Electronic Supplementary Information (ESI)

Digitally encoded silica microparticles for multiplexed nucleic acid detection

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Contents:

S1. HPV DNA contained Plasmids

Plasmids containing HPV DNA (complete genomes for HPV types 6, 18, 31, 33, 35, 39, 45, 56, 58, 59, 73, 82 and 83; 1600bp L1 DNA for HPV types 16, 51, 52 ; MY09/MY11 fragment for HPV types 43, 53, 69, 87) were used for evaluation of HPV genotyping.

S2. Multiplexed nested asymmetric PCR amplification

The PCR was performed in an ABI 9700 PCR thermocycler. The reaction mixture (10 μL) contained 1× PCR Buffer for UNG plus, 0.25 unit hot-start Taq DNA polymerase (Takara,R013), 0.2 unit uracil-DNA glycosylase (UNG), 200uM of each deoxynucleoside triphosphate (dATP, dGTP, dCTP), 600uM of deoxyuridine triphosphate (dUTP). Sequences and concentrations of primers mixture used in the multiplexed nested asymmetric PCR amplification were shown in tabel S1. The concentration of plasmids for specificity evaluation and hybridization time optimization was 0.1ng/μL. Before amplification, the mixture was incubated in 25°C for 10 minutes, which was the UNG treatment condition. Then activation of polymerase at 95°C for 2 min(heat inactivation of UNG), followed by 20 cycles of 94°C for 10 s, 55°C for 20 s, and 72°C for 30 s, and 30 cycles of 94°C for 10 s, 50°C for 20 s, and 72°C for 30 s. This was followed by a final extension of 5 minutes at 72°C, and finally cooling to 10°C.

S3. Preparation of carboxylic acid-functionalized silica microparticles

The silica microparticles (5.0×10^6) were amino-functionalized with 3-(2-aminoethyamino)propyl-dimethoxy-methylsilane by adding 95% ethanol/water solution containing 5% (v/v) 3-(2-aminoethyamino)propyl-dimethoxy-methylsilane. After vortexing and sonication for 30 seconds, the mixture was allowed to stir 30minutes at room temperature. The particles were purified by centrifugation and redispersion in ethanol, which was repeated twice. After washing with N.N-dimethyl formamide, the particles were dispensed in 10% (w/v) succinic anhydride that dissolved in N.N-dimethyl formamide. The mixture was left to stir 6 hours at room temperature.

Then the particles were purified by centrifugation and redispersion in ddH_2O, which was repeated three times. The carboxylated microparticles can be purified and resuspended with anhydrous ethanol for storage at 4℃ in the dark.

S4. Coupling of DNA probe to microparticles

The carboxylated particles (5.0 x 10^5) were washed with 0.1 M 2-[N-morpholino] ethanesulfonic acid (MES, pH 4.5) by centrifugation and redispersion, followed by resuspending in 45ul 0.1M MES (pH 4.5). Subsequently, 5ul of 100uM amino-modified DNA probes and 50ul of freshly made 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (200mg/mL in 0.1M MES) were added to the microparticels. Then the mixture was incubated at room temperature in dark under agitation for 30 minutes. The addition of EDC and incubation was repeated. Finally, the microparticles were washed once with 800 μL of 0.02% Tween 20 and once with 800 μL of 0.1% SDS. After washed twice in hybridization solution (5×SSC/0.05% Tween 20), the particles were resuspended in hybridization solution for storage at 4℃.

S5. Quality control assay for newly coupled microparticles

The coupling efficiency of capture probe was assessed by hybridizing a biotin labeled sequence that is complementary to the universal 20-nucleotides (anti-universal sequence) near the 5' termini of the capture probe. Hybridization was performed using 500 of newly coupled microparticles and 10^4 fmol anti-universal sequence in 100 μL of hybridization solution (5 × SSC/0.05% Tween20). The microparticle which has passed the quality control assay and the encoding different from the particle waiting for test, can be used as a positive control. After being incubated for 15- 30minutes at 47°C and shaking at 700 rpm, the microparticles were washed with 200 μL of washing buffer (1×SSC/0.01% Tween 20). Subsequently, the microparticles were resuspended in 50 μL 2ug/ml SA-PE (Life Technologies™) and incubated with shaking at room temperature in the dark for 5-10 minutes. Following being washed twice with washing buffer, the particle was transferred to well plate for imaging. Both bright and fluorescence micrograghs were acquired using Cytation3 Cell Imaging Multi-Mode Reader (Biotek). The decoding and analyzing of the micrograghs were performed using homebrewed software.

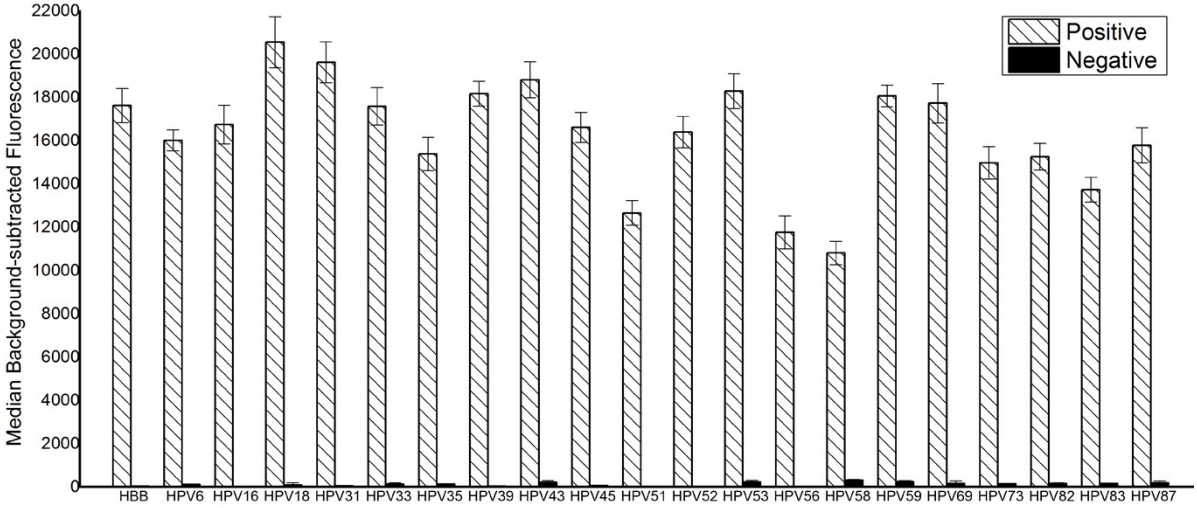

Fig. S1 The coupling efficiency of each microparticles was accessed, showing all the probe-conjugated microparticles can be used.

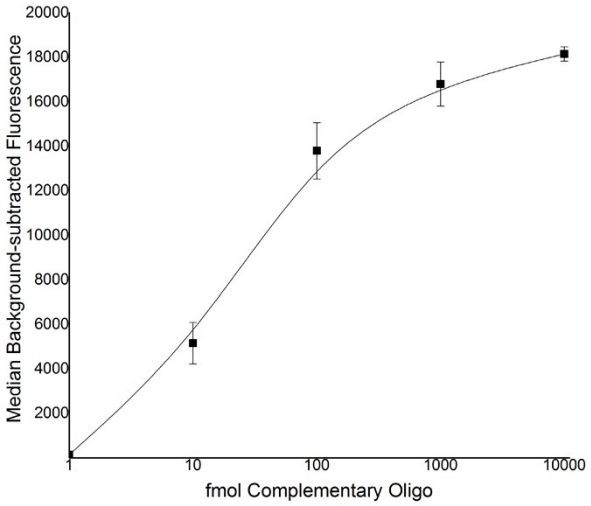

Fig. S2 Detection sensitivity of the particle was investigated by a series of concentrations (1fmol to 10^4 fmol) of the anti-universal sequence.

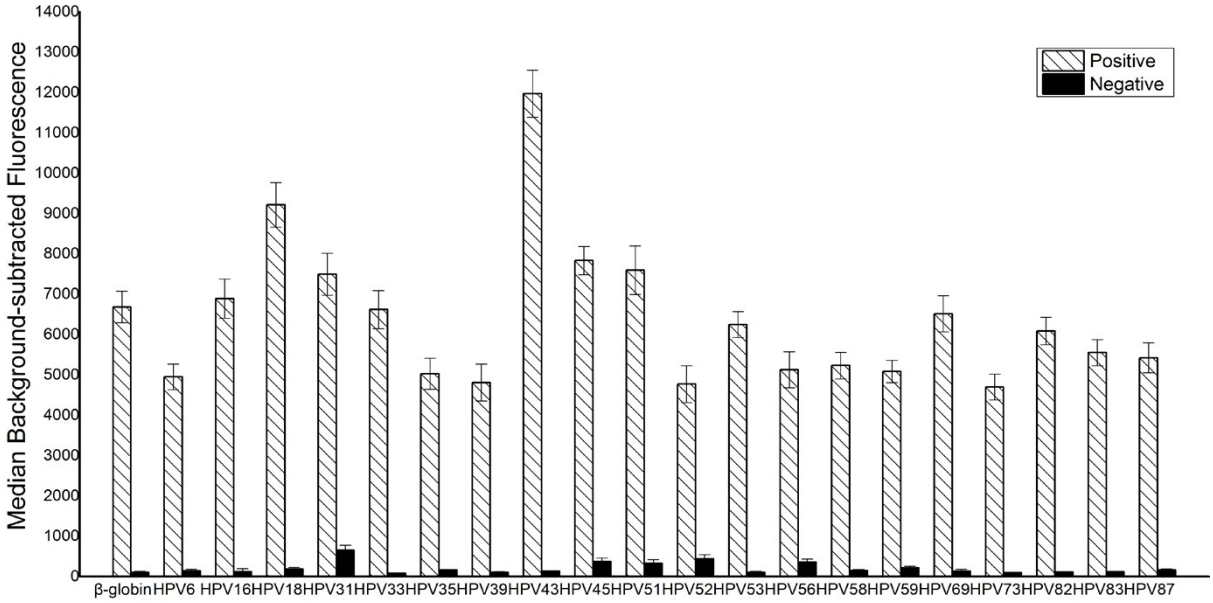

Fig. S3 21 common HPVs types were tested

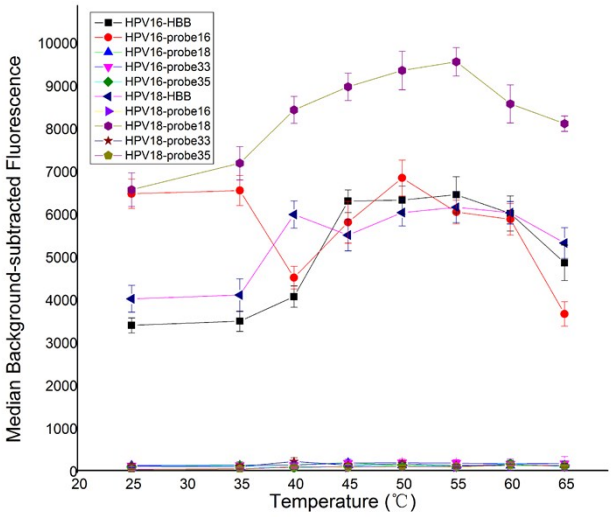

Fig. S4 Optimization of the hybridization temperature with PCR products. The concentration of the HPV plasmids template for PCR was 0.1ng/μL, 1ng/μL of placenta DNA was added to the PCR reaction. The fluorescence intensity of negative assays (data not shown) and cross hybridization were all under 200.

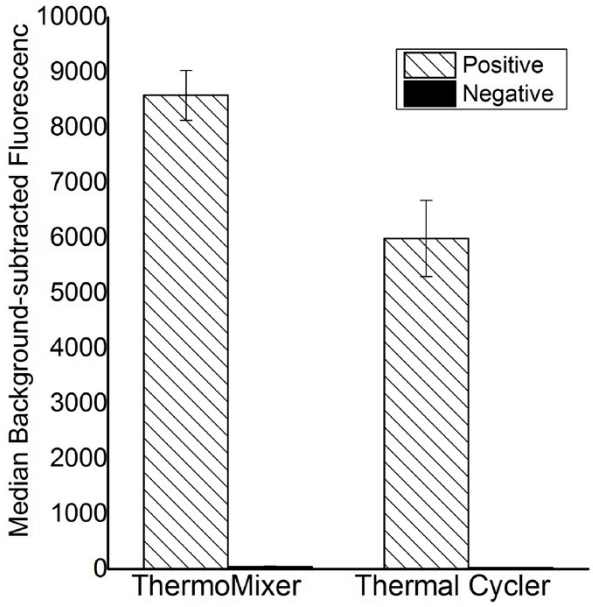

Fig. S5 The contrast of hybridization in Eppendorf ThermoMixer with shaking at 700 rpm (CV=5.2%) and in thermal cycler at static (CV=11.6%). It indicates the hybridization could be performed at static.

	HBB	Probe16	Probe18	Probe31	Probe33	Probe58
ddH_2O	69	138	102	53	48	128
gDNA	6994	181	133	70	125	199
gDNA+HPV16	5115	6529	146	96	105	153
gDNA+HPV18	5460	232	5426	128	169	243
gDNA+HPV31	5744	73	91	7496	60	149
gDNA+HPV33	6185	310	161	101	9071	751
gDNA+HPV58	5812	195	129	161	540	5506

Fig. S6 Five HPVs that most common in China were interrogated. Placenta DNA (containing gDNA) was added to the PCR reaction, it contains β-globin gene that was used as a reference gene in

detection.

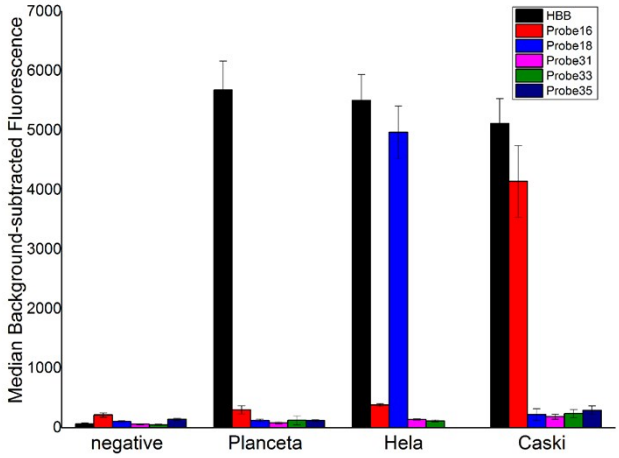

Fig. S7 HPV genetyping in HPV positive cell lines. Showing β-globin was detected in all the biological samples, and HPV18 was contained in Hela cell, HPV16 was contained in the Caski cell.

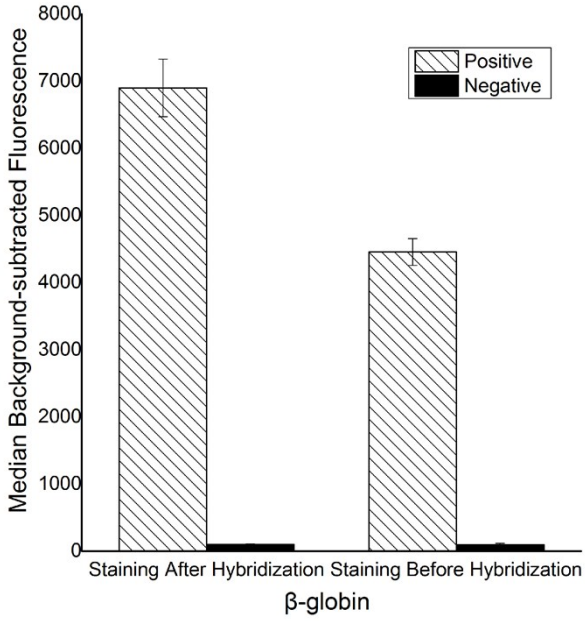

Fig. S8 Compared with staining after hybridization, staining before hybridization is also feasible, even though the fluorescence intensity slightly lower (Perhaps because part of SA-PE has been bound to the biotin on dissociative DNA).

Tabel S1. Sequences of primers

Primer Name	Sequence (5'to3')	Modification	Concentration (nM)	Remarks
MY11	GCM CAG GGW CAT AAY AAT GG	none	200nM	Outer Forward Primer
MY09	CGT CCM ARR GGA WAC TGA TC	none	200nM	Outer Reverse Primer
HMB01	GCG ACC CAA TGC AAA TTG GT	none	50nM	Outer Reverse Primer
Pr-[HPV31-outer-Fw]	GGA TGC AAC GTG CTC AGG GA	none	50nM	Outer Forward Primer
Pr-[HPV31-outer-Rv]	GGA TCT TCC TTG GGC TTT TG	none	50nM	Outer Reverse Primer
Pr-[HPV33-outer-Fw]	CCA TAT TGG CTA CAA CGT GC	none	50nM	Outer Forward Primer
Pr-[HPV33-outer-Rv]	CAG ATG GAG GAG GTG TTA AAC C	none	50nM	Outer Reverse Primer
Pr-[HPV35-outer-Fw]	GGC TCT ATG GTA ACC TCC GA	none	50nM	Outer Forward Primer
Pr-[HPV35-outer-Rv]	CAG AAG GCG GTG GTG TAA G	none	50nM	Outer Reverse Primer
Pr-[43_Rv]	CGC CCT AAG GGA AAC TGG GT	none	50nM	Outer Reverse Primer
Pr-[53_Rv]	CCA TAA CCT CAG CAG ACA GG	none	50nM	Outer Reverse Primer
Pr-[GH2O]	GAA GAG CCA AGG ACA GGT AC	none	50nM	Outer Forward Primer
Pr-[PCO4]	CAA CTT CAT CCA CGT TCA CC	none	50nM	Outer Reverse Primer
Pr-[GP5+]	TTT GTT ACT GTG GTA GAT ACT AC	none	0-50nM	Inner Forward Primer
Pr-[GP6+]-5Bio	GAA AAA TAA ACT GTA AAT CAT ATT C	5'-Biotin	500nM	Inner Reverse Primer
Pr-[GP6-de]-5Bio	GAA AHA YAA AYT GYA ADT CAW AYT C	5'-Biotin	500nM	Inner Reverse Primer
Pr-[GP6-31]-5Bio	GAA ATA TAA ATT GTA AAT CAA ATT C	5'-Biotin	300nM	Inner Reverse Primer
Pr-[GP6-33]-5Bio	GAA AAA CAA ACT GTA GAT CAT ATT C	5'-Biotin	300nM	Inner Reverse Primer
Pr-[GP6-51]-5Bio	GAA AAA TAA ATT GCA ATT CAT ACT C	5'-Biotin	300nM	Inner Reverse Primer
Pr-[GPL-16]-5Bio	GTA AAT CAT ATT CCT CCC CAT G	5'-Biotin	200nM	Inner Reverse Primer
Pr-[GPL-31]-5Bio	GTA AAT CAA ATT CCT CAC CAT G	5'-Biotin	200nM	Inner Reverse Primer
Pr-[GPL-33]-5Bio	GTA GAT CAT ATT CTT CAA CAT G	5'-Biotin	200nM	Inner Reverse Primer
Pr-[β_Rv-inner]-5Bio	GGC AGA CTT CTC CTC A	5'-Biotin	500nM	Inner Reverse Primer
Pr-[Anti-universal]-5Bio	AGAGGTGGGAGGTGAGATTT	5'-Biotin	——	For coupling efficiency assess

Tabel S2. Sequences of capture probe

Probe Name	Sequence (5'to3')	Modification
HBB	AAAAAAAAAA AAATCTCACCTCCCACCTCT ACATTTGCTTCTGACACAACTGTGTTCACT	5'-AminolinkerC6
Probe6	AAAAAAAAAA AAATCTCACCTCCCACCTCT ATCCGTAACTACATCTTCCACATACACCAA	5'-AminolinkerC6
Probe16	AAAAAAAAAA AAATCTCACCTCCCACCTCT GTCATTATGTGCTGCCATATCTACTTCAGA	5'-AminolinkerC6
Probe18	AAAAAAAAAA AAATCTCACCTCCCACCTCT ATATGTGCTTCTACACAGTCTCCTGTACCT	5'-AminolinkerC6
Probe31	AAAAAAAAAA AAATCTCACCTCCCACCTCT CAATATGTCTGTTTGTGCTGCAATTGCAAA	5'-AminolinkerC6
Probe33	AAAAAAAAAA AAATCTCACCTCCCACCTCT TGACTTTATGCACACAAGTAACTAGTGACA	5'-AminolinkerC6
Probe35	AAAAAAAAAA AAATCTCACCTCCCACCTCT AAATATGTCTGTGTGTTCTGCTGTGTCTTC	5'-AminolinkerC6
Probe39	AAAAAAAAAA AAATCTCACCTCCCACCTCT ATCTACCTCTATAGAGTCTTCCATACCTTC	5'-AminolinkerC6
Probe43	AAAAAAAAAA AAATCTCACCTCCCACCTCT AAACTTAACGTTATGTGCCTCTACTGACCC	5'-AminolinkerC6
Probe45	AAAAAAAAAA AAATCTCACCTCCCACCTCT ACACAAAATCCTGTGCCAAGTACATATGAC	5'-AminolinkerC6
Probe51	AAAAAAAAAA AAATCTCACCTCCCACCTCT CCCCAACATTTACTCCAAGTAACTTTAAGC	5'-AminolinkerC6
Probe52	AAAAAAAAAA AAATCTCACCTCCCACCTCT GACTTTATGTGCTGAGGTTAAAAAGGAAAG	5'-AminolinkerC6
Probe53	AAAAAAAAAA AAATCTCACCTCCCACCTCT CGCAACCACACAGTCTATGTCTACATATAA	5'-AminolinkerC6
Probe56	AAAAAAAAAA AAATCTCACCTCCCACCTCT CATGACTATTAGTACTGCTACAGAACAGTT	5'-AminolinkerC6
Probe58	AAAAAAAAAA AAATCTCACCTCCCACCTCT GACATTATGCACTGAAGTAACTAAGGAAGG	5'-AminolinkerC6
Probe59	AAAAAAAAAA AAATCTCACCTCCCACCTCT CTTTCTGTGTGTGCTTCTACTACTTCTTCT	5'-AminolinkerC6
Probe69	AAAAAAAAAA AAATCTCACCTCCCACCTCT ACTGTATCTGCACAATCTGCATCTGCCACT	5'-AminolinkerC6
Probe73	AAAAAAAAAA AAATCTCACCTCCCACCTCT TAGGTACACAGGCTAGTAGCTCTACTACAA	5'-AminolinkerC6
Probe82	AAAAAAAAAA AAATCTCACCTCCCACCTCT GCTGTTACTCCATCTGTTGCACAAACATTT	5'-AminolinkerC6
Probe83	AAAAAAAAAA AAATCTCACCTCCCACCTCT CAGCTGCTGCTACACAGGCTAATGAATACA	5'-AminolinkerC6
Probe87	AAAAAAAAAA AAATCTCACCTCCCACCTCT CAATTTTACTATTAGTGCTGCCACTCAAAC	5'-AminolinkerC6