Electronic Supplementary Material (ESI) for Chemical Communications

New Platform for Convenient Genotyping System

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Supporting information

1. Materials &Instruments

All chemicals were purchased from Sigma-Aldrich Chemicals, Korea. All the oligonucleotides were purchased from Bioneer, Korea. Glass fiber membrane (2.5x7.5 cm) was purchased from Whatman, Springfield, UK**.** All washing solvents for the substrates are of HPLC grade from SK Chemicals, Korea. Ultrapure water (18 M Ω/cm) was obtained from a Milli-Q purification system (Millipore). Oligonucleotides were lined using dispenser (BioDot Technologies, Inc., 2852 Alton Pkwy, Irvine, CA 92606, USA). Hybridization was done at 25^0C. The fluorescence intensities were recorded on the BMT Reader™, Biometrix Technology Inc., South Korea.

2. Composition of different solutions:

a) Immobilization solution (pH = 7.4): 15% glycerol, 50mM butyl amine, 600mM NH_4Cl

b) Blocking buffer solution (pH = 7.4): 0.5% milk casein in 4x SSC

c) Hybridization buffers (pH = 7.4): 25% Formamide, 0.1% Triton X-100, 6x SSC

d) Washing buffer solution (pH = 7.4): 0.1% SDS in 4x SSC

3. Probes and primers

Table S1: Probes and primers

Probes	HPV Type	Sequence
Probe1	HPV16	5'-GGGGGGGGG CTTTAT CCT ACG ACT TGG GGA GG-3'
Probe2	HPV18	5'-GGGGGGGGG CTTTAT TAG CAG ACT TGT TGA GG-3'
Probe3	HPV45	5'-GGGGGGGGG CTTTAT TAG TAG ACA TAT GGA GG-3'
Probe4	HPV33	5'-GGGGGGGGG CTTTAT TAT AAG ACA TGT TGA AG-3'
Probe5	HPV31	5'-GGGGGGGGG CTTTAT TTT AAG ACA TAG TGA GG-3'
Probe6	HC	5'-GGGGGGGGG TTTCCT AGT GGC TCT ATG GTA AC-3'
Probe7	PC	5'-GGGGGGGGG TGA TTT ACA GTT TAT DTT TC-3'
Probe8	PCR	5'-GGGGGGGGG ATTGGC ATG BKG ARG ART WTGA-3'
Target Probes	**HPV Type**	**Sequence**
Probe9	HC-Cy5-T1	3'-GGATCACCGAGATACCATTGGAGACTGCG-Cy5-5'
Probe10	HPV16-Cy5	3'-GG ATG CTG AAC CCC TCC -Cy5-5'
Probe11	HPV18-Cy5	3'- CCT CAA CAT GTC TGC TA-Cy5- 5'
Probe12	HPV45-Cy5	3'-ATC ATC TGT ATA CCT CC-Cy5- 5'
Probe13	HPV33-Cy5	3'-ATA TTC TGT ACA ACT TC-Cy5- 5'
Probe14	HPV31-Cy5	3'-AAA TTC TGT ATC ACT CC-Cy5- 5'
Forward primer	FP	3`-GCMCAGGGWCATAAYAATGG-5'
Reverse primer	RP-Cy5	3`-GAAAHATAAACTGTAAATCATAYTC-Cy5-5'

HC – probe for the Hybridization control, PC – probe for the Primer control (Positive control), PCR – probe for the PCR control, HC-Cy5-T1 – Target oligonucleotide for HC probe GGGGGGGGG – 9G for immobilization of the probes on the AMCA slides CTT TAT – vertical spacer group

4. Clinical samples, DNA extraction, and (PCR) amplification

The standard clinical samples were obtained from the Korea Food and Drug Administration (KFDA), South Korea. The whole HPV genomic DNA in the clinical samples was amplified by PCR to generate amplicons. HPV DNA was amplified with primers RP and FP (**Table 1**). The PCR mixture consisted of 10µl of the extracted DNA, 10µl of each primer (RP, FP), PCR premix (cat# K-2016V1, Bioneer Inc., Daejan, Korea) containing deoxyribonucleotide triphosphate, 2U of Fast Start *Taq* DNA polymerase in an amplification buffer containing 2mM $MgCl_2$, and a tracking dye (Cy5). All tubes were incubated for 2 min at 50℃ before PCR was started. Amplification was performed with the following steps: pre-denaturation for 5min at 94℃, 45 cycles of 30s each for the denaturation at 94℃; 45 cycles of 30s each for annealing at 65℃, 45 cycles of 30s each for elongation at 72℃, and an final elongation step of 7min at 72℃. 5µl of PCR product was subjected to agarose gel electrophoresis, using a 2% agarose standard run in 1X Tris borate EDTA. 5µl of this Cy5 labeled PCR product was used for the further hybridization experiments on the 9G membrane for the HPV detection and genotyping.

5. The statistics of cervical cancers worldwide caused by HPV 16 and HPV 18

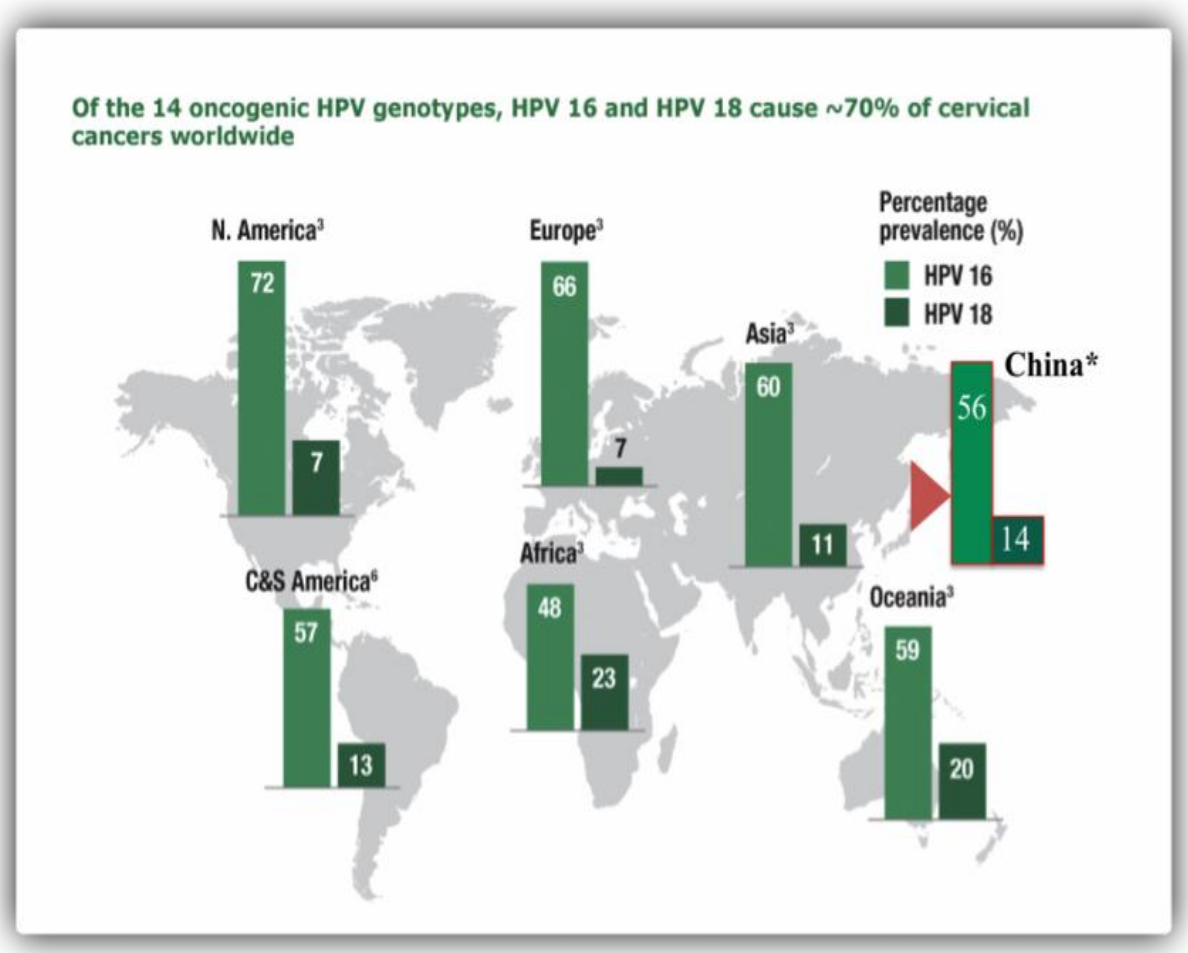
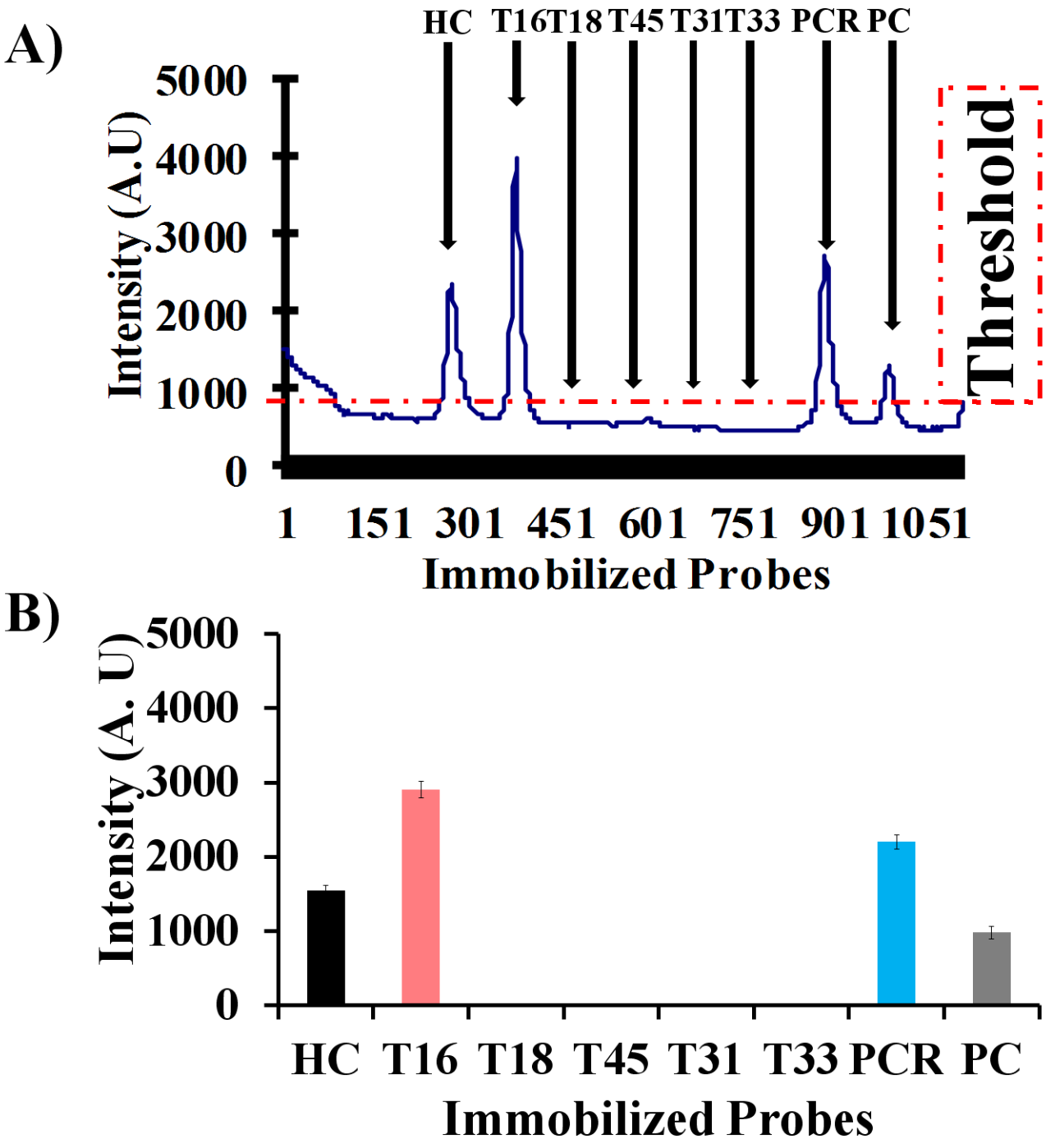


Figure S1: The statistics of cervical cancers worldwide caused by HPV16 and HPV18[1]

6. Scheme of fluorescence intensities corresponding to different probes



Scheme S1: A) Graph of fluorescence intensity upon scanning of the 9G membrane after hybridization in BMT reader, B) Graph of corrected fluorescence intensities in A) corresponding to the HC, T16, T18, T45, T31, T33, PCR, and PC, respectively.

7. Determination of Optimum Washing Time

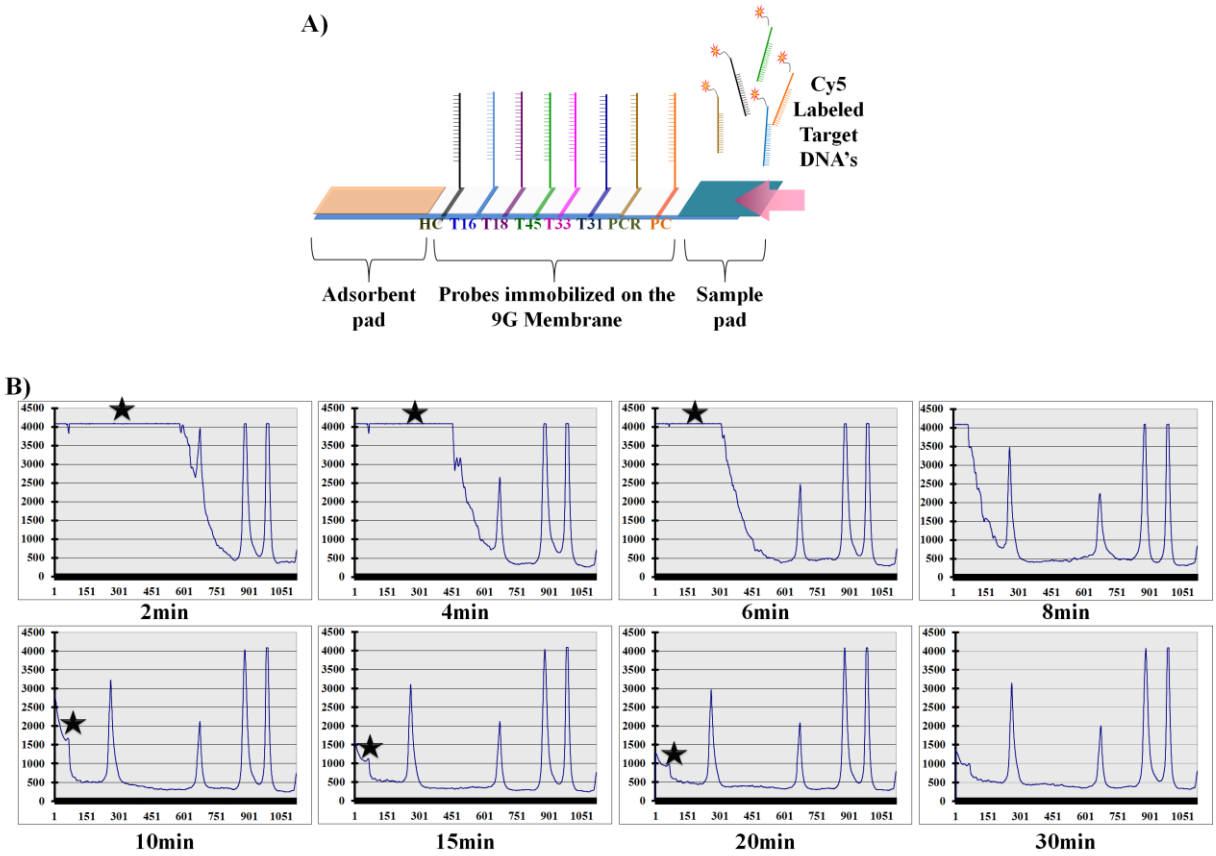


Fig. S2 Determination of optimum washing time, A), Scheme for the immobilization of the Probe1-Probe8, B) Graphs corresponding to the time dependent washing after 20min hybridization, (★) indicates the residue of the excess Cy5 labeled PCR product of HPV33.

To determine the optimum washing time, the immobilized probes on the 9G membrane were allowed to hybridize with the 10^2 copies of Cy5 labeled PCR product of the HPV33 by loading the solution in the sample port. After 20min hybridization, the 9G membranes were washed at different time intervals (2, 4, 6, 8, 10, 15, 20, and 30min) by loading the washing solution in the washing port. After washing, the 9G membranes were scanned by BMT membrane reader to obtain the results (**Figure S2**). As shown in the **Figure S2**, after 2min washing the residue of the excess Cy5 labeled PCR product of the HPV33 was not washed away, indicated by the broad peak on the left side of the chart. However, as the washing time increased from 4 to 8min, the residue of the excess Cy5 labeled PCR product decreases as marked by the sharp decrease of the broad peak on the left side of the chart. Interestingly, most of the excess residue of the Cy5 labeled PCR product is washed away after 10min washing. Moreover, after 15, 20, and 30min washing the excess residue of the Cy5 labeled PCR product is completely washed away indicated by the sharp peaks corresponding to the HC, HPV33, PCR, and PC (from left to right, also see the **Figure S2 A**), respectively. The Therefore, the optimum washing time was considered to be 20min and used for further experiments.

8. Determination of Optimum hybridization Time

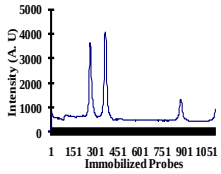
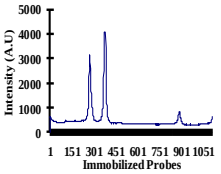
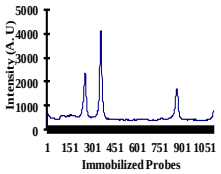
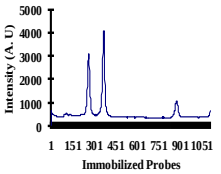
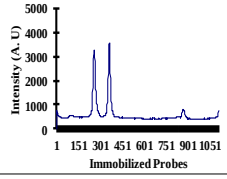
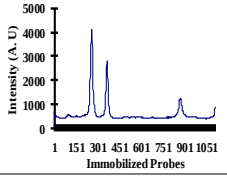
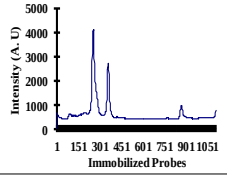
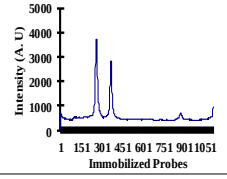
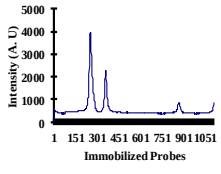
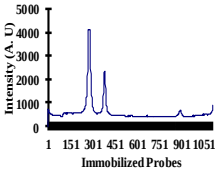
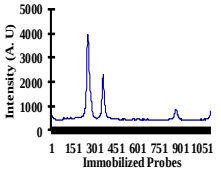
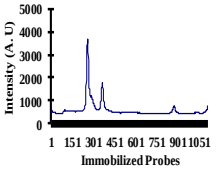
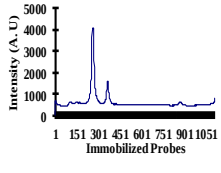
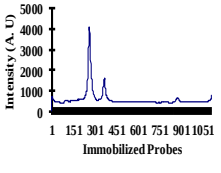
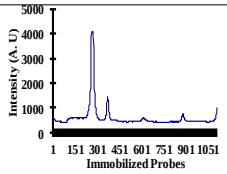
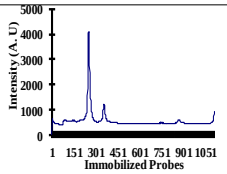
Time / Conc.	20min	15min	10min	5min
40fmol/µL				
20fmol/µL				
10fmol/µL				
5fmol/µL				

Fig. S3 Determination of, Optimum hybridization time, hybridization of Probe1 (HPV 16) with complementary single stranded target Probe10 with the concentration of 5, 10, 20, and 40fm/µL for 5, 10, 15, and 20min.

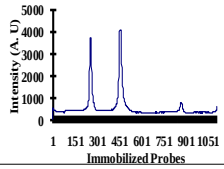
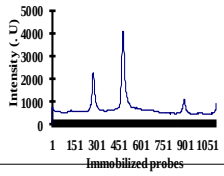
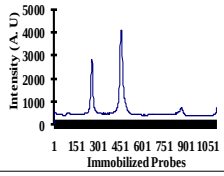
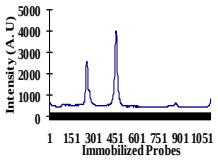
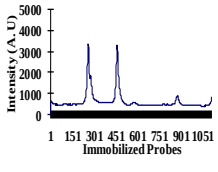
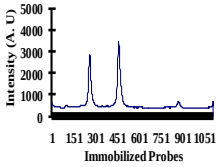
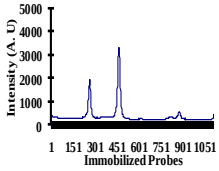
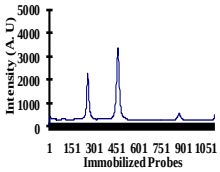
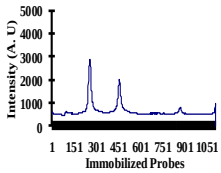
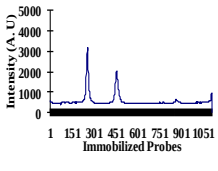
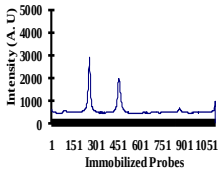
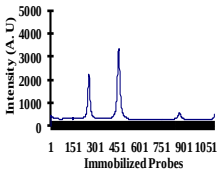
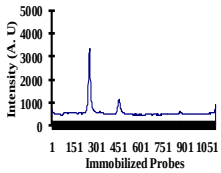
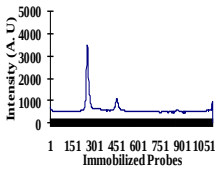
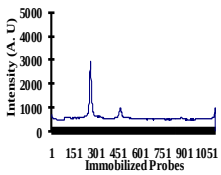
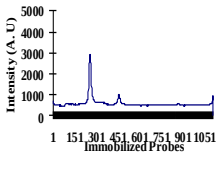
Time / Conc.	20min	15min	10min	5min
40fmol/µL				
20fmol/µL				
10fmol/µL				
5fmol/µL				

Fig. S4 Determination of, Optimum hybridization time, hybridization of Probe2 (HPV 18) with complementary single stranded target Probe11 with the concentration of 5, 10, 20, and 40fm/µL for 5, 10, 15, and 20min. The data is presented in the **Fig. S5.**

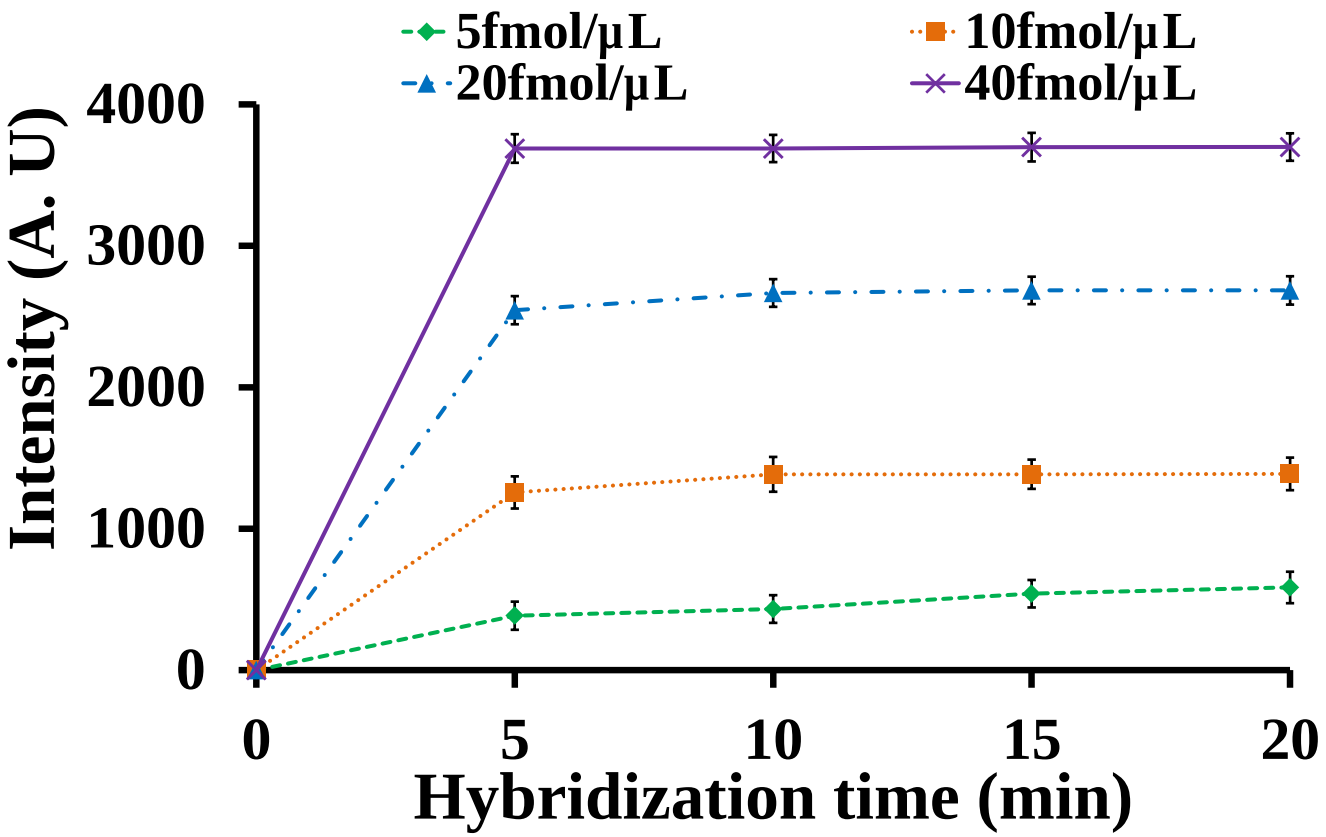


Fig. S5 Determination of, Optimum hybridization time, hybridization with target Probe11 complementary to HPV 18 (Probe2) with the concentration of 5, 10, 20, and 40fmol/µL for 5, 10, 15, and 20min.

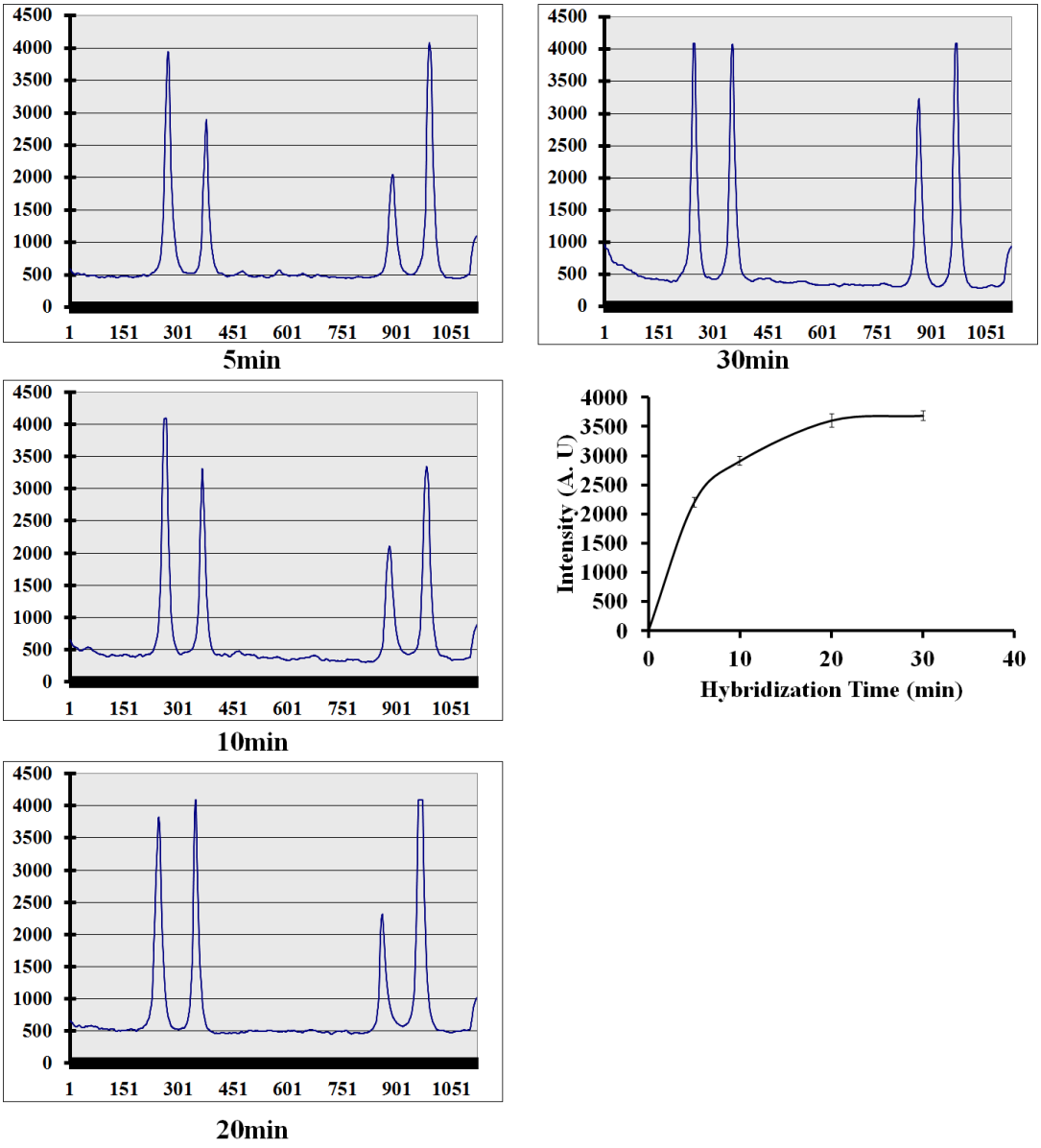


Figure S6: Optimum hybridization time, Graphs of fluorescent intensities corresponding to the hybridization of immobilized probes with the Cy5 labeled PCR products of HPV16. The data is presented in the **Fig. S7**.

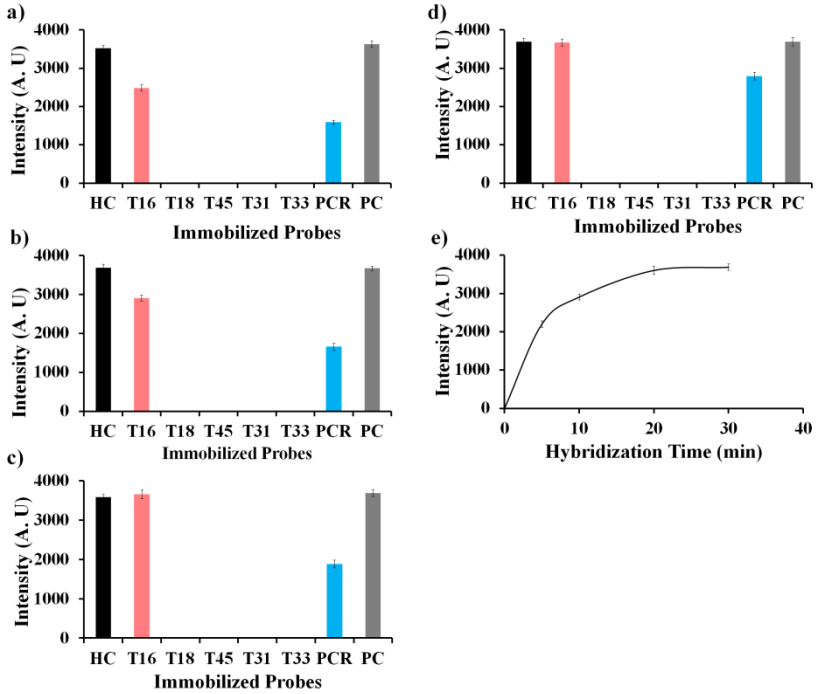


Fig. S7 Determination of optimum hybridization time, a), b), c), and d) graphs represents the respective fluorescent intensity after the 5, 10, 20, and 30min, hybridization of Cy5 labeled PCR product of HPV16, e) Increase in fluorescence intensity with increase in time at the Probe1 corresponding to the HPV16.

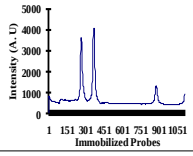
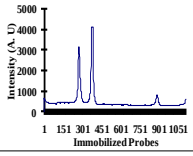
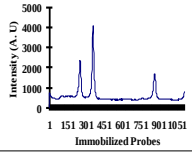
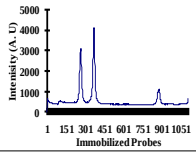
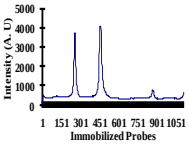
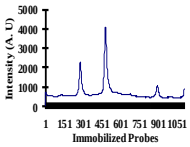
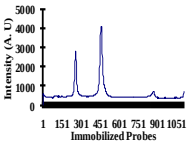
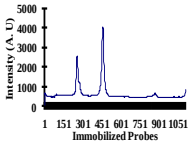
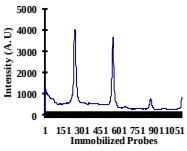
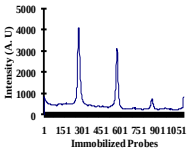
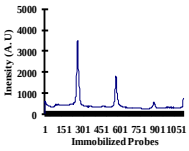
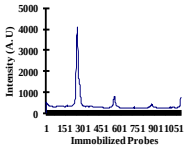
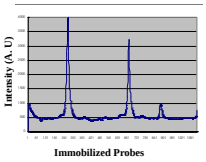
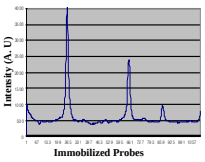
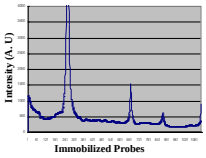
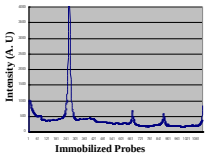
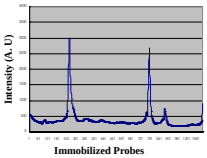
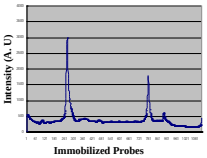
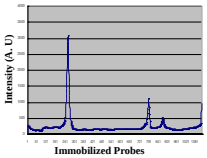
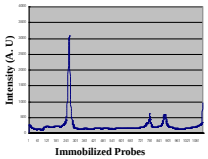
Concentration / HPV Genotype	40fmol/µL	20fmol/µL	10fmol/µL	5fmol/µL
HPV16				
HPV18				
HPV45				
HPV31				
HPV33				

Fig. S8 Optimum concentration, hybridization with single stranded target Probe10 -14 complementary to HPV 16, 18, 45, 31, and 33, respectively, with the concentration of 5, 10, 20, and 40fmol/µL for 20min.

9. Correlation of the fluorescence intensity and the number of HPV DNA copies upon hybridization with the Cy5 labeled PCR products

9.1 Hybridization of the immobilized probes with the Cy5 labeled PCR product of the HPV16

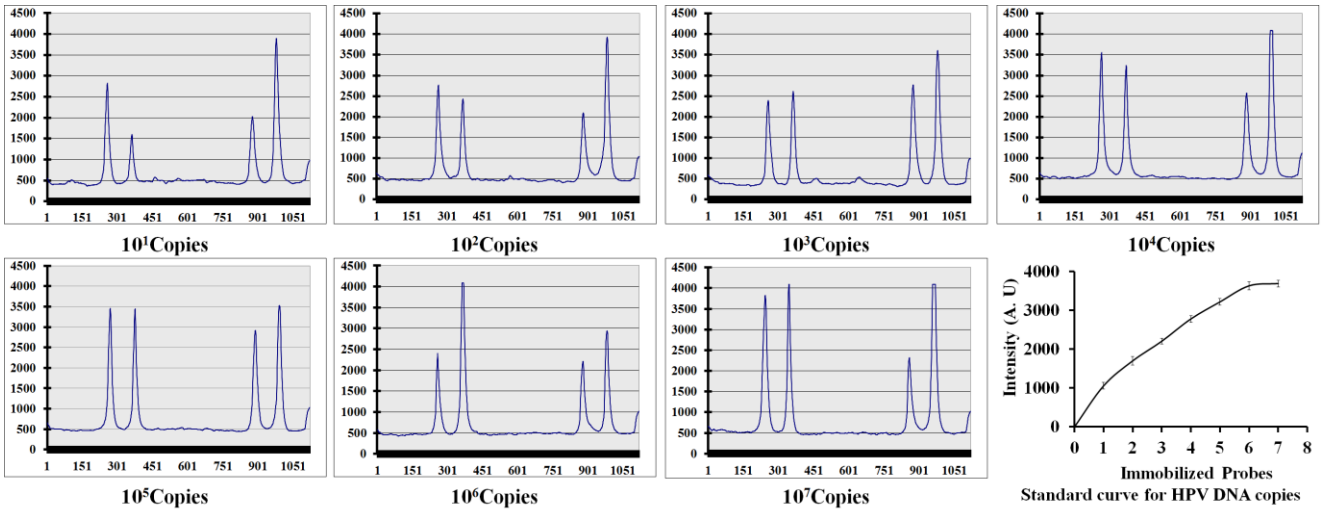
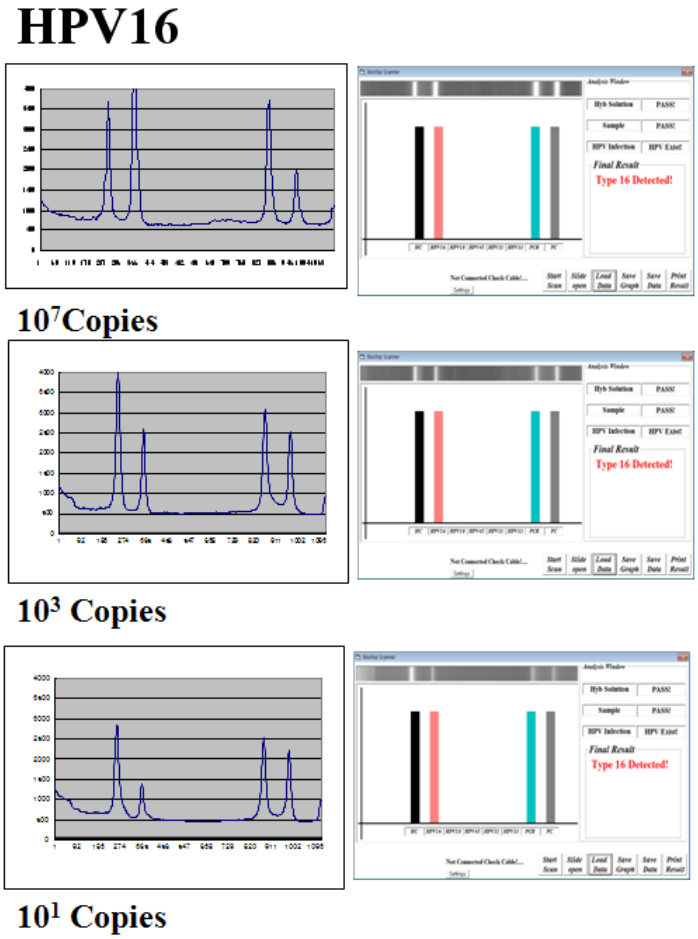
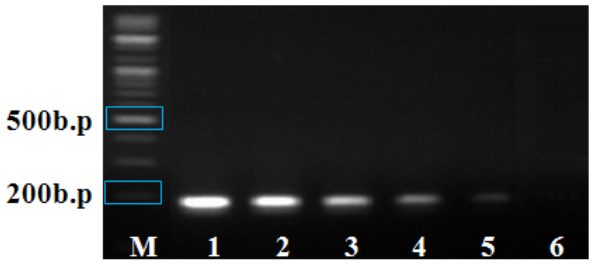


Figure S9: Graphs of the fluorescence intensities upon hybridization of the immobilized probes with the Cy5 labeled PCR product of the HPV16

9.2. Correlation of the fluorescence intensity and the number of HPV DNA copies upon hybridization with the Cy5 labeled PCR products of HPV16.



	HPV16		
	Sample 1	Sample2	Sample 3
Intensity (A.U)	900	2000	3400
No. Copies	~10^1 Copies	~10^3Copies	~10^7Copies



M	100b.p DNA size marker
Lane 1	1.0 x 10^7copies
Lane 2	1.0 x 10^5copies
Lane 3	1.0 x 10^3copies
Lane 4	1.0 x 10^2copies
Lane 5	1.0 x 10^1copies
Lane 6	3.0 copies

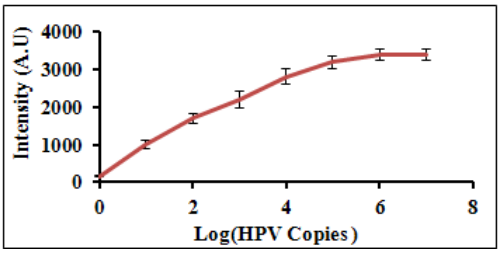
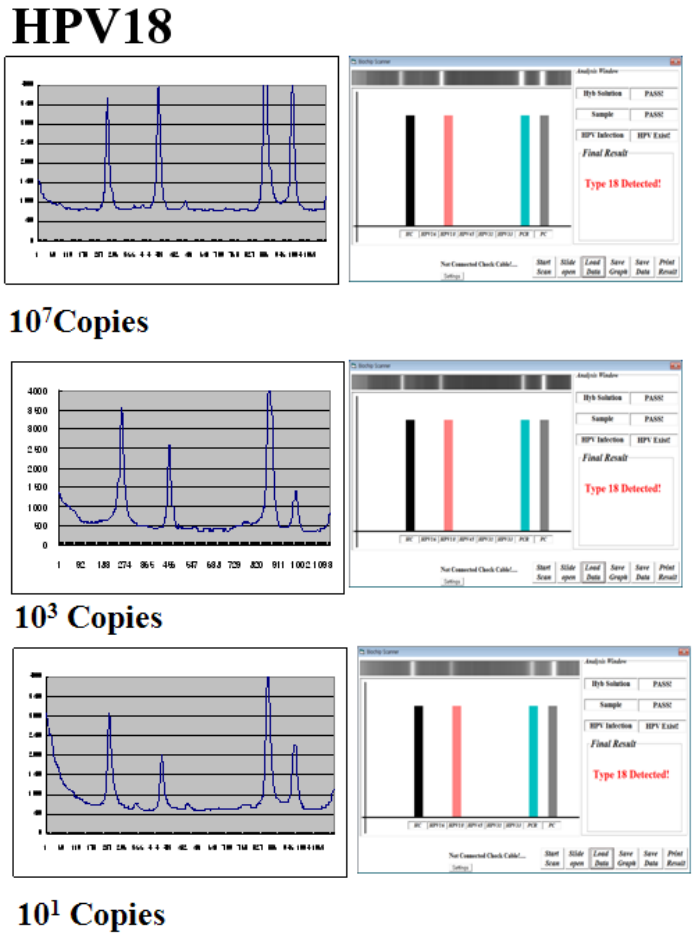
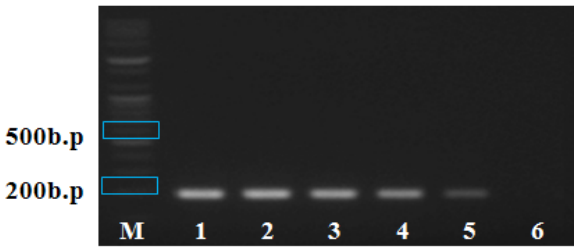


Figure S10: Correlation of the fluorescence intensity and the number of HPV DNA copies upon hybridization with the Cy5 labeled PCR products of HPV16.

9.3. Correlation of the fluorescence intensity and the number of HPV DNA copies upon hybridization with the Cy5 labeled PCR products of HPV18.



	HPV18		
	Sample 1	Sample2	Sample 3
Intensity (A.U)	1100	2100	3300
No. Copies	~10^1 Copies	~10^3Copies	~10^7Copies



M	100b.p DNA size marker
Lane 1	1.0 x 10^7copies
Lane 2	1.0 x 10^5copies
Lane 3	1.0 x 10^3copies
Lane 4	1.0 x 10^2copies
Lane 5	1.0 x 10^2copies
Lane 6	3.0 copies

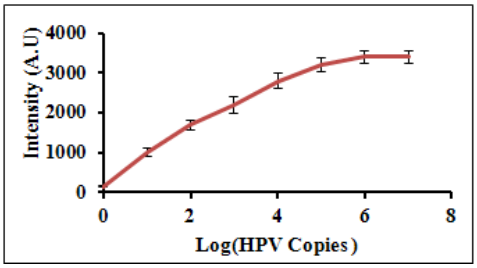
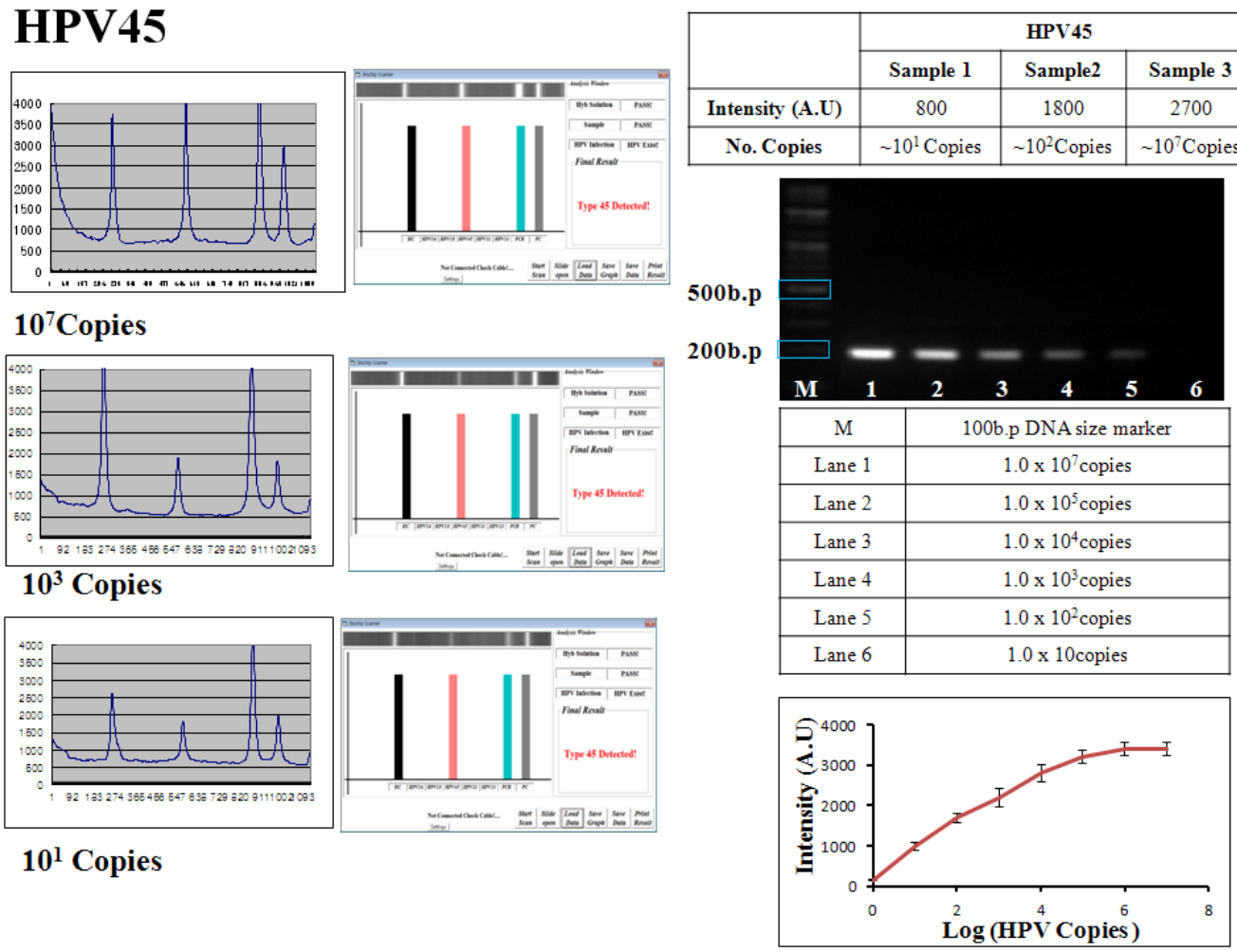


Figure S11: Correlation of the fluorescence intensity and the number of HPV DNA copies upon hybridization with the Cy5 labeled PCR products of HPV18.

9.4. Correlation of the fluorescence intensity and the number of HPV DNA copies upon hybridization with the Cy5 labeled PCR products of HPV45.

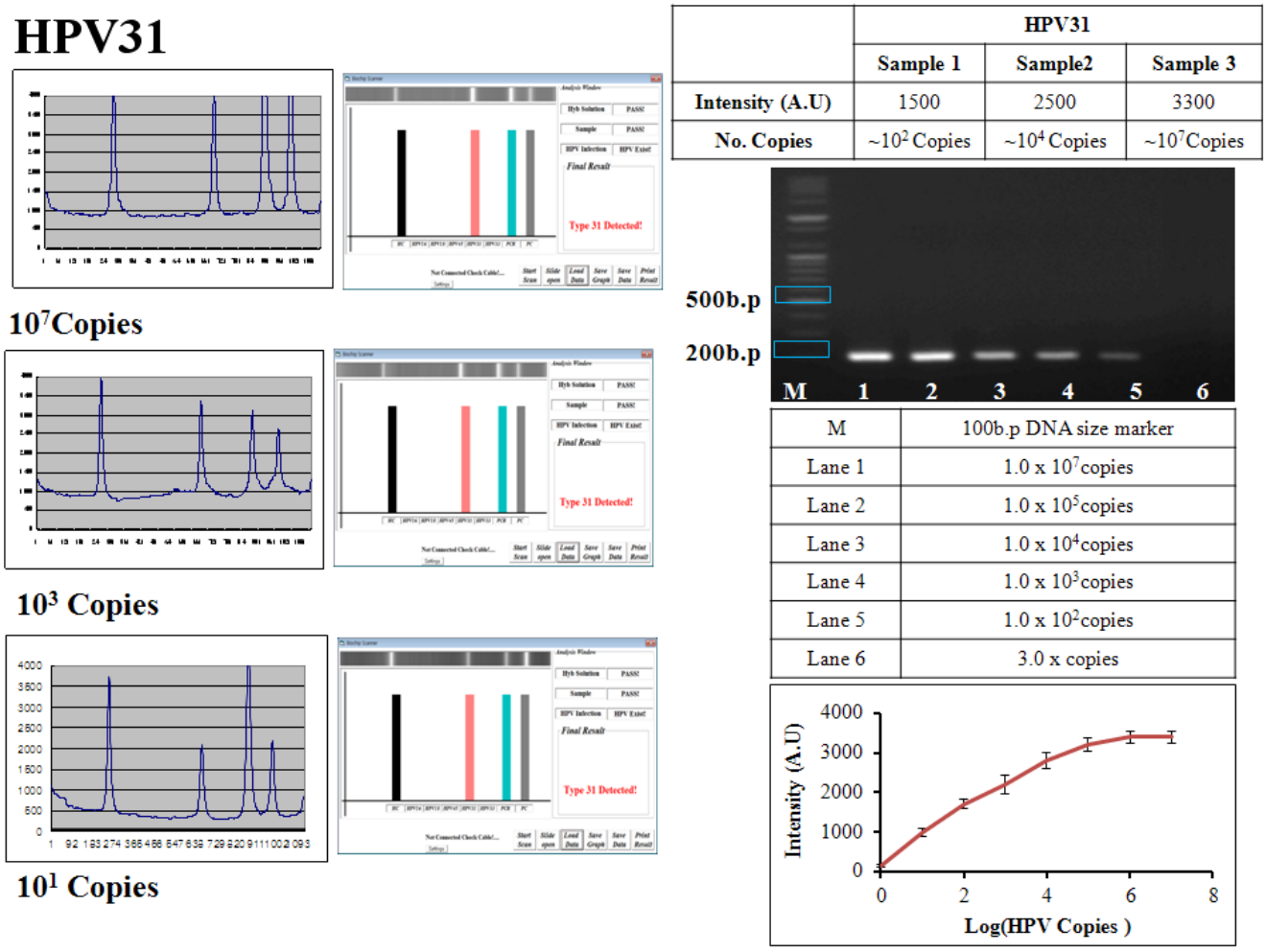


	HPV45		
	Sample 1	Sample2	Sample 3
Intensity (A.U)	800	1800	2700
No. Copies	~10^1 Copies	~10^2Copies	~10^7Copies

M	100b.p DNA size marker
Lane 1	1.0 x 10^7copies
Lane 2	1.0 x 10^5copies
Lane 3	1.0 x 10^4copies
Lane 4	1.0 x 10^3copies
Lane 5	1.0 x 10^2copies
Lane 6	1.0 x 10copies

Figure S12: Correlation of the fluorescence intensity and the number of HPV DNA copies upon hybridization with the Cy5 labeled PCR products of HPV45.

9.5. Correlation of the fluorescence intensity and the number of HPV DNA copies upon hybridization with the Cy5 labeled PCR products of HPV31.

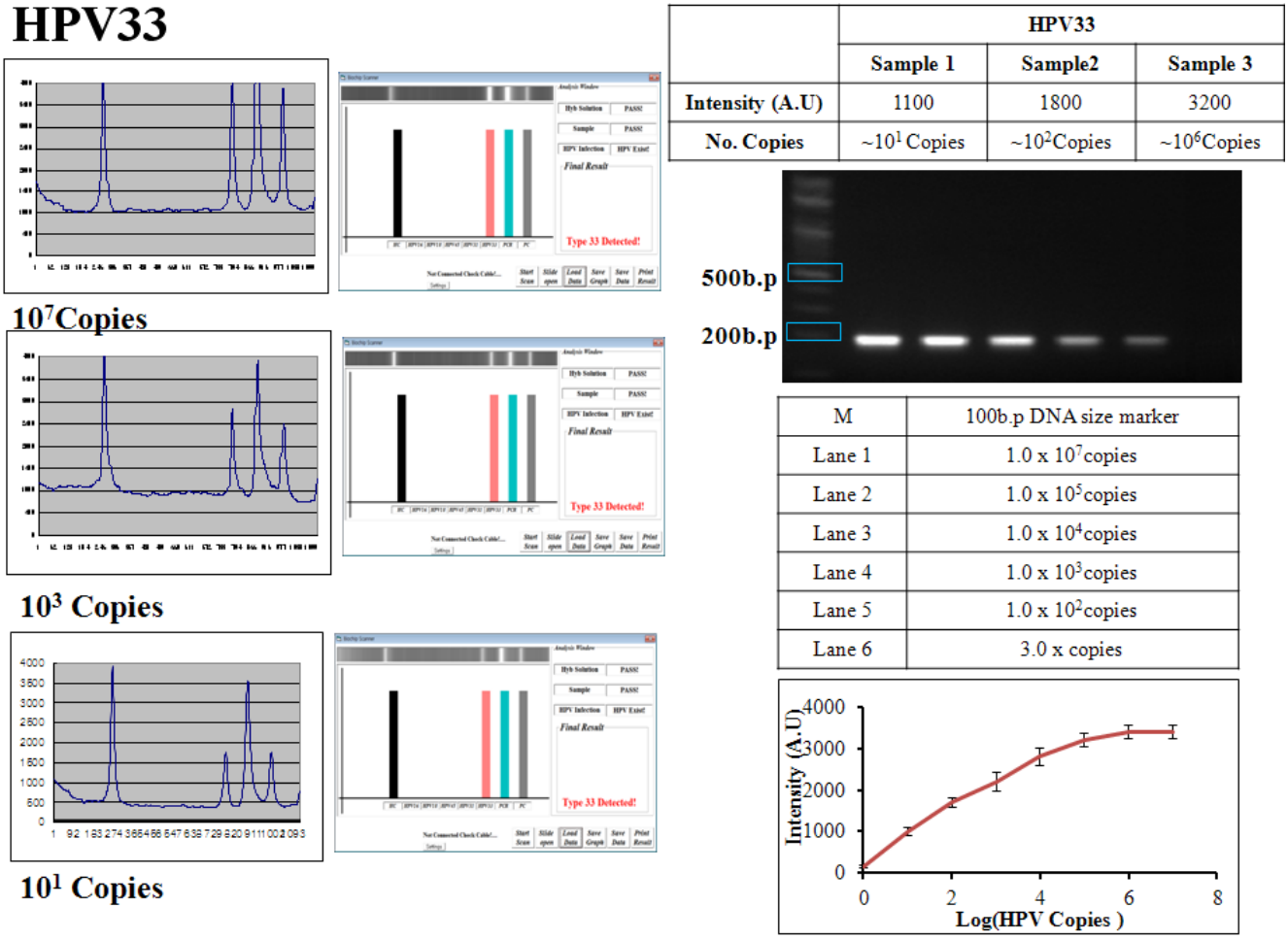


	HPV31		
	Sample 1	Sample2	Sample 3
Intensity (A.U)	1500	2500	3300
No. Copies	~10^2 Copies	~10^4 Copies	~10^7Copies

M	100b.p DNA size marker
Lane 1	1.0 x 10^7copies
Lane 2	1.0 x 10^5copies
Lane 3	1.0 x 10^4copies
Lane 4	1.0 x 10^3copies
Lane 5	1.0 x 10^2copies
Lane 6	3.0 x copies

Figure S13: Correlation of the fluorescence intensity and the number of HPV DNA copies upon hybridization with the Cy5 labeled PCR products of HPV31.

9.6. Correlation of the fluorescence intensity and the number of HPV DNA copies upon hybridization with the Cy5 labeled PCR products of HPV33.



	HPV33		
	Sample 1	Sample2	Sample 3
Intensity (A.U)	1100	1800	3200
No. Copies	~10^1 Copies	~10^2Copies	~10^6Copies

M	100b.p DNA size marker
Lane 1	1.0 x 10^7copies
Lane 2	1.0 x 10^5copies
Lane 3	1.0 x 10^4copies
Lane 4	1.0 x 10^3copies
Lane 5	1.0 x 10^2copies
Lane 6	3.0 x copies

Figure S14: Correlation of the fluorescence intensity and the number of HPV DNA copies upon hybridization with the Cy5 labeled PCR products of HPV33.

9.7. Discrimination of HR-HPV genotypes

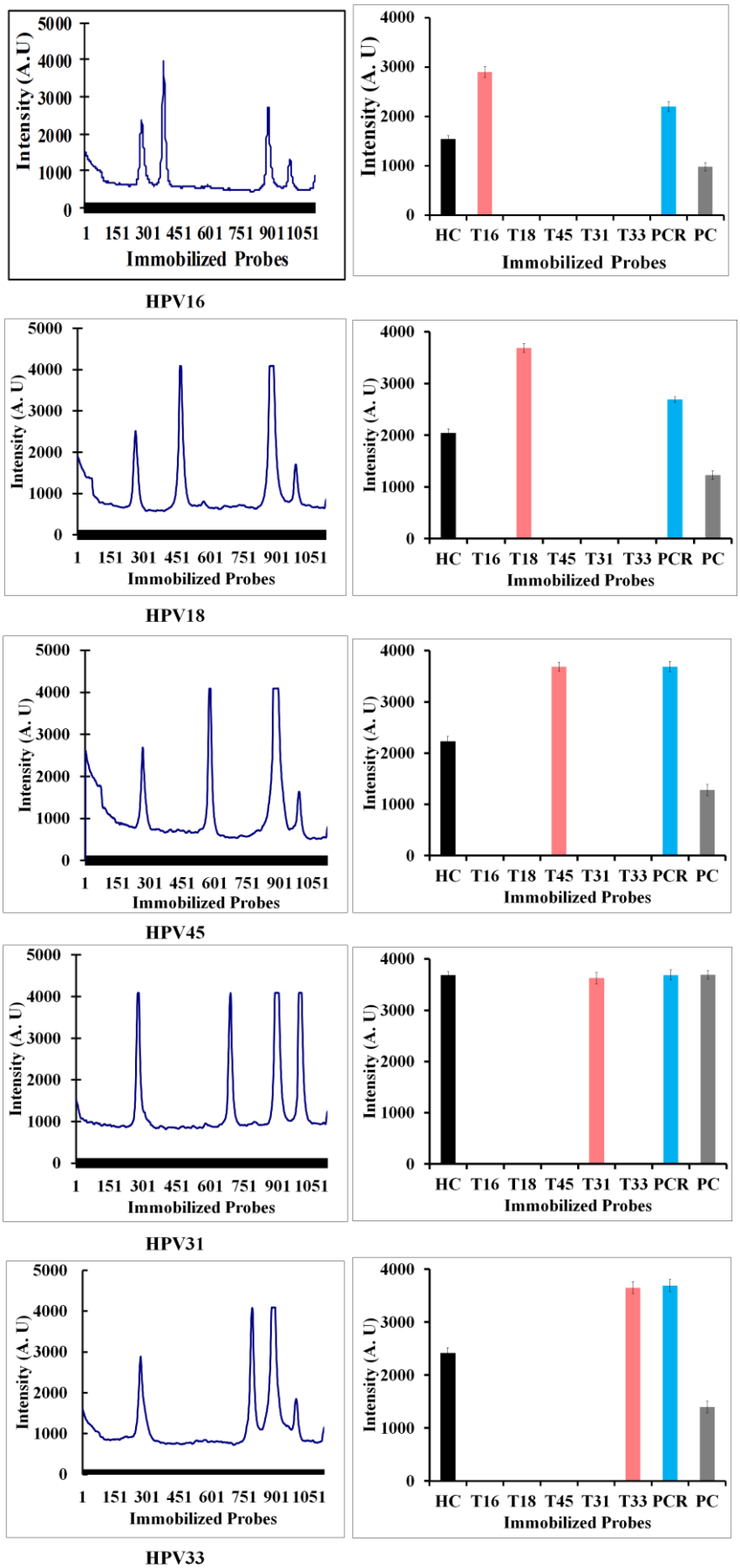


Figure S15: Discrimination of HR-HPV genotypes

10. Easy to use 9G membrane

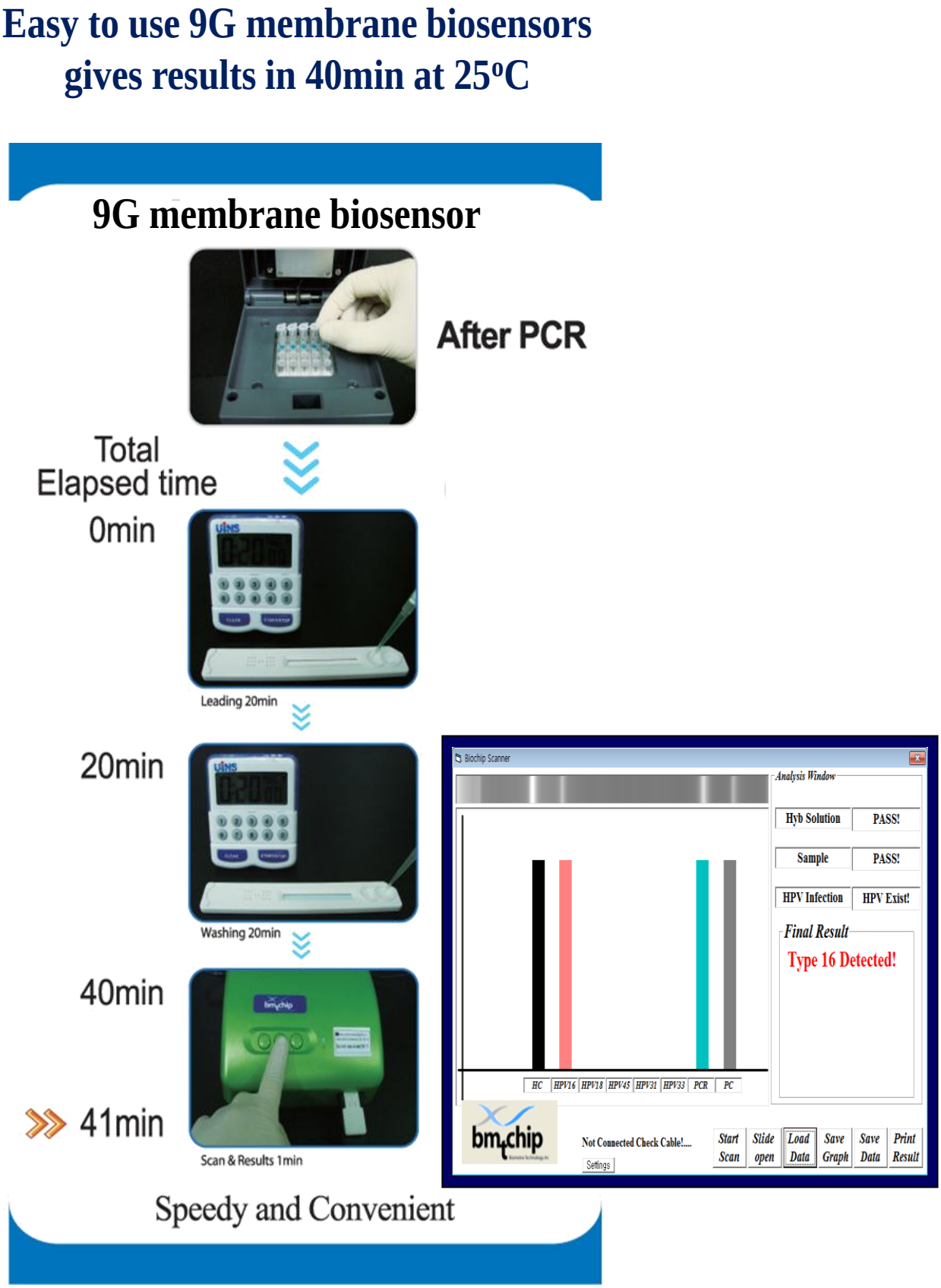
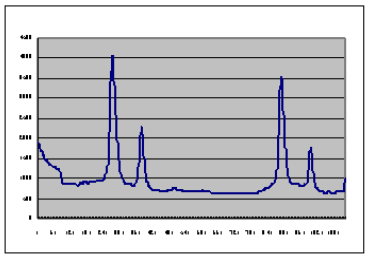


Figure S16: Obtain the genotyping results in 40min using 9G membrane

11. Results in Clinical Samples:

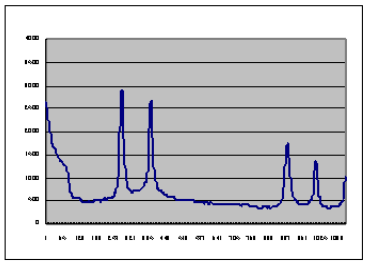
Table S2

Test No.	1
Sample code	AH2009-00969
Smear result	squa. cell ca.
Sequencing result	HPV 16
BMT result (HPV1)	HPV 16 

	intensity
HC	3193
HPV 16	1534
HPV 18	46
HPV 45	20
HPV 31	30
HPV 33	65
PCR	2751
PC	1037

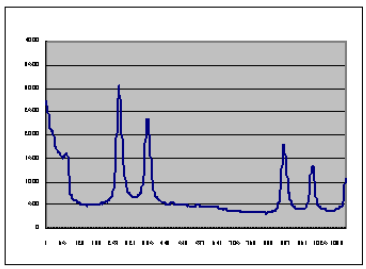
Table S3

Test No.	2
Sample code	AH2009-00956
Smear result	ASCUS
Sequencing result	HPV 16
BMT result (HPV1)	HPV

	 1

	intensity
HC	2285
HPV 16	1961
HPV 18	118
HPV 45	36
HPV 31	20
HPV 33	35
PCR	1266
PC	959

Table S4

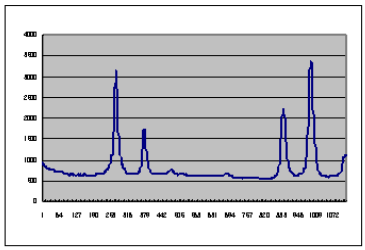
Test No.	3
Sample code	AH2009-00932
Smear result	ASCUS
Sequencing result	HPV 16
BMT result (HPV1)	HPV 16 

	intensity
HC	2421
HPV 16	1723
HPV 18	47
HPV 45	24
HPV 31	52
HPV 33	21
PCR	1411
PC	916

Table S5

Test No.	4
Sample code	AH2009-00862
Smear result	squa. cell ca.
Sequencing result	HPV 16

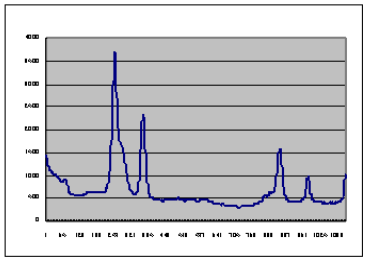
BMT result (HPV1)	HPV 16



	intensity
HC	2467
HPV 16	1060
HPV 18	93
HPV 45	26
HPV 31	65
HPV 33	13
PCR	1621
PC	2777

Table S6

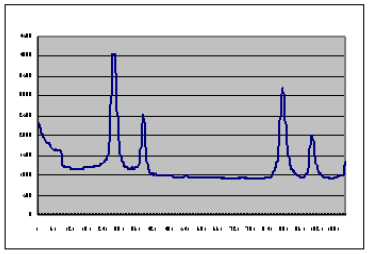
Test No.	5
Sample code	AH2008-00140
Smear result	ASCUS
Sequencing result	HPV 16
BMT result (HPV1)	HPV 16



	intensity
HC	3044
HPV 16	1792
HPV 18	41
HPV 45	49
HPV 31	51
HPV 33	148
PCR	1108
PC	555

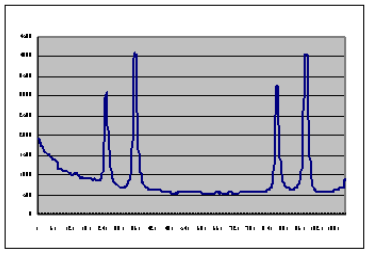
Table S7

Test No.	6
Sample code	AH2007-06177

Smear result	NIL
Sequencing result	HPV 16
BMT result (HPV1)	HPV 16 

	intensity
HC	2863
HPV 16	1446
HPV 18	46
HPV 45	39
HPV 31	35
HPV 33	23
PCR	2180
PC	1032

Table S8

Test No.	7
Sample code	AH2007-06113
Smear result	HSIL
Sequencing result	HPV 16
BMT result (HPV1)	HPV 16 

	intensity
HC	2305
HPV 16	3401
HPV 18	22
HPV 45	38
HPV 31	28
HPV 33	30
PCR	2659
PC	3466

Table S9

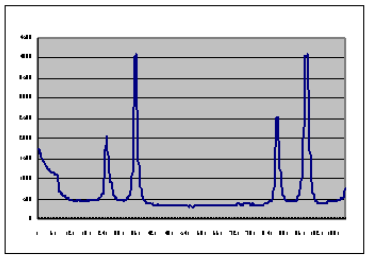
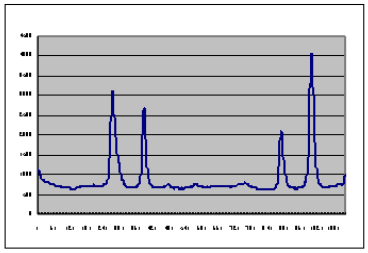
Test No.	8
Sample code	AH2007-06057
Smear result	HSIL
Sequencing result	HPV 16
BMT result (HPV1)	HPV 16  intensity HC 1580 HPV 16 3659 HPV 18 21 HPV 45 6 HPV 31 17 HPV 33 16 PCR 2150 PC 3663

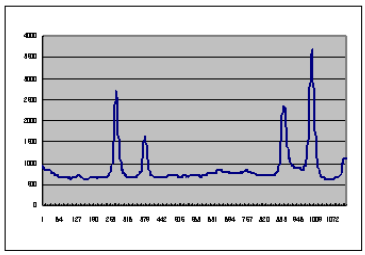
Table S10

Test No.	9
Sample code	AH2007-06009
Smear result	ASC-H
Sequencing result	HPV 16
BMT result (HPV1)	HPV 16

	intensity
HC	2423
HPV 16	2011
HPV 18	88
HPV 45	99
HPV 31	32
HPV 33	122
PCR	1473
PC	3428

Table S11

Test No.	10
Sample code	AH2007-06008
Smear result	infla
Sequencing result	HPV 16
BMT result (HPV1)	HPV 16 

	intensity
HC	2034
HPV 16	993
HPV 18	46
HPV 45	24
HPV 31	110
HPV 33	96
PCR	1549
PC	2939

13. Reference:

1. F. X. Bosch, S. de Sanjosé, *J. Natl. Cancer. Inst. Monogr.*, **2003**, *31*, 3-13.