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PURIFICATION AND SIZE-FRACTIONATION OF MILLI-LITER DNA SAMPLES IN MINUTES USING ELECTROHYDRODYNAMIC MIGRATION

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Introduction

DNA electrohydrodynamic migration in viscoelastic solution proved to be a very versatile technology for high sensitivity DNA sizing with a limit of detection of 10 fg/ μL in microfluidic chip¹ or capillary format². This technology is now commercially applied for analysis of circulating cell-free DNA (cfDNA) in the field of liquid biopsy³. This technology is also suited to perform size fractionation⁴. Indeed, the migration involves a hydrodynamic flow and a counter electric field that results in transverse migration toward the walls of microchannels⁵. Because the transverse force depends on DNA molecular weight, it is possible to switch the migration regime from hydrodynamics-dominated up to a cut-off size to electrophoresis-dominated in the opposite direction above this cut-off. Size fractionation is hence achieved by merely controlling the electric field. However, this operation carried out in a single capillary of micrometer scale is relevant to the processing of samples of a few μL , hampering the development of sample preparation applications, e.g. the library preparation for third generation sequencing. Here, we describe an upscaled technology based on the same separation principle, which is upscaled at a macroscopic scale (0.4 mL/min) with the use of isoporous membranes.

Experimental

This one-channel size fractionation operation is multiplexed using parallel pores etched in a polycarbonate isoporous membranes (it4ip – ipPORE track etched membranes) of thickness between 48 and 123 μm . Each pore measure 2 μm in diameter and their density is $4 \cdot 10^5 \text{ cm}^{-2}$. Hence using a technology based on a surface of 1.13 cm^2 , we have around 450 000 pores in operation. The porous membrane is integrated in a fluidic system fabricated by 3D stereolithography (DWS Digitalwax 029 J+ printer with an acrylic biocompatible resist DWS DS3000), in which platinum electrodes grids are placed to control the electric field.

Solutions are injected with a constant pressure delivered by a pressure controller (Fluigent Flow EZ, 7 bar). Tension is applied with a DC power supply in the range [4 V , 50 V], and current is monitored by a multimeter and registered with a LabVIEW program. We use a DNA 100 bp ladder (New England Bio Labs) at a concentration of 25 pg/ μL in a separation buffer supplemented with Polyvinylpyrrolidone (PVP, 360 kDa) at a mass concentration of 5%. Collected samples are profiled by an Adélis BIABooster system on a Agilent 1600 capillary electrophoresis machine and a Zetalif Laser detector (Adélis). Analyses are processed with a BIABooster DNA 1k kit (Adélis).

Results

We demonstrate that size fractionation can be operated with a very sharp cut-off in the range of 150 to 2,000 bp by merely controlling the electric field in the range of 4 to 50 V. Specifically electrohydrodynamic actuation enables us to elute low MW molecules, and retain high MW molecules which are stacked at the membrane. After stopping the electric field, the retained sample is collected with an enrichment of up to 10-fold. Substantially, a 4 min experiment enables us to process a volume of 1,4 mL containing from 10 to 500 ng of DNA. Further, we combine size fractionation to salt purification of the sample. A salty solution with 75 mM can indeed be injected, stacked at the membrane with the electric field while the hydrodynamic flow enables us to flush the salt. Enriched and size fractionated DNA is eventually collected in low salt buffer.

Conclusion

We present a unique technology to purify and size fractionate milli-liter DNA samples achieving enrichment of 10-fold within few minutes. Our technology is versatile because only operated by an electric field of tens of volts, and requires simple integration of an isoporous membranes. The spectrum of applications of this technology is broad, and we will report preliminary results in the context of molecular medicine with cell-free DNA and third generation sequencing.

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