Supporting Information

Centrifugation-Assisted Immiscible Fluid Filtration for Dual-Bioanalyte Extraction

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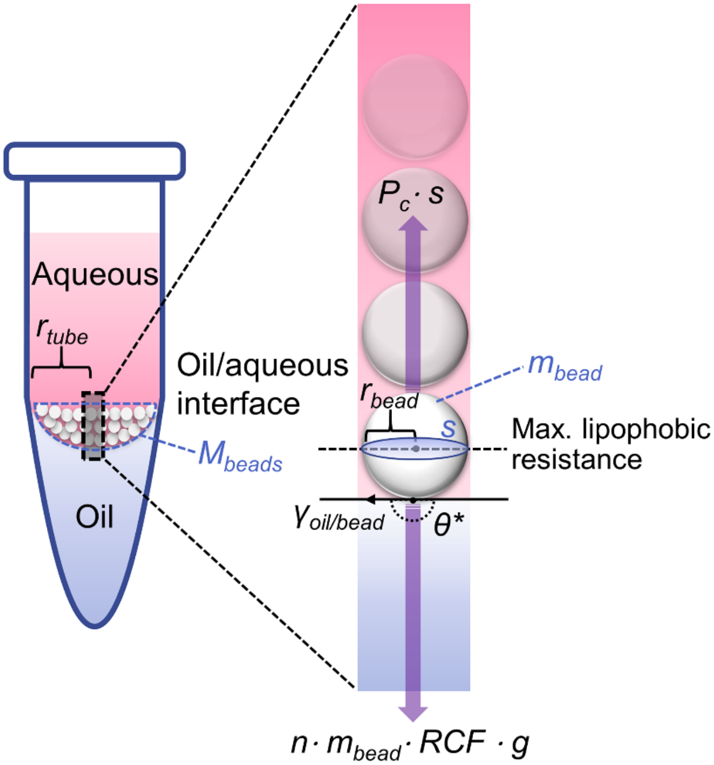

Figure S1. physical variables governing bead jump in CIFF.

The physics governing the conditions of bead jump in CIFF can be written as:

$$n \cdot m_{bead} \cdot RCF \cdot g = P_c \cdot s \quad \textit{(equation 1)}$$

where n is the stacking coefficient estimated as $N_{total} / N_{per\ layer}$ on average, N_{total} is the total number of beads in the tube, $N_{per\ layer}$ is the number of beads in the largest circle in the bead pellet, which is $N_{per\ layer} = (r_{tube}/r_{bead})^2$, where r_{tube} is the radius of the centrifugal tube, and r_{bead} is the radius of a bead. m_{bead} is the mass of a single bead, *RCF* is the relative centrifugal force (a dimensionless unit defined as the ratio of centrifugal acceleration over gravitational acceleration (g) at the Earth's surface), s is the projected area of a single bead at the oil/aqueous interface equal to $\pi\ r_{bead}^2$, P_c is the capillary pressure (lipophobic resistance) applied on a single bead from the oil phase which is equal to $2\gamma_{oil/bead} \cdot cos\theta/r_{bead}$. $\theta = \pi - \theta^*$ and θ^* is Young's contact angle of the fluorinated oil (FC-3283) on the bead (*i.e.*, glass) surface under water estimated from our previous work,[1] and $\gamma_{oil/bead}$ is the oil-bead interfacial tension.

$$\frac{N_{total}}{N_{per\ layer}} \cdot m_{bead} \cdot RCF \cdot g = 2\frac{\gamma_{oil/bead} \cdot cos\theta}{r_{bead}} \cdot \pi\, r_{bead}^{2} \qquad \textit{(equation 2)}$$

$$\left(\frac{N_{total}}{(r_{tube}/r_{bead})^{2}}\right) \cdot m_{bead} \cdot RCF \cdot g = 2\frac{\gamma_{oil/bead} \cdot cos\theta}{r_{bead}} \cdot \pi\, r_{bead}^{2} \qquad \textit{(equation 3)}$$

Here we define $M_{beads} = N_{total} \cdot m_{bead}$

$$M_{beads} \cdot RCF = 2\frac{\gamma_{oil/bead} \cdot cos\theta}{r_{bead}} \cdot \pi\, r_{bead}^{2} \cdot (r_{tube}/r_{bead})^{2}/\, g \approx 12000 \cdot \mathrm{mg} \qquad \textit{(equation 4)}$$

Solving for *equation 4* using constants that represent the actual values or measured values from a previous work,[1] including r_{tube} = 2500 μm, r_{bead} = 20 μm, $\gamma_{oil/bead}$ = 59.0 mN/m,[1] θ^* = 180°, and g = 9.807 m/s^2, gives

$$RCF \approx 12000/M_{beads} \qquad \textit{(equation 5)}$$

By plotting *equation 5* with M_{beads} as the *x* axis and *RCF* as the *y* axis yields the predicted curve shown in Figure 2 of the main text.

Thus, for a given oil/aqueous pair, physical characteristics of bead and centrifugal tube, more beads are added to the tube would result in a larger M_{beads}, and hence a smaller centrifugal force (or *RCF*) would be needed to cause the jumping of beads. It is worth noting that a variance of *n* (the stacking coefficient) across the oil/aqueous meniscus (*i.e.*, larger towards the center and smaller towards the edge) will be seen, especially in cases of smaller M_{beads}. The smaller the *n*, the higher the required *RCF*. In our prediction (*equation 1*), *n* is estimated as an average across the bead pellet, so the predicted RCF is actually smaller than the measured value and the discrepancy between prediction and experiment becomes more noticeable when M_{beads} becomes smaller (Figure 2B). This also explains the trend seen in Figure 2C where smaller M_{beads} values are associated with a higher percentage of residual beads.

As can be seen in *equation 4*, a more hydrophilic (or lipophobic) surface of beads would result in a larger $\gamma_{oil/bead}$ and thus an increased resistance retaining the beads in the aqueous phase. On the other hand, a smaller $\gamma_{oil/bead}$ which can be achieved for example by adding surfactant to the aqueous phase will allow the jumping of beads to occur much more easily. Similarly, if a tube with a smaller r_{tube} is used, for a given M_{beads}, a smaller centrifugal force (or *RCF*) for bead jump can be expected.

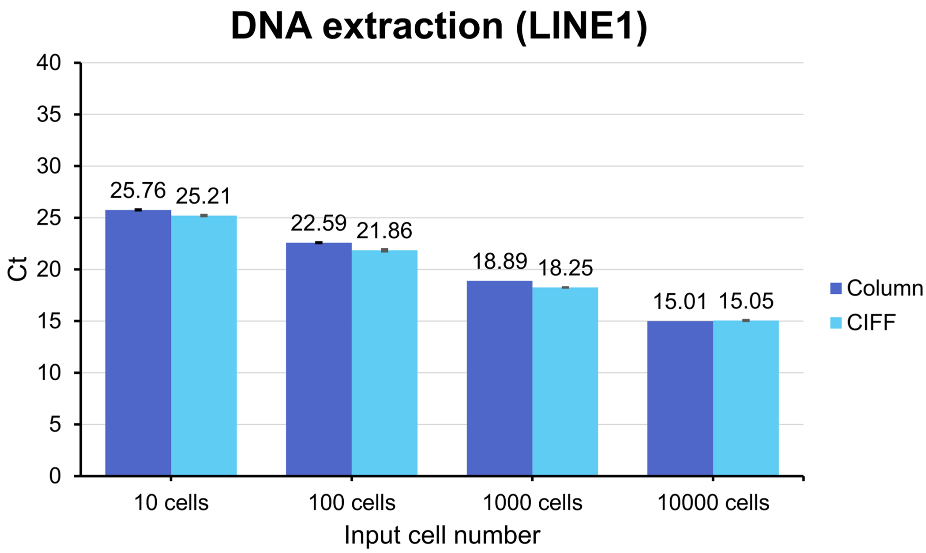

Figure S2. DNA extraction efficiency of CIFF compared to a traditional column-based technique for low input samples. qPCR performance of LINE1 DNA extracted from 10 to 10,000 LNCaP cells using CIFF compared to a traditional column-based technique (Qiagen QIAamp DNA Mini Kit).

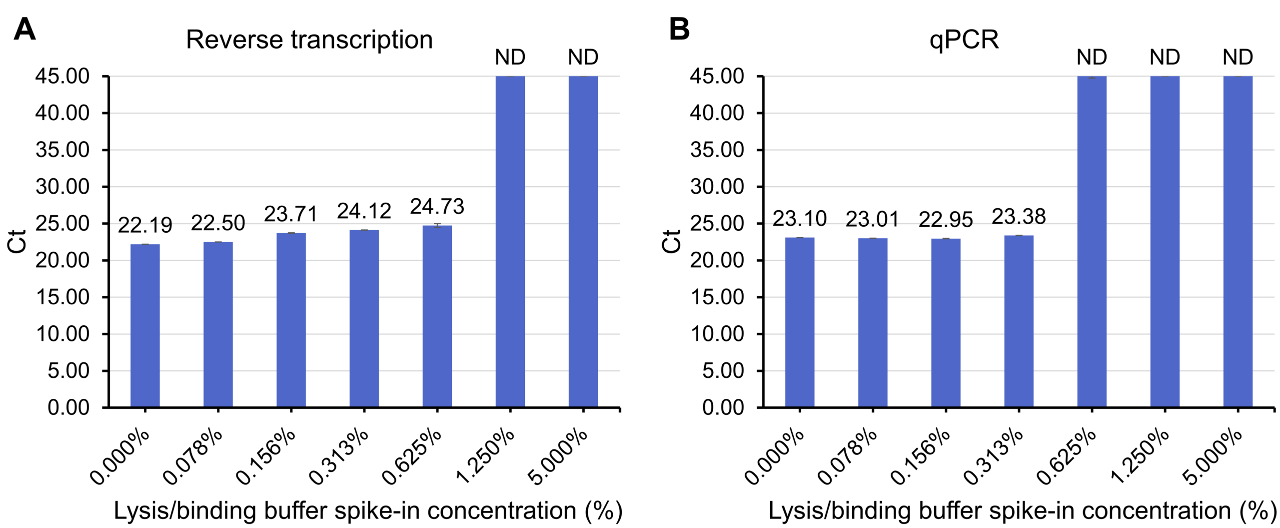

Figure S3. Inhibition of reverse transcription (A) and quantitative PCR (B) reactions at various concentrations of mRNA Lysis/Binding buffer (100 mM Tris-HCl (pH 7.5), 500 mM LiCl, 10 mM EDTA, 1% LiDS, 5 mM dithiothreitol (DTT)) contamination in the reaction.

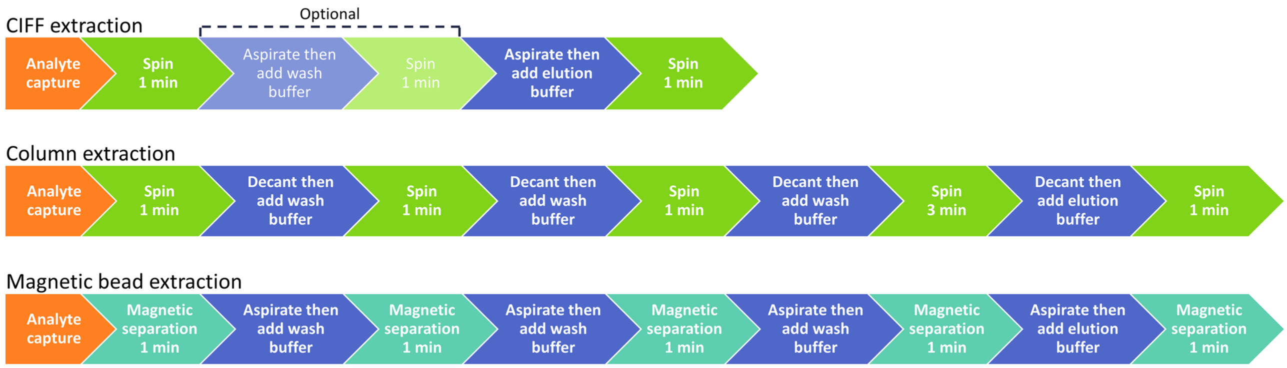

Figure S4. Comparison of operation workflow of CIFF extraction, column-based extraction, and magnetic bead-based extraction.

References:

(1) Li, C.; Yu, J.; Schehr, J.; Berry, S. M.; Leal, T. A.; Lang, J. M.; Beebe, D. J. Exclusive Liquid Repellency: An Open Multi-Liquid-Phase Technology for Rare Cell Culture and Single-Cell Processing. *ACS Appl. Mater. Interfaces* **2018**, *10* (20), 17065–17070.