SUPPORTING INFORMATION

Immobilization of Lambda Exonuclease onto Polymer Micropillar Arrays for the Solid-Phase Digestion of dsDNAs

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EXPERIMENTAL

Fabrication of the IMERs. In brief, the fabrication and assembly of this device included hot embossing using a HEX03 hot embossing system (JenOptik, Jena, Germany), which created the microstructured devices. The devices were replicated from a brass mold master that was fabricated using a micromilling machine (Kern, MMP Feinwerktechnik, Murnau-Westried, Germany). The PMMA IMERs consisted of a microreactor bed, which was comprised of a channel (24 mm long and 1.4 mm wide) containing 3,600 micropillars (100 μm height, 100 μm diameter and 50 μm pillar-to-pillar spacing) and a total surface area of 116.9 mm^2 with a 2.9-μL volume. A schematic of the device and a SEM can be found in Figure S1A and S1B, respectively. Post-processing of the microfluidic device included mechanically drilling reservoirs for sample introduction followed by cleaning the device and cover plate with isopropyl alcohol, rinsing with ddH_2O for debris removal and finally, drying in an oven at 70ºC. Ultraviolet (UV) radiation of the immobilization beds using a 254-nm UV light with a power density of 16.0 mW/cm^2 for 15 min was performed so as to activate the polymer surface by producing carboxylic acid groups that could be later functionalized with the enzyme following cover plate assembly.

Thin PMMA sheets, 0.125 mm thick, were used as cover plates to enclose the fluidic network of the IMERs by thermal fusion bonding.[1] The PMMA substrate and cover plate were sandwiched between two borosilicate glass plates (McMaster, Atlanta, GA, USA) and clamped together prior to insertion into a convection oven. The thermal bonding of the PMMA IMERs was performed at 101ºC for 20 min.[2,3]

Enzyme immobilization onto PMMA IMERs. λ-Exo was provided with a 10X reaction buffer (670 mM glycine-KOH, pH 9.4, 25 mM $MgCl_2$, 0.1% (v/v) Triton X-100), which was purchased from Fermentas Life Sciences (Glen Burnie, MD). No purification steps were performed prior to use. Following thermal fusion bonding of the cover plate to the substrate, succinimidyl ester

intermediates were formed to facilitate enzyme attachment. This was carried out by filling the reactor bed with a solution containing 200 mM 3-(3-dimethylaminopropyl) carbodiimide (EDC), and 50 mM N-hydroxysuccinimide (NHS) in 0.1 M 2-(4-morpholino)-ethane sulfonic acid at pH 5.1 (MES, Fisher Biotech, Fair Lawn, NJ) for 15 min at room temperature. The EDC/NHS reagents were then hydrodynamically displaced with a solution consisting of 0.03 μg/μL λ-Exo enzyme; the reaction was allowed to proceed overnight at 4°C. The enzyme-functionalized device was then rinsed with 1X λ-Exo reaction buffer to remove all unbound reagents from the PMMA surface. The schematic for this reaction is depicted in Figure S1C.

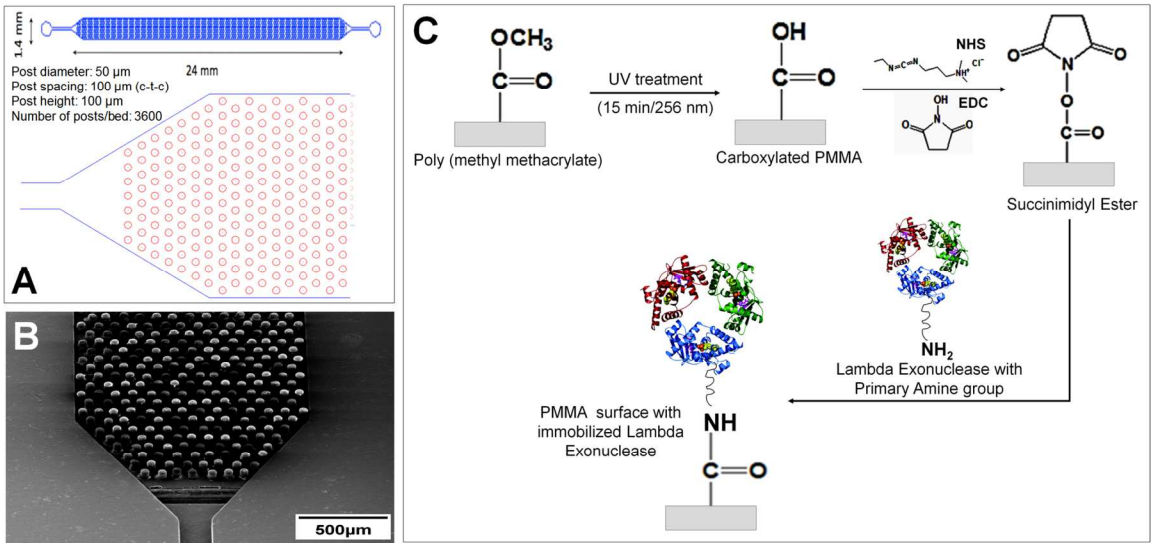

Figure S1. (A) Schematic showing the layout of the IMERs used for λ-Exo digestion of double-stranded DNA. The reactor bed was populated with micropillars that were 100 μm in diameter. The IMERs was made from the thermoplastic, PMMA, via hot embossing. (B) SEM of the IMERs fabricated via hot embossing from a brass-molding tool. (C) Schematic showing the immobilization of λ-Exo onto a PMMA substrate. The substrate was activated by ultraviolet radiation to generate surface confined carboxylic acid groups. This was followed by EDC/NHS coupling chemistry to covalently attach the enzyme to the substrate during an incubation period, which was carried out overnight at 4°C.

CE-LIF. Bare fused silica capillaries from Molex Polymicro Technologies (Phoenix, AZ) were used for the CE (total length = 33 cm, 20 cm effective length) and possessed a 50 μm internal diameter. The CE columns were preconditioned with 0.1 M NaOH for 30 min and rinsed by flushing with 0.5X TBE buffer (pH 8.3). Finally, the capillary surface, prior to the electrophoretic separations, was treated with a dynamic coating containing 2% (w/v) polyvinylpyrrlidone (PVP,

Mr = 40,000; Sigma Aldrich St. Louis, MO) in 0.5X TBE and a sieving matrix of methylcellulose (Sigma Aldrich) that was 0.5% (w/v) in 0.5X TBE buffer (pH 8.3).

Sample introduction was performed by electrokinetic injection at 10 kV for 180 s. DNA digestion products of λ-Exo were electrophoresed at a field strength of 303 V/cm and fragment size analysis was determined by comparing the results to the *Hind III* λ-DNA sizing ladder purchased from New England Biolabs (Ipswich, MA). CE data was acquired and analyzed using a custom designed LabView 6.1 program (National Instruments, Austin, TX) and Origin 8.7 software (OriginLab Co., Northampton, MA), respectively.

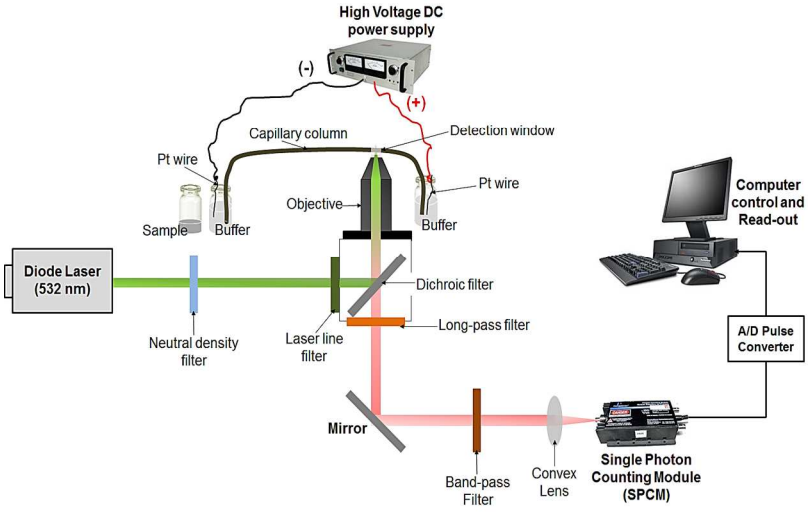

Figure S2. Schematic of the in-house built CE-LIF system utilizing a 532 nm, 20 mW excitation laser with edge filter and 560 nm long pass filter, 532 nm dichroic filter and SPCM-AQR single photon counting module. A 40X high numerical aperture (NA = 0.85) microscope objective was used to focus the laser beam onto the capillary and collect the fluorescence.

The LIF detector depicted in Figure S2 was configured in an epillumination format containing a 532 nm, 20 mW excitation laser (LaserGlow Technologies, Toronto, Ontario, Canada), XF 3085 edge filter (Horiba Scientific, Middlesex, UK), 3RD560LP 560 nm long pass filter (Omega Optical, Brattleboro, VT), a 532 nm dichroic filter (550DRLP, Omega Optical) and a SPCM-AQR single photon counting module (Perkin Elmer Optoelectronics, Waltham, MA). A 40X high numerical aperture (NA = 0.85) microscope objective from Nikon (Natick, MA) was used to focus the laser beam onto the capillary and collect the fluorescence. Prior to CE, the dsDNA was stained with Sytox Orange (547/570, Life Technologies, Grand Island, NY).

Results and Discussion

Data from the 660 nm protein absorbance assay for λ-Exo immobilization and fluorescence assays for determining the percent of dsDNA digestion. Table S1A shows the absorbance loss for the enzyme, λ-Exo, loaded onto a photo-activated PMMA IMERs bed. The amount of λ-Exo in solution was determined using a spectrophotometric assay and consisted of measuring the 660 nm absorbance both before and after passing through the activated bed in the presence of EDC/NHS. The amount (pmol) of enzyme used for Beds 1-3 was 75, 90 and 100, respectively. The enzyme was suspended in 25 µL of reaction buffer containing EDC/NHS and was pumped through the reactor bed at a rate to allow for a reaction time of ~15 min. The effluent was collected and its absorbance measured without dilution. The absorbance difference was ascribed to material covalently immobilized to the reactor surface.

Table S1A. Absorbance loss for λ-Exo, loaded onto an activated PMMA IMERs bed.

Table S1A		660 nm Assay Absorbance Values		
Bed	Pre- Fill Absorbance	Post-Fill Absorbance	Absorbance Difference	Calculated pMoles Lost to Immobilization
1	0.128	0.123	0.005	3.25
2	0.147	0.143	0.004	4.96
3	0.16	0.158	0.002	6.40

Table S1B shows the percent of digestion as a function of enzyme load onto the bed. A 50 µg/mL solution of λ-DNA was introduced into the IMERs and allowed to react for 60 s. The effluent from the bed was stained with PicoGreen to determine the amount of dsDNA remaining following passage through the IMERs. Following staining, the effluent fluorescence was measured using a Fluorolog fluorescence spectrometer.

Table S1B. Percent digestion as a function of enzyme load to the bed.

Table S1B	Varied Enzyme Concentration		
pMoles of Enzyme	3.25	4.96	6.2
% Enzyme Digestion	96.49 ± 1.75	94.69 ± 2.85	84.74 ± 8.80

Table S1C provides data on the percent of dsDNA digestion as a function of reaction time within the IMERs device. This data was collected following the same procedure as that described for Table S1B. The amount of enzyme immobilized to the bed was 4.96 pmol.

Table S1C. Percent of dsDNA digestion as a function of reaction time.

Table S1C	**Varied Reaction Time**		
Reaction Time (s)	60	300	1200
% Enzyme Digestion	95.01 ± 2.69	94.40 ± 1.53	90.20 ± 0.22

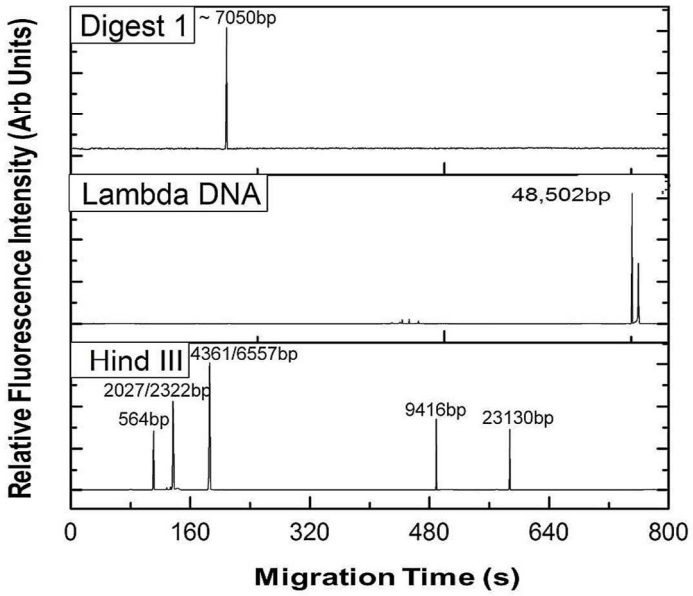

Figure S3. Electropherograms of the *Hind III* ladder, λ-DNA (passed through an enzyme-free IMERs), and product(s) of the IMER digestion. The dsDNA was stained in a 1:5 dye/bp ratio with Sytox orange (547 nm); the staining was accomplished using the effluent from the IMERs. Following staining, the effluent was then introduced into the electrophoresis capillary via 10 kV electrokinetic injection and the CE was carried out using a capillary coated with 2% PVP. The sieving matrix consisted of 0.5% methylcellulose solution in 0.5X Tris-Borate EDTA buffer and the applied separation field strength was 303 V/cm.

Analysis of λ-Exo reaction products using CE-LIF. Electrophoretic analyses of λ-Exo digestion products using the IMERs were accomplished by adaptation of published separation protocols with slight modifications.[4] In Figure S3, an electropherogram of the digestion product(s), the *Hind III* sizing ladder, and λ-DNA transported through an enzyme-free IMERs are shown. Using methylcellulose as the sieving matrix, baseline resolution of five *Hind III* peaks were observed with co-migration of the 2027/2322 bp and 4361/6557 bp fragments. The peak at 125 bp was not observed because its fluorescence intensity was below the detection limit of the LIF system. Using the same CE conditions, the dsDNA remaining after an IMER's reaction was analyzed yielding a peak of ~7 kbp in size when compared to the *HIND III* sizing ladder (see top

electropherogram of Figure S3). Previous work performed on λ-Exo digestion of surface immobilized λ-DNA produced fragments equivalent to ~19 kbp.[5] As can be observed from our data, the digestion reactions were absent of peaks corresponding to intact λ-DNA suggesting that the majority of the λ-DNA was digested upon passage through the IMERs containing immobilized enzyme.

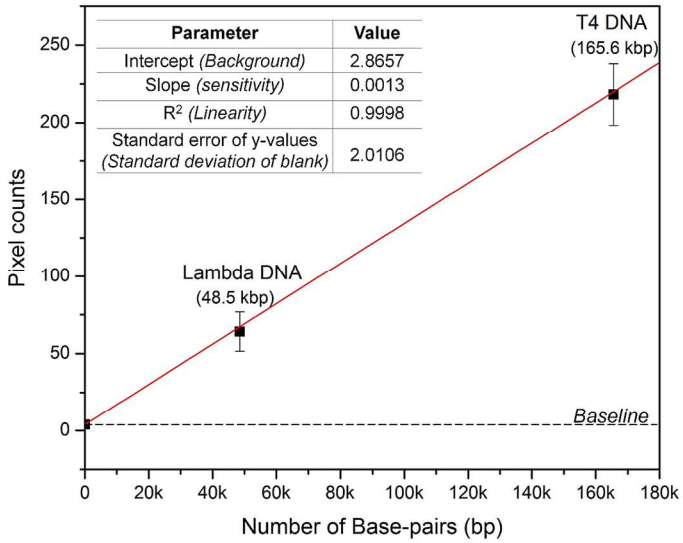

Figure S4. Fluorescence intensity as a function of DNA length (bp) to determine the smallest observable DNA fragment using a 1:50 dye/bp stained standards (staining dye was YOYO-1). The standards consisted of lambda and T4 duplexed DNA. DNA fragments as small as 4.6 kbp could be detected based on the linear plot shown (95% confidence interval; R^2 value of 0.9973). The fluorescence was measured using the Zeiss inverted microscope fitted with an EMCCD camera.

Fluorescence calibration plot of stained dsDNA. To determine the smallest dsDNA fragment that could be observed using the fluorescence microscope (see main text for description) and the staining conditions employed, a calibration plot was created using the fluorescence intensities obtained for lambda and T4 DNA (48,502 and 165,600 bp in length, respectively) stained at 1:50 dye/bp ratio using YOYO-1 (see Figure S4). The insert in Figure S4 shows a table of the relevant parameters obtained from the linear fit of the graph. From the plot, the smallest approximate size of a DNA fragment distinguishable from the background at a 95% confidence interval with the imaging conditions employed was approximately 4.6 kbp.

IMER reusability. We also evaluated the ability to reuse λ-exonuclease when immobilized to a solid support. Figure S5 shows the solid-phase reaction data performed using a single reactor. As can be observed, with the first usage of the IMER at different reaction times, >90% of λ-DNA

digestion was observed, consistent with our data shown in Table S1C. Upon subsequent reuse of the reactor, digestion efficiency decreased with the efficiency dropping to around 80% for the second use and ~53% for the third use.

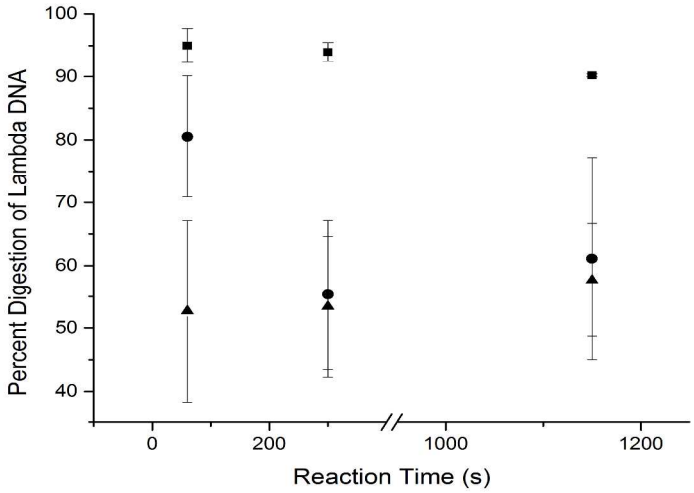

Figure S5. Percent digestion of λ-DNA at 60, 300 and 1200 s for three uses of the same reactor. λ-DNA in presence of reaction buffer was introduced to 4.96 pmol of enzyme immobilized to the reactors at 37ºC and over 3 usages to determine reactor short-term reusability. Squares – first use; Circles – second use and triangles – third use of IMER.

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