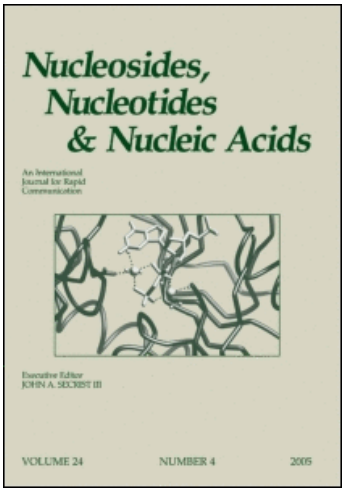

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Labeling During Cleavage of Nucleic Acids for Their Detection on DNA Chips

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ABSTRACT

A new and efficient strategy for labeling of nucleic acids prior to their hybridization on high density DNA chip has been developed. Our approach which combines the fragmentation and the labeling is based on the reactivity of the terminal phosphates of cleaved DNA and RNA fragments with a reporter molecule bearing aryldiazomethane group.

Key Words: DNA chip; Labeling; Cleavage; Aryldiazomethane.

DNA microarrays (or DNA chips), which are miniaturized arrays of oligonucleotide probes,[1] provide an outstanding and rapid means for nucleic acid sequence analysis by hybridization. Nevertheless, the well control of different steps upstream from the hybridization on the chip is fundamental for a specific detection of labeled target. Labeling is usually achieved using the enzymatic incorporation of the

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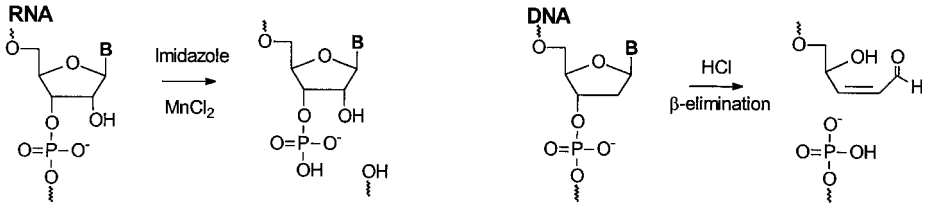

Figure 1. Chemical cleavage of RNA and DNA.

label during the amplification step.[2] Chemical approaches for post-amplification labeling[3] have been used as well. However, these procedures lead to the modifications on the nucleic bases affecting the base pairing rules, fundamental for preserving the specificity during the hybridization. Furthermore, in order to achieve a good signal uniformity and hybridization specificity on high density probe arrays, labeled nucleic acids have to be fragmented.[1,4] Thus, we used a buffer containing metal ion and imidazole to cleave RNA and acidic treatment for depurination and cleavage of DNA (Fig. 1).

Using the cleavage step, we developed a new, efficient and rapid aqueous chemistry for post-amplification labeling of nucleic acid targets for their detection on high density probe arrays.[5] This strategy, so-called Labeling During Cleavage "LDC", uses the reaction of a reporter molecule containing a reactive group which reacts preferentially against terminal phosphates of fragmented nucleic acids. There is no reaction occurring on the nucleobases. LDC approach has been first evaluated on RNA using a reporter molecule containing bromomethyl group, 5-(bromomethyl)fluorescein, but the labeling yields of DNA were very weak.[5,6]

Here, we describe the use of aryldiazomethane reporter molecules that selectively react on the phosphate in DNA and RNA fragments. The diazo-biotins described here (Fig. 2) have been designed to bear an aromatic ring, that modulates the reactivity and stabilizes the diazo function, and biotin as detectable moiety. The performances of diazomethyl based LDC was demonstrated on DNA and RNA targets using the *meta*-BioPMDAM.

DNA amplicons containing T7 RNA polymerase promoter sequences were obtained by PCR amplification of a region of *Mycobacterium tuberculosis* 16S rRNA. RNA transcripts were then generated from PCR amplicons using T7 RNA polymerase. For labeling reaction, RNA transcripts were added in LDC buffer containing 6 mM imidazole, 60 mM $MnCl_2$ and 10 mM *meta*-BioPMDAM and then

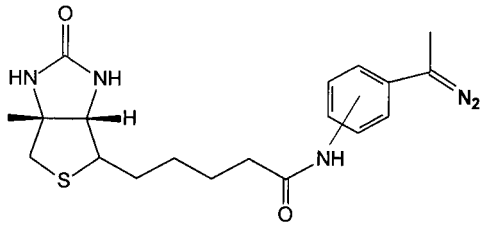

Figure 2. Diazo-biotin derivatives: *ortho*-, *para*-, and *meta*-PMDAM.

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Table 1. Labeling results of amplified DNA and RNA from 16S rRNA of *Mycobacterium tuberculosis*.

Labeling procedure		Chip results		
		Median (RFU)	Median/ Background	Identification (BC%)
DNA amplicons	LDC	13343	21.6	100
	dUTP-biotin	4322	7.1	97.8
RNA transcripts	LDC	31513	53.6	99.0

incubated at 60°C for 10 min. DNA labeling was achieved after the addition of PCR amplicons in LDC buffer containing 3 mM HCl and 10 mM *meta*-BioPMDAM and the incubation at 95°C for 10 min. Aliquot of biotinylated samples were then hybridized on GeneChip® probe array, designed to identify a particular region of 16S rRNA target,[7] stained with a solution of Streptavidin-R-Phycoerythrin. The scan and data analysis were carried out using a GeneArray Scanner (Agilent, Palo Alto, CA) and GeneChip software™ (Affymetrix, Santa Clara, CA).

The chip results showed that both targets, DNA amplicons and RNA transcripts, were successfully labeled with diazomethyl-based LDC (Table 1). In both cases, the median intensity (RFU) of the labeling signals detected on the chip is higher than that obtained with the reference method based on labeled nucleotide incorporation (dUTP-biotin) during the PCR reaction. Moreover, identification percentage of the two targets (percentage of homology between the experimentally derived sequence and the reference sequence tiled on the Chip) is in the neighborhood of 100%. This clearly shows that the diazomethyl labeling chemistry doesn't affect the hybridization efficiency and specificity.

In conclusion, LDC using diazo reporter molecules appears to be an excellent site-selective labeling procedure that allows an efficient labeling of DNA an RNA without any effect on the base pairing capability and hybridization specificity. This makes this new, flexible and very simple strategy a universal labeling technology for DNA and RNA labeling without any effect of the sequence or base composition on the labeling efficacy. It is an excellent chemical labeling approach for nucleic acid based diagnostics using high density microarrays.

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