Supporting information

Photopatterning of poly(N-isopropylacrylamide) membrane for high-level enrichment and cleanup of nucleic acids in microfluidic chips

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SI 1. Experimental Section

SI 1.1. Reagents and Materials

Unless stated otherwise, all chemicals, including photoinitiator, used in the experiments were purchased from Sigma-Aldrich (St. Louis, MO, USA). Labeled DNA oligonucleotides and buffer solutions (1× TE 10 mM Tris-HCl, 1.0 mM EDTA, pH 8.0, and 1× TG 25 mM Tris, 192 mM glycine, pH 8.0) were ordered from Sangon Biotech. Co., Ltd (shanghai, China). The 6-carboxyfluorescein (6-FAM) was labeled directly at the 5′end of 38-nt DNA (5′-C AGT CCG TGG TAG GGC AGG TTG GGG TGA CTT CGT GGA A-3′). All solutions were made with deionized water purified by a Milli-Q Plus System (ca. 18 MΩ cm, Millipore, USA).

SI 1.2. Microchip Fabrication

Glass microchips were designed and fabricated in-house using standard photolithography, wet chemical etching, room temperature bonding and dicing methods.[1] As shown in Fig. 1, the two symmetric microchip channels (typically 25 μm deep and approximately 120 μm wide at half-depth) are chevron-shaped, in mirror image orientation, with their apexes designed to pass within 500 μm of each other, forming a bridge-like section between the channels. The dimensions of the main channels between sample reservoir (SR) and buffer reservoir (BR) are 1.0 cm, and the others are 2.0 cm (between sample waste reservoir (SWR) and buffer waste reservoir (BWR)). Note that, our chevron-shaped design not only facilitates access to quickly remove unpolymerized solution during fabrication, but also alleviates the concentration polarization phenomena on the both sides of the membrane and provides continuous sample flow by gravitational flow during enrichment.

The microchip channels were conditioned prior to membrane photopolymerization following a procedure similar to that described by Herr et al,[2] but a little modification. Using this protocol, the membrane was covalently attached to the inner wall of silica

microchip channels with stability that can stand for positive/negative pressure and voltage. After initial treated with 1.0 M NaOH for 30 min, rinsed thoroughly with deionized water, and purged by N_2, channels were functionalized with acrylate-terminated self-assembled monolayers by conditioning with a 2:5:3 (vol/vol/vol) mixture of 3-(tri-methoxysilyl)propyl methacrylate (TMSPMA, ≥ 98%), glacial acetic acid, and deionized water for 30 min, where all reservoirs were sealed by 5% wt/vol 2-hydroxyethyl cellulose (HEC, average Mv ~720000) drops to prevent the mixture from drying. At the end of the incubating step, the channels were rinsed with a 3:7 (vol/vol) mixture of glacial acetic acid and deionized water, 70% vol/vol ethanol solution, deionized water, thoroughly dried with vacuum and stored dry at 60 ℃ until membrane fabrication.

Polymer membrane was fabricated inside the bridge-like section of microchip channels using in situ spatially controlled photopatterning technology to obtain a high resolution structure and precise location. The microchip was loaded by capillary action with a deaerated solution of 0.545 g N-isopropylacrylamide (monomer, NIPAAm, ≥ 99%), 0.12 g N,N-methylene-bis-arylamide (crosslinker, MBAAm, 99%) (22% wt/wt with respect to NIPAAm), 1.0 g dimethyl sulfoxide (solvent, DMSO, ≥ 99.5%) and 0.0375 g 2-hydroxy-2-methylpropiophenone (photoinitiator, 97%). The four reservoirs were cleared, and then each reservoir was filled with 1.5 μL of the precursor solution, covered with 5% wt/vol HEC drops to stop hydrodynamic flow, and equalized at room temperature for 5 min. At the same time, the microchip was aligned on a collimated UV-illumination system setup (Omni Cure Series 1500, EXFO, Mississauga, Canada), and the membrane was formed by exposing the device to UV light (the intensity was calibrated to 4.6 mW cm^{-2} at a distance of 9.0 cm using a radiometer) from a large area collimating adaptor through a custom chrome photomask (NO.55 Electronic Institution, Nanjing, China) for 30 s. Upon membrane polymerization, the channels were rinsed twice with 1× TE buffer to remove any unpolymerized solution from the device and membrane using a vacuum, and stored submerged in buffer at 4 ℃ when not in use. Microchips were usually tested within three days after fabrication.

SI 1.3. Experimental Operation

Before sample loading, the cured PDMS (19:1 weight ratio mixture of the prepolymer and curing agent, Sylgard 184, Dow Corning, Midland, MI, USA) slabs punched vertically with 4.0 mm diameter holes were boned concentrically over the SR and BR (2 mm diameter holes) for enlarging the volume of reservoirs to 60 μL. The PDMS slabs were passivated with 1% (wt/vol) bovine serum albumin (BSA, ≥ 96%) for 30 min to reduce nonspecific binding of the sample to inner walls. After that, the microchips were flushed and filled with 1× TE buffer by vacuum until the samples

were ready to be loaded. Samples with different concentrations of 10 nM, 100 nM, and 1000 nM were prepared by mixing DNA and TE buffer in plastic vials which were also passivated, and were stored at 4 ℃ before use. Then, each reservoir was cleared, 30 μL of sample and 30 μL of TE buffer were added into the SR and BR respectively, while the others were filled with 10 μL of TE buffer solution. In order to load the sample into the main channel, a brief suction at the SWR was applied carefully. SR and BR was filled with larger volume than SWR and BWR, creating a pressure difference between these reservoirs, generating a continuous gravitational flow without any need for an external pump, and also alleviating the concentration polarization effect.

Two platinum electrodes connected to a laboratory-made high voltage power supply (0 ~ 2000 V) were immersed into SR and BR. The bias voltage could be automatically switched and the current was monitored in real time using a computer and self-compiled software. During the enrichment process, various electric field strengths (10 V/cm, 30 V/cm, 60 V/cm and 100 V/cm) were applied to BR for varying time intervals while SR grounded, and in the clean process, a reverse 50 V/cm electric field was switched immediately at the end of enrichment to elute the enriched sample band. To study the reliability of the membrane, these processes were repeated after removing the contents and replacing with new samples, and it was suffered from high voltage test. Otherwise, in order to increase the utilization efficiency, the used microchips were recycled by immersing in concentrated sulfuric acid over night at 70 ℃ after being thoroughly dried, and the treated membrane could be flushed out easily with large amounts of water.

SI 1.4. Measurement Instruments and Data Analysis

To evaluate the performance of the sample enrichment and clean system, the prepared microchip was placed on the stage of an inverted fluorescence microscope (DMIRE2, Leica, Germany) which was equipped with a mercury lamp for excitation, a fluorescence filter cube, a cooled CCD camera (DP71, Olympus, Japan) on top tube port, and a cooled electron-multiplying CCD camera (EMCCD, QuantEM: 512SC, Photometrics, USA) on lateral port. A plastic holder was used to fix the electrodes and microchip. All experiments were performed using a 5× objective lens for microimaging (bright-field) and movie recording (dark-field). Specially, in enrichment and clean experiments, various capture sequences including frames (1 × 1 binning, 512 × 512 pixels) were recorded at appropriate rate (1 s/frame, 2 s/frame, or 10 s/frame) with exposure time 100 ms and on-chip multiplication gain 15 in darkroom. Fluorescence intensity data were collected near the membrane interface, where the area of interest (AOI) was defined to 25 × 2 pixels, with varied enrichment times and voltages. Image analysis, enrichment factor quantification and cleaning

performance were accomplished using Image-Pro Plus (IPP) software to calculate the mean fluorescent intensity in a small AOI in front of the interface. Remarkably, all fluorescence intensity values were corrected by subtracting the background value at t = 0 s and the effects of photobleaching were neglected in any reported data. Note that, the position in "pixels" has been rescaled to "μm" in main text according to the 5× reference calibration scale supplied by manufacturer, 1 pixel equals about 3.14 μm.

SI 2. Optimization of PNIPAAm membrane

Table S1

Doses of the precursors and correspondingly photopatterned states

Polymer	NIPAAm (g)	MBAAm (g)	DMSO (g)	PI (g)	State
1	0.545	0.030 (5.5%)	0.75 + 0.25 H_2O_2	0.0375	Translucent
2	0.545	0.030 (5.5%)	1	0.0375	Transparent
3	0.545	0.060 (11%)	1	0.0375	Transparent
4	0.545	0.0872 (16%)	1	0.0375	Transparent
5	0.545	0.120 (22%)	1	0.0375	Transparent
6	0.545	0.1635 (30%)	1	0.0375	Opaque

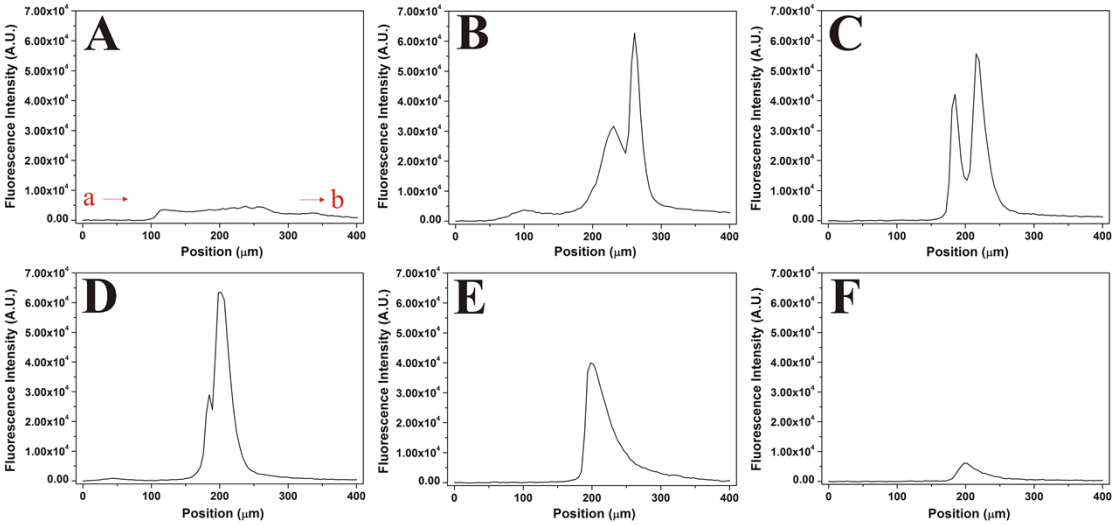

Figure S1. Fluorescence intensity profiles across the membranes corresponding to Fig. 2.

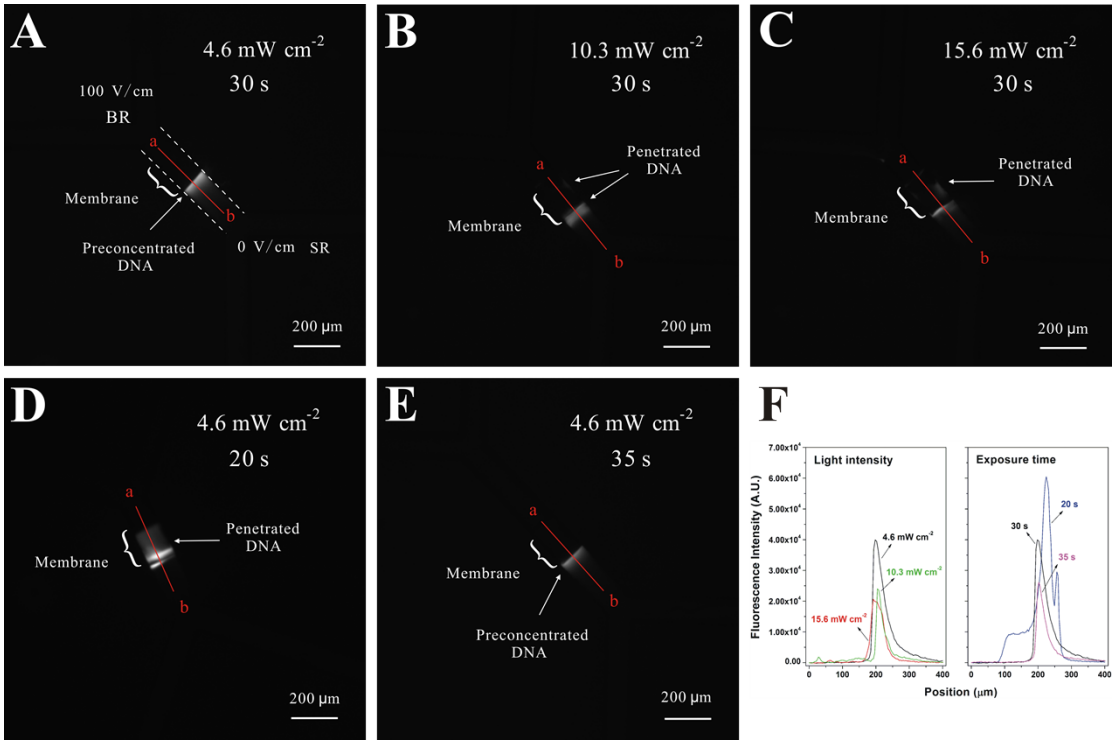

Figure S2. The 90 s enrichment behaviors of membranes photopolymerized from different light intensity, 4.6 mW cm^{-2} (A), 10.3 mW cm^{-2} (B) and 15.6 mW cm^{-2} (C) for 30 s illumination, and different exposure time, 20 s (D), 35 s (E) at fixed light intensity of 4.6 mW cm^{-2}. Figure F showed the corresponding fluorescence intensity profiles across the membranes. Conditions: 1.0 μM initial concentration of DNA, 100 V/cm applied electric field strength, 1× TE buffer.

The UV light intensity and exposure time was a couple of key points to fabricate workable nanoporous membranes. Under the conditions of a three typical light intensity 4.6 mW cm^{-2}, 10.3 mW cm^{-2} and 15.6 mW cm^{-2} for 30s illumination, the results were shown in Fig. S2. When strong light (10.3 mW cm^{-2} and 15.6 mW cm^{-2}) were used (Fig. S2B and C), the DNA samples would go through the membranes, which might be attributed to the uniformity of the membranes generated by over fast photopolymerization. Thus a relatively moderate light of 4.6 mW cm^{-2} was chosen (Fig. S2A) and then taken to inspect the exposure time. As shown in Fig. S2D, DNA migrated from the cathodic to anodic side across the membrane when 20 s exposure time applied, but low concentration intensity was observed from Fig. S2E (all of these could be verified in terms of intensity comparison in Fig. S2F) when 35 s exposure time applied. The enrichment performance of the polymer with 30 s exposure time was superior to the others after comprehensive evaluation. The shorter exposure time (20 s) would result in an incomplete polymerization that the nanopores were not fully formed to block DNA molecules while the longer exposure time (35 s) would result in more crosslinking which diminished the accessability of ions into membrane and slowed down the transport of DNA, therefore led to poor enrichment efficiency.

Moreover, fluorescence intensity profiles (Fig. S2F) corresponding to each frame also showed that 4.6 mW cm^{-2} and 30 s were the best illumination conditions for photopatterning of nanoporous membranes. Similarly, membrane lengths ranging from 100 μm to 400 μm were also tested. 150 ~ 200 μm-long membranes were deemed optimal as they had better stability (withstand 100 V/cm electric field for over 30 minutes) and ion-permeability (the current was recorded to be around 3.0 μA under 100 V/cm) than shorter membranes (easily expanded and damaged below 100 μm, whose current was high to reach 5.5 μA similar to blank channels) and longer membranes (badly efficient transport above 300 μm that the current was decreased around to be 1.0 μA).

SI 3. Optimization of enrichment behavior

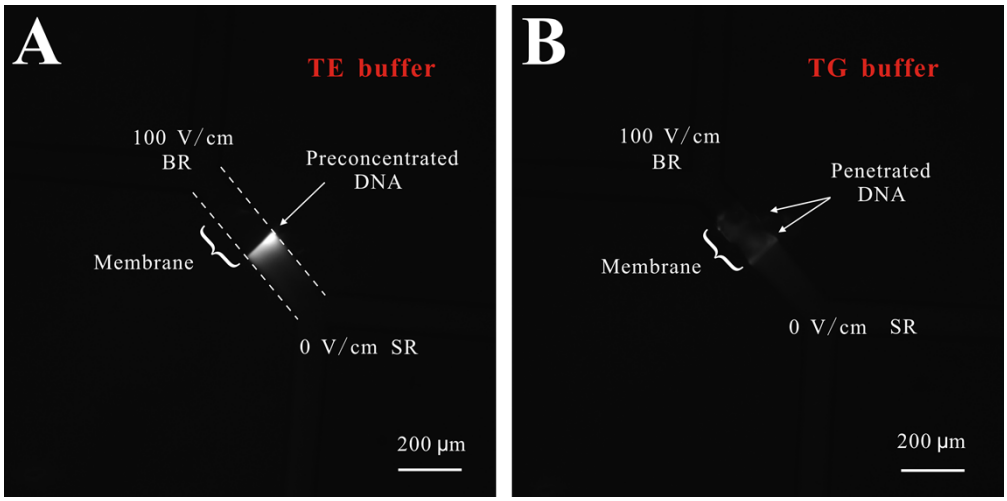

Figure S3. The 180 s enrichment behaviors of membranes in two typical electrophoresis buffers with different ion strength, TE buffer (A) and TG buffer (B). Conditions: 1.0 μM initial concentration of DNA, 100 V/cm applied electric field strength.

In this experiment, Two commercial buffer solutions with standard recipes and different ionic strengths were used to address the concentration efficiency. For a typically low ionic strength TE buffer (10 mM Tris-HCl, 1.0 mM EDTA), the EDL overlapped within the nanoporous membrane whose pore size was smaller than the Debye length (that is, the double-layer thickness),[3] therefore, negatively charged DNA molecules were excluded and enriched from the interface successfully (Fig. S3A), meanwhile, TG buffer (25 mM Tris, 192 mM glycine) with higher ionic strength made the thickness of EDL decreased, resulted in DNA penetration (Fig. S3B).

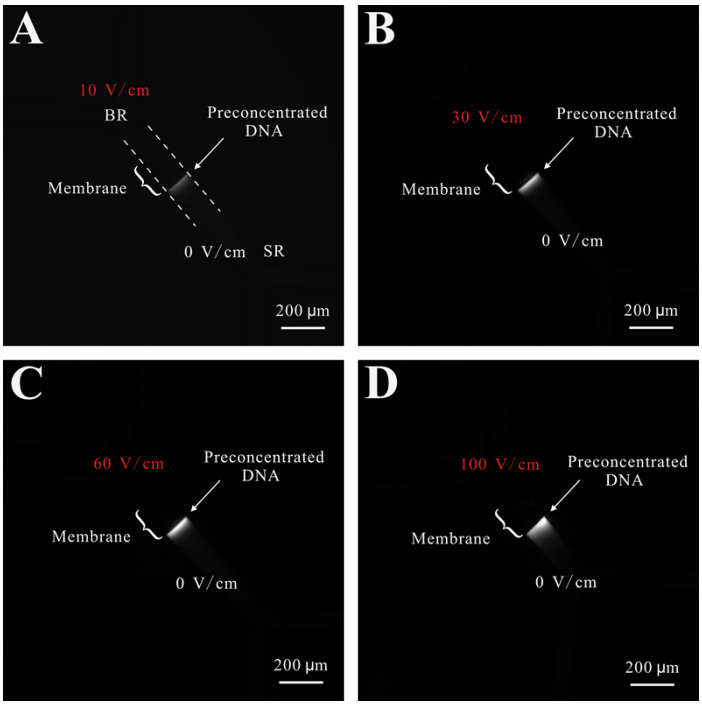

Figure S4. The 180 s enrichment behaviors with different applied electric fields, 10 V/cm (A), 30 V/cm (B), 60 V/cm (C) and 100 V/cm (D). Conditions: 1.0 μM initial concentration of DNA, 1× TE buffer.

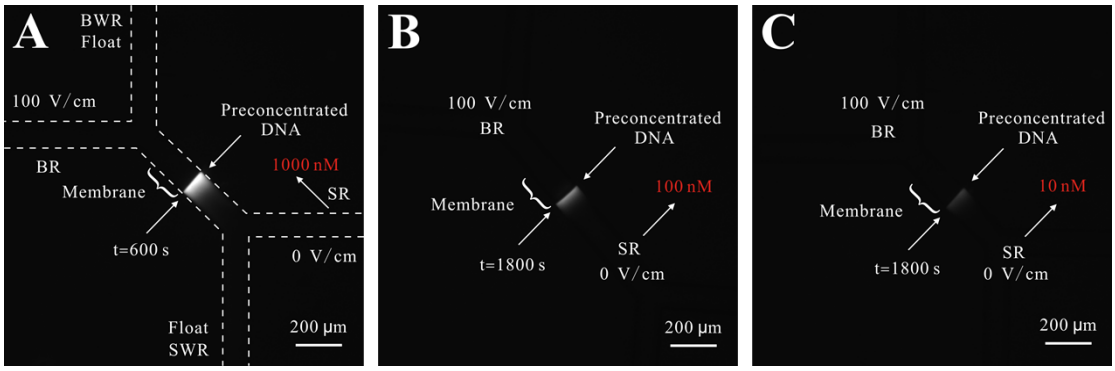

Figure S5. The enrichment behaviors with different initial concentration of DNA, 1000 nM at 600 s (A), 100 nM at 1800 s (B) and 10 nM at 1800 s (C). Conditions: 100 V/cm applied electric field strength, 1× TE buffer.

At the onset of this experiment, DNA solutions with different concentrations were loaded into the sample reservoir respectively, after applying 100 V/cm, trapping DNA was continued for 10 min (initial concentration of 1000 nM) and 30 min (100 nM, 10 nM). The results indicated that the membrane with 22% crosslinker had enough rigidity and stability to endure the electric field as high as 100 V/cm for long time (over 30 minutes). Fig. S5 showed three fluorescence micrographs that demonstrated the enrichment from various initial concentrations of DNA. Although the initial concentrations of 10 nM and 100 nM could not achieve the maximum intensity

visually in contrast to 1000 nM, a brightly enriched band also appeared clearly at the interface of membrane without any penetration.

SI 4. The enrichment and cleanup process

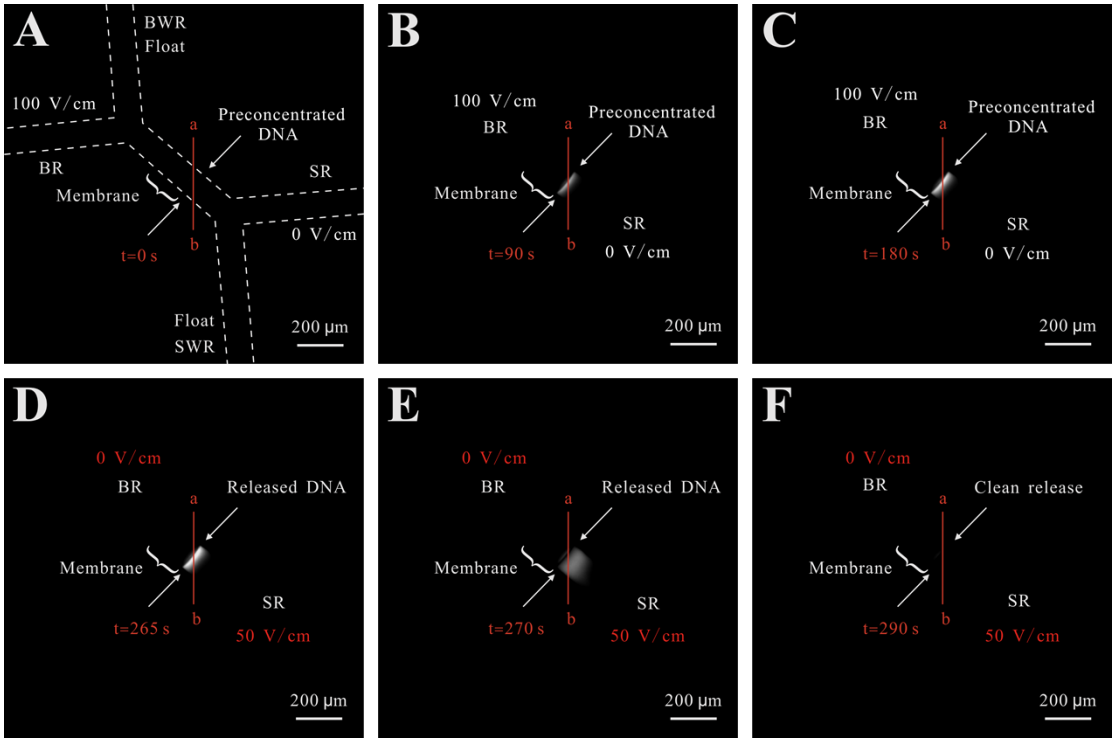

Figure S6. Time sequence images of enrichment and cleanup of 1.0 μM initial concentration of DNA. Conditions: 100 V/cm applied electric field strength for enrichment, 50 V/cm for cleanup, 1× TE buffer.

Reference:

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2 Herr, A. E.; Throckmorton, D. J.; Davenport, A. A.; Singh, A. K. *Anal. Chem.* **2005**, *77*, 585-590.

3 Dhopeshwarkar, R.; Li, S. A.; Crooks, R. M. *Lab Chip* **2005**, *5*, 1148-1154.