

IMPROVED SPOT HOMOGENEITY FOR DNA MALDI MATRICES

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For size measurement of relatively small DNA molecules such as synthetic oligonucleotides and polymerase chain reaction (PCR) products, MALDI-TOF mass spectrometry [1-5] offers potential advantages of speed, accuracy, and automation over conventional electrophoretic or hybridization techniques. However, with commonly used UV matrices, MALDI of DNA is rather labor intensive for several reasons, one of which is the sparse distribution of "sweet spots" in the final dried sample spot.

In contrast to protein matrices such as 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA) and α -cyano-4-hydroxycinnamic acid (CHCA) that yield homogeneous dried spots, well known MALDI matrices for single- and double-stranded DNA such as 3-hydroxypicolinic acid (HPA) and picolinic acid (PA) tend to form the crystals at the rim of their spots. Uneven deposition of DNA-doped matrix crystals on the periphery of the dried spot necessitates a tedious search for sweet spots with the laser. For automated, high throughput MALDI-TOF analysis of short DNA fragments, it is important to obtain homogeneous MALDI spots that yield good signals not only from the periphery but the entire spot.

Several groups [6-8] have shown that substrates such as nitrocellulose, active nafion, and parafilm can be used in order to improve MALDI spot preparation. We have developed a new procedure using polymer substrates and additives to obtain good mass spectra from any location on DNA-doped MALDI spots with a mixed matrix containing HPA and PA. Hydrophilic polymers such as linear polyacrylamide (LPA), poly(ethylene oxide) (PEO), cellulose derivative (methyl cellulose) and other substrates such as Nafion were employed to control the crystal formation of the matrix in order to produce homogeneous spots. Investigation of the DNA distribution in the spot was performed by imaging a synthetic oligonucleotide (20 mer covalently labeled with HEX dye) with a fluorescence microscopy equipped with a CCD camera (see Figure 1).

While DNA/matrix crystals formed only at the spot's rim without any polymer substrate, the use of a combined hydrophilic polymer and Nafion substrate enhanced the homogeneous formation of DNA-doped matrix crystals in the interior of the MALDI spots. Good MALDI spectra were obtained from any region of the spot with high success rate (see Figure 2). Polymer-only or Nafion-only substrate showed limited success, and poor mass spectra were obtained with an anionic polymer (polyacrylic acid, PAA) and a hydrophobic polymer (polydodecyl acrylate, PDA) substrates. We believe that hydrophilic polymer substrates may either reduce transport of the DNA and matrix to the periphery of the spot during the drying process, or influence nucleus formation and growth of HPA and PA crystals. Parameters such as surface tension of the drop, viscosity, molecular weight and hydrophilicity of polymer substrate might also influence crystal formation. Matrix and solvent composition were also studied for the homogeneous spot preparation. Good mass spectra were obtained in the interior of MALDI spot using 50% of acetonitrile with HPA/PA=40:10 mg/mL. Other concentrations of either acetonitrile or HPA/PA did not produce good mass spectra probably due to the different solvent evaporation rate and/or rate of crystal formation.

We are currently working on the application of our spot preparation method to the PCR products (>100 bp) for high-throughput analysis. The goal is to obtain MALDI signal from one location per shot.

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Fluorescence Microscope Images of MALDI spots

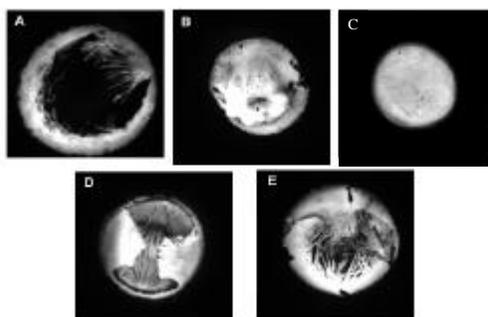


Figure 1

- A. On gold plate, HPA:PA=4:1, HEX-20 mer DNA,
- B. Nafion dried first,
- C. LPA dried first, then Nafion dried next,
- D. LPA and Nafion mixed together,
- E. LPA dried first

Average Success Rate (%) (MALDI signals from one spot)

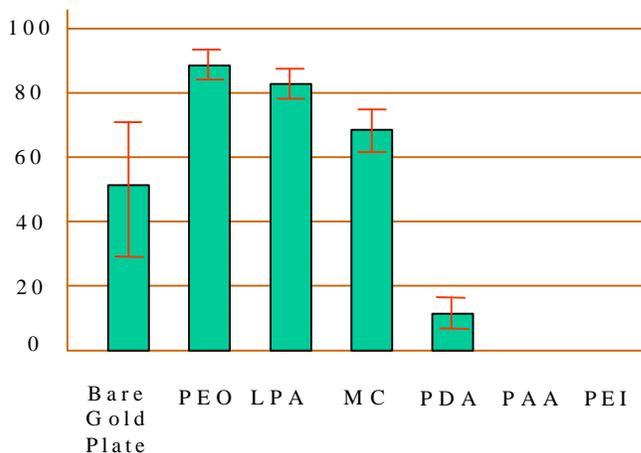


Figure 2