Supporting Information

Detection of microRNA SNPs with ultrahigh specificity by using reduced graphene oxide-assisted rolling circle amplification

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I. Experimental section

Materials and Apparatus. All oligonucleotides used in this research (Table S1) were synthesized by TaKaRa Biotechnology Co., Ltd. (Dalian, China) with HPLC purification. Deoxynucleotide solution mixture (dNTPs) and 6 × loading buffer were also obtained from the TaKaRa Biotechnology. T4 DNA Ligase, Bst DNA polymerase large fragment, and their corresponding buffer were purchased from NEB Inc. SanPrep Column DNA Gel Extraction Kit was purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). SYBR Green I was from Solarbio Technology Co., Ltd. Low-profile 0.2 ml 8-tube strips for PCR were obtained from Bio-Rad Laboratories, Inc. GO and rGO were from Sigma-Aldrich and XFNANO Materials Tech Co., Ltd., respectively.

Isothermal amplification reactions were performed on a ThermoStat plus (Eppendorf China Ltd.). Gel electrophoresis was conducted using a Wide Mini-Sub Cell GT Cell and a Gel Doc XR Imaging System (Bio-Rad, US). Real-time Fluorescent signals were recorded at a constant temperature of 65 °C with an interval of 1 min using a CFX96TMTouch Real-Time PCR Detection System (Bio-Rad, US). The products of RCA were also observed directly by an ex situ Agilent 5500 atomic force microscope system (Santa Clara, CA). Fluorescence intensity was measured using a SpectraMax M3 Multi-Mode Microplate Reader with SoftMax Pro software (Molecular Devices,Sunnyvale, CA, USA). Fourier transform infrared spectra were obtained using a VERTEX70 Fourier infrared spectrometer (Bruker Hongkong Limited, GER).

rGO-assisted RCA reaction. RGO-assisted RCA reaction was conducted in a 25 μL reaction mixture. First, different concentrations of miRNA (either the targets or the mutant variants) working as the template were incubated with rGO for 10min. Then, 1 × polymerase buffer, 100 nM circular probe, 400 μM dNTPs and 8 U Bst DNA polymerase were added. The reaction was allowed to proceed at 65 °C for 30 min and terminate by deactivating the Bst at 95 °C for 10 min.

Gel electrophoresis analysis. Agarose gel electrophoresis was performed for the characterization of the products of rGO-assisted RCA as well as conventional RCA. 5 μL of the products of RCA together with 1 μL 6 × loading buffer was loaded onto a 2% non-denaturing agarose gel. The electrophoresis experiments were carried out in 1 × Tris-acetate-EDTA (TAE) at 80 V for 30 min. Subsequently, the gel was stained with SYBR Green I for 30 min. The imaging of the gel was performed using a Gel Doc XR Imaging System.

AFM characterization. The products of rGO-assisted RCA were also characterized under AFM. The amplified products were firstly separated using gel electrophoresis to exclude the interference of enzymes, dNTPs, and etc. DNA fragments with a length of 2000~3000 bp were extracted from the agarose gel by the SanPrep Column DNA Gel Extraction Kit. Subsequently, the DNA fragments dissolved in 10 mM $MgCl_2$ were spread onto a fresh mica slice (Yunfeng Co. Ltd., China) and allowed to incubate for 20 min under room temperature. The mica slice was then rinsed with double distilled water and blown completely dried with nitrogen gas. Morphological analysis of the RCA products on the mica slice was achieved by using an ex situ Agilent 5500 AFM system. Samples were imaged at a scan rate of 0.5-1 Hz in a tapping mode. AFM tips with resonant frequency in a range 160-260 kHz were used. Images were acquired at a resolution of 512×512 pixels.

Real-time PCR detection. Real-time fluorescent signals (SYBR Green I, $\lambda_{excitation}$: 470 nm, $\lambda_{emission}$: 540 nm) of the rGO-assisted RCA system were recorded at 65 °C with an interval of 1 min by using a CFX96TM Touch Real-Time PCR Detection System. RGO-assisted RCA reaction was conducted as described above with the only difference of the addition of 10 × SYBR Green I.

Fluorescent analysis. Fluorescence spectra were recorded for quantitative analysis purpose. 25 μL of rGO-assisted RCA products and 5 μL of 20 × SYBR Green I were added into 70 μL of double distilled water, followed by incubation at room temperature for 10 min. Fluorescence spectra were obtained using a SpectraMax M3 Multi-Mode Microplate Reader. The excitation wavelength was fixed at 470 nm; and the emission spectra were measured in the range from 500 nm to 650 nm. The fluorescence intensity of the peak was recorded at the emission wavelength of 540 nm.

Fourier transform infrared spectroscopic characterization. Interaction between rGO and components of RCA was investigated using FT-IR. First, certain amounts of the components of RCA each (miRNA template, circular probe, dNTPs, or Bst DNA polymerase) were allowed to incubate with rGO for 10min. Then, the mixture was centrifuged at 13000 rpm for 15min and the precipitate was collected and dried under an infrared lamp for 30min. After that, infrared spectra were investigated using a VERTEX70 Fourier infrared spectrometer.

II. Supporting Figures

Table S1 Sequences of nucleic acids.

Name	Sequences
circular probe I	5'-CAC GCG ATC CGC ***CAC AGG TTA AAG GGT CTC AGG GA***C ACC CTC CAA CCA CCA AGG CAA TGT ACA CGA ATT CGC CGA ACG-3'
circular probe II	5'- CAC GCG ATC CGC ***AAC TAT ACA ACC TAC TAC CTC A***AC ACC CTC CAA CCA CCA AGG CAA TGT ACA CGA ATT CGC CGA ACG-3'
linear probe	5'- ACC AAG GCA ATG TAC ACG AAT TC-3'
miR-125a	5'-UCC CUG AGA CCC UUU AAC CUG UG-3'
miR-125a-U_8	5'-UCC CUG A***U***A CCC UUU AAC CUG UG-3'
miR-125a-U_8G_{16}	5'-UCC CUG A***U***A CCC UUU ***G***AC CUG UG-3'
miR-125a-A_{23}	5'-UCC CUG AGA CCC UUU AAC CUG U***A***-3'
let-7a	5'-UGA GGU AGU AGG UUG UAU AGU U-3'
let-7c	5'-UGA GGU AGU AGG UUG UAU ***G***GU U-3'
let-7b	5'-UGA GGU AGU AGG UUG U***G***U ***G***GU U-3'
let-7d	5'-***A***GA GGU AGU AGG UUG ***C***AU AGU U-3'

Note: The italic and underlined bases of the circular probe I and II are complementary with the target miR-125a and let-7a, respectively. The italic and underlined bases of the variants of the target miRNA are the mutant sites. The double underlined bases of the circular probe is the same as the sequence of the linear probe.

Table S2. Ability of some P-RCA methods for the discrimination of single-base mutation.

Name	Target sequence (5'-3')	1-base Mutant	Discrimination
RNA: Let-7a	UGA GGU AGU AGG UUG UAU AGU U	$-G_{19}$	6.2:1[14a]
RNA: Let-7a	UGA GGU AGU AGG UUG UAU AGU U	$-G_{19}$	ca. 8:1[18a]
DNA: β-thalassemia gene	ATC AGG GCT GGG CAT AAA AGT CAG GGC AGA GTA	$-G_{17}$	ca. 10:1[18b]
DNA: human *p53* gene	GCG TGT TTG TGC CTG TCC TGG GAG AGA CCG GCG CAC AGA GGA AGA G	$-T_{29}$	ca. 6.5:1[18c]
DNA: human *p53* gene	TCA TCA CAC TGG AAG ACT C	$-A_{17}$	ca. 3:1[18d]
DNA: *BRCA*1 gene	CAC AGT GTC CTT TAT GTA AGA ATG ATA TAA C	$-G_{15}$	19:1[18e]

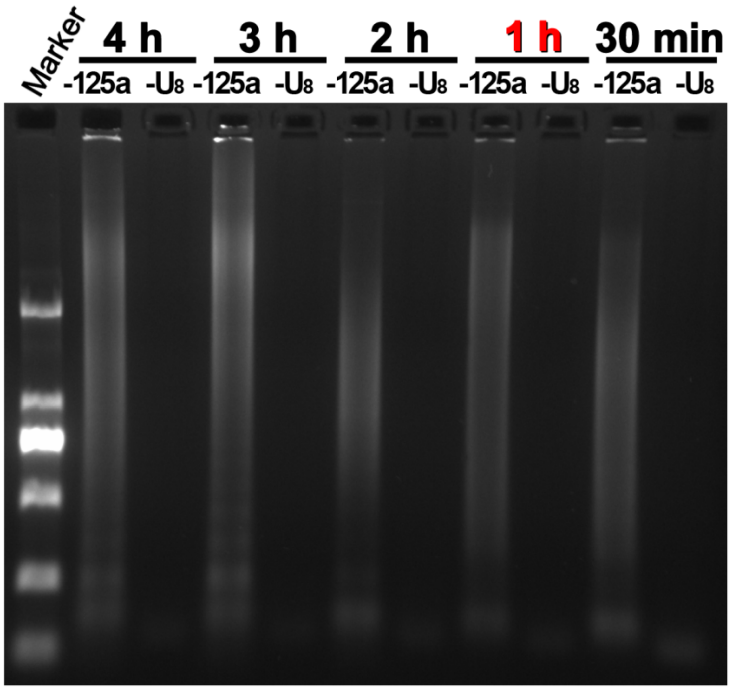

Fig. S1 Agarose gel electrophoresis patterns of rGO-assisted RCA products using miR-125a (1 μM) and miR-125a-U_8 (1 μM) as the template. RCA times varied from 30 min to 4 h for different lanes.

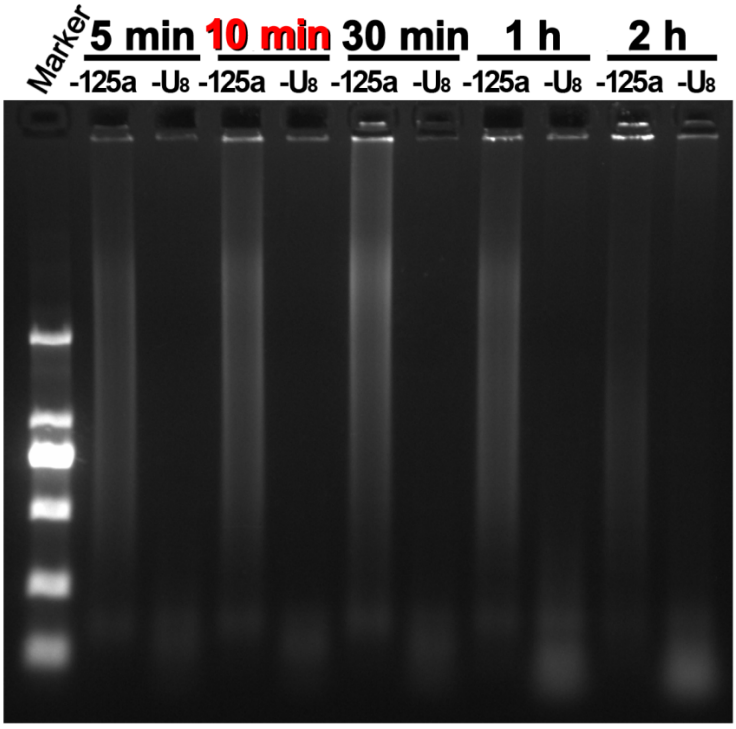

Fig. S2 Agarose gel electrophoresis patterns of rGO-assisted RCA products using miR-125a (1 μM) and miR-125a-U_8 (1 μM) as the template. The miRNA templates were pre-incubated with rGO for different times from 5 min to 2 h before the proceeding of RCA.

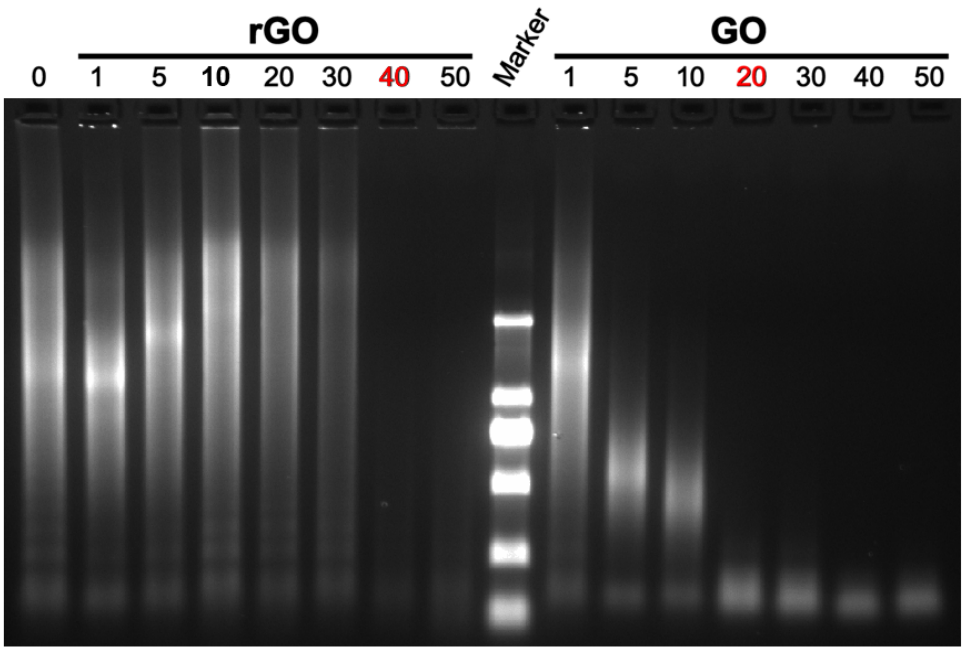

Fig. S3 Agarose gel electrophoresis patterns of RCA products in the presence of rGO or GO. MiR-125a (1 μM) works as the template. The numbers 1 to 50 indicate the concentrations of rGO/GO with a unit μg/ml.

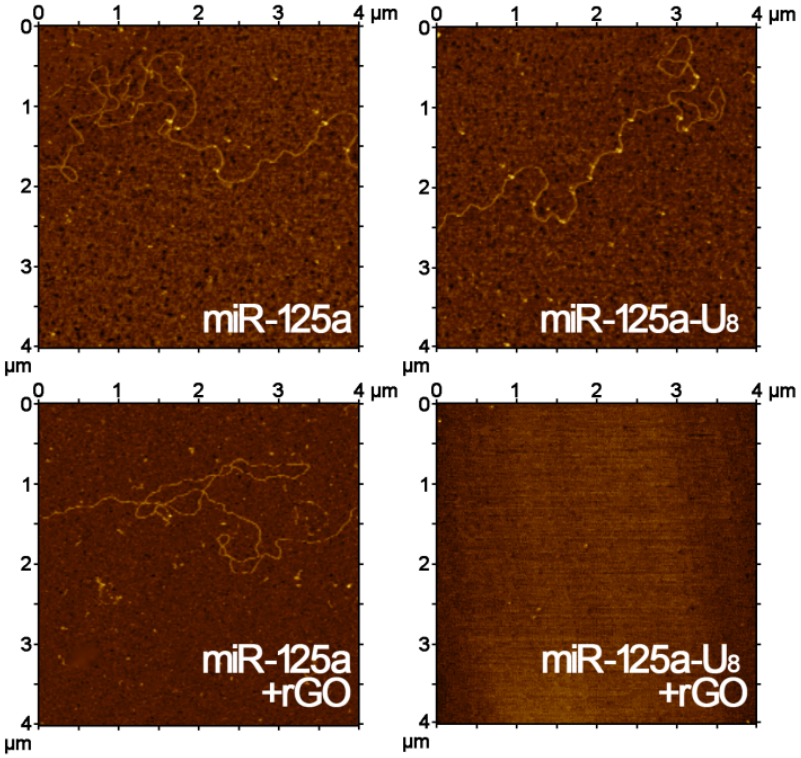

Fig. S4 AFM images of RCA products, in the presence (bottom) or absence (top) of rGO, and using miR-125a (left) or miR-125a-U8 (right) as the template.

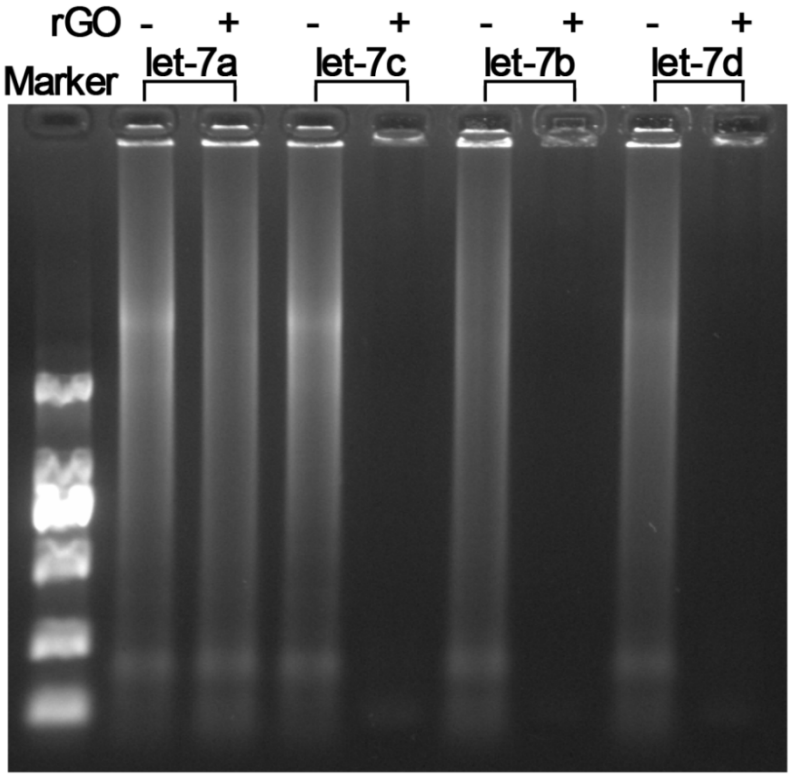

Fig. S5 Agarose gel electrophoresis patterns of rGO-assisted RCA products using Let-7a (1 μM) and Let-7c (1 μM) as the template. The symbols "+" and "-" indicate that rGO is present and absent, respectively.

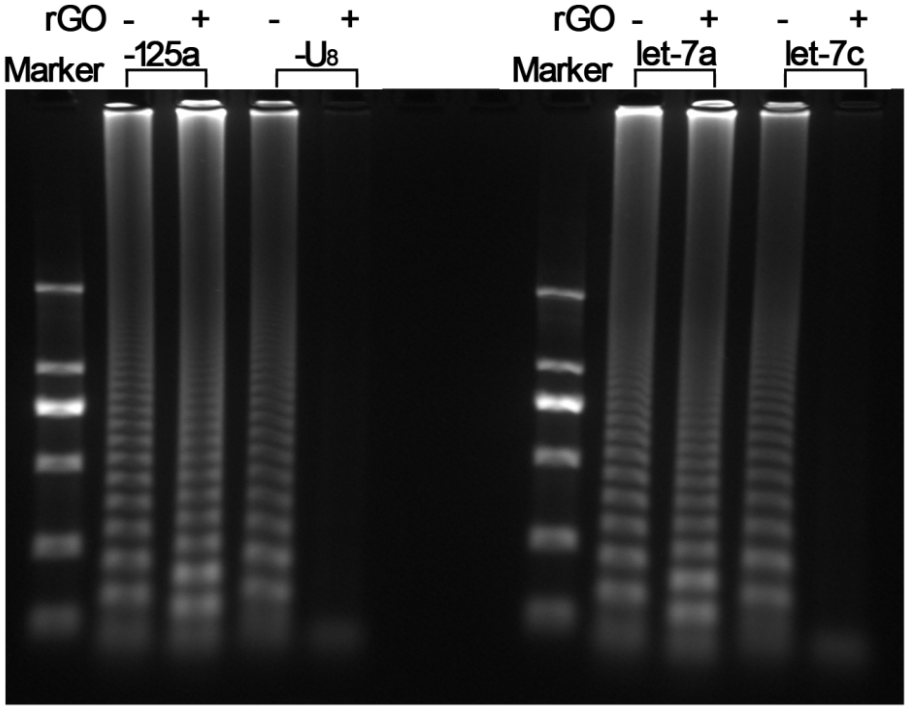

Fig. S6 Comparison of the rGO-assisted discrimination of SNPs of miR-125 and Let-7a. Others same as in Fig. S3.

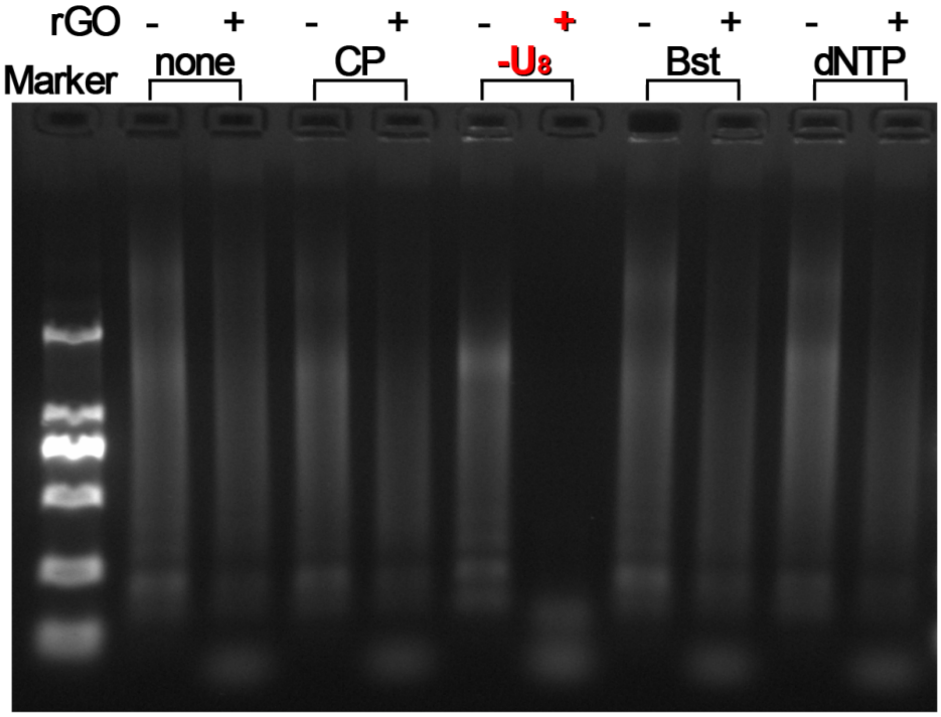

Fig. S7 Agarose gel electrophoresis patterns of rGO-assisted RCA products using miR-125a-U_8 (1 μM) as the template. Before the proceeding of RCA, rGO was pre-incubated with nothing, or circular probe (abbr. as CP), or miR-125a-U_8 (abbr. -U_8), or Bst DNA polymerase, or dNTPs. The symbols "+" and "-" indicate that rGO is present and absent, respectively.

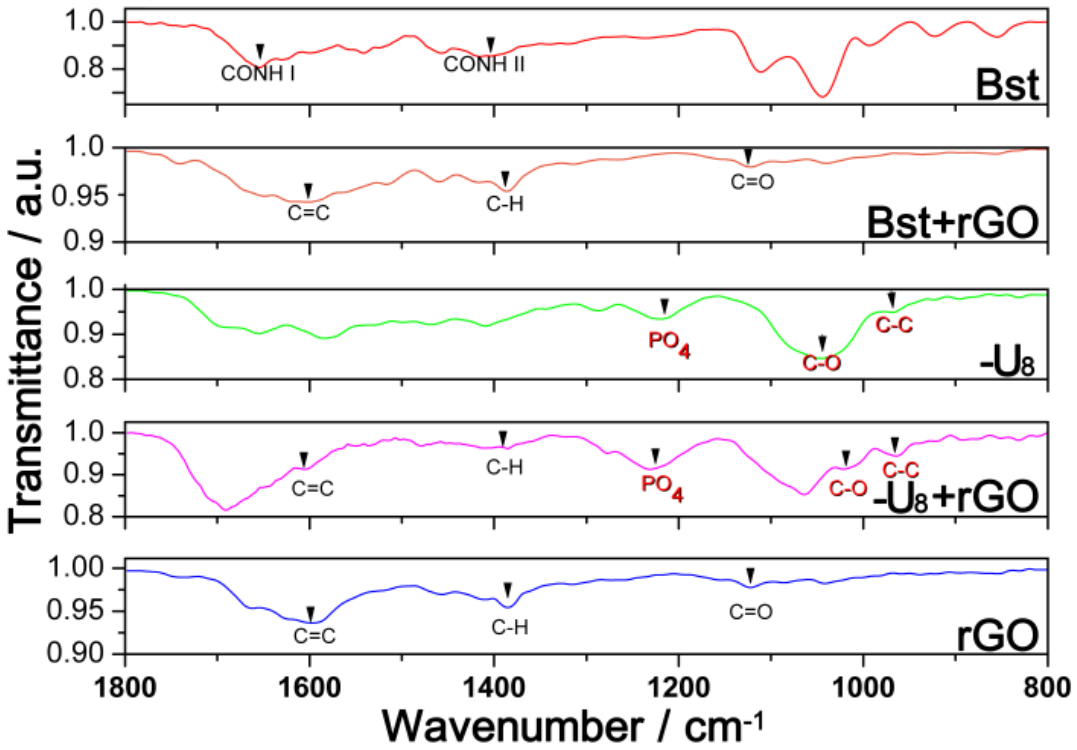

Fig. S8 FT-IR spectra of Bst DNA polymerase (abbr. Bst), miR-125a-U8 (abbr. -U8), rGO, rGO pretreated with Bst, and rGO pretreated with -U8.

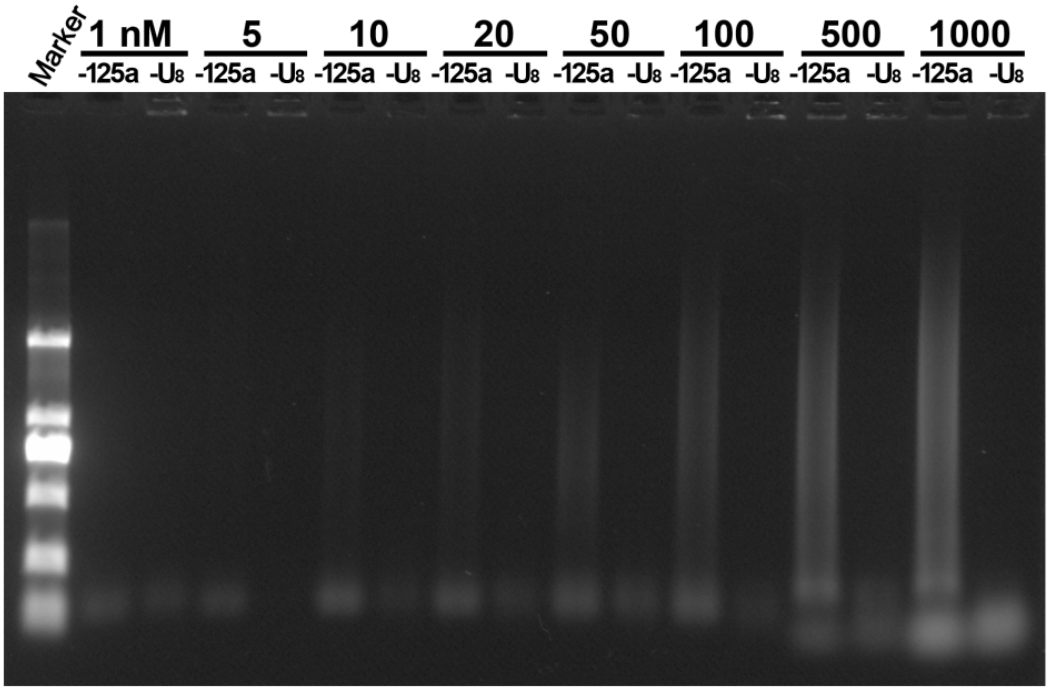

Fig. S9 Agarose gel electrophoresis patterns of rGO-assisted RCA products using miR-125a and miR-125a-U_8 as the template. Concentration of the miRNAs varied from 1 nM to 1000 nM.

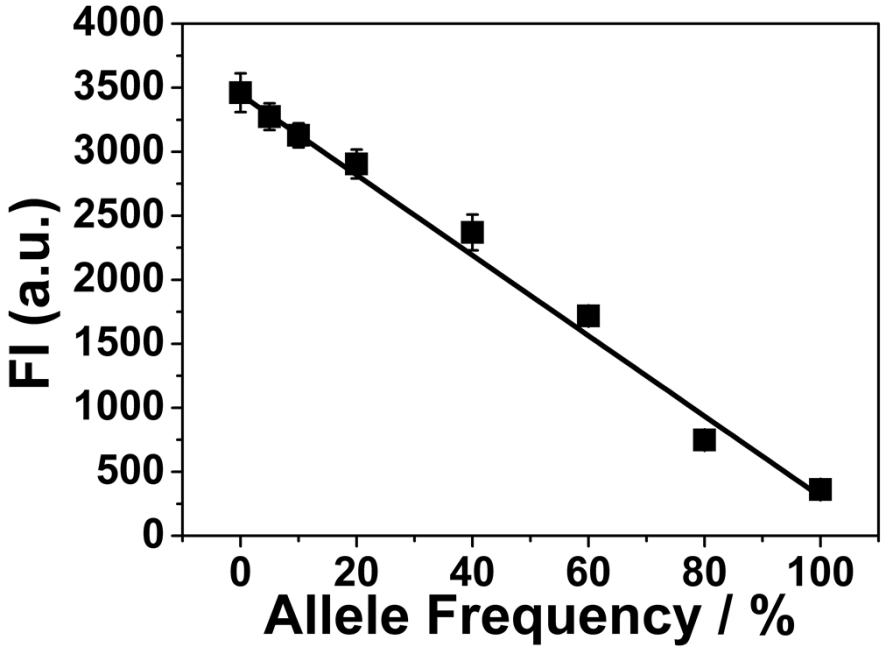

Fig. S10 Peak fluorescence intensities of rGO-assisted RCA products under different allele frequencies miR-125a-U8/(miR-125a + miR-125a-U8): 0, 5%, 10%, 20%, 40%, 60%, 80%, and 100%. The total miRNA (including miR-125a and miR-125a-U8) was 100 fM.

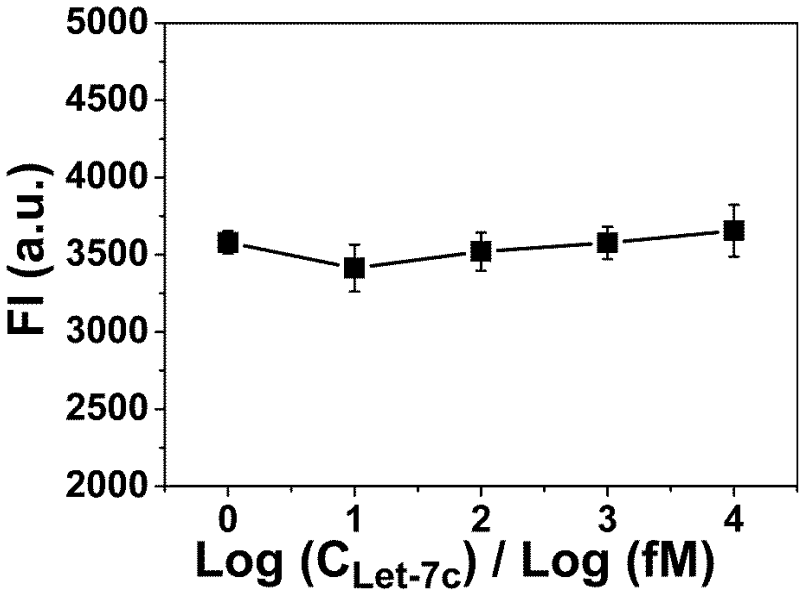

Fig. S11 Peak fluorescence intensities of rGO-assisted RCA products using 100 fM Let-7a as the template. Different concentrations of Let-7c (from 1 fM to 10 pM) working as interference were also presented with the target Let-7a.