

METHOD AND KIT FOR DETECTING A WILD-TYPE AND/OR A MUTATED
TARGET DNA SEQUENCE

ABSTRACT

5 The present invention relates to a method for detecting a
first and/or a second target DNA sequence from a DNA library,
differing in that a mutation generates/eliminates a restriction
site for a restriction endonuclease, comprising the steps of: (a)
providing the DNA library, in which each of the DNA sequences
10 comprises a first sequence segment, a second sequence segment of
genomic DNA as cleaved by the restriction endonuclease, and a
third sequence segment reverse complementary to the union of said
first sequence segment and 5' overhang, if any, of the
restriction endonuclease; (b) amplifying the library of DNA
15 sequences by PCR using: a first reverse primer which hybridises
to the 3' end region of the second sequence segment of the first
or second target sequence positive strand; a second forward
primer which hybridises to the 3' end region of the second
sequence segment of the first target sequence antipositive
20 strand; a third forward primer comprising a first portion
hybridising to the 5' end region of the third sequence segment of
the second target sequence antipositive strand and a second
portion hybridising to the 3' end region of the second sequence
segment of the second target sequence antipositive strand,
25 wherein the first portion of the third forward primer has a
length from 20% to 80% with respect to the total length of
the third forward primer; (c) detecting DNA sequences
amplified in step (b).

30 Figure 4A