Supplementary Information: One-step purification and concentration of DNA in porous membranes for point-of-care applications

Figure S1. SEM images of glass fiber and nitrocellulose used to determine membrane surface area for polymer adsorption studies. SEM imaging and sputter coating work was performed at the University of Washington Nanotech User Facility (NTUF), a member of the NSF-sponsored National Nanotechnology Infrastructure Network (NNIN). A) Glass fiber. B) Nitrocellulose.

Figure S2. Pulse field gel electrophoresis to determine fragment size of DNA purified by chitosan in porous membranes.
Figure S3. Sensitivity of the ldh-1 qPCR to red dye and blood. A) Addition of red dye to the qPCR standards does not interfere with output fluorescence signal. B) Addition of up to 0.5% blood to the qPCR standards does not significantly interfere with the output fluorescent signal at concentrations of 1x10^5 copies and below of input DNA.
**A.** Chitosan
5000 MW - 34 monomers
1 monomer = 0.5 nm long
Total polymer = 17 nm long

**B.**
**Nitrocellulose**
Surface area ratio $\rightarrow 200 \mum^2/\mum^3$
SA to volume ratio $\rightarrow \frac{200 \mum^2}{\mum^3} \times \frac{1}{\mum} = 2.0 \mum^{-1}/\mum^3$

Total volume of membrane punch = thickness $\times$ area
$\text{volume} = (0.1 \mum) \times (2.4 \mum)^2$
$\text{volume} = 1.8 \times 10^5 \mum^3$

Total $SA$ in patterned membrane volume
$1.8 \times 10^5 \mum^3 \times \frac{2.0 \mum^2}{\mum^3} = 3.6 \times 10^5 \mum^2$ surface area

Minimum number of chitosan molecules adsorbed
\[
\text{Surface Area} = \frac{3.6 \times 10^5 \mum^2}{8.5 \times 10^{-6} \mum^2/\text{molecule}} = 4.2 \times 10^{14} \text{[molecules]}
\]

\[
\frac{4.2 \times 10^{14} \text{[molecules]}}{N_A \text{[molecules/mole]}} \times 5000 \left(\frac{\mum}{\text{mole}}\right) = 3.6 \left(\frac{\mum}{\text{mole}}\right) \left[\mum\text{chitosan}\right]
\]

Maximum number of chitosan molecules adsorbed (per 1000 $\mum^2$)
\[
\text{Surface Area} = \frac{3.6 \times 10^9 \mum^2}{25 \times 10^{-11} \mum^2/\text{molecule}} = 1.4 \times 10^{16} \text{[molecules]}
\]

\[
\frac{1.4 \times 10^{16} \text{[molecules]}}{N_A \text{[molecules/mole]}} \times 5000 \left(\frac{\mum}{\text{mole}}\right) = 120 \left(\frac{\mum}{\text{mole}}\right) \left[\mum\text{chitosan}\right]
\]

Concentration of chitosan on pore surface (per 1000 $\mum^2$)
Minimum = $3.5 \left[\mum\text{chitosan}\right] = 9.7 \times 10^{-10} \left(\frac{\mum}{\mum^2}\right)$
Maximum = $120 \left[\mum\text{chitosan}\right] = 3.3 \times 10^{-8} \left(\frac{\mum}{\mum^2}\right)$

**Theoretical Range** = $9.7 \times 10^{-10} - 3.3 \times 10^{-8} \left[\mum/\mum^2\right]$

**Glass Fiber**
Surface area ratio $\rightarrow 70 \mum^2/\mum^3$
SA to volume ratio $\rightarrow \frac{70 \mum^2}{\mum^3} \times \frac{1}{\mum} = 0.19 \mum^{-1}/\mum^3$

Total volume of membrane punch = thickness $\times$ area
$\text{volume} = (0.37 \mum) \times (2.4 \mum)^2$
$\text{volume} = 6.7 \times 10^5 \mum^3$

Total $SA$ in patterned membrane volume
$6.7 \times 10^5 \mum^3 \times \frac{0.19 \mum^2}{\mum^3} = 1.3 \times 10^4 \mum^2$ surface area

Minimum number of chitosan molecules adsorbed
\[
\text{Surface Area} = \frac{1.3 \times 10^4 \mum^2}{8.5 \times 10^{-6} \mum^2/\text{molecule}} = 1.5 \times 10^{14} \text{[molecules]}
\]

\[
\frac{1.5 \times 10^{14} \text{[molecules]}}{N_A \text{[molecules/mole]}} \times 5000 \left(\frac{\mum}{\text{mole}}\right) = 1.2 \left(\frac{\mum}{\text{mole}}\right) \left[\mum\text{chitosan}\right]
\]

Maximum number of chitosan molecules adsorbed (per 1000 $\mum^2$)
\[
\text{Surface Area} = \frac{1.3 \times 10^9 \mum^2}{25 \times 10^{-11} \mum^2/\text{molecule}} = 5.1 \times 10^{15} \text{[molecules]}
\]

\[
\frac{5.1 \times 10^{15} \text{[molecules]}}{N_A \text{[molecules/mole]}} \times 5000 \left(\frac{\mum}{\text{mole}}\right) = 42 \left(\frac{\mum}{\text{mole}}\right) \left[\mum\text{chitosan}\right]
\]

Concentration of chitosan on pore surface (per 1000 $\mum^2$)
Minimum = $1.2 \left[\mum\text{chitosan}\right] = 9.5 \times 10^{-10} \left(\frac{\mum}{\mum^2}\right)$
Maximum = $42 \left[\mum\text{chitosan}\right] = 3.2 \times 10^{-8} \left(\frac{\mum}{\mum^2}\right)$

**Theoretical Range** = $9.5 \times 10^{-10} - 3.2 \times 10^{-8} \left[\mum/\mum^2\right]$

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Experimental Capacity (ug/um²)</th>
<th>Theoretical Capacity (ug/um²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrocellulose</td>
<td>$\sim 3.3 \times 10^4$</td>
<td>$9.7 \times 10^{-10} - 3.3 \times 10^4$</td>
</tr>
<tr>
<td>Glass Fiber</td>
<td>$3.6 \times 10^9 - 7.2 \times 10^9$</td>
<td>$9.5 \times 10^{-10} - 3.2 \times 10^9$</td>
</tr>
</tbody>
</table>

Figure S4. Membrane capacity for chitosan. A) Schematic, B) calculations, and C) experimental data.
A. **Nitrocellulose**

DNA capacity: $1.9 \times 10^6$ c/μg chitosan = $3.8 \times 10^{12}$ bp/μg chitosan

Chitosan available on membrane after accounting for loss: $8.08 \mu g$

$$8.08 \, \text{[\mu g]} \times \frac{1 \, \text{[mole]}}{5000 \, \text{[g]}} \times N_A \left[\frac{\text{molecules}}{\text{mole}}\right] = 9.73 \times 10^{14} \, \text{[molecules]}$$

Total (+) charges available

$$9.73 \times 10^{14} \, \text{[molecules]} \times 34 \, [\text{+charges}] = 3.31 \times 10^{16} \, [\text{+charges}]$$

Measured DNA capacity = $3.8 \times 10^{11}$ bp/μg chitosan

$$3.8 \times 10^{11} \left[\frac{\text{bp}}{\text{μg chitosan}}\right] \times 8.08 \, \text{[μg chitosan]} = 3.07 \times 10^{12} \, [\text{bp}]$$

$$3.07 \times 10^{12} \, [\text{bp}] \times 2 \left[\frac{\text{charges}}{\text{bp}}\right] = 6.14 \times 10^{13} \, [-\text{charges}]$$

Because there are far more (+) charges available when the DNA capacity has been reached in nitrocellulose, it can be assumed that the chitosan is covered with less than a monolayer of DNA.

B. **Glass Fiber**

DNA capacity: $9.9 \times 10^6$ c/μg chitosan = $2.0 \times 10^{12}$ bp/μg chitosan

Chitosan available on membrane after accounting for loss: $5.05 \mu g$

$$5.05 \, \text{[μg]} \times \frac{1 \, \text{[mole]}}{5000 \, \text{[g]}} \times N_A \left[\frac{\text{molecules}}{\text{mole}}\right] = 6.08 \times 10^{14} \, \text{[molecules]}$$

Total (+) charges available

$$6.08 \times 10^{14} \, \text{[molecules]} \times 34 \, [\text{+charges}] = 2.07 \times 10^{16} \, [\text{+charges}]$$

Measured DNA capacity = $2.0 \times 10^{12}$ bp/μg chitosan

$$2.0 \times 10^{12} \left[\frac{\text{bp}}{\text{μg chitosan}}\right] \times 5.05 \, \text{[μg chitosan]} = 1.01 \times 10^{13} \, [\text{bp}]$$

$$1.01 \times 10^{13} \, [\text{bp}] \times 2 \left[\frac{\text{charges}}{\text{bp}}\right] = 2.02 \times 10^{13} \, [-\text{charges}]$$

Because there are far more (+) charges available when the DNA capacity has been reached in glass fiber, it can be assumed that the chitosan is covered with less than a monolayer of DNA.

**Figure S5.** Calculation of less than a monolayer of DNA coverage when chitosan capacity is reach.  A) Nitrocellulose  B) Glass fiber.
Figure S6. Dispersion of sequentially delivered fluids in nitrocellulose (NC) and glass fiber (GF).
A) Capture buffer, 50 mM MES at pH 5, with bromothymol blue is first flowed through the membranes. At pH values below 6.0, the indicator is yellow. As elution buffer, 50 mM Tris at pH 9, flows into the membranes, the interface of the buffers exchange ions. At pH values above 7.6, the indicator turns blue. In nitrocellulose, which has a relatively homogenous pore size distribution, the interface between two sequentially delivered fluids is sharply defined, which limits dispersional mixing and produces a sharp pH change. The relatively inhomogeneous pore feature size distribution in glass fiber, however, results in a poorly defined buffer interface, which increases mixing and causes a more gradual pH gradient to develop. B) Elution profile of DNA from chitosan capture in nitrocellulose and C) glass fiber.
Figure S7. Sequential delivery of buffers to enable a single end-user step. A) Schematic. B-D) The images are taken from a video where three buffers were sequentially delivered to nitrocellulose. Yellow: capture buffer; red: wash buffer; blue: elution buffer. Using this method, our DNA concentration and purification system can be directly integrated with a simple user interface which requires only one step for activation$^{62,63}$. 