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Reversible phospholipid nanogels for deoxyribonucleic acid fragment size determinations up to 1500 base pairs and integrated sample stacking

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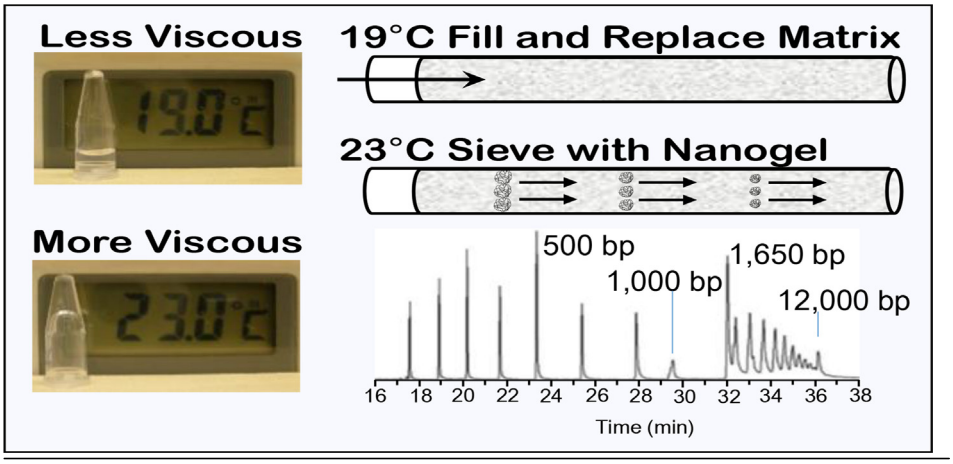
HIGHLIGHTS

- A matrix is devised to size deoxyribonucleic acid fragments up to 1500 base pairs.
- The separation matrix is used to distinguish invasive strains of *S. pyogenes*.
- A thermally-reversible stacking gel is integrated in capillary sieving separations.

GRAPHICAL ABSTRACT



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ABSTRACT

Phospholipid additives are a cost-effective medium to separate deoxyribonucleic acid (DNA) fragments and possess a thermally-responsive viscosity. This provides a mechanism to easily create and replace a highly viscous nanogel in a narrow bore capillary with only a 10 °C change in temperature. Preparations composed of dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) self-assemble, forming structures such as nanodisks and wormlike micelles. Factors that influence the morphology of a particular DMPC–DHPC preparation include the concentration of lipid in solution, the temperature, and the ratio of DMPC and DHPC. It has previously been established that an aqueous solution containing 10% phospholipid with a ratio of [DMPC]/[DHPC] = 2.5 separates DNA fragments with nearly single base resolution for DNA fragments up to 500 base pairs in length, but beyond this size the resolution decreases dramatically. A new DMPC–DHPC medium is developed to effectively separate and size DNA fragments up to 1500 base pairs by decreasing the total lipid

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Aspergillus
Streptococcus

concentration to 2.5%. A 2.5% phospholipid nanogel generates a resolution of 1% of the DNA fragment size up to 1500 base pairs. This increase in the upper size limit is accomplished using commercially available phospholipids at an even lower material cost than is achieved with the 10% preparation. The separation additive is used to evaluate size markers ranging between 200 and 1500 base pairs in order to distinguish invasive strains of *Streptococcus pyogenes* and *Aspergillus* species by harnessing differences in gene sequences of collagen-like proteins in these organisms. For the first time, a reversible stacking gel is integrated in a capillary sieving separation by utilizing the thermally-responsive viscosity of these self-assembled phospholipid preparations. A discontinuous matrix is created that is composed of a cartridge of highly viscous phospholipid assimilated into a separation matrix of low viscosity. DNA sample stacking is facilitated with longer injection times without sacrificing separation efficiency.

1. Introduction

Estimating the size of DNA is critical in genetic analysis associated with human identification [1], species identification [2,3], detecting personal biomarkers [4], analyzing food [5], and categorizing pathogenicity [6]. Moreover, following sample preparation, accurate determination of the size of DNA fragments is a mandatory analytical step in sophisticated high-throughput sequencing techniques. It is also a critical endpoint for methods based on PCR amplification, where the length of the product is used to determine the presence of a specific target sequence in the DNA template. Confirmation of size is achieved by comparing the fragment migration time during electrophoretic sieving using various sieving agents. Slab gel electrophoresis, the workhorse of modern analytical science, is a commonly used tool for DNA sizing due to the simplicity and low cost of the method. Sizing is achieved by comparing the migration time of the targeted DNA with a DNA ladder that contains a mixture of fragments of known size. The DNA ladder standard is run in a separate lane. A serious disadvantage of slab gels is the poor separation efficiency and the reduced throughput even when completed in parallel lanes in the gel [7,8]. Although more expensive than slab gel methods, capillary gel electrophoresis provides a size discrimination that is improved substantially, and higher sample throughput. Sequencing of the human genome provides direct proof of this [9].

Capillary gel electrophoresis separations of DNA fragments are accomplished using polymer solutions that provide a size-based separation of DNA fragments by creating networks or matrices that form dynamic pores for sieving [10–12], or separation through DNA entanglement [13]. DNA fragments ranging from 50 to 1500 bases are often present in an electropherogram, but the resolution obtained for the larger DNA fragments is poor and accurate sizing is limited to DNA fragments that are shorter than 500 bases in length. Ultra-high resolution separations were achieved with 0.75% hydroxyethyl cellulose used to size a 256 base pair (bp) DNA fragment [14]. Although larger fragments could not be sized, 1078 bp and 1353 bp DNA were baseline resolved [14]. A commercial system utilized hydroxyethyl cellulose to separate 50 bp to 10 kbp DNA and to accurately size 275 to 815 bp DNA [15]. In another case, a matrix of 2% linear polyacrylamide was used to sequence a DNA fragment 1300 bases long [16]. Several polymer properties dictate the utility of the material and the useful size range of the separation [17]. Increasing the upper fragment size limit of the separation can be accomplished by increasing the length of the polymer and maintaining the concentration of the gel at or above the entanglement threshold [10]. This increase in concentration is accompanied by an increase in viscosity [11], which provides challenges for the introduction to or replacement of the gel in the separation capillary.

Temporary gels, which rupture and reform during the separation, are an exciting alternative to permanent gels because the upper size limit increases when the rate of gel rupture is optimized [18]. Wormlike micelles form large self-assembled structures that dynamically rupture and reform. Aqueous solutions of the long-chain phospholipid dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and the short-chain phospholipid 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) are known to form wormlike micelles [19,20]. A dynamic sieving matrix of 10% phospholipid with [DMPC]/[DHPC] = 2.5 was optimized for single-base pair resolution that was adequate for detection of short tandem repeats (STR) in selected loci utilized for human identification using a 100 cm long capillary within a 30 min run time [21]. This is comparable to separations on commercial capillary electrophoresis instruments for forensic STR analyses [22]. What makes this particularly attractive, is that the phospholipid nanogel is substantially more cost-effective at roughly one-fourth the cost of commercial gels utilized for human identification [23–25]. A significant advantage of the dynamic sieving matrix is the thermally-responsive viscosity. The sieving matrix has low viscosity below the gel-phase transition temperature of the phospholipids and becomes gel-like at higher temperatures. As a result, the sieving agent is easily introduced into or expelled from the narrow bore separation capillary at a temperature of 19 °C. Prior to the separation, the matrix is transformed into a viscous gel in-capillary at a separation temperature of 30 °C.

While a phospholipid additive has been previously described for the separation of DNA fragments below 500 bp [21], we show that the DNA sizing range of the separation can be extended by reducing the concentration of wormlike micelles in solution. The size, shape, and stability of the phospholipid structures depend upon the phospholipid concentration, the ratio of DMPC and DHPC, and the temperature. The effect of these parameters on the size limit of DNA separations was evaluated, and the linear range for size separation was extended to 1500 bp by tuning the concentration of the phospholipid nanogel and the ratio of [DMPC]/[DHPC]. This preparation was used to compare the size of the DNA fragments to internal standards. The effectiveness of the sieving matrix was demonstrated with the analysis of PCR-amplified DNA sequences specific to select pathogens. The difference between the true size of a DNA fragment and the size calculated from internal standards is less than 2.3% for fragment sizes between 200 and 500 bp. For fragments that range from 600 to 1500 bp this difference between the true size and measured size is less than or equal to 4%. Different properties of the high and low concentration phospholipids were exploited by integrating both dynamic sieving matrices into a single capillary to create a discontinuous gel in-capillary used to concentrate DNA samples through gel stacking.

2. Materials and methods

2.1. Chemicals and reagents

The fluorescently labeled DNA ladder (catalog # MM-1000-FAM, 50–1000 bp) was purchased from BioVentures (Murfreesboro, TN). The fluorescent intercalating dye SYBR Green I nucleic