Supporting Information for

A three-dimensional microfluidic device embedded within a thermal cycler tube for electrokinetic DNA extraction

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In this supporting document, we provide the following complementary information and results: a detailed description of three-dimensional (3D) printing materials and structure compatibility; experimental setup details and image analysis of isotachophoresis (ITP) visualization; quantification of ITP including the typical voltage curves and extraction time distributions; experimental details and results of SYBR Green-based real-time PCR detection; a comparison of PCR performance with and without leading electrolyte (LE) buffer; a comparison with commercial nucleic acids isolation kits regarding manual steps; and a supplementary movie that illustrates ITP migration of focused fluorescein dye.

Contents

S.1 Complementary details of three-dimensional (3D) printing

We here provide additional details about the 3D printed device and materials.

S.1.1 3D printing materials. The 3D printing was performed with a commercial printer, Profluidics 285D 3D printer (CADworks3D). We specified the commercially available resin used in this model in Section Design and fabrication of 3D-printed microfluidic structure (the Clear Microfluidics Resin V7.0a resin, Product ID: CW3D-R-CMV7A-1KG). The Safety Data Sheet (SDS) provided by CADworks3D lists the main components as methacrylate oligomers (15-40%), modified acrylate monomers (15-50%), and photoinitiators (0.5-4%), with more specific chemical specifications marked as proprietary.

S.1.2 Compatibility with other tube designs. The microfluidic insert described in this work is likely compatible with a variety of polymerase chain reaction (PCR) tubes. For the data in the main paper, we used a PCR tube manufactured by Applied Biosystems™ (MicroAmp™ Fast Reaction Tube with Cap). In Figure S1, we show the same 3D printed microfluidic device can be mated with three other brands.

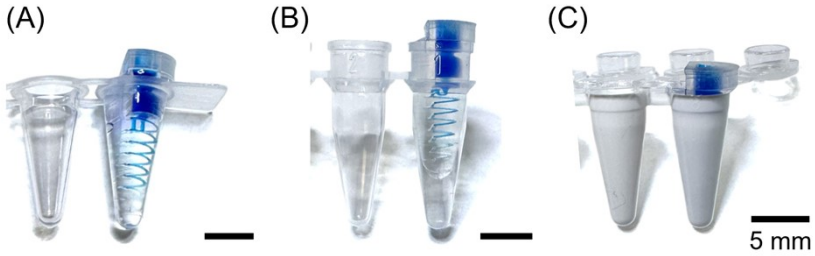

Fig. S1. Images of 3D microfluidic device compatible with and inserted into three different commercially available PCR tubes. Each image shows two tubes: one empty (standard) tube on the left, and one PCR tube with the inserted microfluidic part on the right. The microfluidic structure was filled with blue dye to visualize internal structures. (*A*) Device inserted into Thermo Scientific Strip tube. These low-profile tubes are used to reduce dead space and increase PCR efficiency. (*B*) Example insertion into Labcon PurePlus 0.2 mL PCR tube. These support reaction volumes up to 125 µL. (*C*) Insert in a USA Scientific TempAssure 0.1 mL PCR Tube. These tubes are opaque with individually attached optically clear caps. (Scale bars are each 5 mm.)

We note that this size fit may be insufficient to evaluate the efficacy of these tubes. The images of Fig. S1 are provided only to show size compatibility with common models.

S.2 Isotachophoresis (ITP) visualization

We here provide additional information on the optical setup for ITP visualization, fluorescent intensity analysis of the images, and plume visualization of ITP zone at the end of the extraction. We used 10 μM of fluorescein in the sample reservoir as a marker to visualize the process (same as shown in Figure 2A in the main text). Note we only need the setup in Fig. S2 (B) and (C) for DNA purification, the visualization setup of Fig. S2 (D) is not necessary when performing the DNA extraction function.

S.2.1 Support structure and optical setup. During the ITP extraction process, electrodes and tube-embedded 3D device are held and secured in the support structure. And we use a 45-degree mirror to image the fluorescein migration in the vertical plane.

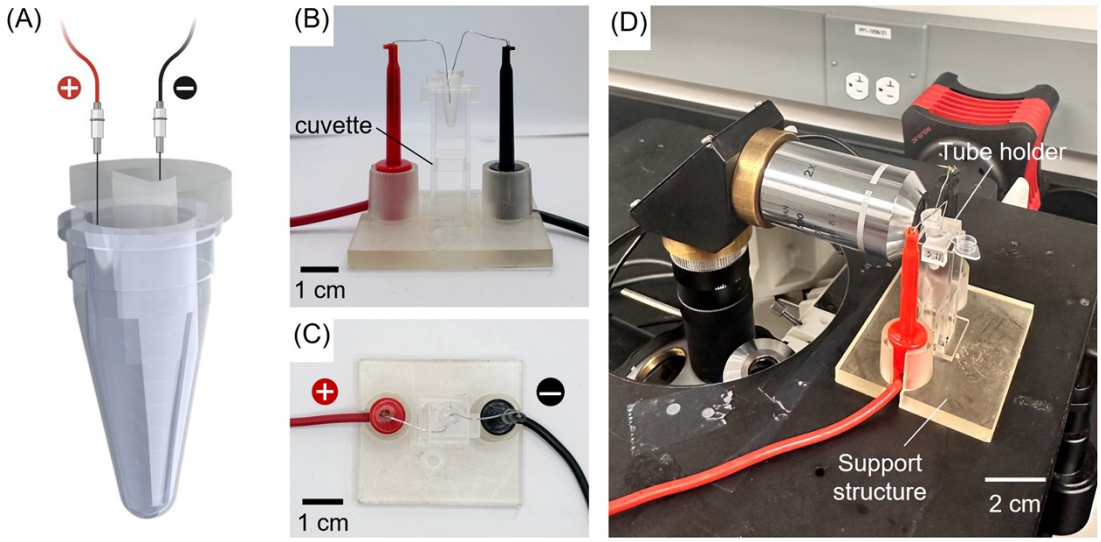

Fig. S2. Schematic and images of device, support structure, and optical setup used to visualize ITP. (*A*) Schematic of electrodes layout. Positive and negative electrodes are connected to the peripheral LE reservoir and the top TE reservoir, respectively. (*B*–*C*) Front and top views of the ITP extraction setup. The PCR tube/microfluidics assembly is immersed into a polystyrene cuvette (BRAND, BrandTech) that is supported by a 3D-printed structure. The cuvette is filled with glycerol to approximately match the index of refraction of the 3D printed part, and thereby facilitate enhanced optical access and visualization. The bracket is designed to also secure the electrodes as shown. (*D*) Image of the ITP extraction setup integrated with an optical microscope for visualization. We used an epifluorescence inverted microscope (Nikon Eclipse TE300). We also used a connection with a mirror at 45 degrees to the optical access to transmit light from/to a horizontally oriented microscope objective. The microscope objective is a Nikon 2X Plan Apo with a numerical aperture (NA) of 0.10. The camera is connected to a 0.63 demag lens to increase the field of view and visualize the entire spiral channel. This setup was specially designed to visualize migration of the ITP zone within the spiral channel. This optical setup is not required to perform the assay.

S.2.3 Fluorescent intensity of the ITP zone. Based on Movie S1, we applied a temporal median subtraction to decrease background image noise of each image frame. We then performed an automated detection of ITP zone (using a custom MATLAB code) and then summed intensities over a 61 x 41 pixel region centered on the detected ITP zone for each frame. This integrated intensity is then plotted versus time.

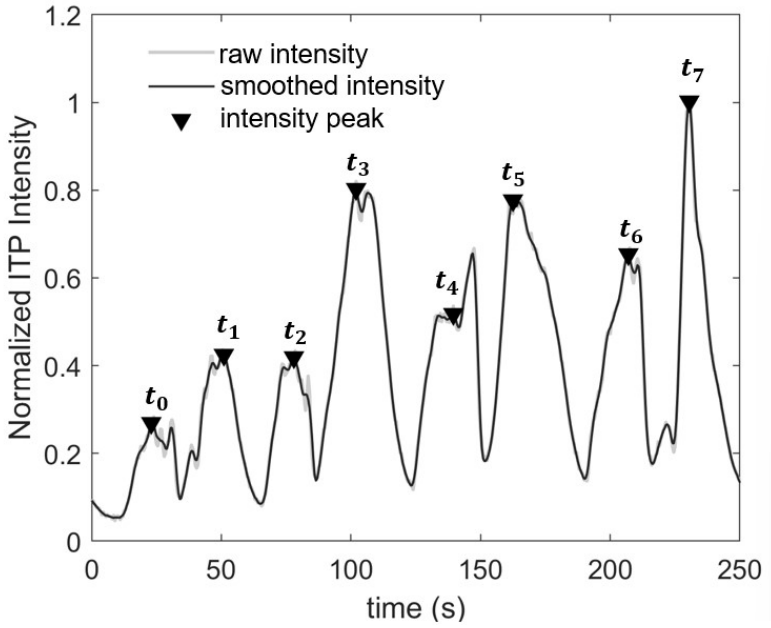

Fig. S3. Measured fluorescence intensity of the moving ITP zone. See also Movie S1. The intensity fluctuates versus time as the fluorescence emission is refracted and absorbed by the complex 3D structure as the ITP migrates in a tightening helix. The local minima in the signal correspond to highly refracted images as the ITP zone migrates into and out of the plane of the image. The triangle tick marks t_0 through t_7 correspond to the annotated time points in Figure 2A of the main manuscript.

S.2.3 Plume of ITP zone exiting into the LE reservoir. We used 1 μM of fluorescein to visualize the buoyant plume created as the ITP zone exits the spiral channel and enters the LE reservoir. This plume helps mix the sample within the LE reservoir. After observing that the voltage curve stopped increasing linearly, we continued recording for about 200 s before stopping the current.

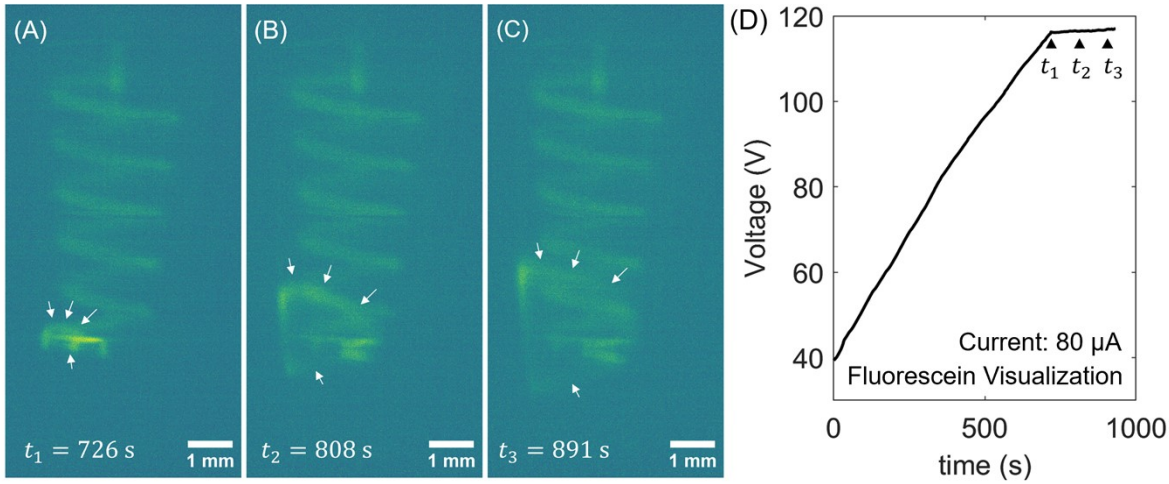

Fig. S4. Plume of fluorescent sample exiting the spiral channel after ITP and corresponding voltage curve. (*A*–*C*) Images captured at three time points with an equal interval of $\Delta t = 82\ s$. These images depict the spatiotemporal evolution of a fluorescein-labeled zone under the combined influence of buoyancy-driven mixing and an applied electric field. Images are processed with the false color scheme Viridis (imageJ) to increase the contrast. The white arrows indicate the top and bottom edges of the fluorescein plume. We observed that under the influence of buoyant force and electric field, the plume tends to asymmetrically disperse upward. (*D*) Voltage curve versus time. The voltage initially increases linearly as the ITP zone spirals down the microchannel, then reaches a plateau once the ITP zone exits the spiral channel. The triangle tic marks in (D) correspond to annotated time in (A)–(C).

S.3 Quantification of ITP extraction

Here, we provide additional information to quantify the ITP extraction process with spiked-in serum, including sample voltage curves and the distribution of ITP extraction times.

S.3.1 Voltage curve versus time for serum extraction. During ITP DNA purification from serum, we applied a constant current of 80 μA using a Keithley 2410 and recorded the voltage curve with a custom MATLAB script. Consistent patterns were observed across experiments.

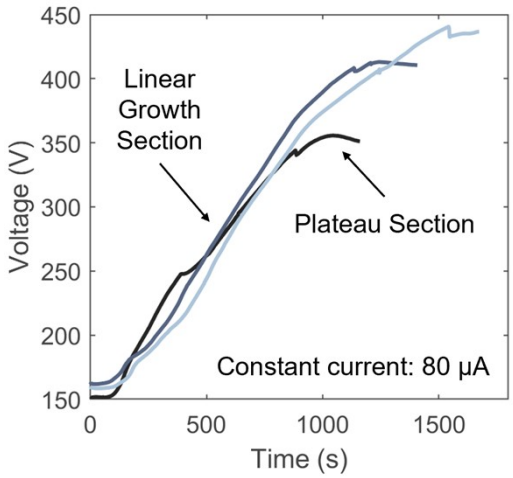

Fig. S5. Example traces of measured voltage versus time during ITP extraction. For each ITP extraction, we imposed a constant current of 80 μA. This drives the ITP process and extraction and purification of nucleic acids from human serum samples. Traces reach a characteristic plateau (where voltage levels off with time) when the ITP zone exits the 3D insert and enters the LE reservoir zone (see also Figure 1E of the main paper). We detected this plateau in the voltage traces and used this signal to terminate the applied current. In this way, our assay does not require real-time visualization of any kind to control the process.

S.3.2 Distribution of extraction time. We here quantify the variation of the extraction time and present a histogram.

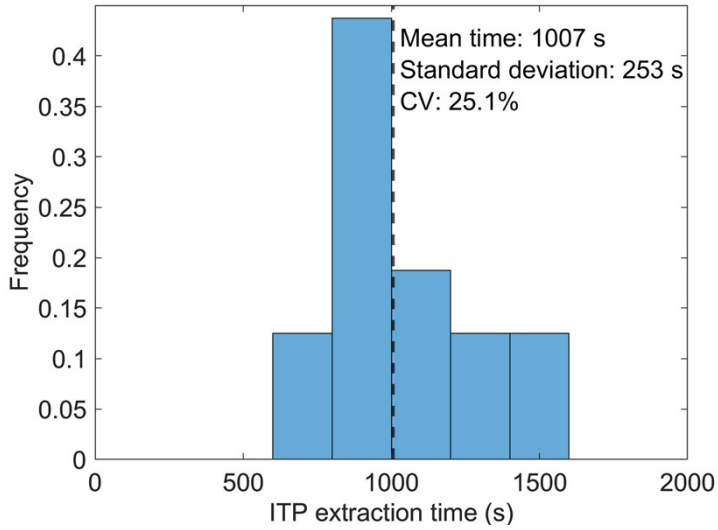

Fig. S6. Histogram of ITP completion time with a constant current of 80 μA. The times when a drop of voltage occurred (indicating the sample has entered the LE reservoir) ranged between 600 to 1500 s, with the mean and standard deviation of extraction times to be 1010 s and 250 s, respectively, over 16 experiments. We attributed this variability mostly to variability in the loading of the sample chamber, including pipetting variability.

S.4 SYBR Green-based qPCR detection

We used two different qPCR methods to demonstrate the versatility of our system. TaqMan probe-based detection method is described in **Methods and Materials** in the main text. We also performed a SYBR Green-based qPCR assay to demonstrate one additional type of PCR protocol. For the latter, each reaction was performed in a total volume of 40 µL, consisting of 20 µL of master mix (PowerUp™ SYBR™ Green Master Mix for qPCR, Applied Biosystems™), 1 µL of 20 µM forward primer, 1 µL of 20 µM reverse primer, and 18 µL of DNA sample. For pure DNA samples (gray curves), the DNA sample consisted of the target DNA at the desired concentration, diluted in LE buffer. For serum samples with ITP purification (red curves), the DNA sample consisted of the ITP-extracted product. For non-template controls (NTC), the reaction contained 18 µL of pure LE buffer as the sample input, without DNA spike-in. Post-amplification melting curve analysis was used to confirm the specificity of the amplicon.

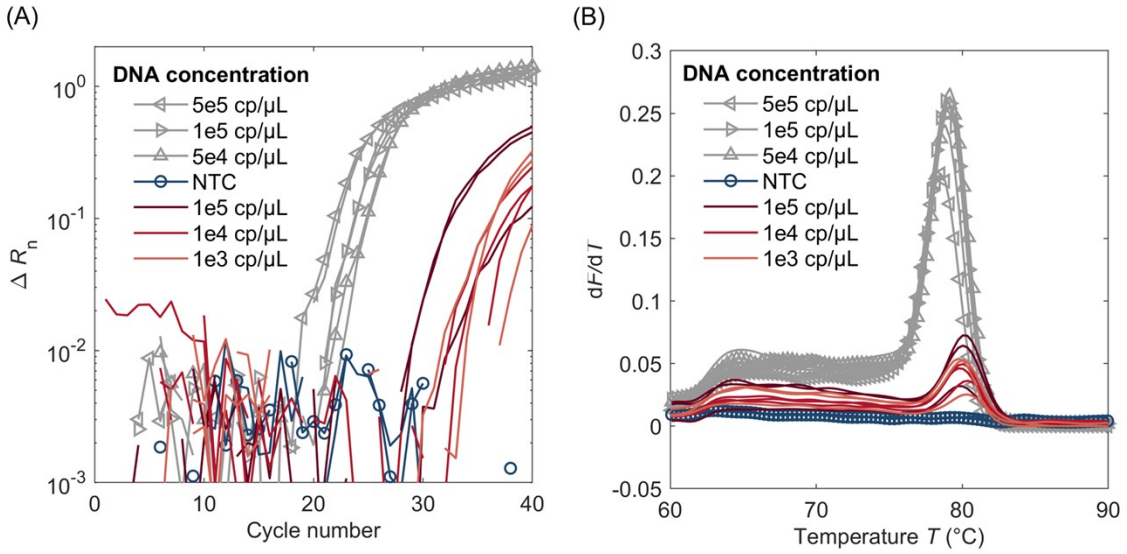

Fig. S7. Real-time PCR results using SYBR Green at various nucleic acids concentrations in serum and associated controls. (*A*) Real-time amplification curves of qPCR results. We plotted normalized dye fluorescence ΔR_n versus cycle number. SYBR Green qPCR for SARS-CoV-2 N gene shows successful amplification enabled by ITP extraction. Note how no template control (NTC, circles) shows only background noise. (*B*) Melting curves plotted as the derivative of normalized reporter fluorescence intensity with respect to measured temperature as a function of temperature. These post-PCR melting-curve analyses confirm the presence of a single qPCR amplicon.

S.5 LE effect on PCR

We compared real-time PCR reactions with and without LE buffer in a 20-μL reaction system and observed no significant effect of LE on the PCR Cq number and amplification behavior. Each 20 μL reaction contained 10 μL TaqMan Master mix (TaqMan™ Universal PCR Master Mix, ThermoFisher Scientific), 0.5 μL of 20 μM forward primer, 0.5 μL of 20 μM reverse primer, 0.5 μL of 10 μM FAM-labelled probe, 1 μL DNA template, and 7.5 μL of either DNase-free deionized water or LE buffer. A Student's *t*-test of Cq values showed no significant difference for DNA concentrations larger than 10^3 cp/rxn between conditions with or without LE buffer ($p > 0.05$), indicating that LE buffer does not reduce amplicon yield. These results confirm the compatibility of PCR amplification with the ITP buffer.

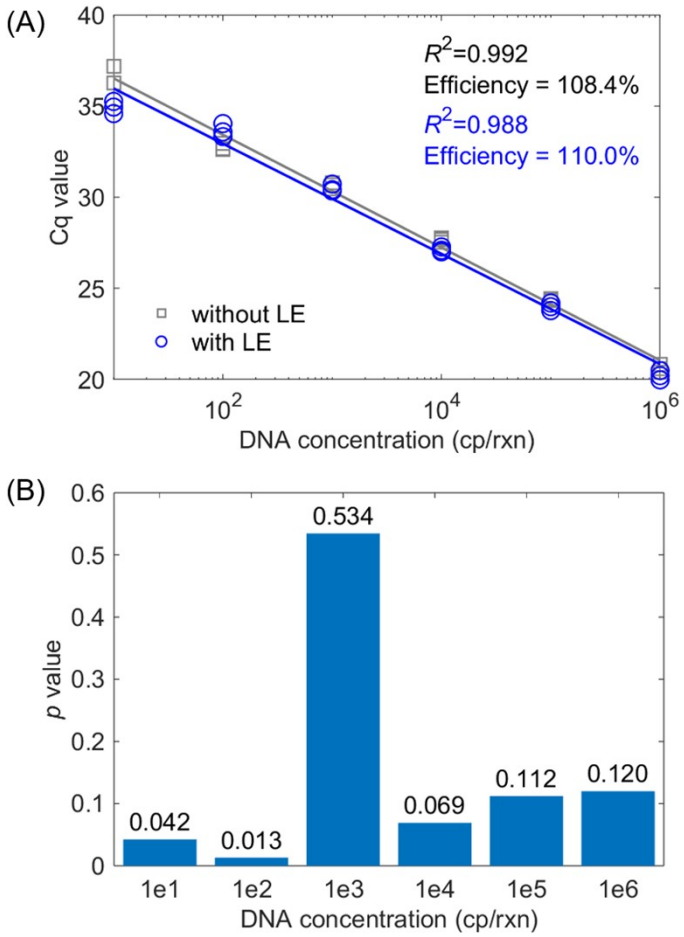

Fig. S8. Real-time PCR results with or without LE buffer. (*A*) Measured Cq values as a function of DNA copies per reaction. The qPCR efficiency of the standard curves with LE (blue) and without LE (gray) are within the acceptable range. The standard curve without LE exhibits a higher R^2 value. (*B*) Calculated *p*-values of Student's *t*-test comparing conditions with and without LE buffer as a function of DNA concentration and across a broad DNA concentration range (10^3-10^6 cp/rxn) *p*-values typically exceed 0.05, indicating that negligible difference between presence and absence of LE buffer. We conclude that LE buffer chemistry does not significantly impact PCR performance. At low DNA concentrations (10^1 and 10^2 cp/rxn), PCR yields very slightly lower Cq values in the presence of LE buffer.

S.6 Comparison of manual steps with commercial purification kits

We present a step-by-step comparison of the manual sample preparation procedures for three nucleic acid extraction methods: the column-based QIAamp Circulating Nucleic Acid Kit by Qiagen, the magnetic bead-based MagMAX Nucleic Acid Isolation Kit by Thermo Fisher, and our ITP-based extraction assay. The QIAamp kit requires 16 manual steps, including two centrifugation steps and four vacuum-based buffer rinses, with a total hands-on time of ~68 min. The MagMAX protocol involves 23 manual steps with a total time of ~67 min. The combined time required for traditional solid-phase nucleic acid purification and subsequent qPCR detection often exceeds 2 h. Our assay involves fewer discrete manual steps and a total preparation time of ~25 min in the extraction process.

Table S1. Comparison of manual steps involved in ITP-based assay and commercially available extraction kits

	QIAmp Circulating Nucleic Acid Kit		**MagMAX Nucleic Acid Isolation Kit**		**Our ITP-based assay**	
Step No.	Steps	Time (min)	Steps	Time (min)	Steps	Time (min)
1	Pipet Proteinase K	1	Add Proteinase K and SDS to serum	1	Add Proteinase K and Triton X to serum	1
2	Add serum	1	Incubate at 60°C; prepare the Binding Solution/Beads Mix	20	Incubate sample at 65°C; place TE-Pluronic mixture on ice	5
3	Add Buffer ACL and vortex	0.5	Cool the tubes containing the serum sample to room temperature	5	Dispense LE into spiral channel	0.5
4	Incubate at 60°C	30	Vortex the tubes to bind the cfDNA to the beads, and centrifuge	10	Dispense treated sample in the sample reservoir	0.5
5	Add Buffer ACB and vortex	0.5	Place the tube on the magnet	5	Dispense TE-Pluronic mixture in TE reservoir	0.5
6	incubate on ice	5	Carefully discard the supernatant	1	Dispense LE in PCR tube, and insert 3D printed part	0.5
7	Connect to vacuum and insert tube extender		Keep the tube on the magnet and remove the residual supernatant	1	Secure the tube with embedded structure and reagents on the rack	1
8	Add ACB mixture and apply vacuum	10	Remove the tube from the magnet, then suspend the beads in cfDNA Wash Solution	1	Insert electrodes	1
9	Remove the		Vortex, then centrifuge	0.5	Apply current and perform	15

	tube extender				ITP extraction	
10	Add wash buffer (ACW1) and vacuum	1	Place the tube on the magnet	2		
11	Add wash buffer (ACW2) and vacuum	1	Carefully discard the supernatant	0.5		
12	Add ethanol and vacuum	1	Keep the tube on the magnet, tap the stand, then remove any residual liquid	1		
13	Spin at 14,000 rpm	3	Remove the tube from the magnet, add 80% ethanol	1		
14	Incubate at 56°C	10	Vortex, then centrifuge	0.5		
15	Add buffer AVE and incubate	3	Place the tube on the magnet	1		
16	Spin at 14,000 rpm	1	Remove the supernatant	0.5		
17			Keep the tube on the magnet, tap the stand, then remove any residual liquid	1		
18			Repeat for a second wash with 80% ethanol	4		
19			Keep the tube on the magnet, air dry the beads	3		
20			Keep the tube on the magnet, tap the stand, then remove any residual liquid	1		
21			Add cfDNA elution solution			
22			Vortex, then centrifuge	5		
23			Place the tube on the magnet (the supernatant contains the purified cfDNA)	2		
	Total time (min)	**68**	**Total time (min)**	**67**	**Total time (min)**	**25**

Movie S1 (separate file). ITP migration of focused fluorescein dye as it travels through the three-dimensional extraction (spiral) channel. We applied a constant current of 80 μA to extract the analyte from the sample reservoir. Fluorescein concentration in the sample reservoir is 10 μM. The spiral microchannel outline is overlaid for reference.