Supplementary information

Nanotip for single-step preparation of DNA for qPCR analysis

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1. Inhibition test of nanotips in qPCR

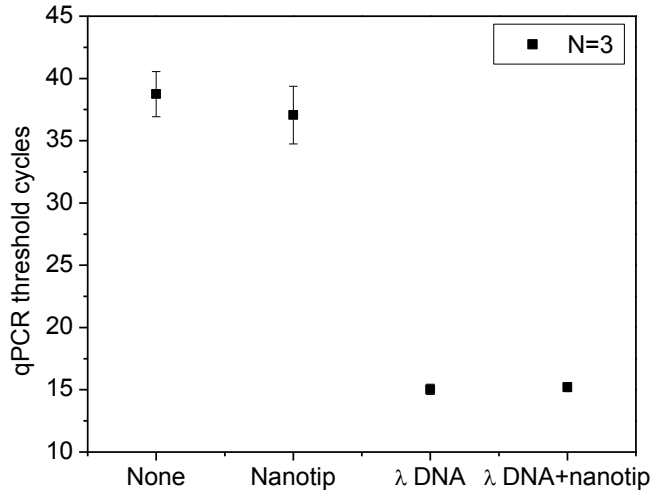
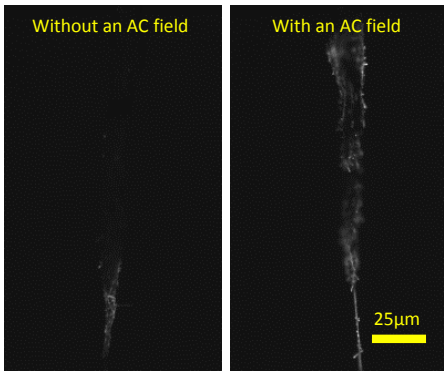


Fig. S1. qPCR results of the control tests with and without a nanotip. A nanotip does not inhibit qPCR analysis (N=3).

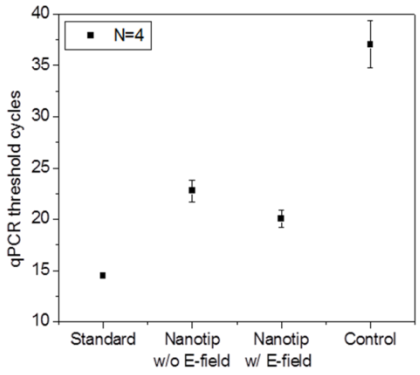
The inhibition test of a nanotip in qPCR reaction was conducted to study two potential issues. When a nanotip is dissolved in the PCR soltuion, the nanotip could interfere with the polymerization and depolymerization process. A nanotip can also interfere with the fluorescence measurement in qPCR analysis. Four different combinations of the inhibition tests were conducted to address both interferences. In the first test of the control experiment, qPCR analysis was conducted without a nanotip or λ-DNA. In the second test, only a nanotip was added in qPCR analysis without λ-DNA. The purpose of the first and second tests was to examine any cross-reaction among dissolved nanotips and primers. The third test was to inject λ-DNA solution (10 ng/mL at 2.5 μL) directly into qPCR analysis without a nanotip. The fourth test was to run qPCR together with a nanotip and λ-DNA. The purpose of the third and fourth tests was to observe any inhibition effect on DNA amplification potentially caused by a nanotip. Each experiment was conducted three times (N=3).

In the experimental results, neither qPCR reaction nor fluorescence measurement in qPCR was inhibited by the nanotips (Fig. S1). The threshold cycles for the negative controls without λ-DNA were the same in an error range with and without dissolved nanotips. In a qPCR tube with λ-DNA, the threshold cycles were the same with and without nanotips. When a nanotip was dissolved in PCR buffer, SWCNTs and SiC nanowires did not inhibit the PCR reaction and the fluorescence measurement.

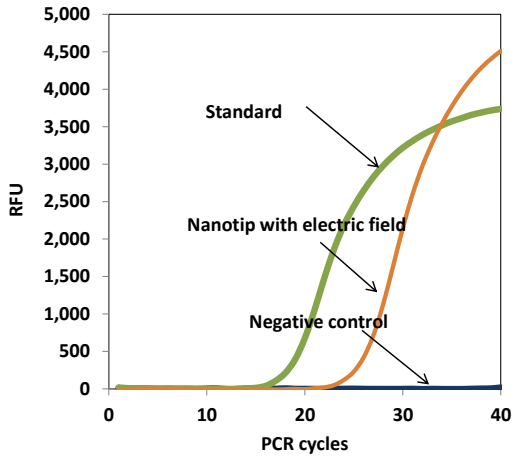
2. Recovery of λ-DNA with and without an electric field



(a)



(b)



(c)

Fig. S2. (a) Fluorescence images of the nanotip without and with an AC electric potential. (b) qPCR threshold cycles of recovered λ-DNA without and with an AC electric potential (N=4) (c) qPCR amplification curve for standard (2.5 µL of 10 ng/mL λ DNA), a nanotip with an electric field, and negative control (2.5 µL of 1x TE buffer),

To study the recovery of DNA, λ-DNA (10 ng/mL) stained with PicoGreen was captured with an AC electric potential. The concentration time was 5 minutes. The nanotips were analyzed by both fluorescence microscopy and qPCR. The control test was performed without an AC potential.

The fluorescence measurement on the nanotip after DNA capture is shown in Fig. S2(a). The fluorescence image with an electric field shows a higher intensity than that without an electric field. Thus, it is found that more DNA is attracted using an electric field. The fluorescence intensities with and without an electric field show 7464 a.u and 412 a.u., respectively, which shows more than one order of magnitude

difference. The corresponding qPCR results are shown in the Fig. S2(b). The absence and presence of an electric field shows a difference of 2.7 cycles, which corresponds to about 6 fold in terms of the number of DNA copies. Based on the standardization curve in Fig. 2 of the paper, an average of 3.6 cycles correspond to 10 fold concentration difference, and therefore 2.7 Ct cycles correspond to 6 fold. According to the result, DNA could be captured by both an electric field and no-electric field. However, the nanotip result with an electric field was more dominant than that without an electric field.

The amplification curves of qPCR for standard and control samples are shown in the Fig. S2 (c). The standard value means the threshold cycle of qPCR using 2.5 μL of λ-DNA (10 ng/mL) in 1x TE buffer. The control refers to the threshold cycle of qPCR using 2.5 μL of 1x TE buffer without λ-DNA. 1x TE buffer is the elution buffer for λ-DNA, which is used for negative control. 1x TE buffer is 10mM Tris and 1mM of EDTA.

Evaporation was observed in the capture both with an electric field and without an electric field. However, the nanotip was always submerged in the solution during the 5 minutes of concentation. Therefore the effect of evaporation was minimal in all of the experiments. The PCR signal of a nanotip without an electric field could be caused by non-specific binding of DNA to single walled carbon-nanotubes and nanowires[1]. Thus, DNA could be nonspecifically captured by both an electric field and diffusion on the nanotip surface. An electric field could enhance the capturing efficiency in comparison to the diffusion case.

3. Concentration time test

An electric field was used to concentrate DNA on to the nanotips. The electric potential was applied between a nanotip and a coil holding the sample solution by surface tension. At 5 MHz, dielectrophoresis was dominant while electroosmosis and electrophoresis were significantly reduced in 1x TE buffer. The concentration time varied for 0.5, 1, 2, 3.5 and 5 minutes. The upper bound of 5 minutes was due to the complete evaporation of the solution from the coil in the application of an electric field. As the control experiment, the same concentration of λ-DNA (10 ng/mL at 2.5 μL) was directly injected into qPCR analysis. Three experiments were conducted for each concentration time.

As the concentration times increased from 0.5 to 5 minutes, the qPCR threshold cycles were reduced along with the errors among the tests (Fig. S3). It was found that 5 minutes yielded lower qPCR cycles with the smallest standard deviation. In comparison with the standard value (control sample), the threshold cycle at 5 minutes was lagged by 6.5 cycle. The standard value is the qPCR threshold cycles for 2.5 μL of 10 ng/mL concentration of λ-DNA. Due to the small area of the nanotip, a limited number of DNA molecules were captured and recovered for qPCR analysis.

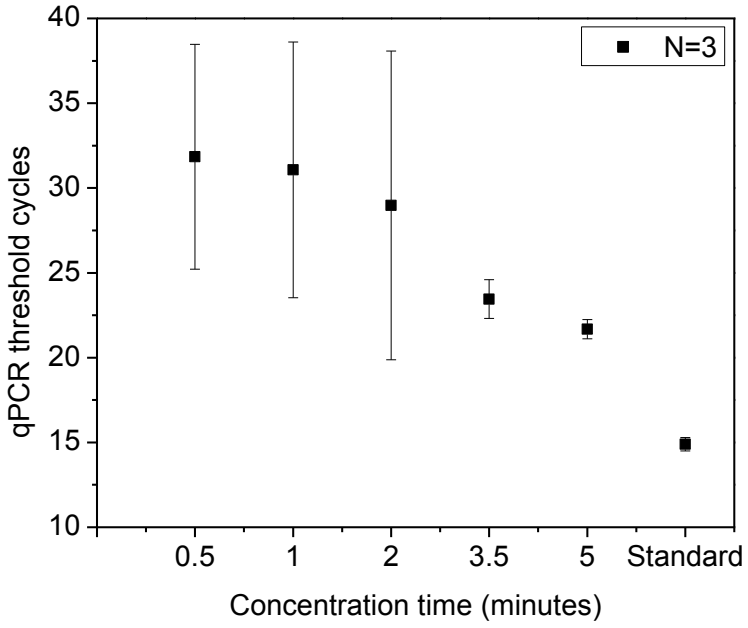


Fig. S3. qPCR threshold cycles of recovered λ-DNA from various concentration times from 0.5 to 5 minutes

4. qPCR analysis

For fluorescence measurement on a nanotip, λ-DNA (New England Biolabs, Ipswich, MA) was stained with an intercalating dye of PicoGreen® (Invitrogen, Carlsbad, CA). After mixing λ-DNA and PicoGreen, the mixture was incubated for 5 minutes. The nanotips after DNA capture were analyzed by a fluorescence microscope (Olympus BX-41, Olympus America Inc., Melville, NY). The excitation and emission wavelengths were 480 nm and 520 nm, respectively.

For quantification of DNA, qPCR was used (CFX 96 from BioRAD, Hercules, CA). Sybr GREEN Express® (Invitrogen, Carlsbad, CA, USA) mastermix was used for qPCR analysis. The primers for λ-DNA experiments were 5' – GAT GAG TTC GTG TCC GTA CAA CTG G-3' (25 bases) and 5'- GGT TAT CGA AAT CAG CCA CAG CGC C-3' (25 bases). For human genomic DNA, the sequence of β actin gene was used. The primer sequences were: forward primer: 5' -ACC CAC ACT GTG CCC ATC TAC-3' (21 bases) and reverse primer: 5' - TCG GTG AGG ATC TTC ATG AGG TA - 3' (23 bases).

For qPCR procedure, 14 µL of master mix, and 3 µL of the forward and reverse primers at 2 µM concentration were used. The qPCR thermocycle was conducted by the following sequence: pre-incubation at 50 °C for 2 minutes, incubation at 95 °C for 10 minutes, denaturation at 95 °C for 15 seconds, annealing and extension at 60 °C for one minute. The denaturation and annealing/extension

cycles were repeated 40 times. The fluorescence intensity was automatically measured at the end of each annealing/extension cycle, which was processed to identify a threshold cycle.

5. Recovery of λ-DNA spiked in buffer

Various concentrations of λ-DNA from 1 μg/mL to 10 pg/mL were prepared by serial, 10-fold dilutions using 1x TE buffer. Under an AC potential, DNA was captured with the concentration time of 5 minutes. The nanotips were injected into qPCR tubes after capture. The qPCR analysis was performed for the nanotips having λ-DNA recovered from various concentrations. The dose response of λ-DNA recovered from buffer is shown in Fig**.** S4. The threshold cycles of the original λ-DNA aliquot are shown as the red circles. The amount of the recovered λ-DNA using the nanotip were proportional to the initial concentrations of λ-DNA in buffer.

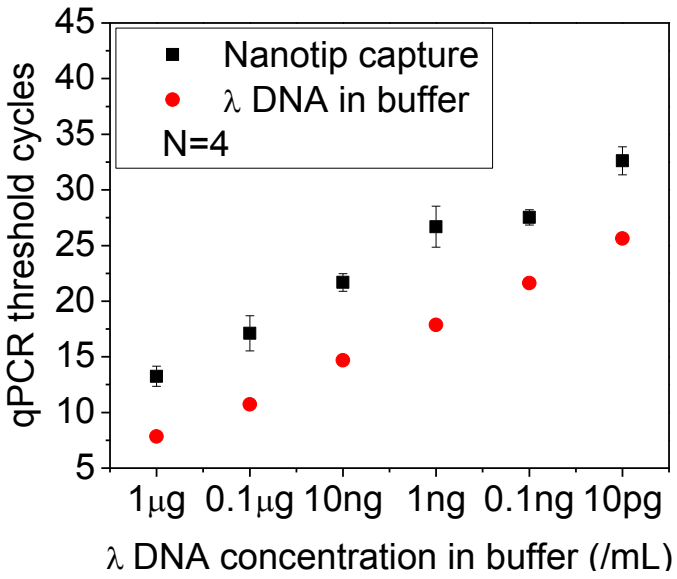
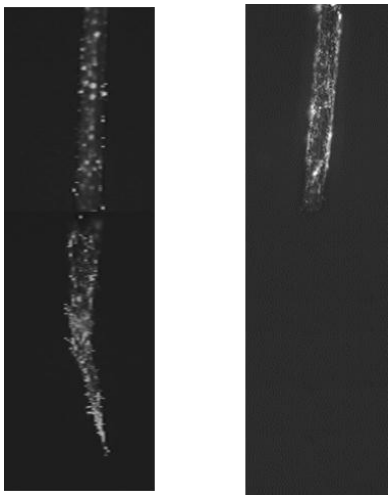


Fig. S4. Recovery of various concentrations of λ-DNA from buffer

6. Desorption of λ DNA from the nanotips in PCR

To study the desorption of DNA from the nanotips in PCR reaction, we conducted the following experiment. The nanotip was inspected for DNA using fluorescence before and after PCR. For this experiment, 2.5 μL λ DNA in 1x TE buffer (10ng/mL) was used. After DNA capture and before PCR, a fluorescence image was taken as shown in Fig. S5 (a). The fluorescence image after PCR is shown in Fig. S5 (b). During fabrication of the nanotip, a tungsten microwire was wrapped with SiC nanowires and SWCNTs. During PCR reaction, nanotips were dissolved in PCR liquid, and the microwire wrapped with nanomaterials could be observed under fluorescence microscopy. Fluorescence signals were observed on

the nanotip before PCR while fluorescence signals were still observed on the microwire. It was speculated that DNA could not be completely desorbed from the nanowires or the nanotubes. For the nanotips in Fig. S5, the average threshold cycles were 21.03 with standard deviation of 0.7.



(a) (b)

Fig. S5. Fluorescence images of nanotips (a) before and (b) after PCR.

References:

[1] P. C. Ke and R. Qiao, "Carbon nanomaterials in biological systems," *Journal of Physics: Condensed Matter*, vol. 19, p. 373101, 2007.