Supporting Information: Quantification of global microRNA abundance by selective isotachophoresis

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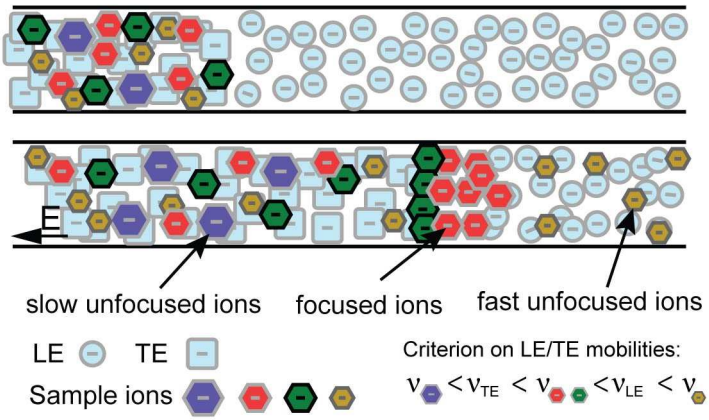

Figure S1: Schematic of process by which ITP selectively focuses some analytes but not others. Shown is a sample containing four different ions initially dissolved into TE. Two ions (mid-sized red and green hexagons) have mobilities bound by those of the TE and LE. Upon application of an electric field, these two targeted ions focus (and may eventually self-segregate into distinct zones). Ions with mobilities too low (large blue hexagons) or too high to focus (small yellow hexagons) are respectively left behind or overspeed the ITP zone. In the case of selective focusing of miRNA, small RNA will focus at the TE-LE interface while longer RNA remain in the TE zone as their electrophoretic mobility is smaller than the TE.

Supplementary description of methods

We here provide details of our experimental setup, whose main components are shown in Figure s2.

Optical setup

We performed visualizations on an inverted epifluorescence microscope (Eclipse TE200, Nikon, Japan) equipped with 488 nm diode laser (Stradus 488, Vortran, Sacramento, CA), a filter cube (exciter/emitter 482/536 nm, model FITC-3540B, Semrock, Rochester, NY) and a 60x water immersion objective (N.A. = 1.0, Fluor, Nikon, Japan). For quantitative miRNA detection, we assembled a custom

confocal setup by coupling to a 150 µm pinhole placed at the image focal plane to reduce noise created by out of focus light. We measured fluorescence intensity using a photomultiplier tube (H7422-40, Hamamatsu Photonics, Japan) set to 900 V. Signal was converted using an amplifier/converter unit (C7319, Hamamatsu, Japan), filtered using a low pass RC circuit (RC = 1.2 ms) and acquired using a DAQ card (NI USB-6211, National Instruments, Austin, TX) controlled with Matlab (The Mathworks, Natick, MA). We performed all measurements at 90 kS/s and filtered signal with a 1500 points moving average. We calculated fluorescence intensity by integrating the signal in the ITP peak over 3.0 standard deviations (we integrated the raw data under a Gaussian function fitted to the peak).

Alternately, for the spatiotemporal diagrams of Figure 1, we used a 488 nm collimated diode light source for illumination (Thorlabs, Newton, NJ), a 4x objective (N.A. = 0.2, Plan Apo, Nikon) and acquired images using a cooled CCD camera (cascade 512F, Photometrics, Tucson, AZ) controlled with Winview32 (Princeton Instruments, Trenton, NJ).

Oligo length (name)	Sequence (5' to 3')
23 nt (miR-17)	CAAAGUGCUUACAGUGCAGGUAG
40 nt	CUGUGACACUUCAAACUCGUACCGUGAGUAAUAAUGCGCC
60 nt	CAUUAUUACUUUUGGUACGCGCUGUGAC ACUUCAAACUCGUACCGUGAGUAAUAAUGCGC
22 nt (miR-126)	UCGUACCGUGAGUAAUAAUGCG
22 nt (complementary to miR-126)	CGCAUUAUUACUCACGGUACGA

Table s1. Sequences of oligoribonucleotides used in this work for calibration experiments.

Injection protocol

We here describe the strategy to prepare the chip for miRNA quantitation. The off-the-shelf chip design (model NS260, Caliper LS, Mountain View, CA) is shown on Figure 2. Before each set of experiments, we first precondition the chip by rinsing the channels successively with 100 mM sodium hydroxide for 5 min, deionized (DI) water for 1 min, 100 mM hydrochloric acid for 5 min, and DI water for 1 min. We then add the different LEs to reservoirs 1 to 8 as described in step 1 of table s3 and apply vacuum to reservoirs 3 and 7 for 5 min. These initial rinsing and filling steps are useful to reduce and stabilize electroosmotic flow in the borosilicate chip.

Before each experiment, all reservoirs are rinsed with DI water. We then add the different LEs to reservoirs 1 to 8 as described in step 1 of the table below and apply vacuum to reservoirs 3 and 7 for 2 min. Applying vacuum at 3 creates an interface between LE1 and LE2 at the intersection A (see Figure 2) and applying vacuum at 7 creates an interface between LE2 and LE3 at the intersection B. We then release vacuum, rinse and empty reservoir 1, and add the mixture of TE and sample. Finally, we apply a 3 kV potential difference between reservoir 8 and 1 to start the ITP process using a high voltage power supply (Labsmith, Livermore, CA) and stop voltage after the ITP interface has reached the detector.

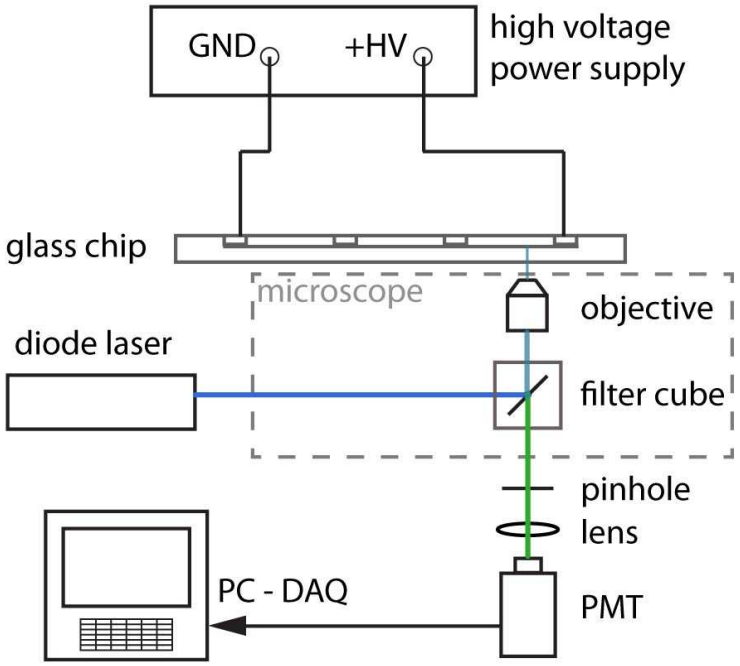

Figure s2. Schematic summarizing the ITP experimental setup for miRNA quantification. The primary components of the optical setup are diode laser, microscope (objective and filter cube), pinhole, lens, and PMT. The optical visualizes the ITP interface in the glass microchip generated with a high voltage power supply.

Reservoir	1	2	3	4	5	6	7	8
Step 1	LE1, 5 µl	LE1, 5 µl	LE1, 5 µl	LE2, 5 µl	LE2, 5 µl	LE2, 5 µl	LE3, 5 µl	LE3, 10 µl
Step 2			Vacuum 2 min				Vacuum 2 min	
Step 3	Empty, rinse, add 10 µl TE+sample							
Step 4	GND							+ 3 kV

Table s2. Summary of the injection protocol