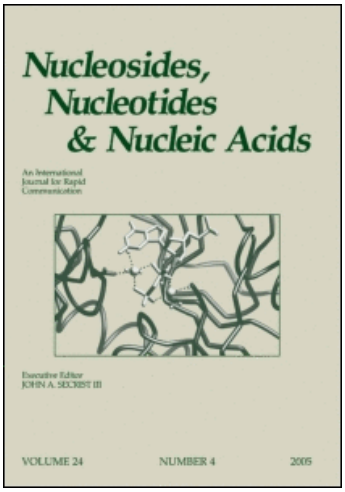

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Universal Labeling Chemistry for Nucleic Acid Detection on DNA-Arrays

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ABSTRACT

We show here a new and efficient aqueous chemistry for labeling of any class of nucleic acids for their detection on DNA chip. The labels contain a diazo function as reactive moiety and biotin as detectable unit. The highly selective reaction of diazo group on the phosphate does not disrupt base pairing recognition and hybridization specificity.

Key Words: Labeling; Nucleic acids; Diazomethyl; DNA-chip.

The use of high density DNA arrays (or DNA chips) for the analysis of populations of nucleic acids in biological samples requires a well-controlled labeling chemistry. This includes uniform fragmentation of the target as well as maintaining its hydrogen bonding ability. We have described a method for post-amplification labeling of RNA, the so-called LDC-Labeling During Cleavage-. By this technique, 5-(bromomethyl)fluorescein (5-BMF) is made to react with terminal phosphates

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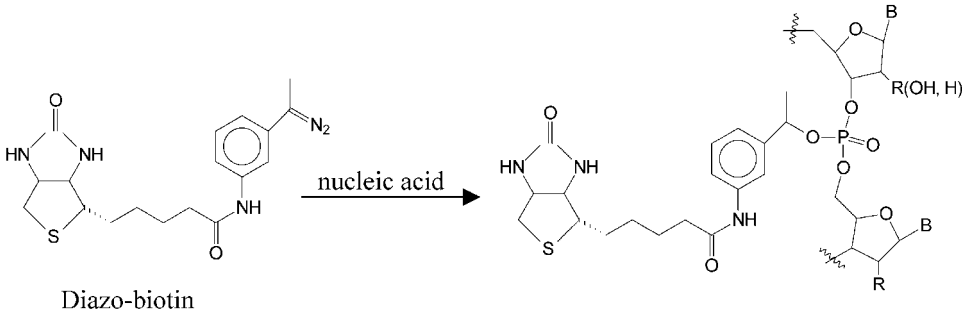

Figure 1. Labeling of nucleic acids with diazo-biotin.

originated by controlled RNA fragmentation. This technology allows the detection of amplified RNA targets without affecting the amplification efficiency and fidelity.[1] Moreover, labeling on the phosphates does not affect the hybridization specificity.

We have now developed a new strategy for universal labeling of nucleic acids for their detection on high density Genechip® microarrays.[2] This chemistry is based on the reactivity of the diazomethyl group toward the phosphates. The reaction is highly selective, there is no alkylation on the nucleophilic sites of the nucleic bases, and works equally with DNA and RNA targets.

Thus, we designed a diazomethyl derivative carrying a biotin as reporter group (diazo-biotin, Fig. 1), and used it for labeling different nucleic acid targets. Streptavidin-fluorescein conjugate was used for biotinylated DNA and RNA detection on Genechip® probe arrays (Affymetrix, Santa Clara, CA).

Labeling of amplified DNA and RNA from *Mycobacterium tuberculosis* with diazo-biotin was assayed using a sequence of the 16S rRNA locus, that was amplified by PCR and in-vitro transcription.[4] DNA and RNA fragments were labeled and detected on *Mycobacterium tuberculosis* -Genechip® arrays.[4] dUTP-biotin incorporation during PCR and UTP-fluorescein incorporation during transcription were used as controls. The obtained results show a higher labeling efficiency as compared to those obtained with 5-BMF. Strong specific signals, 5 to 20-fold higher than those obtained with enzymatic incorporation of the label, where observed. With both targets (DNA and RNA) sequence identification on the chip was higher than 99%.

Compared to commercially available nucleic acids labeling methods, diazo-biotin based labeling generates higher signals with both RNA and DNA, without any effect of the sequence or base composition on the labeling yield. The amplification efficiency and specificity are not disturbed, and the selectivity of the phosphate labeling preserves the hybridization specificity. This new fast, versatile and universal labeling technology is an excellent tool for use in diagnostic tests aiming at the detection and identification of pathogens and genetic disorders by the use of high density microarrays. The labeling protocol is also easily adaptable to other detection supports (micro plates, low density arrays, ...).

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