Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

for

Retooling Phage Display with Electrohydrodynamic Nanomixing and Nanopore Sequencing

Authors

Lyndon J Raftery[1#], Christopher B Howard[1,2#], Yadveer S Grewal[1,2#], Ramanathan Vaidyanathan[1,2#], Martina L Jones[1], Will Anderson[1,2], Darren Korbie[1,2], Tania Duarte[3], Minh Duc Cao[3], Son Hoang Nguyen[3], Lachlan JM Coin[3], Stephen M Mahler[1,4], Matt Trau[1,2,5*]

Affiliations

[1]Australian Institute for Bioengineering and Nanotechnology (AIBN), University of Queensland, Brisbane, Australia

[2]Centre for Personalised Nanomedicine, AIBN, University of Queensland, Brisbane, Australia

[3] Institute for Molecular Bioscience, University of Queensland, St Lucia, Brisbane, Australia

[4] School of Chemical Engineering, University of Queensland, Brisbane, Australia

[5] School of Chemistry and Molecular Biosciences (SCMB), University of Queensland, Brisbane, Australia.

[*]Email: m.trau@uq.edu.au

[#] Authors contributed equally to the article

Supplementary Materials

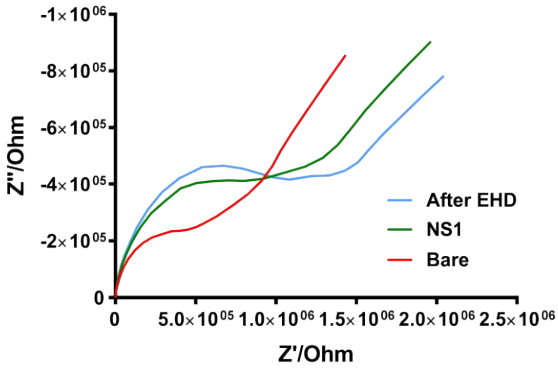
Equation for double layer calculation

The application of an alternating potential difference across an asymmetric microelectrode pair creates a non-uniform electric field, which induces charges within the double-layer of each electrode. These induced charges are then subjected to a force (F) created by the tangential component of the field ($F = \rho E_t$, where ρ = charge density and E_t = tangential component of E). Due to the asymmetric nature of the microelectrodes, a stronger force is created from the larger electrode (F_L) compared to the smaller electrode (F_S) ($F_L > F_S$). Consequently, a fluid flow towards the larger electrode is generated[11].The distance from the interface at which this effect occurs is governed by the Debye length:

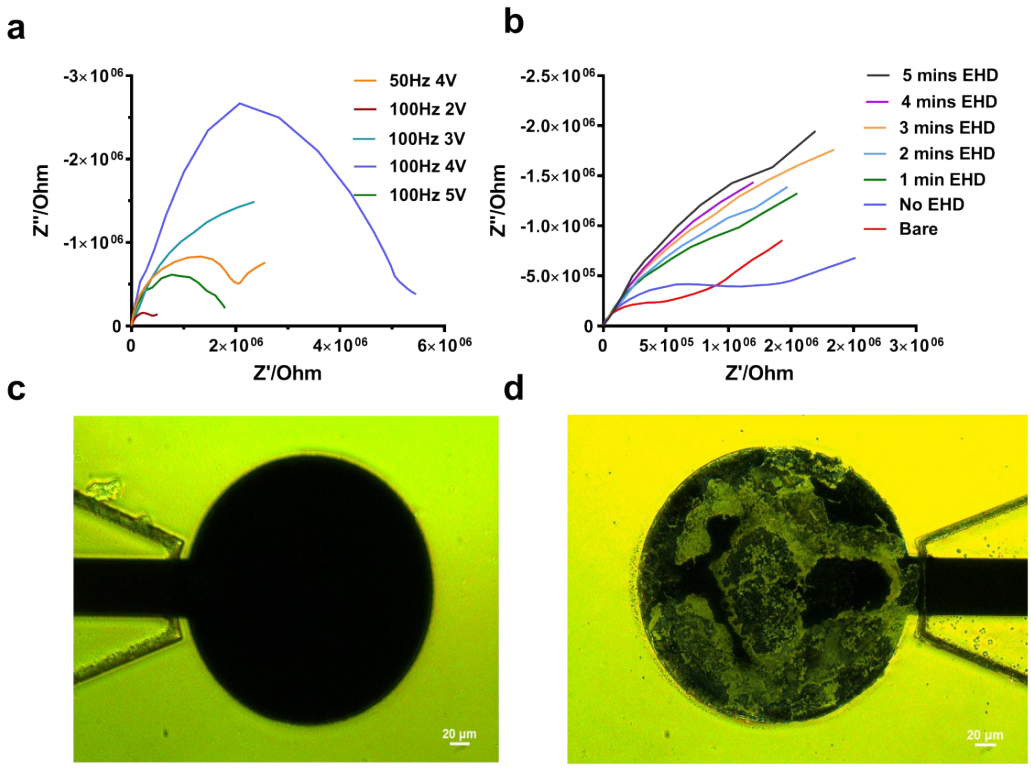
$$\kappa = \left(\frac{2000F^2}{\varepsilon_0 \varepsilon_r kT} \right)^{\frac{1}{2}} \sqrt{I} \quad \text{in units of m}^{-1}$$

$$= 3.288\sqrt{I}\left(\text{nm}^{-1}\right)$$

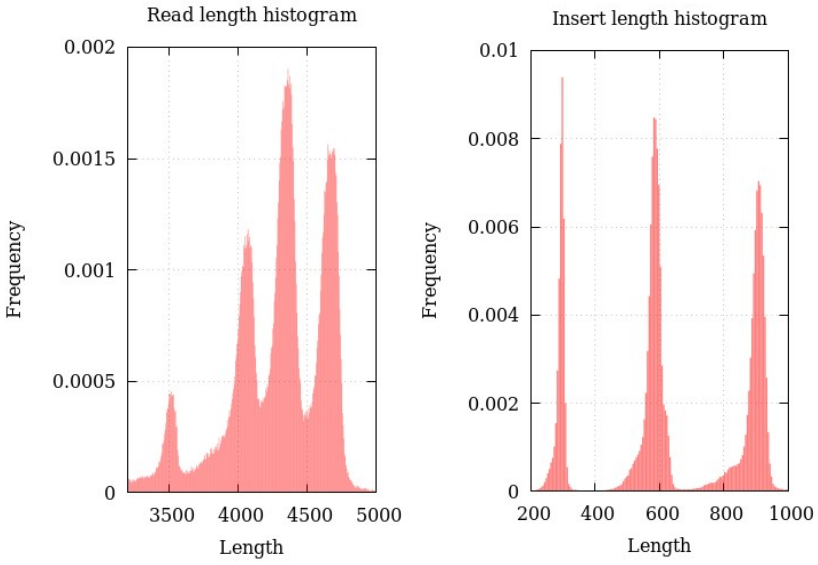
Where $\frac{1}{\kappa}$ = double layer thickness, F is the Faraday constant, I is the ionic strength of the solution ($= \frac{1}{2}\sum c_i z_i^2$ where c_i is the ionic concentration in mol L^{-1} and z_i = valency)[12]. Given the 10 mM phosphate buffer saline solution (PBS) utilized during our experiments, the Debye length was calculated to be 3.07 nm. This calculation suggests that the outlined fluid flow engenders maximum flow velocities within nanometers of the interface, resulting from a flow-profile that increases exponentially from the boundary layer. Additionally, the curvature of the fluid velocity profile under the applied electric field induces a shear gradient lift force (F_{LS}), consequently directing particles away from the channel centre and enhancing micromixing.



Supplementary Figure 1: Nyquist diagram displaying impedance measurements of incubated DENV NS1 before and after EHD. Each measurement conducted on separate electrodes. All measurements in 10 mM phosphate buffer containing 2.5 mM $K_3[Fe(CN)_6]$, 2.5 mM $K_2[Fe(CN)_6]$ and 0.1 M KCl.

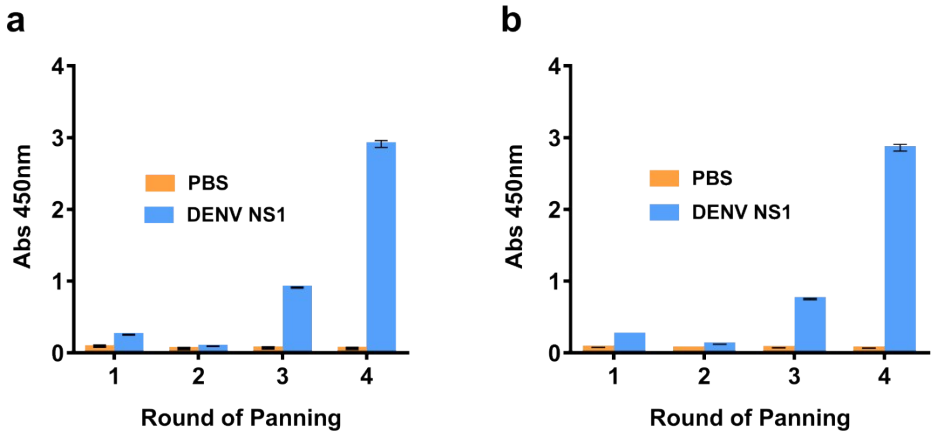


Supplementary Figure 2: Nyquist diagrams displaying AC-field conditions and EHD exposure time for optimal capture of phage. (**a**) AC-field conditions and correlated impedance measures. Field conditions are indicated in figure legend. (**b**) Nyquist diagram displaying impedance spectrum for EHD time-dependent phage capture at 100Hz, 4V. EHD times are indicated in figure legend. 'No EHD' measurement is impedance response following 5 mins of passive incubation. All measurements in 10 mM phosphate buffer containing 2.5 mM $K_3[Fe(CN)_6]$, 2.5 mM $K_2[Fe(CN)_6]$ and 0.1 M KCl utilising F5 (DENV NS1-specific phage particles). (**c, d**) Confocal microscope images of microelectrodes after EHD. (**c**) 3 mins of EHD exposure and (**d**) 6 mins of EHD exposure. Scale bar of 20µm is shown in bottom right corner.

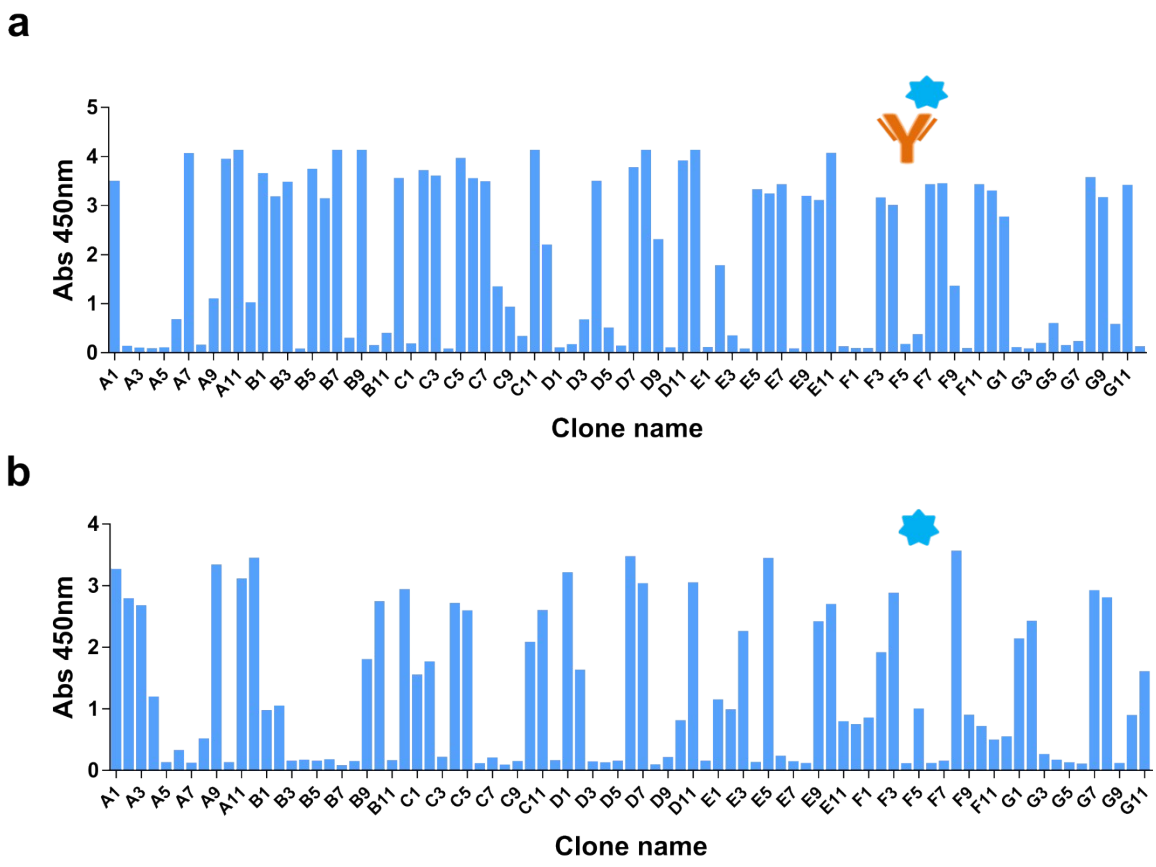


(a) (b)

Supplementary Figure 3: DNA Length distribution of (**a**) reads and (**b**) insert sequences. Lengths are indicated as number of base pairs.



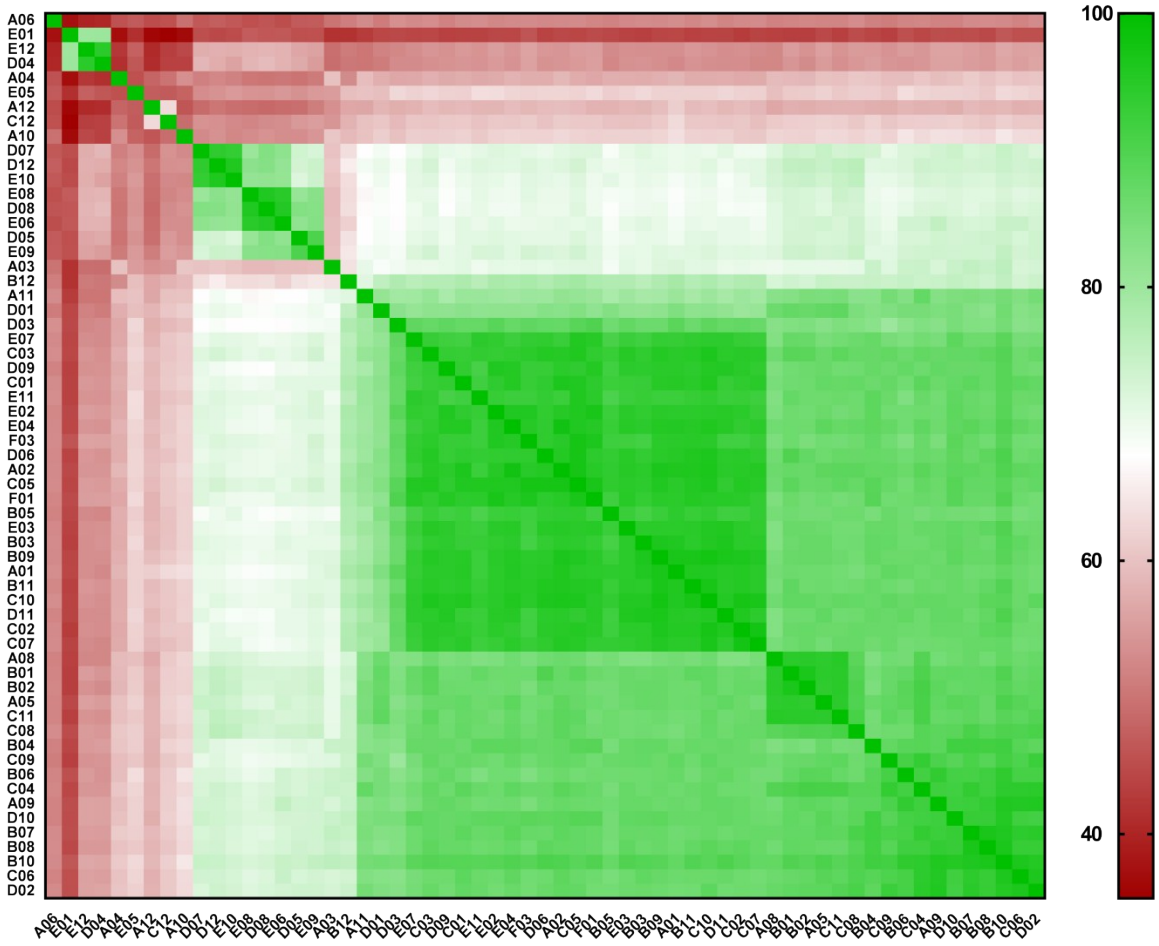
Supplementary Figure 4: Polyclonal ELISA of phage particles at each round of traditional biopanning. (**a**) *Antibody-captured NS1* was coated on ELISA plate and respective phage pool rounds as indicated in graph legend were added. (**b**) *NS1 antigen only* was coated on ELISA plate and respective phage pool rounds as indicated in graph legend were added. Bound phages were detected using anti-M13 phage HRP and read at 450nm on both plates. Error bars represent standard error of the mean absorbance from duplicate analysis (n=2).



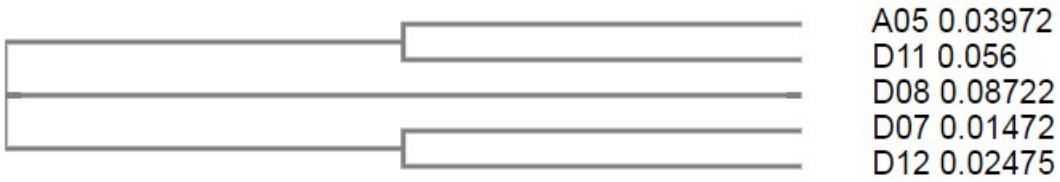
Supplementary Figure 5: Monoclonal ELISA of phage particles against DENV NS1. (**a**) Phage particles from *antibody-captured NS1* strategy. (**b**) Phage particles from *NS1-only* strategy. Bound phages were detected using anti-M13 phage HRP and read at 450nm on both plates. A cut-off >3 absorbance (450 nm) was used to determine 'positive' phage clones.

Supplementary Table 1: Sanger clones from traditional biopanning grouped based on sequence homology.

Group name	**Number of sequences within group**	**Name of clones within group**
A02	8	A02, B09, B11, C01, C02, C05, C07, C10
A03	1	A03
A05	6	A05, A08, B01, B02, C11, D01
A06	1	A06
A07	1	A07
A09	3	A09, C06, D02
A10	1	A10
A12	1	A12
B07	5	B07, B08, B06, C09, C04
B10	1	B10
C08	1	C08
D05	1	D05
D06	1	D06
D07	1	D07
D08	4	D08, E06, E08, E09
D10	1	D10
D11	11	D11, E07, E11, D03, E02, E04 F01, D09, E03, C03, B03
D12	1	D12
E10	1	E10



Supplementary Figure 6: Heat map displaying percentage identity matrix data for homology between Sanger sequences.



Supplementary Figure 7: Cladogram of 5 Sanger sequences that mapped to MinION data at >1000 reads. This figure represents the homology of sequences between groups. Divergence of the line further to the left represents a *lesser* homology between sequences.

Supplementary Table 2. Percentage identity matrix of Sanger sequences which exhibited >1000 reads in MinION data. Percentages were rounded to the nearest integer.

	A05	**D11**	**D08**	**D07**	**D12**
A05	100	90	74	77	76
D11	90	100	72	74	76
D08	74	72	100	84	82
D07	77	74	84	100	95
D12	76	76	82	95	100