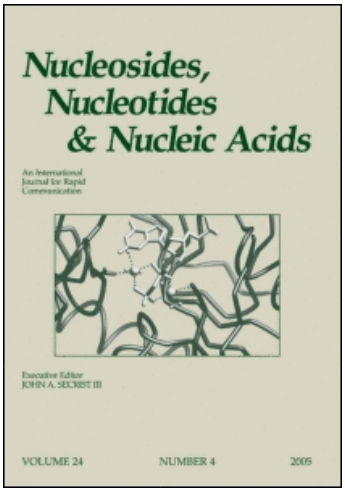

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LABELING DURING CLEAVAGE (LDC), A NEW LABELING APPROACH FOR RNA

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ABSTRACT

A new and efficient strategy for labeling of RNA sequences 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 3′-phosphate of cleaved RNA fragments with a fluorescent molecule bearing aromatic bromomethyl function.

Recent progress in nucleic acids detection for diagnostic purposes has clearly established the importance of labeling strategy to reach high sensitivity and specificity. One of the most commonly applied systems involves incorporation of nucleotides bearing reporter groups (1). Nevertheless, these bulky reporter groups can affect the amplification efficiency and specificity during their enzymatic incorporation.

A new strategy called ≪Labeling During Cleavage (LDC)≫, which is a chemical labeling approach, has been developed as an alternative procedure for labeling amplified RNA targets without affecting the amplification efficiency and fidelity. LDC is a universal labeling technology which is able to achieve at once the two functions: labeling and fragmentation of RNA sequences prior to their hybridization and detection on DNA chip. First feasibility of LDC approach has been demonstrated using 16S rRNA targets from Mycobacteria species. Hybridization of LDC-labeled

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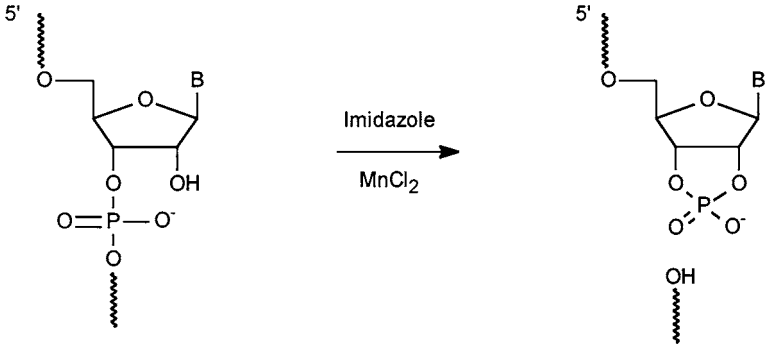

Figure 1. Chemical cleavage of RNA.

targets on GeneChip® probe arrays (Affymetrix, Santa Clara, CA) demonstrated the robustness and the sensitivity of this chemistry. In order to achieve a good signal uniformity and hybridization specificity, on Genechip® probe arrays labeled RNA targets have to be fragmented to an average size of <100 nucleotides. This can be obtained using a cleavage buffer containing metal ions and imidazole (2) (Fig. 1).

Interaction of divalent metal cations, in particular Mn^{2+}, with RNA backbone neutralizes negative charges of the phosphate and therefore facilitates RNA cleavage using imidazole as a catalyst. The produced RNA fragments contain a 2′,3′-cyclic phosphate end. In LDC labeling chemistry, the 2′,3′-cyclic phosphate RNA fragments react with aromatic methyl halide groups according to the phosphate esterification mechanism described by Furuta (3) (Fig. 2).

DNA amplicons containing T7 RNA polymerase promotor 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 (pH 7.5) containing imidazole (30 mM), $MnCl_2$ (30 mM) and 5-(bromomethyl)-fluorescein label (1 mM) and then incubated at 60°C for 30 min. Aliquot of labeled RNA solution was then hybridized on a GeneChip® probe array designed to identify a particular region of 16S rRNA target (4). The scan and data analysis were carried

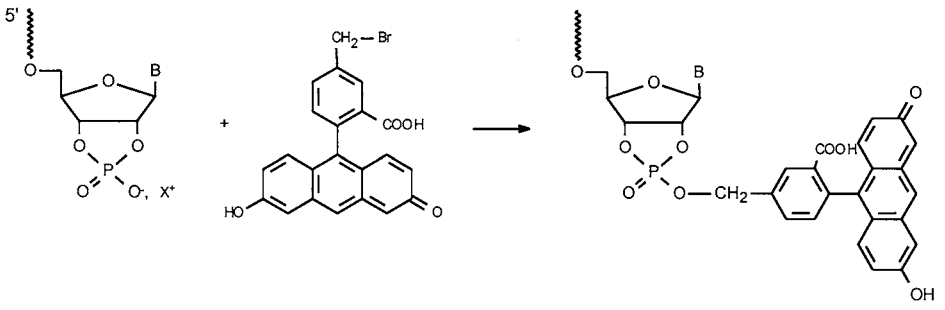

Figure 2. Labeling during cleavage of RNA using 5-(bromomethyl)fluorescein.

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out using Affymetrix GeneChip software™. Labeling results with LDC show that the percentage of identification of the target sequence is in the neighborhood of 100% (>99.8%). The median intensity (RFU) is higher than that obtained with the reference method based on labeled nucleotide incorporation during the post-PCR transcription.

In conclusion, LDC chemistry appears to be an efficient labeling procedure which is very convenient for labeling and detection of RNA targets on high density GeneChip® probe arrays.

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