Supporting Information

Mass Spectrometry Genotyping of Human Papillomavirus Based on High-Efficiency Selective Enrichment of Nanoparticles

Li Zhu[†,§,] Lihui Yin[§], JinJuan Xue[‡], Zhihua Wang[*,†], Zongxiu Nie[*,‡]

[†] State Key Laboratory of Chemical Resource Engineering, and Beijing Advanced Innovation Center for Soft Matter Science and Engineering, Beijing University of Chemical Technology, Beijing 100029 China

[‡] Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190 China

[§] National Institutes for Food and Drug Control, Beijing 102629 China,

Corresponding author:

Prof. Zongxiu Nie; E-mail: znie@iccas.ac.cn;

Prof. Zhihua Wang; E-mail: zhwang@mail.buct.edu.cn.

Experimental section

PCR amplification

Liquid-based cytological (LBC) samples were centrifuged at 1500 rpm in an Eppendorf Microfuge 5417C to obtain cells. Then, the target DNAs were extracted and purified to store in 20 ml distilled water at -20℃ before further use. The details of the RFMP assay protocol were described previously. Briefly, The first PCR mixture contained 4.0μL of template DNA and 16 μL of a mixture of 2.5 mM MgCl2, 10 pmol of each primer, 2 mM deoxynucleoside triphosphates (dNTPs), and one U of Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA). The PCR was performed as follows: initial denaturation at 94℃ for 5 min; 38 cycles of denaturation at 94℃ for 15 s, annealing at 60℃ for 15 s, and extension at 72℃ for 30 s, and a final extension at 72℃ for 5 min. The second PCR carried out with 1.0μL of first PCR product in 10×PCR buffer, containing 2.5 mM MgCl2, 12.5 pmol of each primer, 2 mM of each deoxynucleoside triphosphate and 1 unit of Taq DNA polymerase, and ddH_2O were used to make up a deficiency. The PCR was performed as follows: initial denaturation at 94℃ for 5 min; 38 cycles of denaturation at 94℃ for 10 s, annealing at 45℃ for 10 s, and extension at 72℃ for 30 s, and a final extension at 72℃ for 5 min. The second round PCR primer pairs

consisted of an sense primer specific to bases 6584–6603 (5′-GCMCAGGGHCAYAA GGATG AATGG-3′) and an antisense primer specific to bases 6657–6626 (5′-GTACTDCKDGTRGTATCHACMAC GGATG TAACAAA-3′), where underlined bases indicate *FokI* site (a neoschizomer of *BseGI*).

Adsorption of oligonucleotides

The amount of DNA oligonucleotides adsorbed on SP-diamond NPs was obtained by measuring the DNA supernatant before and after treatment using UV-vis spectroscopy and by the calibration curve of the oligonucleotides.

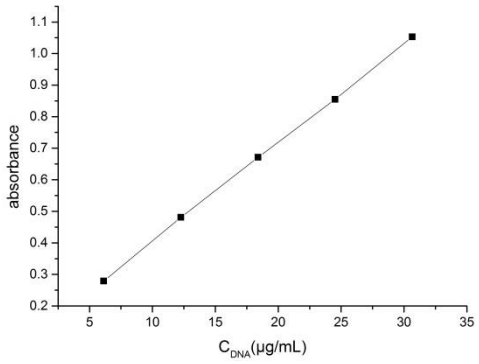

Fig.S-1. Standard curve of d (CAACTATTTGTTA)

The following equation was used.

$$Ae = \frac{(C_0 - C_e)\mathrm{V}}{\mathrm{W}}$$

C_o and C_e are the initial and equilibrium oligonucleotides concentrations(μg/mL) respectively. V is the volume of solution (mL) and W is the weight of the added SP-diamond NPs (g).

We performed spermine to modify 100 nm and 5 nm diamond nanoparticles. We found FT-IR spectrum of 100 nm carboxylated/oxidized nanodiamonds (Fig.S-2 red line) shows the peak at 1780 cm-1, which may contribute to the C=O vibrations of anhydride. Fig.S-2 blue line shows 100nm diamond nanoparticles modified with spermine. The peaks at 1800 cm-1, 1761cm-1 are not obviously, the results indicate the modification of Spermine is incomplete. As we know the amidation reaction is a reaction between a carboxyl group and an amino group. Anhydride does not react with amino groups. FT-IR spectrum of 5nm carboxylated/oxidized nanodiamonds (Fig.S-3 black line) shows the peak at 1724cm-1, which may contribute to the C=O vibrations of carboxylic group.Fig.S-3 blue line indicate spermine totally covers surfaces of 5 nm nanodiamonds.

The supernatant UV absorbance of oligonucleotides at 260 nm before and after pretreated with 100 nm SP-NDs is 1.031 and 0.875 respectively. It shows that the 100 nm SP-NDs have a low enrichment ability of DNA oligonucleotides.

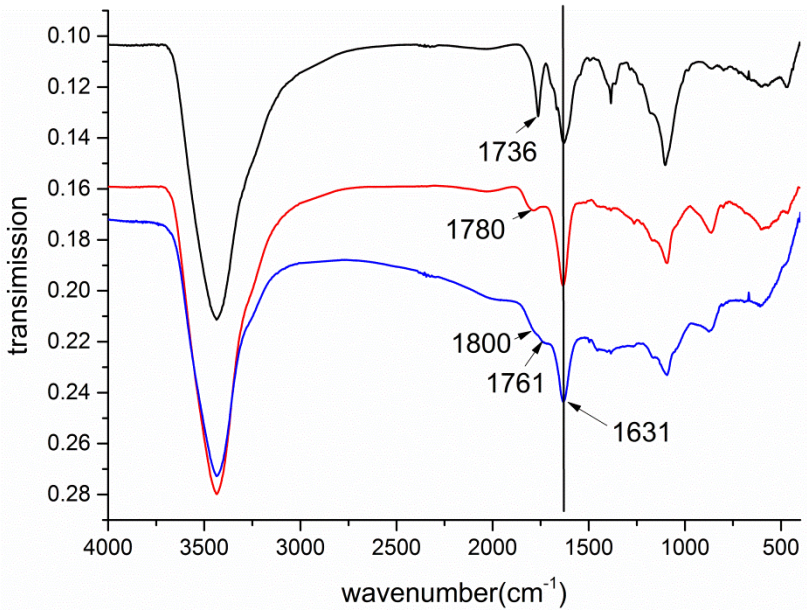

Fig.S-2. Infrared spectra of carboxylated/oxidized diamond NPs (100nm) (red line) , SP-NDs (blue line)and nano-diamond (blank line) (only baseline correction)

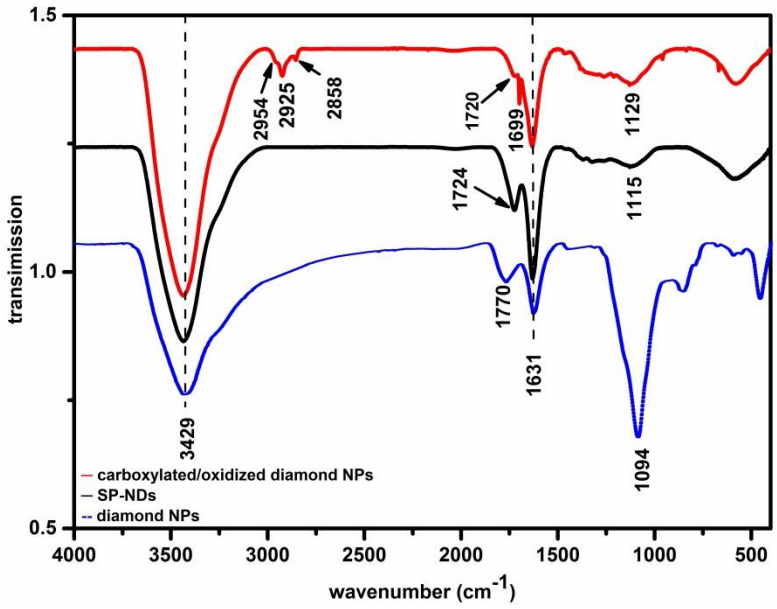

Fig.S-3. Infrared spectra of carboxylated/oxidized diamond NPs (5nm) (black line) , SP-NDs (red line)and nano-diamond (blue line) (only baseline correction)