

Low-Cost Fabrication of Centimeter-Scale Periodic Arrays of Single Plasmid DNA Molecules

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Electronic Supplementary Information (ESI)

Materials

Sylgard 184 polydimethyl siloxane (PDMS) kit was purchased from Dow Corning. 3-Aminopropyltriethoxysilane (APTES), sodium salt of polyacrylic acid (PAA, molecular weight = 5,100 g/mol), polyvinyl alcohol (PVA, 87–90% hydrolyzed, molecular weight = 30,000–70,000 g/mol), and Tween 20 were purchased from Sigma-Aldrich. Glass slides, 2-(N-Morpholino) ethanesulfonic acid (MES) buffer, toluene, fluorescein isothiocyanate (FITC) were purchased from VWR. YOYO-1 fluorescence dye and 1 kilobase pairs (kbp) DNA ladder were purchased from Invitrogen. CX-Rhodamine Label IT® nucleic acid labeling kit was purchased from Mirus Bio LLC (Madison, WI). DNaseI, EcoRI and λ DNA-Monocut Mix were purchased from New England Biolabs. An 11,371 bp plasmid, pCR-XL-TOPO-LD-B1, was constructed by David M. Gilbert's group.

Methods

Fabricating PDMS stamps. PDMS prepolymer and curing agent were cast on a silicon master at 10:1 weight ratio and incubated at 37°C for 24 hr. The cured PDMS was peeled off from the master and cut into 2 cm × 1.5 cm pieces as stamps.

Fabricating APTES nanopatch arrays. (1) Glass slides were cleaned by sonication in deionized (DI) water for 20 min followed by another rinse with DI water and dried thoroughly using a stream of nitrogen. (2) The slides were exposed to oxygen plasma (PDC-32G plasma cleaner, Harrick Plasma, Ithaca, NY) for 3 min at <500 mTorr and High power level. (3) The slides were placed in a vacuum dessicator with ~100 μ L APTES in a centrifuge tube. Vacuum was generated in the desiccator and kept for 24 hr in an oven set at 65°C. After that, the slides were taken out, washed with water, and dried under a stream of nitrogen. (4) A stamp was manually dipped in an aqueous solution of PAA and immediately brought into contact with an APTES-coated glass slide. Slight pressure was applied manually to the stamp to ensure full contact between the stamp and the slide. The stamp was then removed from the slide manually. (5) The slide was exposed to argon plasma for 30 sec at 300 mTorr and medium power level. (6) The slide was sonicated in DI water for 10 min to remove the residual PAA nanoparticles, dried under a stream of nitrogen, and immediately used in DNA patterning experiments. It is of note that PAA printing was affected by relativity humidity. Good printing was generally associated with humidity above 40% measured

by a VWR hygrometer. A household humidifier was used to increase humidity when the ambient humidity was low.

Fabricating 1 μm -wide APTES patches. A 3% (wt/v) PAA solution and a PDMS stamp bearing circular pillars (7 μm diameter, 3.4 μm height, and 8.3 μm center-to-center distance, square lattice) were used following the same procedure as above.

Labeling APTES nanopatches with FITC. A glass slide bearing the APTES nanopatches was immersed in 100 nM FITC in water for 5 min at room temperature. The slide was then washed with DI water to remove unbound FITC. The slide was dried under a nitrogen stream before being imaged.

Immobilizing and stretching plasmid DNA on featureless APTES-coated glass slides. Immobilizing the plasmid DNA on a stationary featureless APTES-coated slide was performed by dropping 20 μL 0.01 ng/ μL DNA solution (150 mM MES, 20 mM NaCl, pH 4.5) on the slide, keeping the solution on the slide briefly (~30 sec), rinsing the slide with DI water, and finally drying it under a nitrogen stream. Stretching the plasmid DNA was performed by dropping 20 μL 0.1 ng/ μL DNA solution in 150 mM MES and 20 mM NaCl (pH 4.5) on an APTES-coated slide spinning at 5500 rounds per minute (G3P-8 spincoater, Specialty Coating Systems) and stopping the spincoater after 2 min. The slide was then rinsed with DI water and dried under a nitrogen stream.

Labeling DNA. DNA was either labeled with YOYO-1 or rhodamine. For YOYO-1 labeling, the dye was mixed with DNA at a dye/base pair ratio of 1:20 and incubated overnight at 4°C. For rhodamine labeling, DNA was treated with the CX-Rhodamine Label IT® nucleic acid labeling kit following the protocol recommended by the manufacturer.

Preparing DNA arrays. 20 μL 0.1 ng/ μL DNA solution (150 mM MES, 20 mM NaCl, pH 4.5) was added on an array substrate and covered with a 2 cm-wide square coverslip that was cleaned with oxygen plasma. After a specified period of time, the coverslip was removed; the slide was rinsed with DI water; and dried under a nitrogen stream. For the time-course experiment, micrographs were recorded at the specified time points with the DNA solution and coverslip on the substrate. For preparing the two-color DNA arrays, equal volumes of YOYO-1-labeled DNA (1 ng/ μL) and rhodamine-labeled DNA (1 ng/ μL) in the above buffer were mixed and immediately added on an array substrate following the above procedure with the incubation time of 60 min.

Stretching plasmid DNA on nanopatches. 20 μL 0.1 ng/ μL DNA solution (150 mM MES, 20 mM NaCl, pH 4.5) was added on an array substrate and kept for 60 min to form a single-DNA array as described above. 400 μL solution of 2% (wt/v) PAA and 0.1% (v/v) Tween 20 was added on the array and kept for 3 min. The array and the solution was then placed in a container filled with 250 μL DI water and immediately pulled out at a speed of 166 $\mu\text{m}/\text{sec}$ controlled by a computer numerical control milling machine (Sherline 8021 CNC Mill System, Vista, CA).

DNaseI treatment of DNA array. DNA array was gently washed with DI water. 2 μL of a DNaseI solution (1U DNase in 1 \times DNaseI Reaction buffer) was spotted on the array and kept for 10 min. The inactivation of DNaseI was accomplished by heating the solution to 70°C for 20 min followed by the addition of 0.5 M ethylenediaminetetraacetic acid (EDTA). The solution of the inactivated DNaseI was applied to the DNA array and kept for 10 min. Regeneration of a

DNA array on an array that was previously wiped off by DNaseI was done by the protocol described above.

Microscopy. The optical micrographs were obtained using an inverted Nikon Ti epifluorescence microscope equipped with an Andor iXonEM+ 885 EMCCD camera. A Nikon B-2E/C filter set was used for imaging FITC and YOYO-1. A Nikon G-2E/C filter set was used for imaging rhodamine. Colors of the images were assigned through software with red to rhodamine and green to YOYO-1 and FITC. Background fluorescence in Figures 3, S2b and S2c was subtracted using Nikon NIS elements program. Atomic force microscopy images were obtained using a Bruker Dimension Icon system operated at tapping mode in air and silicon cantilevers (MikroMasch-Ultrasharp cantilevers) with a tip radius of <10 nm.

Quantitative analysis of DNA arrays. Nikon NIS elements program was used for data analysis. For every fluorescence micrograph, a background area was identified manually. The fluorescence intensity of the area was subtracted from the entire micrograph. To measure fluorescence intensity of a fluorescent dot, the dot was manually enclosed by a standardized circle and average intensity was generated by the program.

Gel electrophoresis. Plasmid DNA (~50 ng) and ladder DNA were run on a 0.8% agarose gel in $1\times$ Tris-acetate-EDTA (TAE) buffer for 2 hr at 50 V. A 1 kbp ladder was used for the unlabeled plasmid DNA and a lambda DNA-Monocut Mix ladder was used for the rhodamine-labeled plasmid DNA. After electrophoresis the gels were stained with SYBR Gold (Invitrogen) for 2 hr, rinsed with water for 20 min and imaged using a BioSpectrum[®] 500 Imaging System (UVP).

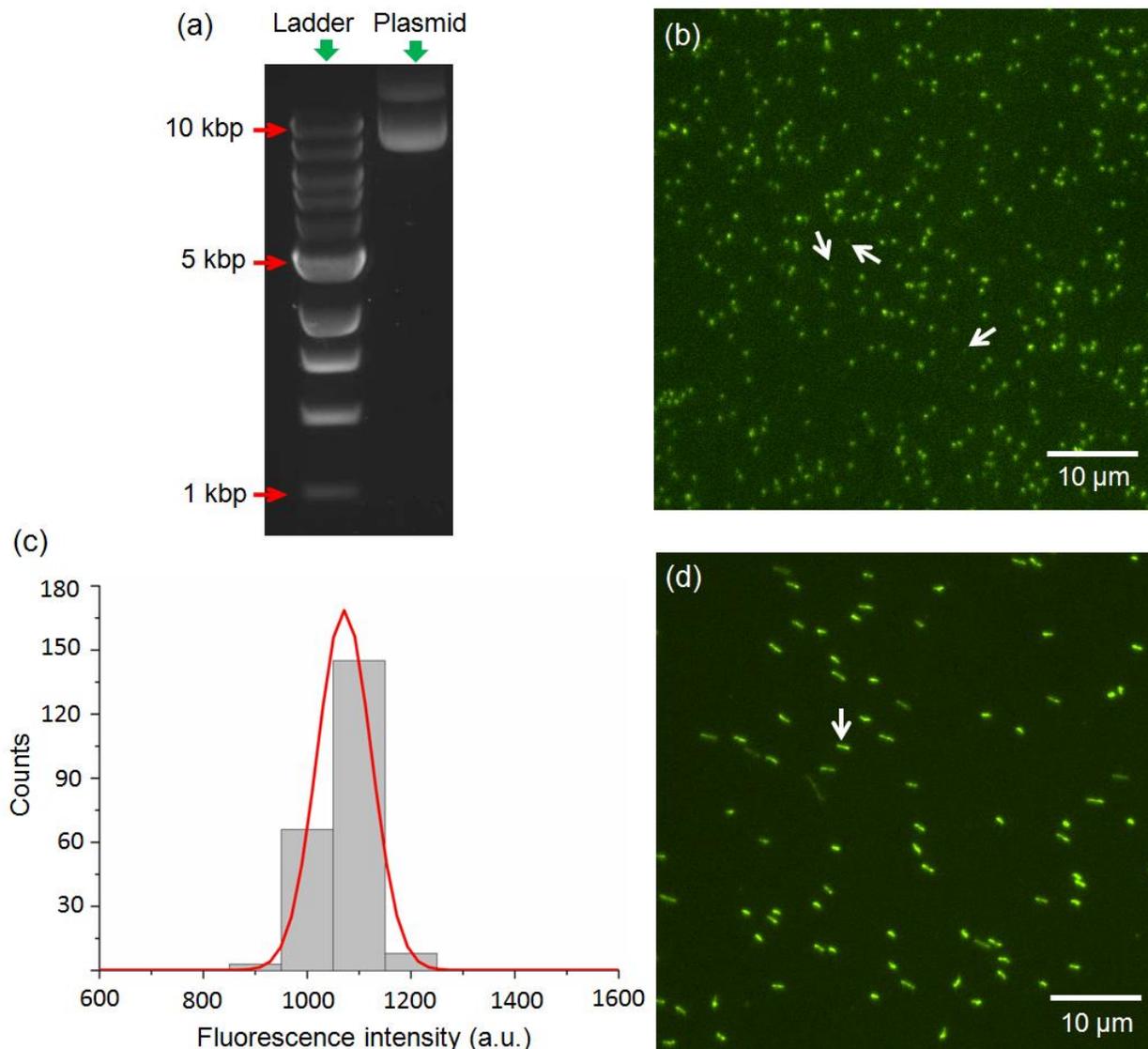


Figure S1. Characterization of plasmid DNA. (a) Gel electrophoresis of the 11 kbp circular plasmid DNA, showing supercoiled (lower) and open-circular (upper) bands. (b) Fluorescence micrograph of plasmid DNA molecules immobilized on a stationary featureless APTES-coated glass slide. The arrowheads point selected dots with fluorescence intensities significantly lower than the majority of the dots. (c) Histogram of fluorescence intensity of 222 molecules along with a Gaussian fit curve (red line) from (b). Center of peak: 1071. (d) Fluorescence micrograph of stretched plasmid DNA molecules prepared by dropping DNA solution on a rapidly spinning featureless APTES-coated glass slide. The arrowhead points a stretched DNA molecule with length of $\sim 1.3 \mu\text{m}$. DNA in (b) and (d) was labeled with YOYO-1 dye.

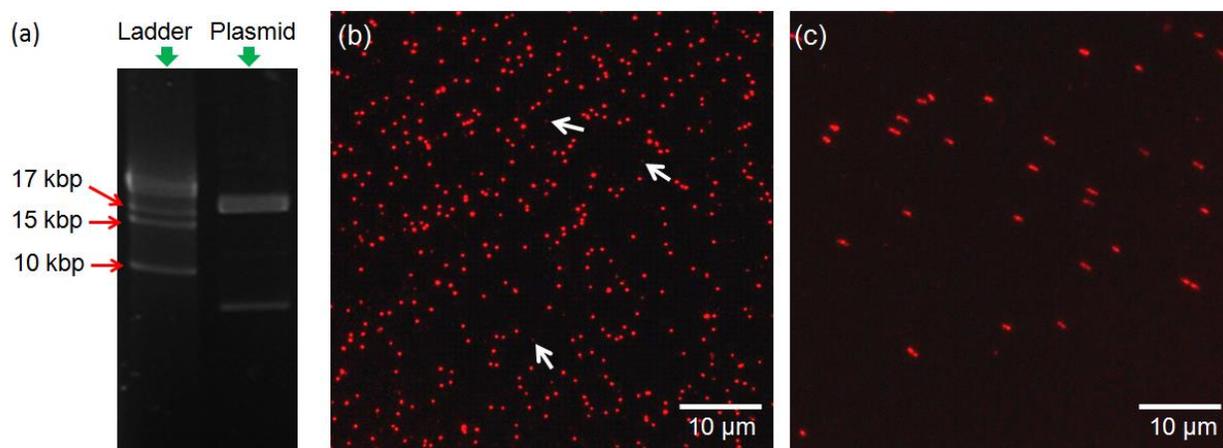


Figure S2. Characterization of rhodamine-labeled plasmid DNA. (a) Gel electrophoresis of rhodamine-labeled 11 kbp plasmid DNA, showing supercoiled (lower) and open-circular (upper) bands. Fluorescence micrographs of rhodamine-labeled plasmid DNA immobilized on (b) a stationary and (c) a rapidly spinning featureless APTES-coated glass slide. The arrowheads in Figure (b) point selected dots with fluorescence intensities significantly lower than the majority of the dots.