Electronic Supplementary Information

Rapidly-prototyped microfluidic device for size-based nucleic acid fractionation using isotachophoresis

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S1. Fabrication protocol for microfluidic devices

We here describe the fabrication protocol for the rapidly-prototyped devices used in this work:

1. Design the three layers (reservoir, channel, and cover layers) using AutoCAD. Alternatively, the layers can be designed using similar tools that are compatible with the particular laser cutter used. In the design, draw circular holes at each edge in the same location on each layer, like the ones shown in Figure S1a. These holes will be used for alignment.
   Note: We recommend designing the three layers adjacent to each other. This will allow the use of a single laser cutting protocol on the same PMMA sheet.

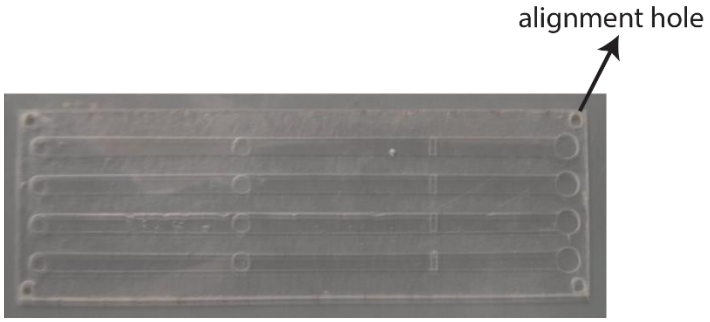

Figure S1. Schematic of completed device highlighting the alignment holes at each edge.

2. Attach two-sided pressure adhesive to the top of the PMMA sheets that will contain the channel and cover layers (as shown in Figure 2a of the main text). Do not peel the cover on the other side of the tape.

3. Laser cut the three layers, making sure to remove any remaining debris.

4. Wipe the bottom of the reservoir and channel layers carefully with ethanol or isopropanol in order to ensure tight sealing with the pressure adhesive.

5. Carefully peel the tape cover off the pressure adhesive.

6. Using alignment posts (which need to have a diameter slightly smaller than the alignment hole, and a length sufficient to comfortably hold all three layers), gently bond the top of the cover layer to the bottom of the channel layer, followed by the top of the channel layer to the bottom of the reservoir layer. We have found that 20 µL pipette tips, or small-diameter screws (*e.g.*, #2-56), work well as alignment posts, and can be affixed to a base in the same arrangement as the alignment holes on the device.

7. Remove the device from the alignment posts. Using a roller (*e.g.*, PCR sealing film roller) or similar tool, apply consistent and significant pressure to the entire layer to form a water-tight seal.

8. Finally, perform air, water, and ethanol washes to cleanse the device and remove any remaining dust or debris.

S2. Approximating pressure head at the loading slit

Pressure drop across a microchannel is given by:

$$\Delta P = QR \tag{S1}$$

Where Q is the flow rate and R is the channel resistance, which are given by:

$$Q = uwh$$

$$R \cong \frac{12\mu L}{wh^3} \tag{S2}$$

Here, u is the average velocity, μ is the dynamic viscosity, L is the channel length, w is the channel width, and h is the channel height. We can rewrite eq S1 as:

$$\Delta P \cong \frac{12u\mu L}{h^2} \tag{S3}$$

We load our device by first dispensing LE from the LE reservoir and allowing it to wick through the channel until it reaches the extraction reservoir and then the loading slit. We approximate that for this loading process, $\Delta P \approx 6$ Pa. The pressure head at the loading slit is given by:

$$\psi = \frac{\Delta P}{\rho g} \approx 600 \text{ μm} \tag{S4}$$

Here, g is the gravitational constant and ρ is the density. We therefore estimate that the pressure head is approximately 600 μm of water in our device.

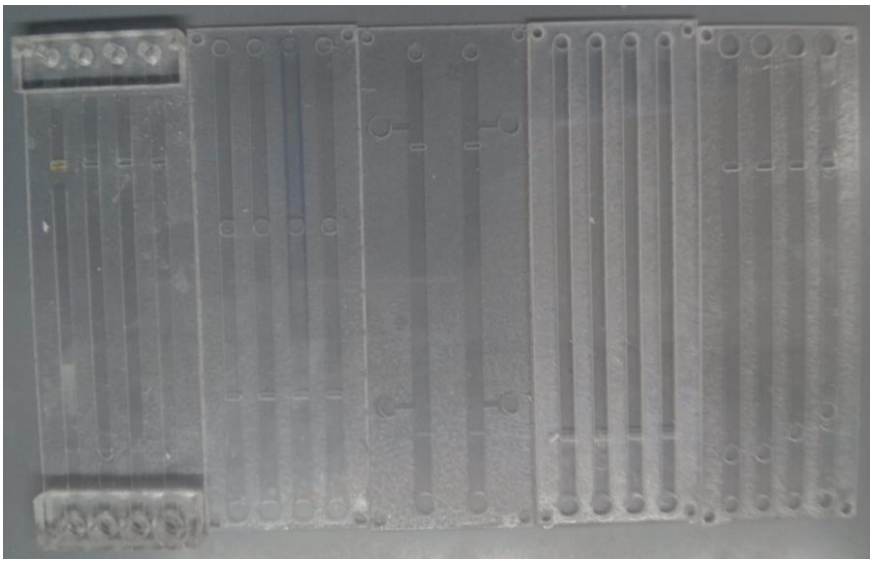

Figure S2. Select device designs generated with rapid prototyping approach. During the development of this assay, we explored different device designs and geometries to optimize ITP preconcentration and fractionation. Our rapid-prototyping approach involved using AutoCAD software to design, and a CO2 laser cutter to generate microfluidic channel geometries in PMMA sheets. This approach allowed to test several designs with minimal turnaround time.

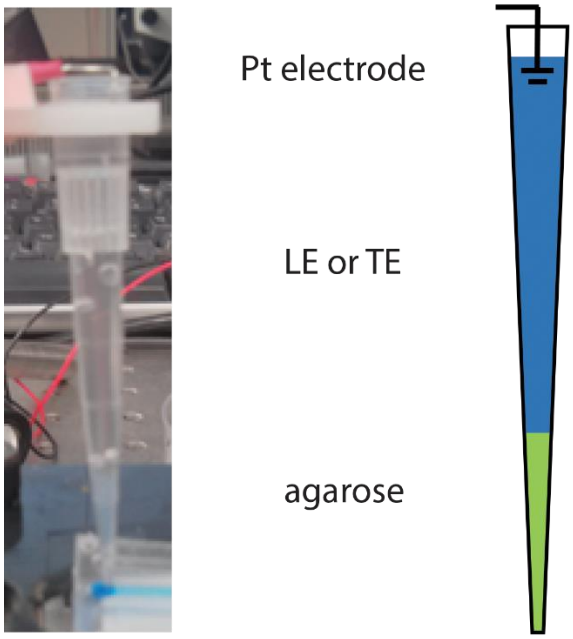

Figure S3. Buffering reservoir design consisting of a 1 mL pipette tip filled with LE or TE buffer, and solidified agarose on the bottom to prevent leakage. A platinum wire electrode is connected to an external voltage source. This reservoir design allows sufficient buffering capacity and prevents bubbles from entering the channel.