Electronic Supplementary Material (ESI) for Lab on a Chip.

A Bifurcated Continuous Field-Flow Fractionation (BCFFF) Chip for High-Yield and High-Throughput Nucleic Acid Extraction and Purification

Supplementary Information

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Fluorescence-labeled ssDNA Sequence	**5'- TAG CCC TAA AGC TAT TTC GGA GAG AAC CA –FAM**
Enterococcus Forward Primer	**5'-GAGAAATTCCAAACGAACTTG**
Enterococcus Reverse Primer	**5'-CAGTGCTCTACCTCCATCATT**
Enterococcus TaqMan® Probe	**[6-FAM]-5'-TGGTTCTCTCCGAAATAGCTTTAGGGCTA-TAMRA**
DNA Template for dsDNA Fragment	**5'-TCATGCAAGTCGAGCGATG**GAGAAATTCCAAACGAACTTG**GGGGTTCTGAGAGGAA GGTGGTAGAGCACTGTTTCGGCATCTGAGGAGCACGAGACGGCAGGCTCGAG**AAT GATGGAGGTAGAGCACTGA**AAAGGAAGATTAATACCGCATAGAGAATGTTATCACG GGAGACAAGTAGCGTGAAGGATGACGG**
rDNA Forward Primer	**5'- ACG AAT TCG TGC CAG CAG CCG CGG TAA**
rDNA Reverse Primer	**5'- TGG AAT ICG GTT ACC TTG TTA CGA CTT**
Ampicillin-resistance gene Forward Primer	**5'- GCT CAC CCA GAA ACG CTG GTG AAA GTA**
Ampicillin-resistance gene Reverse Primer	**5'- CGC AAC GTT GTT GCC ATT GCT ACA GGC**

Table S1 oligonucleotide and gene sequences used in this study (Red: forward primer sequence, Blue: complementary sequence of reverse primer) .

Stability of the Depletion Front

To investigate the influence of flow on the depletion generated by the CEM, ionic current going through the cross channel is recorded. Without flow, the current initially drops down following a diffusive $\sqrt{t}$ law and then approaches a zero-current steady state. Figure S1 shows that as the flow rate increases, a steady-state current is still observed, suggesting a stabilized depletion. However, a higher steady-state current under high flow rate indicates a lower resistance inside the depletion region and, consequently, a lower electric field, which is also true when the ionic strength of the loaded sample is increased. Low electric field cannot achieve high-yield extraction. There is hence an optimum flow rate for a given voltage.

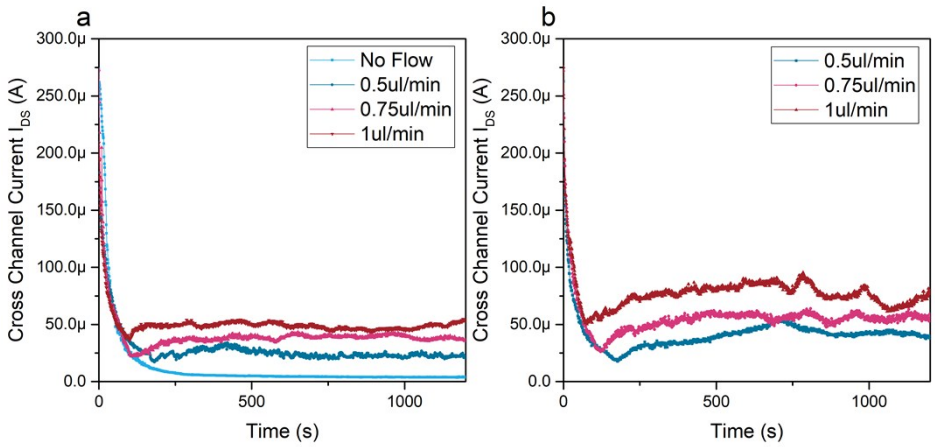

Fig. S1 The change of cross channel current Ids over time at different flow rate with loaded buffer (a) 0.1xPBS, (b) 0.4xPBS. Vd = 1V, Vg = 80V

Proof-of-Concept of Calcium Removal by Purification of E. coli DNA from High Calcium Concentration Buffer

To prove the ability of extracting nucleic acid from inhibitors, a proof-of-concept experiment is done with thermally lysed E. coli spiked into 0.1xPBS buffer with 20mM of added-in calcium chloride (Sigma-Aldrich). The E. coli (Modern Biology Inc. IND-21) is cultured in LB Broth Media (Teknova) for 2 day. The media is centrifuged under 5000g for 15 minutes. The supernatant is removed, and the pellet is resuspended in 0.1xPBS buffer with 20mM of added-in calcium chloride and thermally lysed in 95℃ water bath for 20 minutes. The lysed E. coli is pretreated with our device using the same DNA protocol described in the main article. PCR amplification of both pretreated and untreated sample is carried out using the primer sets and thermal cycling condition in the IND-21 kit targeting an ampicillin-resistance gene on plasmid pUC18. 1% agarose gel is prepared by mixing 0.5g agarose I (Bioscience) and 1.2uL SYBR® Green I Nucleic Acid Stain (Lonza) in 50mL 1×TAE buffer (VMR). After casting the gel, 10μL of amplicons of the sample is mixed with 2μL of 10×loading buffer and loaded onto the gel. Electrophoresis was run at 120V for 1 hour. the gel was visualized by Dark Reader Transilluminator (Clare Chemical). As shown in FigS2, successful amplification is achieved after isolation of DNA on BCFFF chip comparing to no target band for untreated sample.

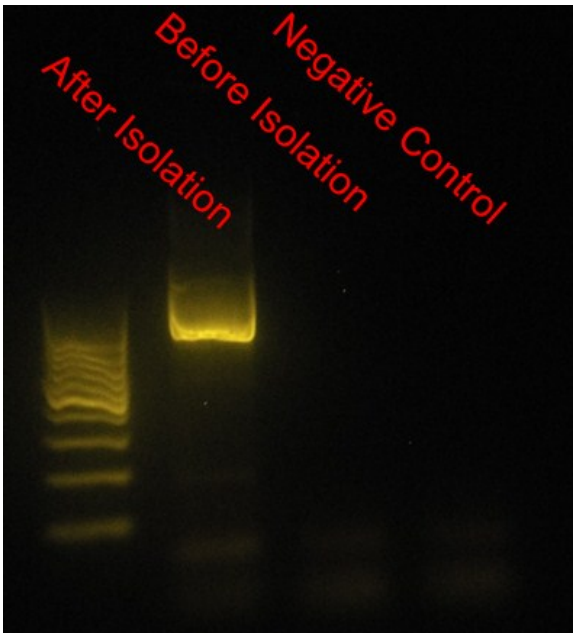

Fig. S2 Gel electrophoresis image of PCR amplicons of both treated and untreated E. Coli DNA in 0.1xPBS with 20mM calcium chloride.

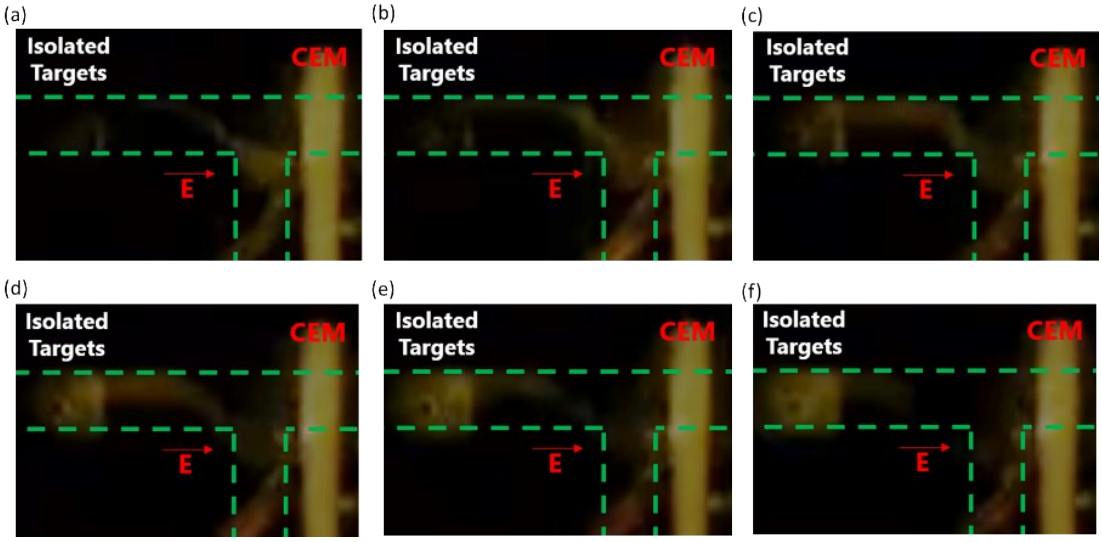

Fig. S3 Real-time fluorescence images shows the isolation of dsDNA from plasma sample: (a) 4min, (b) 6min, (c) 8min, (d) 10min, (e) 12min, (f) 14min.

Supplementary Video 1: Fluorescence Imaging of ssDNA Extraction

Supplementary Video 2: Fluorescence Imaging of dsDNA Extraction