 5' portion hybridising to the 5' end region of the third sequence segment of the at least first cleaved second

target sequence antipositive strand and a second 3' portion hybridising to the 3' end region of the second sequence segment of the at least one first cleaved second target sequence antipositive strand, wherein the first portion of
5 the at least one third forward primer has a length from 20% to 80% with respect to the total length of the at least one third forward primer;

(c) detecting DNA sequences amplified in step (b).

2. The method according to claim 1, wherein step (b)
10 further uses at least one fourth reverse primer which hybridises to the 3' end region of the second sequence segment of the at least one first cleaved second target sequence positive strand.

3. The method according to claim 1 or 2, wherein the
15 library of DNA sequences is obtained by deterministic restriction site whole genome amplification.

4. The method according to any of claims from 1 to 3, wherein step (c) is performed by a DNA sequencing method.

5. The method according to claim 4, wherein the DNA
20 sequencing method is Sanger sequencing or sequencing by synthesis.

6. The method according to any of the preceding claims, wherein the first portion of the at least one third forward primer has a length from 40% to 60% with respect to
25 the total length of the at least one third forward primer.

7. The method according to any of the preceding claims, wherein said second portion of the at least one third forward primer has a length in bases comprised between a minimum corresponding to the consensus sequence
30 of said restriction endonuclease minus, if any, the 5' overhang generated by the restriction endonuclease, all divided by two, and a maximum of 30 bases.

8. The method according to any of the preceding claims, wherein at least one of said primers further

comprises a 5' end region which does not hybridize to any of said first or second target sequence, positive or antipositive strand.

5 9. The method according to any of the preceding claims, wherein the restriction endonuclease is MseI.

10 10. A kit for detecting at least one of at least one first target DNA sequence and at least one second target DNA sequence from a library of DNA sequences, wherein the first target DNA sequence differs from the second target DNA sequence in that a single or multiple nucleotide substitution or deletion or insertion in the second target sequence generates a restriction site for a restriction endonuclease, giving rise - if cleaved by the restriction endonuclease - to a first cleaved second target sequence 15 3' of the generated restriction site and a second cleaved second target sequence 5' of the generated restriction site, and wherein each of the DNA sequences of the library comprises, respectively from the 5' end to the 3' end, a first sequence segment having a length from 15 to 50 20 nucleotides, a second sequence segment of genomic DNA as cleaved by the restriction endonuclease, and a third sequence segment reverse complementary to the union of the first sequence segment and, if any, the 5' overhang generated by the restriction endonuclease, comprising:

25 - at least one first reverse primer which hybridises to the 3' end region of the second sequence segment of the at least one first target sequence positive strand or at least one first cleaved second target sequence positive strand;

30 - at least one second forward primer which hybridises to the 3' end region of the second sequence segment of the at least one first target sequence antipositive strand;

- at least one third forward primer comprising a first 5' portion hybridising to the 5' end region of the third

sequence segment of the at least one first cleaved second target sequence antipositive strand and a second 3' portion hybridising to the 3' end region of the second sequence segment of the at least one first cleaved second target sequence antipositive strand, wherein the first portion of the at least one third forward primer has a length from 20% to 80% with respect to the total length of the at least one third forward primer.

11. The kit according to claim 10 for use in the diagnosis of ALK or EGFR or PIK3CA mutations.