Title (en)

SINGLE STEP AMPLIFICATION AND SEQUENCING OF NUCLEIC ACIDS

Publication

EP 0595982 A4 19951011 (EN)

Application

EP 92916411 A 19920723

Priority

- AU 9200372 W 19920723
- AU PK740091 A 19910724

Abstract (en)

[origin: WO9302212A1] Method for the amplification and sequencing of DNA or RNA. The method comprises the steps of (i) melting a double stranded nucleic acid to yield a pair of complementary nucleic acid strands, (ii) hybridising a primer to each of the strands, the primers being so chosen that the primer annealing to the sense strand is 3' to the position of the primer on the antisense strand, one of the primers being labelled so as to be capable of being visualized independently of the other primer, (iii) causing a polymerase enzyme to amplify the nucleic acid in the presence of a dideoxynucleotide analogue of one of the nucleotides present in the nucleic acid, the dideoxy analogue being present in such a concentration that a majority of the newly synthesised nucleic acid strands are terminated by incorporation of dideoxynucleotides without extending far enough to act as templates for synthesis of the opposite strand using the second primer, (iv) repeating steps (i) to (iii) sequentially a number of times, (v) repeating the steps (i) to (iv) using at least another two dideoxynucleotide analogues of the other three nucleotides present in the nucleic acid, and (vi) electrophoretically separating the reaction products of each of the repetitions of steps (i) to (iv) and visualizing the labelled strands. The other of the nucleotides of at least a part of the strand of the nucleic acid to which the labelled primer annealed between the binding sites may be determined by comparing the separated and visualized gels for each of the nucleotide analogues used.

IPC 1-7

C12Q 1/68

IPC 8 full level

C12Q 1/68 (2006.01); C12Q 1/6869 (2018.01)

CPC (source: EP)

C12Q 1/6869 (2013.01)

Citation (search report)

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- See references of WO 9302212A1

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