ADVANCED MATERIALS

Supporting Information

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DNA Nanotweezers and Graphene Transistor Enable Label-Free Genotyping

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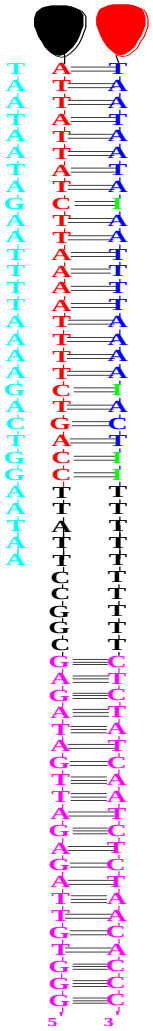

Figure S1. The structure of DNA tweezers with specific sequences. The aqua color strand is complementary target strand, and strand with single mismatch has T instead of G in the middle of the sequence. The red strand with the black toehold is normal side (N) and the counterpart is weak side (W), which contains 4 inosine (I), indicated with green letter. The quencher (black balloon) and Texas red fluorophore (red balloon) are shown.

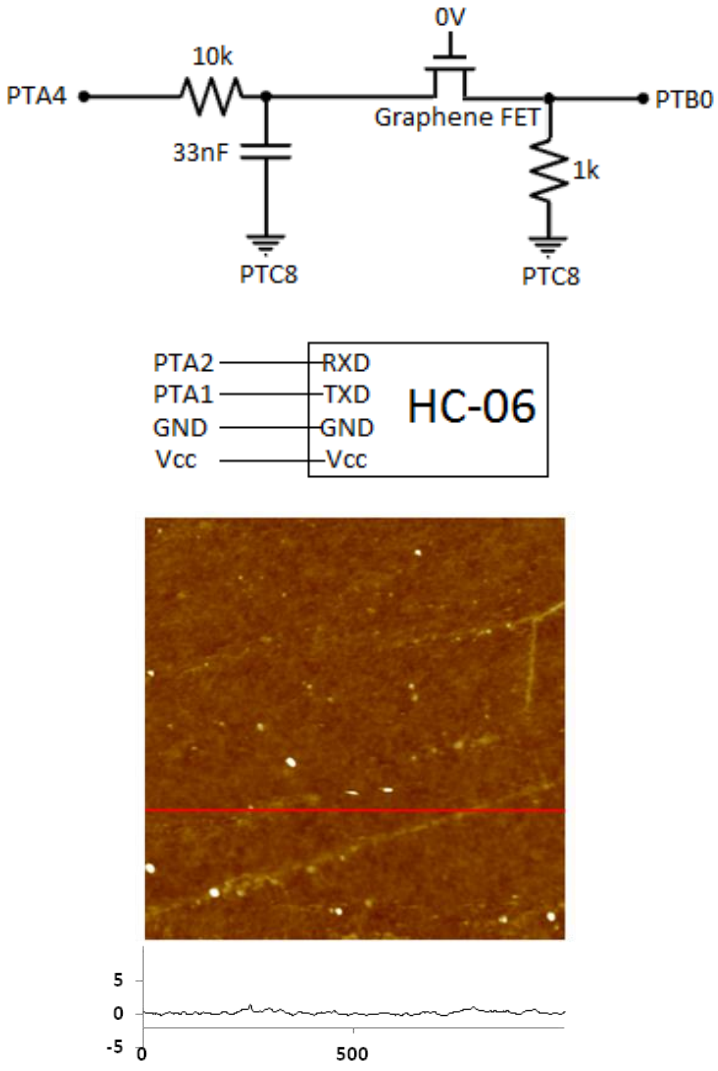

Figure S2. AFM images of graphene transistor surface without the DNA sensor in air. The graphene surface is mostly flat with some wrinkles. Surface height profiles at the red line are plotted at the bottom of each image. Unit is nm.

Figure S3. Low-level circuit design.

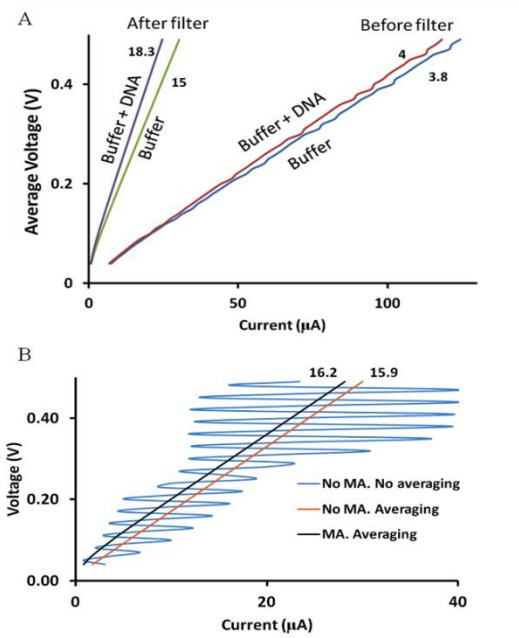

Figure S4. I-V graphs depend on various filter conditions. (A) I-V graphs comparing change in resistance when DNA was added, and data collected before and after low-pass filtering was performed. Before a low-pass filter, similar resistance values (3.8 and 4 kΩ) was observed. This is due to the nature of PWM signals, subjecting the DNA to 3.3 V regardless of the 'analog' signal generated. Significant change in resistance values from 18.3 kΩ to 15 kΩ was measured after passing the PWM signal through a low-pass filter. This implies that a voltage of 3.3 V caused DNA to detach from the graphene surface. Filtering is to ensure accurate measurement. RC low-pass filter with τ = 330 μs was used. (B) I-V curve of 5k resistor was recorded before and after applying a 2nd order Moving-Average (MA) filter. Filtering techniques improve measurement accuracy. Final device design has implemented both averaging and the Moving-Average filter. Standard deviation is calculated as an estimator of measurement noise. Device measured a total resistance of 16.2 kΩ and 15.9 kΩ, while 5.2 kΩ and 4.9 kΩ was detected by removing the resistance contributions from the low-pass filter. The numbers beside the lines represent resistance in kΩ.

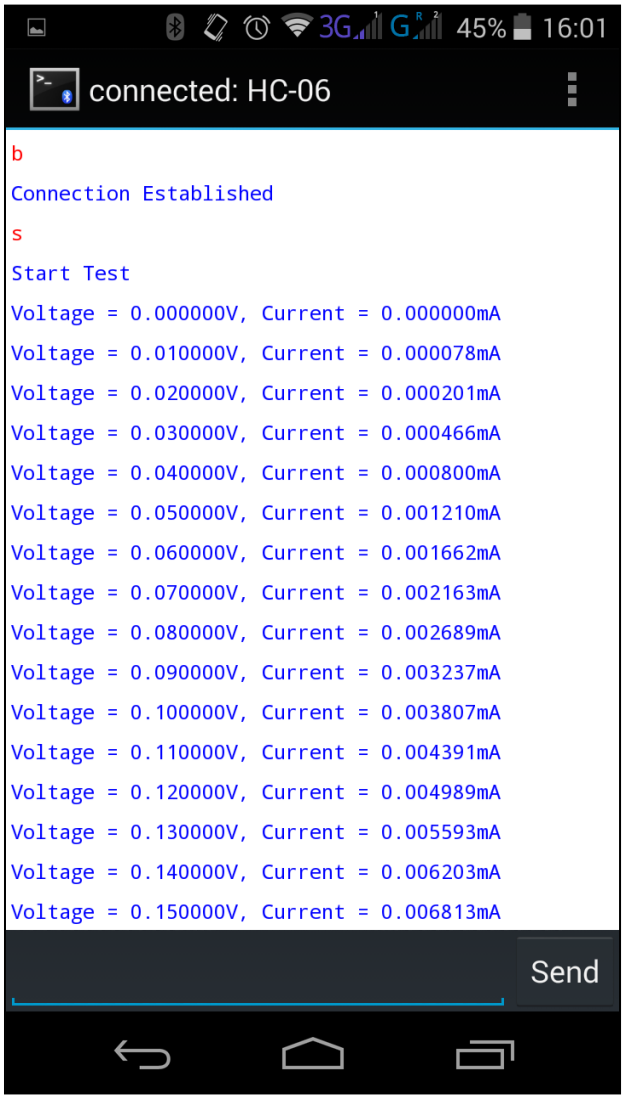

Figure S5. A screenshot of Bluetooth terminal shows data received by the Smartphone during a test demonstrating communication between device and phone.

Table S1. DNA sequences used in experiments.

	Sequence
N	GGGTGTTAGAGATTGATAGAGCGGCC-NH_2-TTATTCCAGTCTTTTAAAATTCTATTATTA
W	TAATAATAIAATTTTAAAAIACTIITTTTTTTTTTTCTCTATCAATCTCTAACACCC
Perfect match DNA	TAATAATAGAATTTTAAAAGACTGGAATAA
Single mismatch DNA	TAATAATAGAATGTTAAAAGACTGGAATAA
Random DNA for the background test	CTAGTCCGTAACACCCAGTCACACACATTGAT

Table S2. Sequences used in the heat map.

	5'-3'
Target strand	TAATAATAGAATTTTAAAAGACTGGAATAA
Normal strand	GGGTGTTAGAGATTGATAGAGCGGCCTTATTCCAGTCTTTTAAAATTCTATTATTA-**Q**
Weak strand	**FLUO**-TAATAATA**I**AATTTTAAAA**I**ACT**II**TTTTTTTTTTTCTCTATCAATCTCTAACACCC
A mismatch on T	TAATAATAGAA**A**TTTAAAAGACTGGAATAA
G mismatch on T	TAATAATAGAA**G**TTTAAAAGACTGGAATAA
C mismatch on T	TAATAATAGAA**C**TTTAAAAGACTGGAATAA
T mismatch on N	GGGTGTTAGAGATTGATAGAGCGGCCTTATTCCAGTCTTTTAAA**T**TTCTATTATTA-**Q**
G mismatch on N	GGGTGTTAGAGATTGATAGAGCGGCCTTATTCCAGTCTTTTAAA**G**TTCTATTATTA-**Q**
C mismatch on N	GGGTGTTAGAGATTGATAGAGCGGCCTTATTCCAGTCTTTTAAA**C**TTCTATTATTA-**Q**

Table S3. Free energy between N/T strands.

	Free energy using NUPACK (kcal/mol)				
		N strand			
		A	G	C	T
T strand	A	-29.96	-29.02	-27.53	-31.06
	G	-29.02	-29.44	-31.90	-28.40
	C	-27.53	-31.90	-26.52	-27.81
	T	-31.06	-28.40	-27.81	-27.80

EXPERIMENTAL PROCEDURES

Materials

Conducting silver paste was obtained from Sigma Aldrich (Saint Louis, MO). Chemical vapor deposition (CVD) grown graphene was purchased from ACS material (Medford, MA) and Graphenesquare (Korea). Graphene for some experiments was grown by CVD in Dr. A.T. Charlie Johnson's lab by the established method. Silicone rubber was purchased from Dow Corning (Midland, MI). PBS solution was purchased from Thermo Fisher Scientific (Waltham, MA). Poly (methyl methacrylate) (PMMA) was purchased from MicroChem (Westborough, MA). DNA gels were purchased from Lonza (Walkersville, MD). Ultrapure water was obtained from a Millipore A10 water purification system and had a resistance of 18.2 MΩ. The FRDM KL-25Z microcontroller board was purchased from Freescale Semiconductor, Inc. A Bluetooth HC-06 module was purchased from Guangzhou HC Information Technology Co., Ltd. All DNA oligonucleotides were purchased from IDT (Coralville, IA) and all DNA sequences are listed in supporting materials (Table S1).

Fluorescence Test

The fluorescence quencher tagged normal strand (N) and Texas red labeled weak strand (W) were mixed in 1:1 ratio in PBS buffer. The N and W strand annealing process was performed for 4 h using PCR machine from 20 ˚C to 90 ˚C and cooled to 4 ˚C. The hybridization kinetic of perfect match strand and single mismatch strand with probe (DS) prepared using N and W strand was performed by exciting sample at 590 nm and emission recorded at 620 nm using Tecan Infinite 200 M plate-reading spectrometer at 27 ± 1.5 °C. To, perform each experiment 20 nM (20 nM DNA

tweezers; 20 and 100 nM target (T)) was used in black 96-well plates. Clear microplate sealing films were applied over the sample wells to avoid evaporation.

Generation of Wireless Signal

The FRDM-KL25Z microcontroller board can generate a digital approximation of an analog signal using pulse-width-modulation (PWM). Typically, PWM signals give a relatively good representation of an analog signal because most electronic appliances do not react to relatively small voltage changes. However, because of the electrolysis of aqueous solutions, the graphene system is sensitive to significant voltage changes. The microcontroller board creates the PWM signal by switching the voltage digitally (between only two modes) off (0 V) and on (3.3 V) producing an analog signal determined by the time averaging 0 V and 3.3 V over each period interval. For example, to produce a PWM analog signal of 1 V, the microcontroller board generates a signal of 0 V for 70 % of the time and 3.3 V signal for 30 % of the time. The nature of PWM signals can therefore cause the DNA-chip to experience a voltage of 3.3V regardless of how small the 'analog' signal generated by the microcontroller board. This bias voltage can cause electrolysis of the aqueous electrolytes and its effect on the system is evident in I-V plot of the system before low-pass filtering (7A). The effect from the 3.3 V spikes was removed from the system by implementing a simple 1st order Resistance-Capacitor (RC) low-pass filter with the shown configuration used [37]. The RC filter is constructed with a 10 kΩ resistor and a 33 nF capacitor. Its smoothening effect on the PWM signal is illustrated in Fig S5 where filtering reduced the peak voltage experienced by the graphene FET to 0.14 V instead of the original 3.3 V.

2nd Order Moving Average Filter

$$V(t) = \frac{[V(t) + V(t-1) + V(t-2)]}{3} \qquad (1)$$

Post Measurement Processing Algorithm and Simulation:

The operation of this algorithm is simulated using the 25 accurate measurements made through the course of this research.

$$Noise = \frac{Device\ measurement}{Accurate\ measurement}$$

$$E(Noise)_{new} = \frac{\left[\left(E(Noise)_{previous} * (Data\ Points - 1)\right) + Noise\right]}{Data\ Points}$$

Measurement noise is obtained and removed from device measurements for subsequent measurements.

$$Estimated\ measurement = \frac{device\ measurement}{E(Noise)}$$

Simulation was conducted using 2000 – 6000 Ω resistance values. The values follow a Gaussian distributed to simulate measurements from human populations. Based on all the measurements done during this research, noise of -2.2 % to 10.91 % was added to these resistances. Measurement noise is assumed to be uniformly distributed.

Heat map experimental details:

1) Quencher (BHQ2) tagged N strand (or mismatched N strands), and Texas red-labeled W strand was mixed in a ratio of 1:1 in 1× PBS solution.
2) The mixture was annealed at 95℃ for 10 min and cooled to 4℃ for 4 h for hybridization.
3) Real-time fluorescence measurements were performed on a Synergy H1 Hybrid Multi-Mode Reader (BioTek) after adding T strand (or mismatched T strand). Excitation and emission of

Texas red were observed at 590 and 620 nm, respectively with the temperature controlled at 27℃ (For each base combination, fluorescence was measured in triplicates).

4) 100 μL sample volume with a device concentration of 20 nM (20 nM DNA tweezers and 100 nM T strand) was mixed in a black 96-well plate, and the real-time fluorescence was monitored for 6 hours. The heat map was illustrated with the average fluorescence data of each base combination at 6-hour time point using the HemI heatmap illustrator.

NUPACK Analysis:

The signal difference for different base combinations revealed that the DNA tweezers with the original N strand showed higher selectivity towards the perfect match T strand.

We analyzed the free energy of N/T strands with different base combinations using NUPACK (see Table S3, perfect match labeled in bold). By comparing experimental results with theoretical data, we conclude that the observed fluorescence intensity is generally in positive correlation with the bonding strength between N and T.

Fabrication of Graphene FET Chip:

The graphene film was cut into ~ 2 mm × 7 mm size with scissors. Graphene films were obtained from the maker prepared on thin copper substrates. The graphene was on the both side of the copper foil, and only the top side was used for FET fabrication. To separate the graphene from the copper substrate, PMMA was spin-coated on the top (carbon) surface of graphene/copper substrate to protect the graphene while the copper bottom was etched away. The back side of graphene was removed by oxygen plasma etching. Copper was etched by floating on 0.1 M of ammonium persulfate for about 5 hours and rinsed in deionized (DI) water overnight. PMMA acted as a supporting layer to the graphene once the copper was removed. The graphene supported by PMMA was then transferred onto a silicon dioxide coated wafer followed by

removal of the PMMA layer with acetone at 60 ˚C for 1 hour. The sample was then annealed at 300 ˚C for 2 hours under hydrogen/argon atmosphere. To fabricate a transistor, conducting silver paste was used as source and drain electrodes at two ends of the graphene. Silicone rubber was then applied to insulate source and drain electrodes from liquid and used as a solution reservoir.

Immobilization of DNA tweezers:

Graphene was treated for 1 h using Pyrenebutanoic acid succinimidyl ester (PASE) (5 mM) dissolved in dimethylformamide (DMF) and rinsed with DMF as well as DI water. The amino modified DNA tweezers probe conjugation on PASE modified graphene surface was performed. After 2 h of incubation at room temperature DNA tweezers functionalized graphene surface was rinsed using 1× PBS. The unsaturated amino group on PASE was treated using ethanolamine solution and further rinsed by using 1× PBS buffer.

Visualization of DNA and Graphene Surface:

Multimode AFM equipped with a Nanoscope V controller (Bruker) was used to acquire DNA topographic images on graphene surface. The imaging was performed in air as well as fluid using silicon cantilevers with a spring constant of 42 N/m (PPP-NCHR; Nanosensor), and silicon nitride cantilevers with spring constants of 0.08 N/m (OMCLTR400; Olympus), respectively. The AFM images were acquired in both tapping mode and peak force-tapping mode. Images were analyzed using Nanoscope Analysis 1.50.

Strand Displacement on the Chip:

The strand displacement reaction was performed using perfect match and single mismatch T in the range of 100 pM-100 nM in 50 µL reaction volume. The reaction sample were incubated for overnight in the reservoir on the graphene FET chip and unbound T was rinsed using PBS solution.

Electrical Measurements:

A semiconductor parameter analyzer equipped with a probe station was used to measure the I-V curves and resistance. To apply gate voltage (Vg) through 12.5 mM $MgCl_2$/30 mM Tris buffer solution, silver wire was used and Vg was swept from −0.5 to 1 V. Drain–source current (Ids) was measured at an assigned Vds. resistance was calculated for that plot. After measurements on the probe station, resistance measurements were simultaneously performed using a standard digital multi-meter (DMM) (Fluke 175 True RMS multimeter) and a smartphone connected via the wireless system. The source voltage was swept between 0, and 0.5 V. Current values were converted into a voltage signal by introducing a pull-up resistor of 1 kΩ. Thus the resulting potential difference (V=IR) will be 1000 times the current value measured by the device. This data was sent to a smartphone for further data processing. Voltage values were plotted against their respective current values with a trend line. The gradient of the trend line represents the resistance of the entire circuit with the resistance of device obtained by subtracting the resistance of the other components, 1 kΩ pull-up resistor and 10 kΩ filtering resistor.

Wireless Communication:

Communication was established through the HC-06 Bluetooth module. The HC-06 module communicated with the FRDM-KL25Z using the Serial RS-232 communication protocol while pairing with user electronics, phones, and computers, over the Bluetooth communication standard (Fig. 2). The HC-06 module supports Bluetooth communication baud rates up to 115200 bps, a baud rate of 9600 bps was used for communication with the smartphone device.