SIZE-BASED SEPARATION OF DNA FRAGMENTS BY ION-PAIR REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Sizing of DNA fragments is a routine analysis traditionally performed on agarose or polyacrylamide gels. Electrophoretic analysis is a labor-intensive method with only limited potential for automation. Isolation of DNA fragments from gels after size-based separation is cumbersome. Here, we present data on size-based separation of DNA fragments by Ion-Pair Reversed-Phase High Performance Liquid Chromatography (IP RP HPLC) on the WAVE™ DNA Fragment Analysis System with the DNASep® cartridge. This system is suitable for accurate and rapid sizing of double stranded (ds) DNA fragments from 10 to ca. 2000 base pairs. The use of fluorescently labeled DNA fragments is compatible with the technology. Length-dependent separation of ds DNA fragments is sequence independent and retention times are highly reproducible. The capabilities of the technology are illustrated by the analysis of multiple DNA size markers, labeled and unlabeled, and in specific applications that depend on accurate determination of PCR fragment sizes. Size-fractionated ds DNA fragments are easily collected and are suitable for downstream applications such as sequencing, cloning, and digestion with restriction enzymes. Size based analysis of restriction fragments and PCR products are reliable and can be fully automated. The technology of IP RP HPLC offers a reliable and fully automated alternative to conventional labor intensive analysis of DNA fragments by gel electrophoresis.