Spectrometry senses more than a small difference

Michael Egholm

Do you sometimes wish that you would never have to pour another gel or perform another dot blot? Thanks to advances in mass spectrometry and DNA probing technology, relief may be at hand. Work presented in this issue by Lloyd Smith and colleagues[1] and elsewhere by Ross et al.[2] may move us one step closer to the "hands-off" molecular biology laboratory. Both groups have demonstrated that the DNA mimic peptide nucleic acid (PNA)[3] hybridized to an immobilized DNA target can be analyzed directly by mass spectrometry, thereby facilitating rapid genotyping. The technique, developed independently by groups at the University of Wisconsin[1] and the Armed Forces Institute of Pathology[2], holds the promise of becoming the most direct, and very possibly the fastest, method for single nucleotide polymorphism (SNP) genotyping.

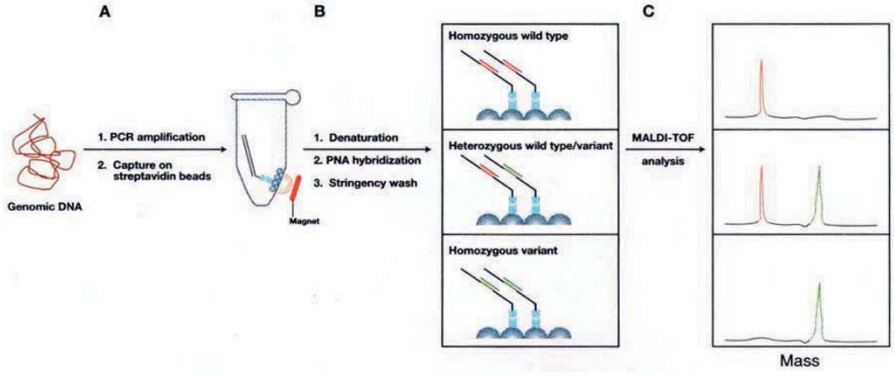

Figure 1. A scheme showing the steps of PNA affinity MALDI-TOF approach of Smith and colleagues[1]. (A) Target DNA is amplified and captured on beads. (B) Following denaturation, allelic PNA probes are added. (C) MALDI-TOF analyzes the probes directly.

The abolishment of gels and dot blots has been the allure of groups around the world striving to apply matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry to the analysis of DNA fragments[4]. Mass spectrometry separates and detects DNA fragments nearly instantly by their mass-to-charge ratio, and thereby allows facile determination of DNA fragment length. For single-base differences, the mass difference is easily measured on instruments that employ the so-called delayed extraction (DE) technique[5].

Although intrinsically powerful, the application of MALDI-TOF to routine DNA analysis is, however, hampered by certain limitations. In particular, fragments approaching 100 bases and above are difficult to analyze because of increased fragmentation and poorer sensitivity of the instruments for larger DNA[6].

Continued research and development will most likely keep pushing the upper barrier for the length of DNA fragments that can routinely be analyzed by MALDI-TOF mass spectrometry. While we await further improvements in instrumentation, indirect mass measurements can be employed for DNA fragments greater than about 100 bases in length. Among the most successful uses of MALDI-TOF are assays that use mass analysis of products from the ligase chain reaction (LCR)[7], single-base primer extension[8], or Sanger dideoxy cycle sequencing[9,10]. These techniques are all enzyme-based assays, and all exploit the sequence specificity of the enzymes, rather than that of the DNA probes.

In the new reports[1,2], both groups opt to use PNA as genotyping probes for amplified DNA products because of their higher affinity and their known ability to discriminate single base pair differences better than DNA probes[3]. In addition, PNA probes "fly" better than DNA under MALDI conditions and, most importantly, do not require salt for binding to their complementary DNA target. The latter concern is a most important one in MALDI-TOF analysis, where the presence of salt strongly interferes with the signal.

The first step in the Wisconsin team's technique (see Fig. 1A) is amplification of the target region from genomic or mitochondrial DNA. The amplification step is performed with one of the primers biotinylated such that the strands of the amplified products can be immobilized by capture on steptavidin-coated magnetic beads. Capture onto the beads is followed by denaturation (Fig. 1B), and washing to remove the nonbiotinylated strand. The single-stranded DNA target is then incubated with the allelic PNA probe variants followed by washing steps to allow only the Watson–Crick complementary PNA probes to stay bound. The beads with the DNA target and the bound complementary PNA probes are then analyzed directly by MALDI-TOF mass spectrometry (Fig. 1C).

The PNAs are designed such that there is a significant mass difference between them (100–500 Da), and they are, therefore, easily distinguished in the resulting mass spectrum. As illustrated in Figure 1, this leads to facile genotyping, simply by knowing the mass of the PNA probe for each allelic variant. This "mass tagging" approach also opens the possibility of carrying out multiplexing (i.e., simultaneous analysis for several mutations in the same loci, using several sets of PNA probes, all with different masses).

How likely is this method to be adopted in screening for polymorphisms? Its amenability to automation is strong card in its favor. Robotics are rapidly becoming part of the landscape in molecular biology laboratories and several platforms for automated DNA extraction and PCR amplification are available today. It is very easy to envisage this new method directly merged with these platforms. The entire procedure can be carried out in a single tube, and lends itself to easy automation with liquid handlers and magnetic separators. As the result of an analysis is a precise value of mass, it is easy to envisage complete automation of data collection as well. Modern MALDI-TOF instruments can accept hundreds to thousands of samples per plate. Together with the ability to multiplex each sample, the technique should allow unheard of rates of genotyping—and make life in the molecular biology laboratory a little easier.

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