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OPTIMIZATION OF OBTAINING THE HIGH-LABEL SINGLE STRAIN PROBE BASED ON THE M13 PHAGE

A method is proposed to obtain high-label single strain probe on the basis of the M13 phage. This is based on the use of a specific primer and the procedure of purification of the synlhesized probe using nitrocellulose.

DNA fingerprinting is known to be widely used in medical expertise, investigations needing genetic markers, population studies, practical selection etc [1, 2]. A probe based on M13 phage sequences belongs to those adapted for genomic profiles researches [3]. This probe is convenient to detect hypervariable genome regions in representatives of different taxo-nomic groups because of its universality [2].

To obtain clear hybridization data, it is necessary to have probes of high specific radioactivity. To obtain such a label of M13 DNA, the reaction of incomplete synthesis on the single-stranded phage form is often used, 17 nucleotides containing primer being introduced into this system [4].

Such an approach, however, is meets some difficulties, optimal conditions for probe synthesis and hybridization are still to be found. Therefore we propose a protocol permitting synthesis of a high labelled one-stranded probe using a specific primer. For this aim, a 15-meric oligonucleotide 5'-TTCATAATCAAAATC-3', being a homo-log of a region localized at the 3'-end of phage gene III has been synthesized; in such a way we obtain the possibility to synthesize exclusively a fragment containing hypervariable repetitive sequences using Klenow fragment [5]. The purification of the single-stranded probe has been made, as usually, in denaturative polyacrylamide gel followed by elution.

To simplify this protocol, we propose an approach described below. After completion synthesis probe, 8 M ammonium acetate (up to 2 M) as well as yeast tRNA (50—100 µg/ml) and ethanol (2.5 volumes) are added.

The mixture is to be incubated at room temperature (15 min) and run at the Eppendorf centrifuge during 10 min. The pellet is to be washed with ethanol solution (70°), partly air-dried, dissolved in water and put on nitrocellulose filter. This material is of significant binding capacity for single-stranded DNA (80—100 *µg/cm*^{2}*)*, hence little pieces of nitrocellulose (0.1—0.2 *cm*^{2}*)* can to be used. The air-dried filters are to be incubated in the vacuum at 80 °C during I—2 hours. These conditions lead to the irreversible .single-stranded fragment adsorbtion on the filter, with the two-stranded phage fragment being non-adsorbed; the latter can be separated from the matrix after boiling of the filters in distilled water (5 min). The probe separated may be later used for hybridization [6].

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ОПТИМЗАЦІЯ УМОВ ОДЕРЖАННЯ ВИСОКОМІЧЕНОГО ОДНОЛАНЦЮГОВОГО ЗОНДА НА ОСНОВІ ФАГА М13

Р е з ю м е

Наведено методику одержання високоміченого одиоланцюгового зонда на основі фага МІЗ, що базується иа використанні специфічного праймеру та процедурі очищення синтезованого зонду за допомогою нітроцелюлози.

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