Purification of Nucleic Acids from Whole Blood Using Isotachophoresis

Supplementary information

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We here provide supplementary figures and information further describing our ITP-based purification method. We present a schematic explaining the principle of ITP (Figure S-1); the method for on-chip nucleic acid quantitation (Figure S-3); a figure showing two methods for localizing extracted nucleic acids during ITP (Figure S-2); and the experimental procedure for post purification PCR analysis (Figure S-4). We also give primer sequences for the PCR amplification of BRCA2 gene fragment.

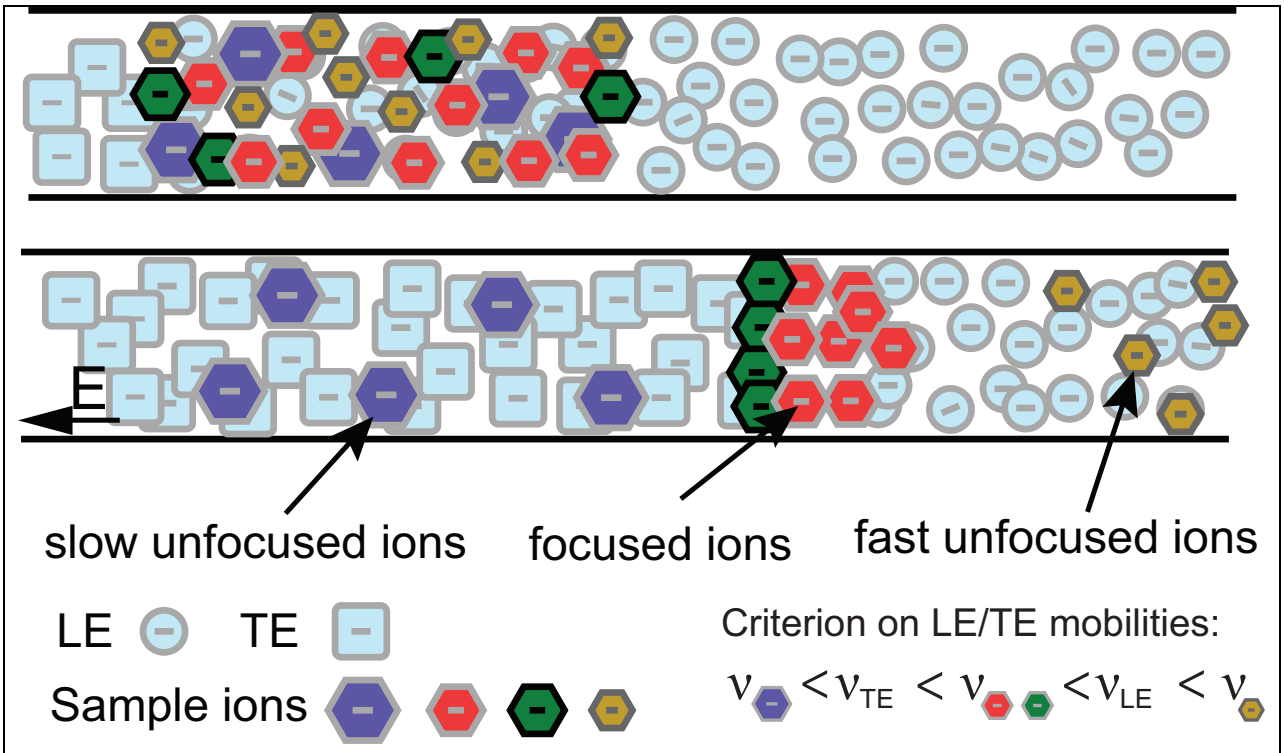


Figure S-1: Schematic representation of ITP with sample containing four different ions injected within the leading electrolyte (LE). Upon application of an electric field, two targeted ionic species (whose mobility is bound by the LE and TE) self-segregate into contiguous zones (generally in order of increasing electrophoretic mobilities). Here, we depict the selective focusing of two target sample analytes (mid-sized green and red hexagons). Species which have mobilities lower than the TE (here large blue hexagons) or higher than the LE (small yellow hexagons) do not focus.

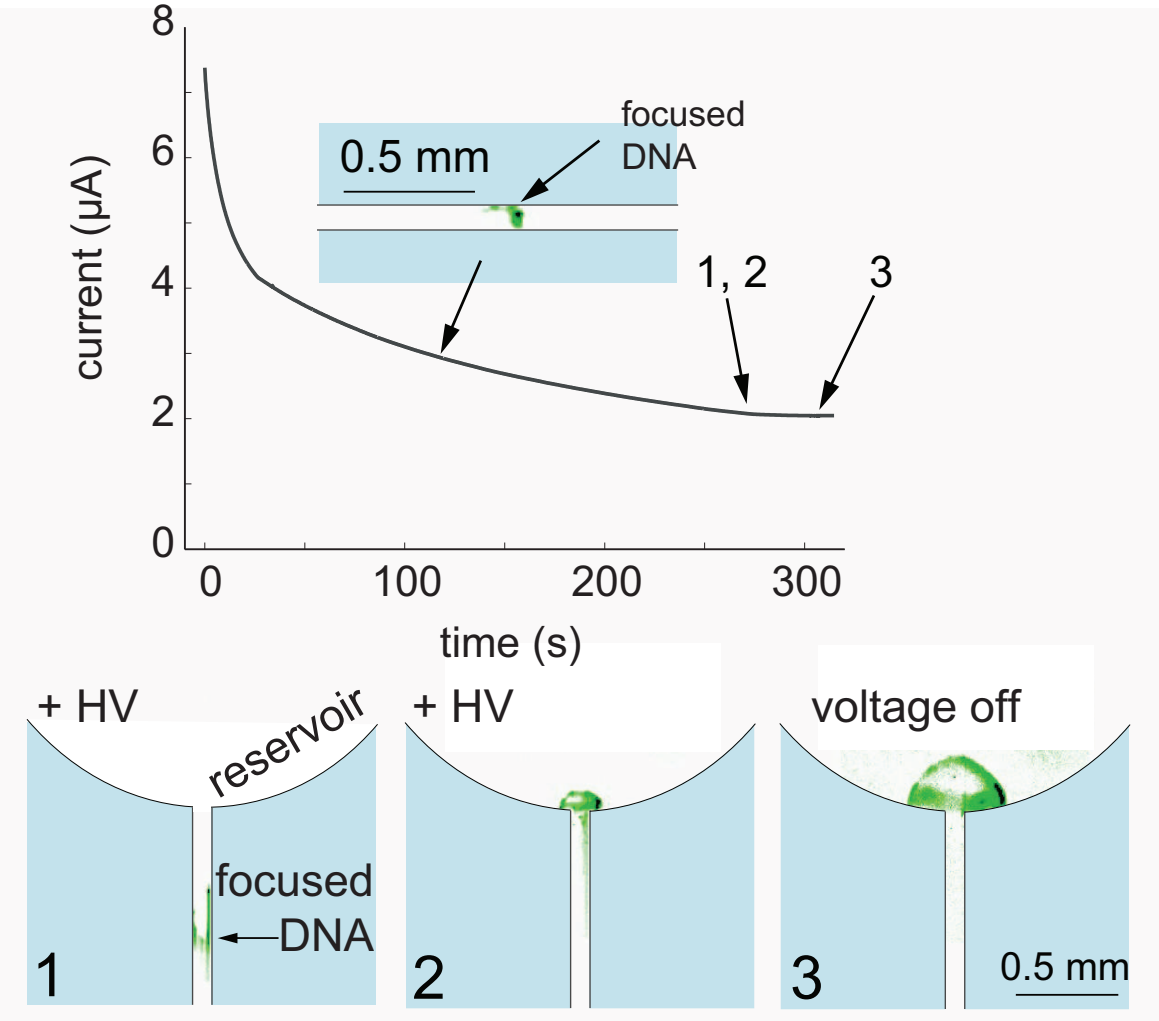
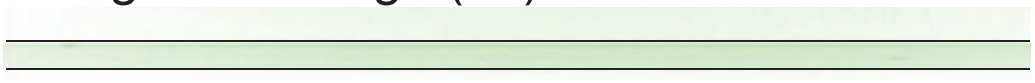


Figure S-2: Localization and extraction of the ITP purified NA. We track the location of the focused NA ITP zone by either monitoring ionic current or by fluorescence visualization. Above, we show a measured current trace obtained from a constant voltage ITP extraction experiment where the sample is a solution containing 7 ng.μL^{-1} of lambda λ-DNA. We acquired the current trace by interfacing the sourcemeter with MATLAB using a GPIB card (National Instruments, TX). The current decreases monotonically as the ITP interface advances within the channel, as the relatively low conductivity TE replaces the high conductivity LE. At the moment where the current reaches a plateau (here near t = 260 s), the purification channel is entirely filled with TE and the ITP interface has reached the anode reservoir. Above the current plot, is an actual image of focused DNA in the microchannel (with a superposed schematic of walls). At the bottom, we show actual fluorescence images corresponding to the same experiment. Image 1 shows the focused DNA approaching the reservoir. Image 2 shows an image of the same location just after the interface enters the reservoir. Image 3 shows the reservoir about 20 s later, where the purified NA has migrated into the reservoir. These three instances in time are highlighted in the current plot. Either or both current monitoring and fluorescence visualization can be used to track the position of the NA during the purification process.

ITP-focused DNA (I)

Channel filled with standard DNA solution (I_{std})

Background image (I_{bgd})



integrated, normalized intensity $F = \sum_{\text{all pixels}} \frac{I - I_{bgd}}{I_{std} - I_{bgd}}$

focused DNA mass = F x ρ_{std} x V_{pixel}

ρ_{std} = concentration of standard solution (pg/nL)

V_{pixel} = pixel volume

= pixel width x pixel height x channel depth

Figure S-3: On-chip fluorescence based quantitation of purified DNA. The top image shows an example of raw data I for a DNA ITP zone from a DNA purification experiment from blood lysate. The middle image shows the fluorescence profile I_{std} of the channel filled with the standard DNA solution (here 1.42 µg.mL^{-1} of human genomic DNA). The last image shows the background fluorescence I_{bgd}, where the channel is filled with deionized water. We correct both ITP-focused DNA and standard images with the background, and then take their ratio. This yields an image where the value of each pixel is in units of standard. Summing over all pixel yields the integrated, normalized intensity F in terms of these standard units. To convert this number to DNA mass, we take its product with the standard concentration and the volume of each pixel (pixel area times the channel depth, assuming an approximately rectangular channel cross section). We perform all calculations using MATLAB (The Mathworks, MA).

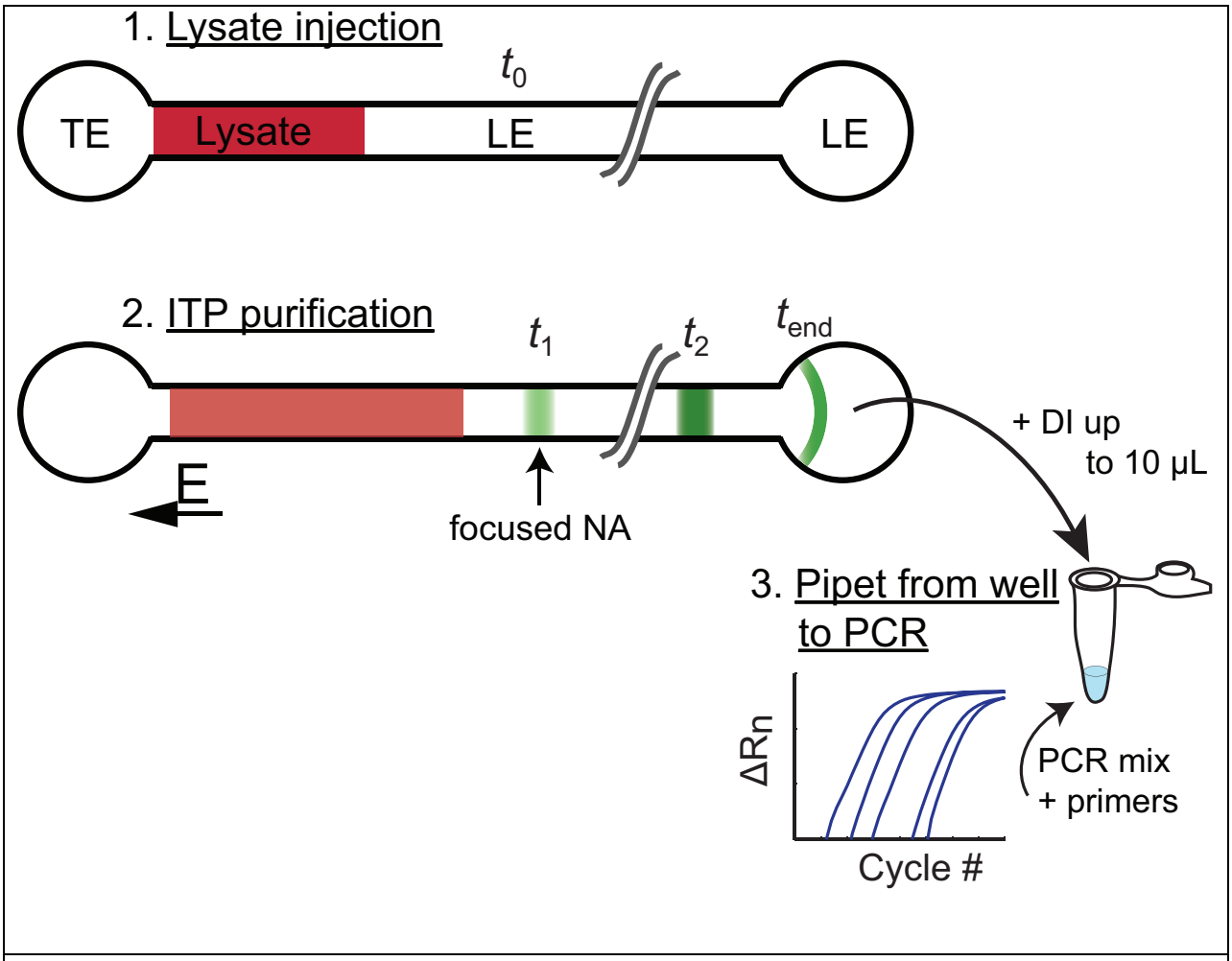


Figure S-4: Schematic of the workflow of the ITP-based purification with off-chip PCR. The lysate is initially hydrodynamically injected between LE and TE. Upon application of an electric field, NAs focus and migrate towards the anode in a sharp concentrated zone. When the focused NA enter the anode reservoir, we pipet all of the solution out of the reservoir (~ 2 µL), add it to the PCR mix, and perform real-time PCR.

The primer sequences for the BRCA2 201 bp fragment amplification are the following:

Forward primer: CAC CTT GTG ATG TTA GTT TGG A

Reverse primer: TGG AAA AGA CTT GCT TGG TAC T