Lab on a Chip

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

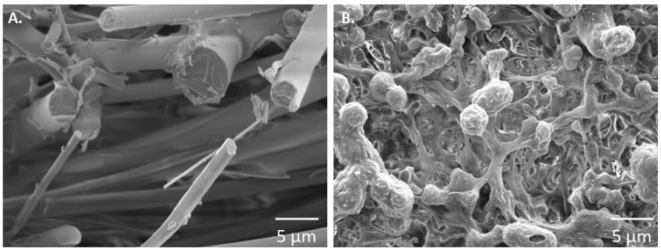


Figure S1. SEM images of glass fiber and nitrocellulose used to determine membrane surface area for polymer adsorption studies. SEM imaging and sputter coating work was performed at the University of Washington Nanotech User Facility (NTUF), a member of the NSF-sponsored National Nanotechnology Infrastructure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

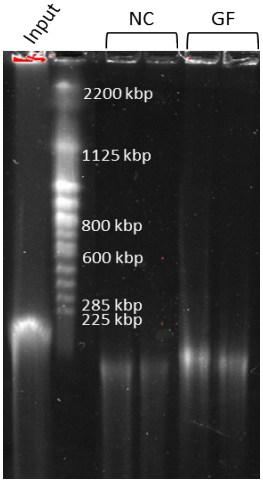


Figure S2. Pulse field gel electrophoresis to determine fragment size of DNA purified by chitosan in porous membranes.

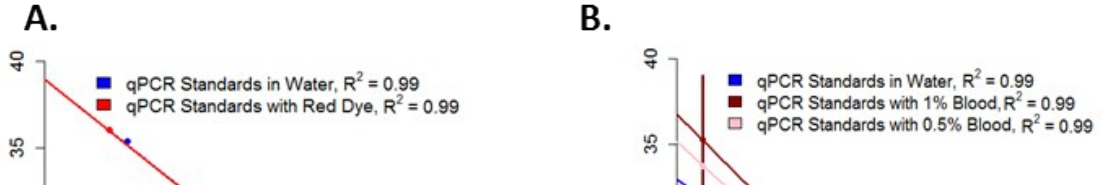


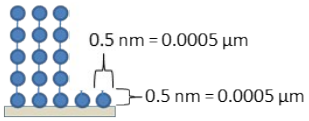
Figure S3. Sensitivity of the *ldh*-1 qPCR to red dye and blood. A) Addition of red dye to the qPCR standards does not interfere with output fluorescence signal. B) Addition of up to 0.5 % blood to the qPCR standards does not significantly interfere with the output fluorescent signal at concentrations of $1x10^5$ copies and below of input DNA.

A.

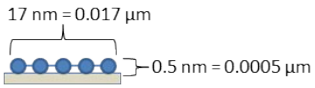
Chitosan
5000 MW ~ 34 monomers
1 monomer = 0.5 nm long
Total polymer = 17 nm long

Membrane Punch =

Single monomer attachment area = 2.5×10^{-7} µm² (Theoretical maximum adsorbed)



Full polymer attachment area = 8.5×10^{-6} µm² (Theoretical minimum adsorbed)



B.

Nitrocellulose

Surface area ratio → 200 µm²/µm²

SA to volume ratio → $\frac{200\ \mu m^2}{\mu m^2} * \frac{1}{100\ \mu m\ thick} = 2.0\ ^{\mu m^2}/_{\mu m^3}$

Total volume of membrane punch = thickness * area
volume = (0.1 mm)*π(2.4 mm)²
volume = 1.8×10^9 µm³

Total SA in patterned membrane volume

$$1.8\ x\ 10^9\ [\mu m^3] * \frac{2.0\ [\mu m^2]}{[\mu m^3]} = 3.6\ x\ 10^9\ [\mu m^2]\ surface\ area$$

Minimum number of chitosan molecules adsorbed

$$\frac{Surface\ Area}{Area\ of\ Attachment} = \frac{3.6\ x\ 10^9\ [\mu m^2]}{8.5\ x\ 10^{-6}\ [\frac{\mu m^2}{molecule}]} = 4.2\ x\ 10^{14}\ [molecules]$$

$$\frac{4.2\ x\ 10^{14}\ [molecules]}{N_A[\frac{molecules}{mole}]} * 5000\ [\frac{g}{mole}] = 3.5\ [\mu g\ chitosan]$$

Maximum number of chitosan molecules adsorbed (per 1000 µm³)

$$\frac{Surface\ Area}{Area\ of\ Attachment} = \frac{3.6\ x\ 10^9 [\mu m^2]}{2.5\ x\ 10^{-7}[\frac{\mu m^2}{molecules}]} = 1.4\ x\ 10^{16}\ [molecules]$$

$$\frac{1.4\ x\ 10^{16}\ molecules}{N_A[\frac{molecules}{mole}]} * 5000\ [\frac{g}{mole}] = 120\ [\mu g\ chitosan]$$

Concentration of chitosan on pore surface (per 1000 µm³)

$$Minimum = \frac{3.5\ [\mu g\ chitosan]}{3.6\ x\ 10^9\ [\mu m^2]} = 9.7\ x\ 10^{-10}\ [\mu g/\mu m^2]$$

$$Maximum = \frac{120\ [\mu g\ chitosan]}{3.6\ x\ 10^9\ [\mu m^2]} = 3.3\ x\ 10^{-8}\ [\mu g/\mu m^2]$$

$$Theoretical\ Range = 9.7\ x\ 10^{-10} - 3.3\ x\ 10^{-8}\ [\mu g/\mu m^2]$$

Glass Fiber

Surface area ratio → 70 µm²/µm²

SA to volume ratio → $\frac{70\ \mu m^2}{\mu m^2} * \frac{1}{370\ \mu m\ thick} = 0.19\ ^{\mu m^2}/_{\mu m^3}$

Total volume of membrane punch = thickness * area
volume = (0.37 mm)*π(2.4 mm)²
volume = 6.7×10^9 µm³

Total SA in patterned membrane volume

$$6.7\ x\ 10^9\ [\mu m^3] * \frac{0.19\ [\mu m^2]}{[\mu m^3]} = 1.3\ x\ 10^9\ [\mu m^2]\ surface\ area$$

Minimum number of chitosan molecules adsorbed

$$\frac{Surface\ Area}{Area\ of\ Attachment} = \frac{1.3\ x\ 10^9\ [\mu m^2]}{8.5\ x\ 10^{-6}[\frac{\mu m^2}{molecule}]} = 1.5\ x\ 10^{14}\ [molecules]$$

$$\frac{1.5\ x\ 10^{14}\ [molecules]}{N_A[\frac{molecules}{mole}]} * 5000\ [\frac{g}{mole}] = 1.2\ [\mu g\ chitosan]$$

Maximum number of chitosan molecules adsorbed (per 1000 µm³)

$$\frac{Surface\ Area}{Area\ of\ Attachment} = \frac{1.3\ x\ 10^9\ [\mu m^2]}{2.5\ x\ 10^{-7}[\frac{\mu m^2}{molecule}]} = 5.1\ x\ 10^{15}\ [molecules]$$

$$\frac{5.1\ x\ 10^{15}\ molecules}{N_A[\frac{molecules}{mole}]} * 5000\ [\frac{g}{mole}] = 42\ [\mu g\ chitosan]$$

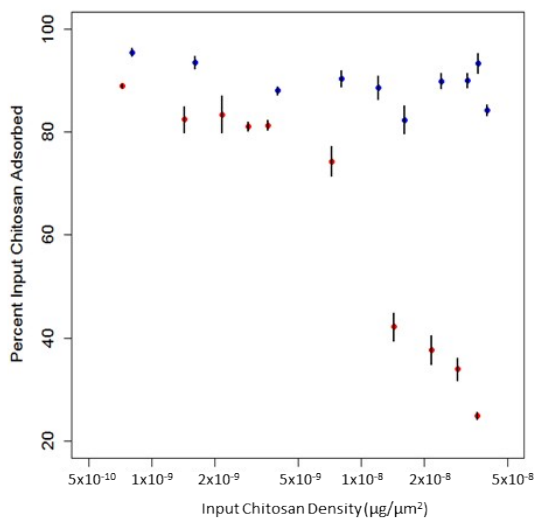
Concentration of chitosan on pore surface (per 1000 µm³)

$$Minimum = \frac{1.2\ [\mu g\ chitosan]}{1.3\ x\ 10^9\ [\mu m^2]} = 9.5\ x\ 10^{-10}\ [\mu g/\mu m^2]$$

$$Maximum = \frac{42\ [\mu g\ chitosan\ ]}{1.3\ x\ 10^9\ [\mu m^2]} = 3.2\ x\ 10^{-8}\ [\mu g/\mu m^2]$$

$$Theoretical\ Range = 9.5\ x\ 10^{-10} - 3.2\ x\ 10^{-8}\ [\mu g/\mu m^2]$$

C.



Membrane	Experimental Capacity (µg/µm²)	Theoretical Capacity (µg/µm²)
Nitrocellulose	~ 3.3×10^{-8}	$9.7 \times 10^{-10} - 3.3 \times 10^{-8}$
Glass Fiber	$3.6 \times 10^{-9} - 7.2 \times 10^{-9}$	$9.5 \times 10^{-10} - 3.2 \times 10^{-8}$

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

A.

Nitrocellulose

DNA capacity: 1.9x10^{6} c/µg chitosan = 3.8x10^{11} bp/µg chitosan

Chitosan available on membrane after accounting for loss: 8.08 µg

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

Total (+) charges available

$$9.73\ x\ 10^{14}\ [molecules] * 34\ [+charges] = 3.31\ x10^{16}\ [+charges]$$

Measured DNA capacity = 3.8x10^{11} bp/µg chitosan

$$3.8\ x\ 10^{11}\ \left[\frac{bp}{\mu g\ chitosan}\right] * 8.08\ [\mu g\ chitosan] = 3.07\ x\ 10^{12}\ [bp]$$

$$3.07\ x\ 10^{12}[bp] * 2\ \left[\frac{-\ charges}{bp}\right] = 6.14\ x\ 10^{12}\ [-charges]$$

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: 9.9x10^{6} c/µg chitosan = 2.0x10^{12} bp/µg chitosan

Chitosan available on membrane after accounting for loss: 5.05 µg

$$5.05\ [\mu g] * \frac{1\ [mole]}{5000\ [g]} * N_A \left[\frac{molecules}{mole}\right] = 6.08\ x\ 10^{14}\ [molecules]$$

Total (+) charges available

$$6.08\ x\ 10^{14}\ [molecules] * 34\ [+charges] = 2.07\ x10^{16}\ [+charges]$$

Measured DNA capacity = 2.0x10^{12} bp/µg chitosan

$$2.0\ x\ 10^{12}\ \left[\frac{bp}{\mu g\ chitosan}\right] * 5.05\ [\mu g\ chitosan] = 1.01\ x\ 10^{13}\ [bp]$$

$$1.01\ x\ 10^{13}[bp] * 2\ \left[\frac{-\ charges}{bp}\right] = 2.02\ x\ 10^{13}\ [-charges]$$

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.

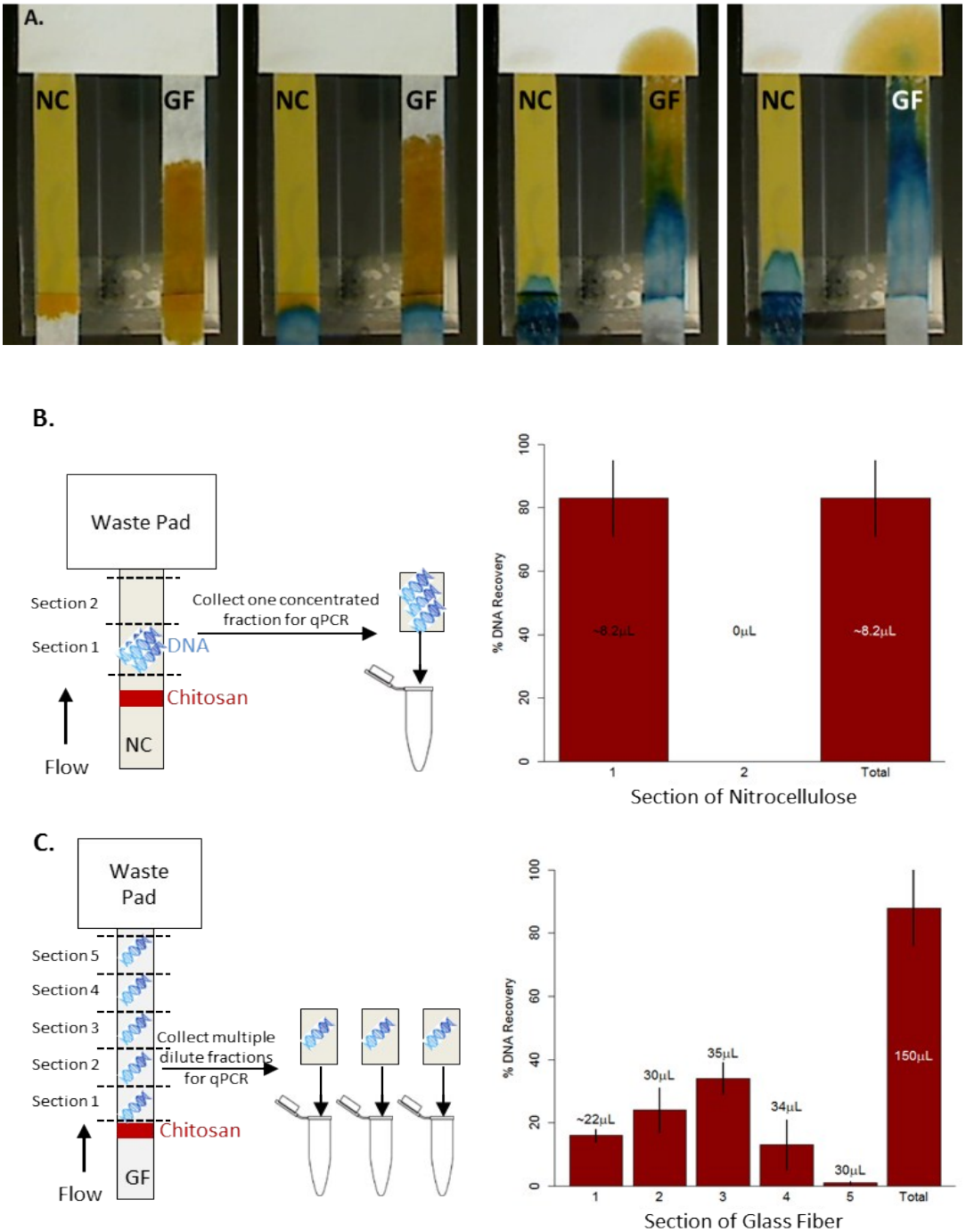


Figure S6. Dispersion of sequentially delivered fluids in nitrocellulose (NC) and glass fiber (GF). A) Capture buffer, 50 mM MES at pH 5, with bromothymol blue is first flowed through the membranes. At pH values below 6.0, the indicator is yellow. As elution buffer, 50 mM Tris at pH 9, flows into the 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.

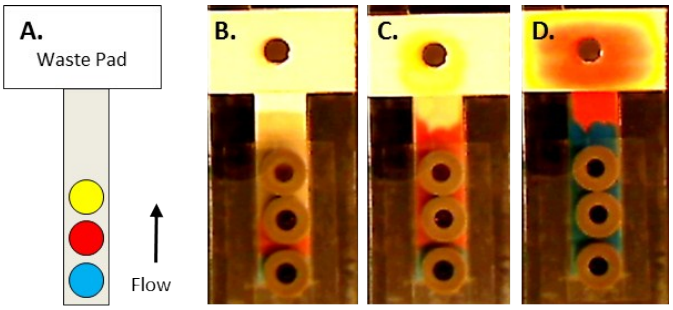


Figure S7. Sequential delivery of buffers to enable a single end-user step. A) Schematic. B-D) The images are taken from a video where three buffers were sequentially delivered to nitrocellulose. Yellow: capture buffer; red: wash buffer; blue: elution buffer. Using this method, our DNA concentration and purification system can be directly integrated with a simple user interface which requires only one step for activation[62,63].