

Generalized probe selection method for DNA chips

Satish Balasaheb Nimse^a, Keum-Soo Song^b, Junghoon Kim^b, Junbae Kim^a, Van-Thao Ta^a,
Van-Thuan Nguyen^a, Taisun Kim^{a*}

*To whom correspondence should be addressed. E-mail: tskim@hallym.ac.kr

Supporting information

1. Materials and Methods	S2
2. Composition of different solutions used	S2
3. Probes and target oligonucleotides	S3-S5
4. Amplification of the HPV DNA by PCR	S6
5. Selection of length of the Probes	S7
6. Selection of the starting position of the probes	S8
7. Probe length and the Probe sequence	S9
8. Results for hybridization of the probes (without mutations)	S10-28
9. Artificial mutation(s) in the probes and its effect on the hybridization with target	S29
10. Comparison of the results after hybridization of the original probes (without mutation) and final probes (with one artificial mutation)	S30-37
11. Comparison of the probes with and without artificial mutations	S38
12: Application of Proposed method for detection and discrimination HPV genotypes	S39-40
13. References	S40

1. Material and Methods:

All chemicals were purchased from Sigma-Aldrich Chemicals, Korea. All the oligonucleotides were purchased from Bioneer, Korea. Glass slides (2.5x7.5 cm) were purchased from Paul Marienfeld GmbH & Co. KG, Germany. The standard viral genomic DNAs were obtained from the Korea Food and Drug Administration. All DNA chips used in this study were obtained by following the previously reported method.¹ Hybridizations were done by using the 100fmol of the Cy5 labeled PCR products of the 19 HPV genotypes at 25⁰C for 30min in the commercial incubator and then the slides were dried using the commercial centrifuge (1000rpm). The fluorescence signal of the microarray was measured on ScanArrayLite (GSI Lumonics), and the images were analyzed by Quant Array software (Packard Bioscience).

2. Composition of different solutions used for hybridization and washing on 9G DNAChips:

1. Immobilization solution (pH = 7.4): 15% glycerol, 50mM butyl amine, 600mM NH₄Cl
2. Blocking buffer solution (pH = 7.4): 0.5% milk casein in 4xSSC
3. Hybridization buffer (pH = 7.4): 25% Formamide, 0.1% Triton X-100, 6xSSC
4. Washing buffer solution A (pH = 7.4): 0.1% SDS in 4xSSC
5. Washing buffer solution B (pH = 7.4): 4xSSC

3. Probes and Target oligonucleotides:

Table S1. Sequences and the nomenclature of probes and target oligonucleotides

Probes	Description	HPV Type	Sequence
(Probe length, 14mer, 17mer, 20mer)			
Probe1	14mer	HPV16	5'-GGGGGGGGGG CTT TAT AC GAC ATG GGG AGG
Probe2	17mer	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC ATG GGG AGG
Probe3	20mer	HPV16	5'-GGGGGGGGGG CTTTAT GT ACC TAC GAC ATG GGG AGG
Probe4	14mer	HPV18	5'-GGGGGGGGGG CTT TAT CA GAC ATG TTG AGG
Probe5	17mer	HPV18	5'-GGGGGGGGGG CTT TAT TA GCA GAC ATG TTG AGG
Probe6	20mer	HPV18	5'-GGGGGGGGGG CTT TAT GT ATA GCA GAC ATG TTG AGG
(Probe position, -14, -11, -8, -5, -1, +3, +7, and +11)			
Probe7	(-14Probe)	HPV16	5'-GGGGGGGGGG CTT TAT GA GGA ATA TGA TTT ACA
Probe8	(-11Probe)	HPV16	5'-GGGGGGGGGG CTT TAT GG GGA GGA ATA TGA TTT
Probe9	(-8Probe)	HPV16	5'-GGGGGGGGGG CTT TAT CT TGG GGA GGA ATA TGA
Probe10	(-5Probe)	HPV16	5'-GGGGGGGGGG CTT TAT CG ACT TGG GGA GGA ATA
Probe11	(-1Probe)	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC ATG GGG AGG
Probe12	(+3Probe)	HPV16	5'-GGGGGGGGGG CTT TAT AG TAC CTA CGA CAT GGG
Probe13	(+8Probe)	HPV16	5'-GGGGGGGGGG CTT TAT TA AGG AGT ACC TAC GAC
Probe14	(+11Probe)	HPV16	5'-GGGGGGGGGG CTT TAT CT TTA AGG AGT ACC TAC
Probe15	(-14Probe)	HPV18	5'-GGGGGGGGGG CTT TAT GA GGA ATA TGA TTT GCA
Probe16	(-11Probe)	HPV18	5'-GGGGGGGGGG CTT TAT GT TGA GGA ATA TGA TTT
Probe17	(-8Probe)	HPV18	5'-GGGGGGGGGG CTT TAT CT TGT TGA GGA ATA TGA
Probe18	(-5Probe)	HPV18	5'-GGGGGGGGGG CTT TAT AG ACT TGT TGA GGA ATA
Probe19	(-1Probe)	HPV18	5'-GGGGGGGGGG CTT TAT TA GCA GAC TTG TTG AGG
Probe20	(+5Probe)	HPV18	5'-GGGGGGGGGG CTT TAT AGT ATA GCA GAC ATG
Probe21	(+8Probe)	HPV18	5'-GGGGGGGGGG CTT TAT AAG CAG TAT AGC AGA C
Probe22	(+12Probe)	HPV18	5'-GGGGGGGGGG CTT TAT ATT TAA GCA GTA TAG C
(Original HPV Probes (without mutations))			
Probe23		HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC ATG GGG AGG
Probe24		HPV18	5'-GGGGGGGGGG CTT TAT TA GCA GAC ATG TTG AGG
Probe25		HPV06	5'-GGGGGGGGGG CTT TAT CA TGC GTC ATG TGG AAG
Probe26		HPV66	5'-GGGGGGGGGG CTT TAT CC TTC GCC ATG TGG AGG
Probe27		HPV39	5'-GGGGGGGGGG CTT TAT TA CCA GGC ACG TGG AGG
Probe28		HPV42	5'-GGGGGGGGGG CTT TAT TT TAA GAC ATG CTG AAG
Probe29		HPV31	5'-GGGGGGGGGG CTT TAT TT TAA GAC ATG GTG AGG
Probe30		HPV45	5'-GGGGGGGGGG CTT TAT TA GTA GAC ATG TGG AGG
Probe31		HPV11	5'-GGGGGGGGGG CTT TAT CA TGC GCC ATG TGG AGG
Probe32		HPV59	5'-GGGGGGGGGG CTT TAT TG CCA GAC ATG TGG AGG
Probe33		HPV51	5'-GGGGGGGGGG CTT TAT TA TTA GGC ATG GGG AAG
Probe34		HPV34	5'-GGGGGGGGGG CTT TAT CC TCA GAC ATG CAG AAG
Probe35		HPV33	5'-GGGGGGGGGG CTT TAT TA TAA GAC ATG TTG AAG

Probe36		HPV52	5'-GGGGGGGGGG CTT TAT CC TTC GTC ATG GCG AGG
Probe37		HPV40	5'-GGGGGGGGGG CTT TAT TT TGC GTC ATG GGG AGG
Probe38		HPV68	5'-GGGGGGGGGG CTT TAT TA TTA GGC ATG TTG AGG
Probe39		HPV56	5'-GGGGGGGGGG CTT TAT CC TTA GAC ATG TGG AGG
Probe40		HPV35	5'-GGGGGGGGGG CTT TAT TT TAA GGC ATG GTG AAG
Probe41		HPV58	5'-GGGGGGGGGG CTT TAT TG TAC GTC ATG TTG AAG
Probe with one mutations (M1), two mutations (M2),and three mutations (M3)			
Probe42	1T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC ATG GGG AGT
Probe43	2T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC ATG GGG ATG
Probe44	3T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC ATG GGG TGG
Probe45	4T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC ATG GGT AGG
Probe46	5T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC ATG GTG AGG
Probe47	6T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC ATG TGG AGG
Probe48	7T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC ATT GGG AGG
Probe49	8A	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC AAG GGG AGG
Probe50	9T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC TTG GGG AGG
Probe51	2,9T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC TTG GGG ATG
Probe52	3,9T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC TTG GGG TGG
Probe53	4,9T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC TTG GGT AGG
Probe54	5,9T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC TTG GTG AGG
Probe55	6,9T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC TTG TGG AGG
Probe56	7,9T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC TTT GGG AGG
Probe57	2,5,9T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC TTG GTG ATG
Probe58	3,6,9T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC TTG TGG TGG
Probe59	4,7,9T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC TTT GGT AGG
(HPV Probes with one artificial mutation except for HPV33 and HPV68)			
Probe60	9T	HPV16	5'-GGGGGGGGGG CTT TAT CC TAC GAC TTG GGG AGG
Probe61	9T	HPV18	5'-GGGGGGGGGG CTT TAT TA GCA GAC TTG TTG AGG
Probe62	7T	HPV06	5'-GGGGGGGGGG CTT TAT CA TGC GTC ATA TGG AAG
Probe63	7T	HPV66	5'-GGGGGGGGGG CTT TAT CC TTC GCC ATA TGG AGG
Probe64	4T	HPV39	5'-GGGGGGGGGG CTT TAT TA CCA GGC ACG TGT AGG
Probe65	9T	HPV42	5'-GGGGGGGGGG CTT TAT TT TAA GAC TTG CTG AAG
Probe66	7T	HPV31	5'-GGGGGGGGGG CTT TAT TT TAA GAC ATA GTG AGG
Probe67	7T	HPV45	5'-GGGGGGGGGG CTT TAT TA GTA GAC ATA TGG AGG
Probe68	7T	HPV11	5'-GGGGGGGGGG CTT TAT CA TGC GCC ATA TGG AGG
Probe69	7T	HPV59	5'-GGGGGGGGGG CTT TAT TG CCA GAC ATA TGG AGG
Probe70	7T	HPV51	5'-GGGGGGGGGG CTT TAT TA TTA GGC ATA GGG AAG
Probe71	9T	HPV34	5'-GGGGGGGGGG CTT TAT CC TCA GAC TTG CAG AAG
Probe72	-	HPV33	5'-GGGGGGGGGG CTT TAT TA TAA GAC ATG TTG AAG
Probe73	8T	HPV52	5'-GGGGGGGGGG CTT TAT CC TTC GTC AAG GCG AGG
Probe74	7T	HPV40	5'-GGGGGGGGGG CTT TAT TT TGC GTC ATA GGG AGG
Probe75	-	HPV68	5'-GGGGGGGGGG CTT TAT TG TTA GGC ATG TTG AGG

Probe76	4T	HPV56	5'-GGGGGGGGGG CTT TAT CC TTA GAC ATG TG ^T AGG
Probe77	8T	HPV35	5'-GGGGGGGGGG CTT TAT TT TAA GGC AA ^G GTG AAG
Probe78	7T	HPV58	5'-GGGGGGGGGG CTT TAT TG TAC GTC AT ^A TTG AAG
Probe79		HC	5'-GGGGGGGGGG TTT CCT AG TGG CTC TAT GGT AAC
Probe80		PC	5'-GGGGGGGGGG TGA TTT ACA GTT TAT DTT TC
Probe81		PCR	5'-GGGGGGGGGG ATT GGC ATG BKG ARG ART WTG A
Probe82		NC	5'-GGGGGGGGGG AAA GCT GCT GCT CGT CGT CGT CGT
Target1		HC-Cy5-T1	3'- GGA TCA CCG AGA TAC CAT TG GAG ACT GCG -Cy5-5'
Forward primer	primer	FP	3'-GCMCAGGGWCATAAAYAATGG-5'
Reverse primer	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
GGGGGGGGGG – 9G for immobilization of the probes on the AMCA slides
CTT TAT – vertical spacer group

4. Amplification of the HPV DNA by PCR:

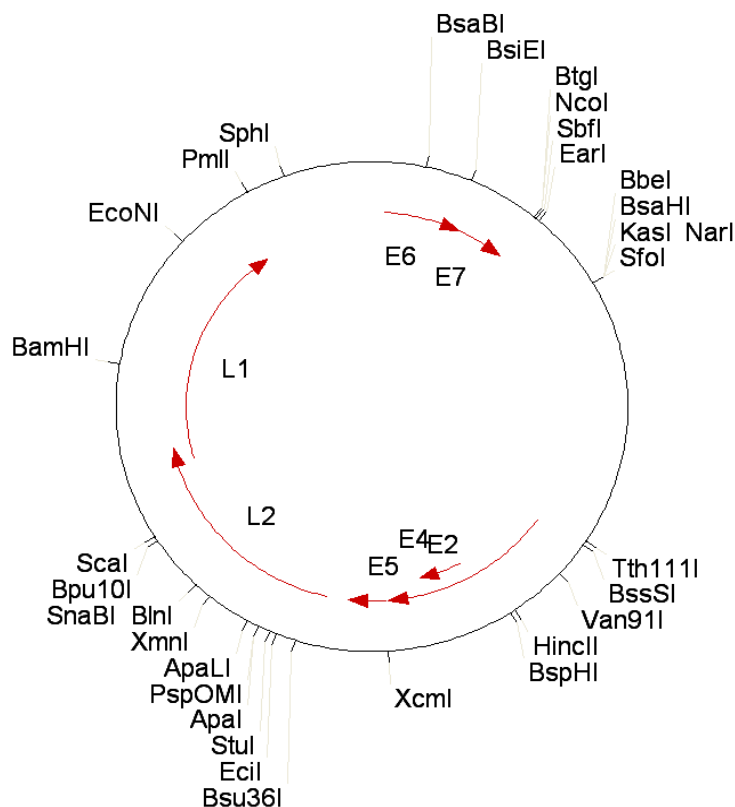


Figure S1: Genomic map with different regions on the HPV 16 genotype, L1 gene position 5559-7154.

The MY09/MY11 primer set-mediated PCR (MY-PCR) and the GP5⁺/GP6⁺ primer set-mediated PCR (GP⁺-PCR) are the most frequently used amplification systems for the detection of HPV DNA in clinical samples, amplifying DNA fragments in the conserved L1 region with approximately 450bp and 150bp, respectively. Further, type-specific PCR primer sets allow the identification of individual genotypes. The MY11/ GP6⁺ primer set consists of a fixed nucleotide sequence for the forward and reverse primers, respectively, and detects a wide range of HPV types by using a lowered annealing temperature during PCR.

(Note: The MY11/GP6⁺ is a primer set is used to amplify a 189 bp fragment of the HPV L1 region by PCR.)

5. Selection of length of the Probes:

5a. Hybridization with Cy5 labeled PCR product of HPV16

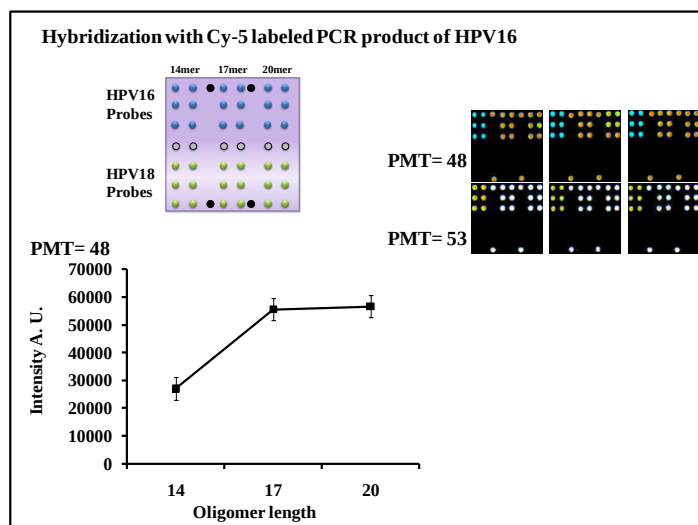


Figure S2: Probe length selection from 14mer, 17mer, and 20mer based on probes complementary to the HPV16

5b. Hybridization with Cy5 labeled PCR product of HPV18

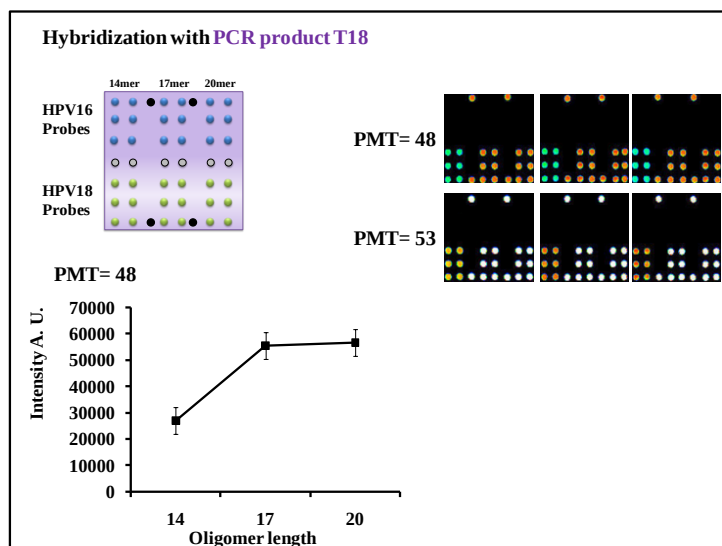


Figure S3: Probe length selection from 14mer, 17mer, and 20mer based on probes complementary to the HPV18

6. Selection of the starting position of the probes:

6a. Hybridization with Cy5 labeled PCR product of HPV16

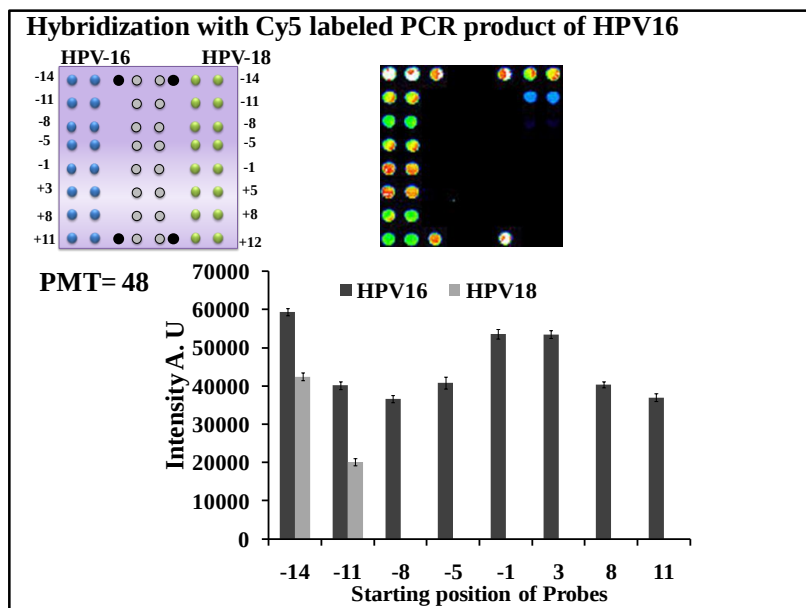


Figure S4: Selection of the starting position of the probes based on probes complementary to the HPV16

6b. Hybridization with Cy5 labeled PCR product of HPV18

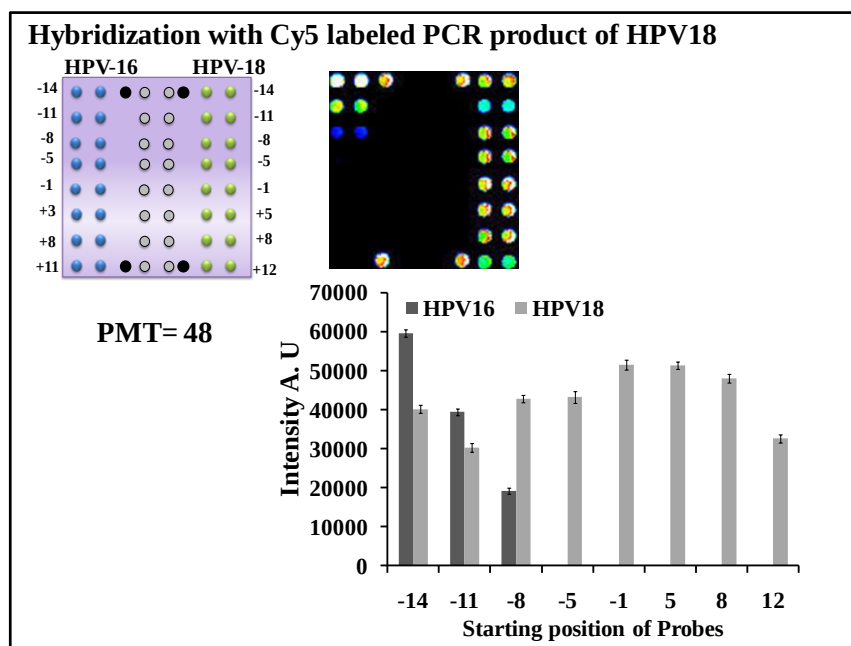


Figure S5: Selection of the starting position of the probes based on probes complementary to the HPV18

7. Probe length and the Probe sequence:

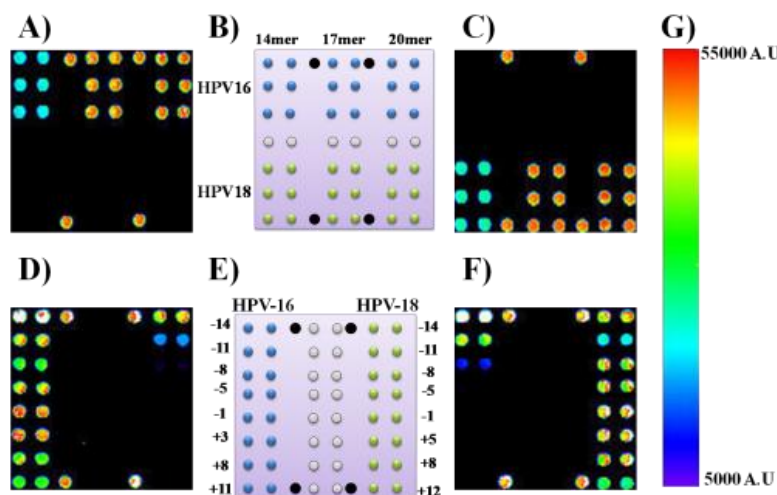


Figure S6: Selection of the Probe length and the Probe sequence. A) Fluorescence image after the hybridization of immobilized probes (Probe1-probe3) with the Cy5 labeled PCR product of HPV16, B) Depicts the scheme for the immobilization of the Probes (Probe1 – Probe6) on the AMCA slide. C) Fluorescence image after the hybridization of the immobilized probes (Probe4 – Probe6) with Cy5 labeled PCR product of HPV18, PMT gain = 48. D) Fluorescence image after the hybridization of the immobilized probes (Probe7 - Probe14) with the Cy5 labeled PCR product of HPV16, PMT gain = 48. E) Depicts the scheme for the immobilization of the probes (Probe7 – Probe22) on the AMCA slide. F) Fluorescence image after the hybridization of the immobilized probes (Probe15 – Probe22) with the Cy5 labeled PCR product of HPV18, G) Fluorescence scale depicting lowest to highest fluorescence. PMT gain = 48.

8. Results for hybridization of the probes (without mutations)

8a. Hybridization with Cy5 labeled PCR product of HPV45

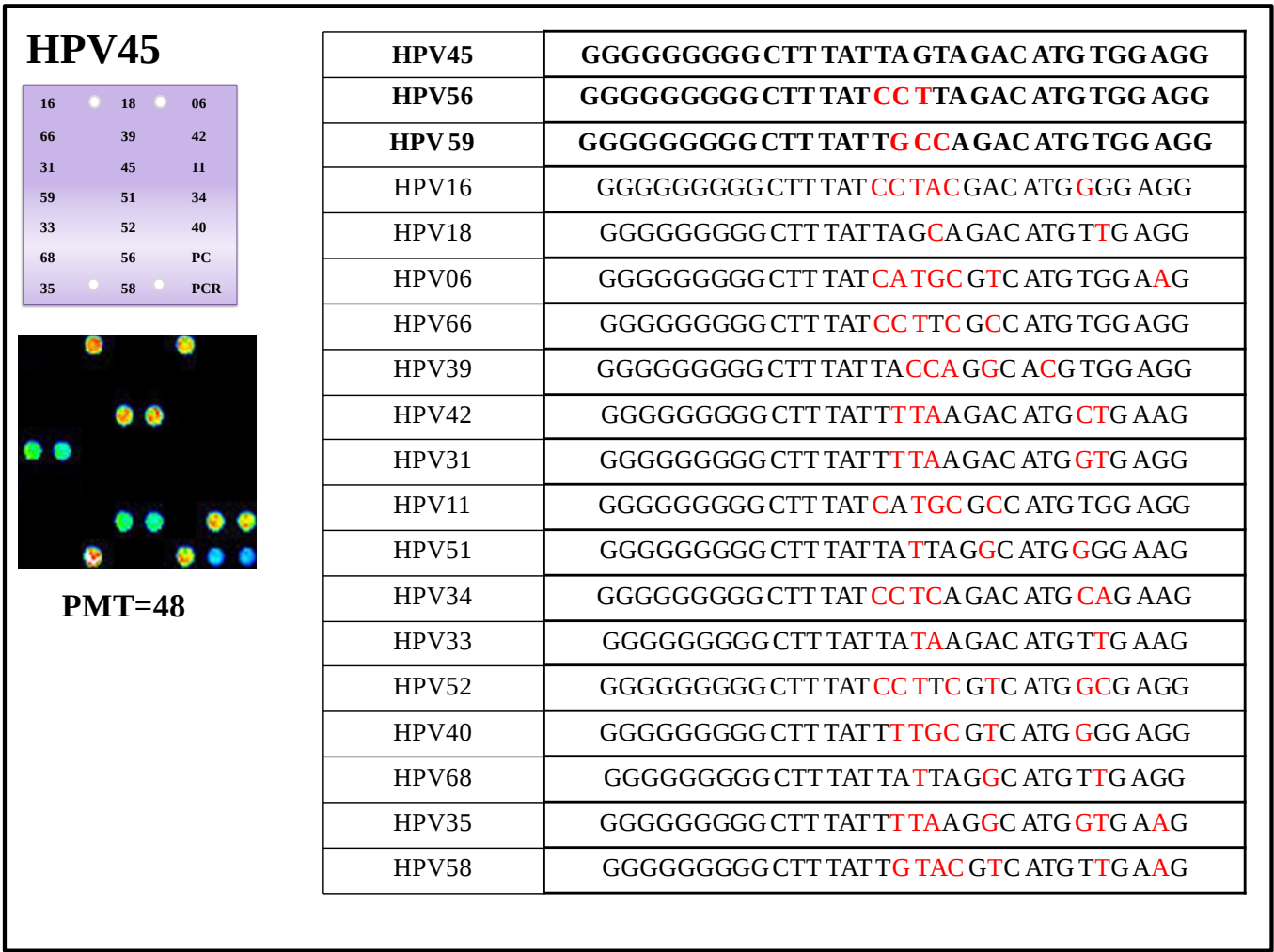


Figure S7: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV45

8b. Hybridization with Cy5 labeled PCR product of HPV33

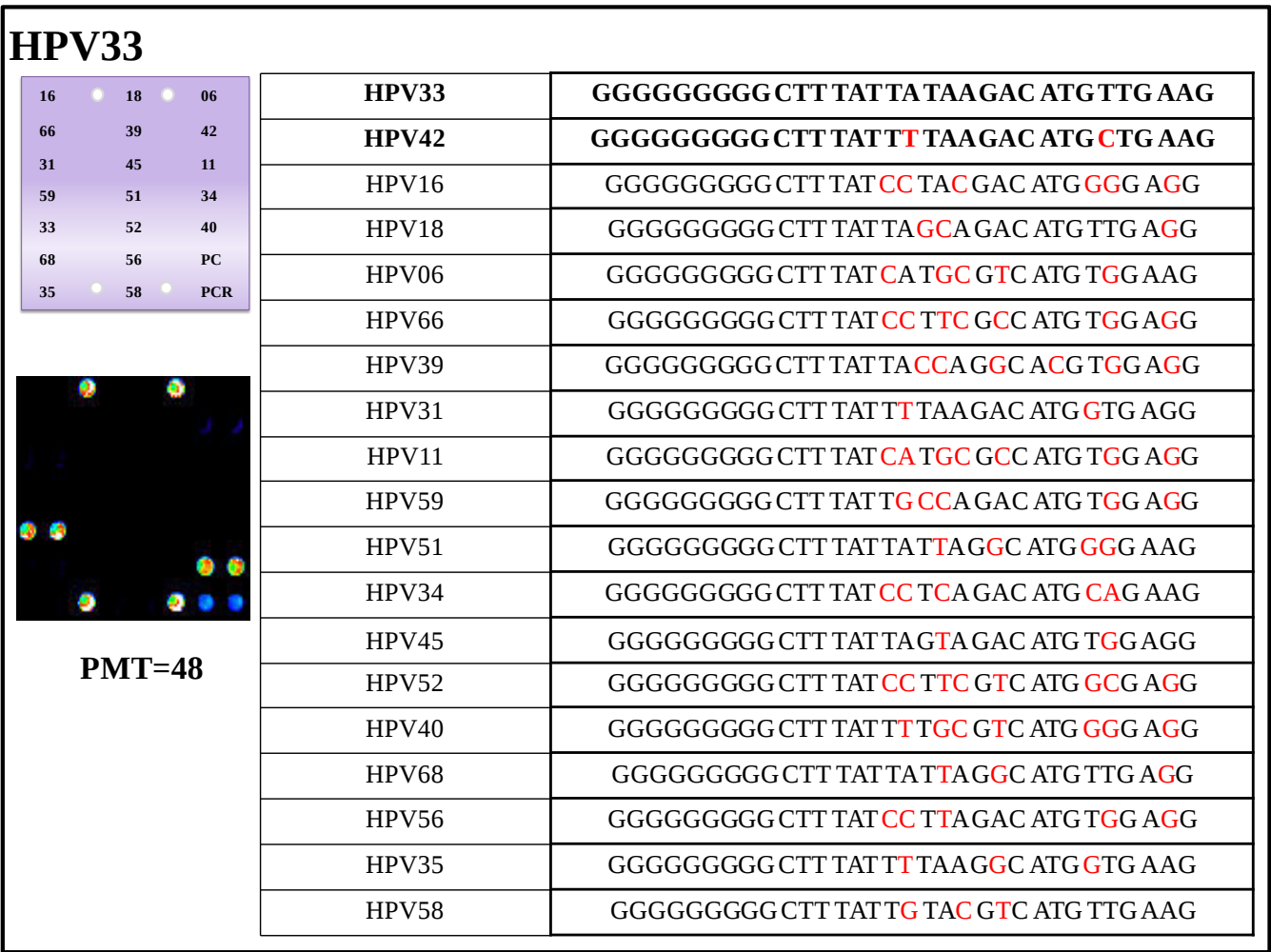


Figure S8: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV33

8c. Hybridization with Cy5 labeled PCR product of HPV31

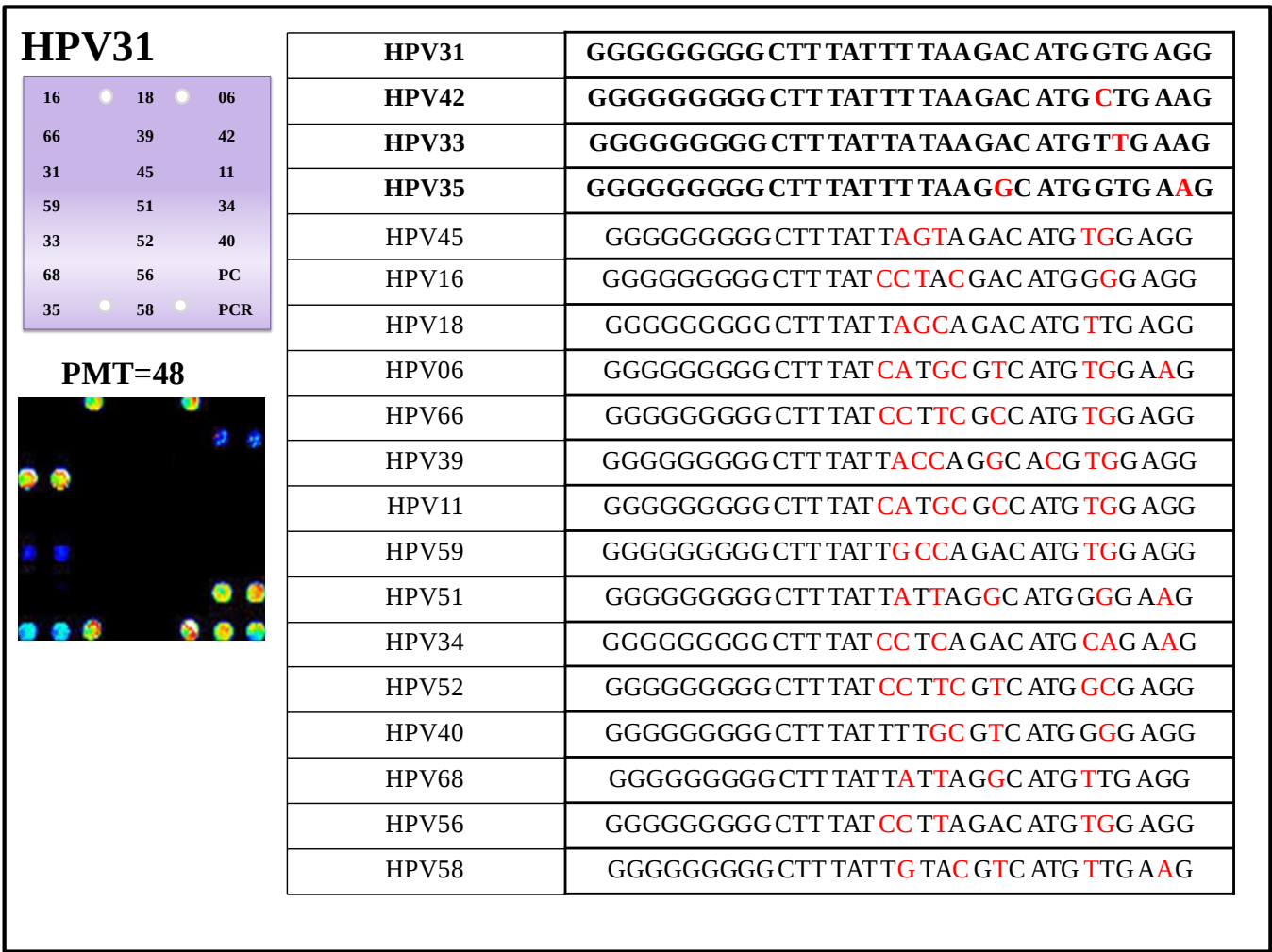


Figure S9: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV31

8d. Hybridization with Cy5 labeled PCR product of HPV42

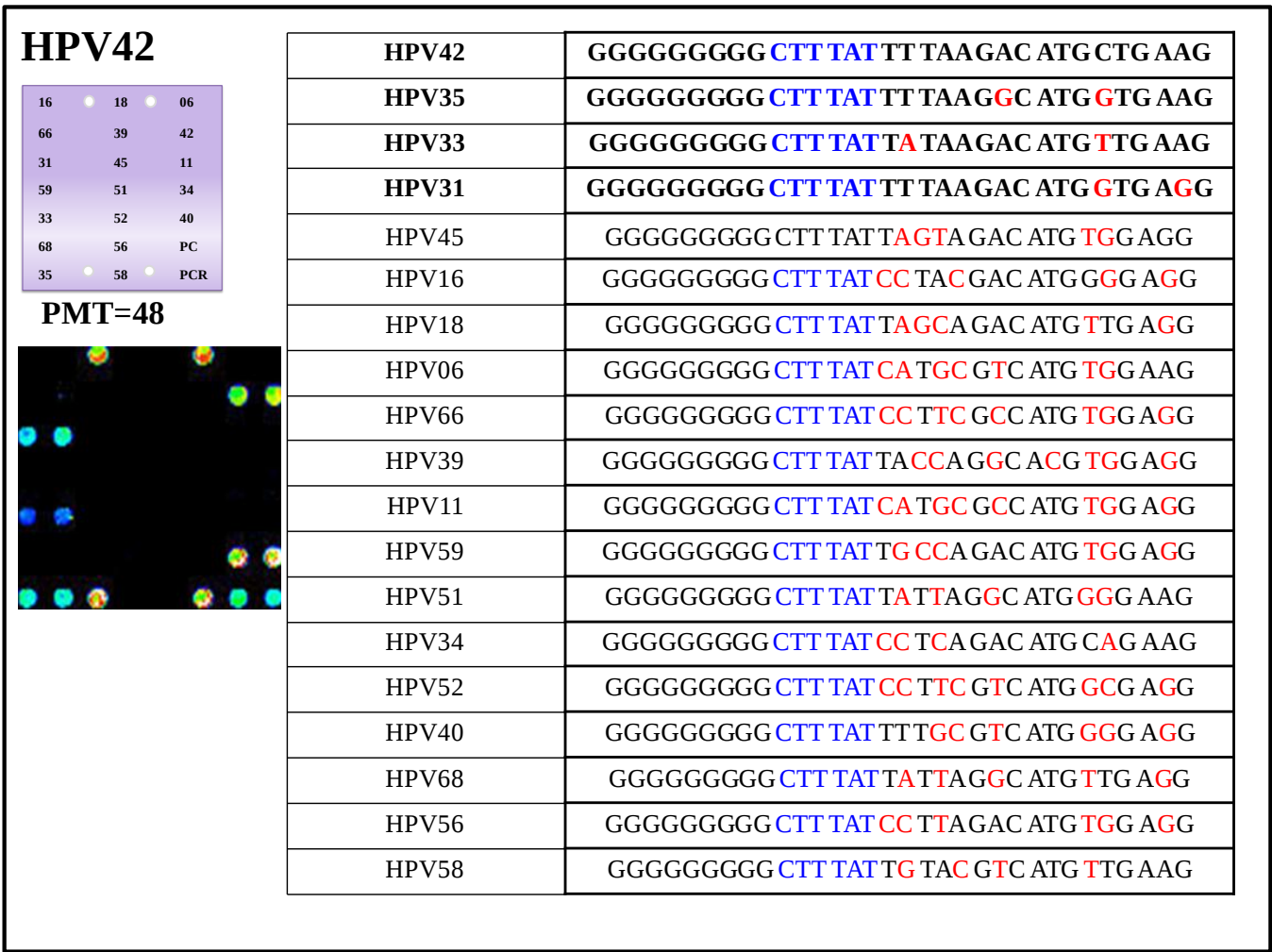


Figure S10: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV42

8e. Hybridization with Cy5 labeled PCR product of HPV59

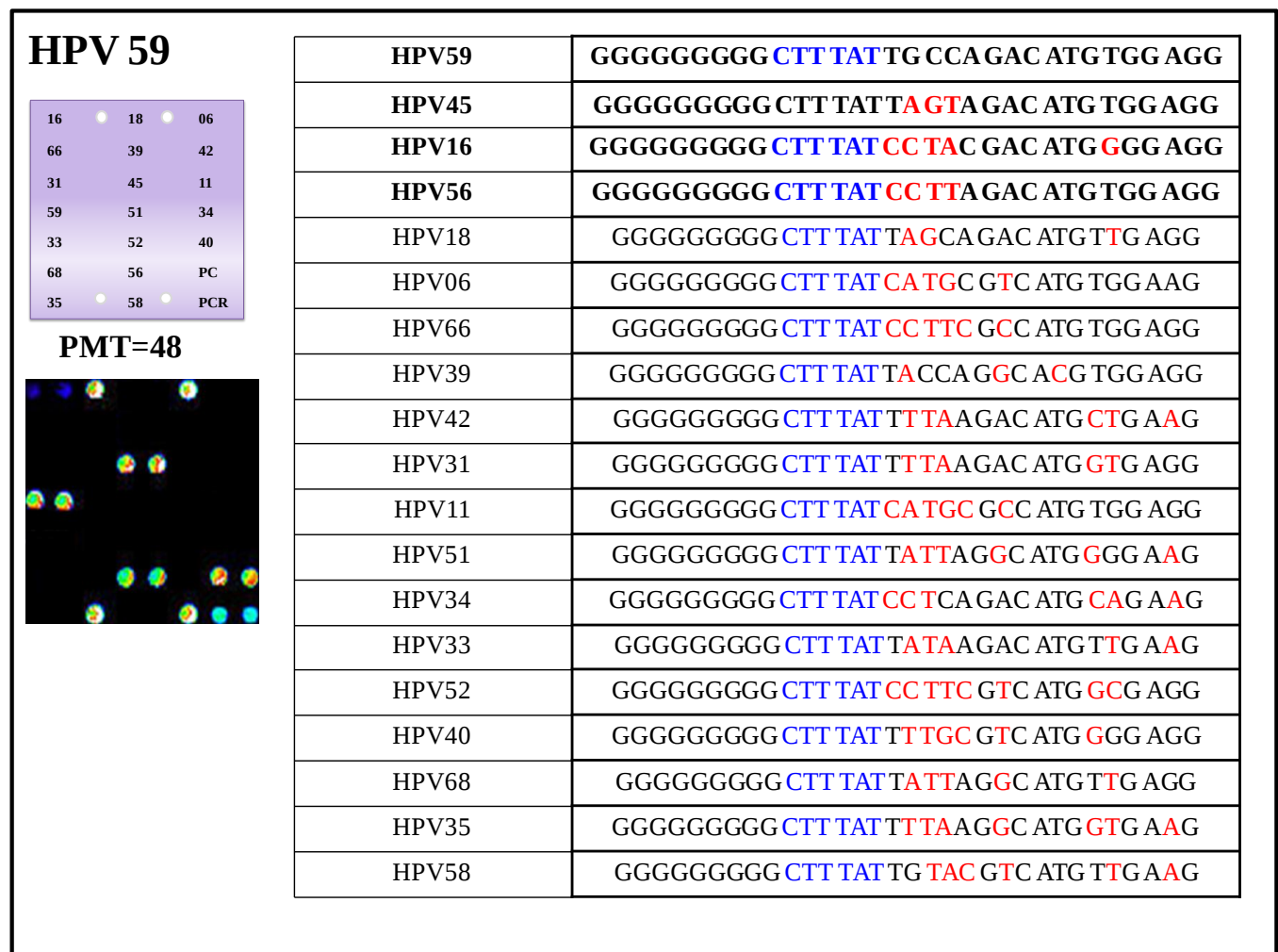


Figure S11: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV59

8f. Hybridization with Cy5 labeled PCR product of HPV51

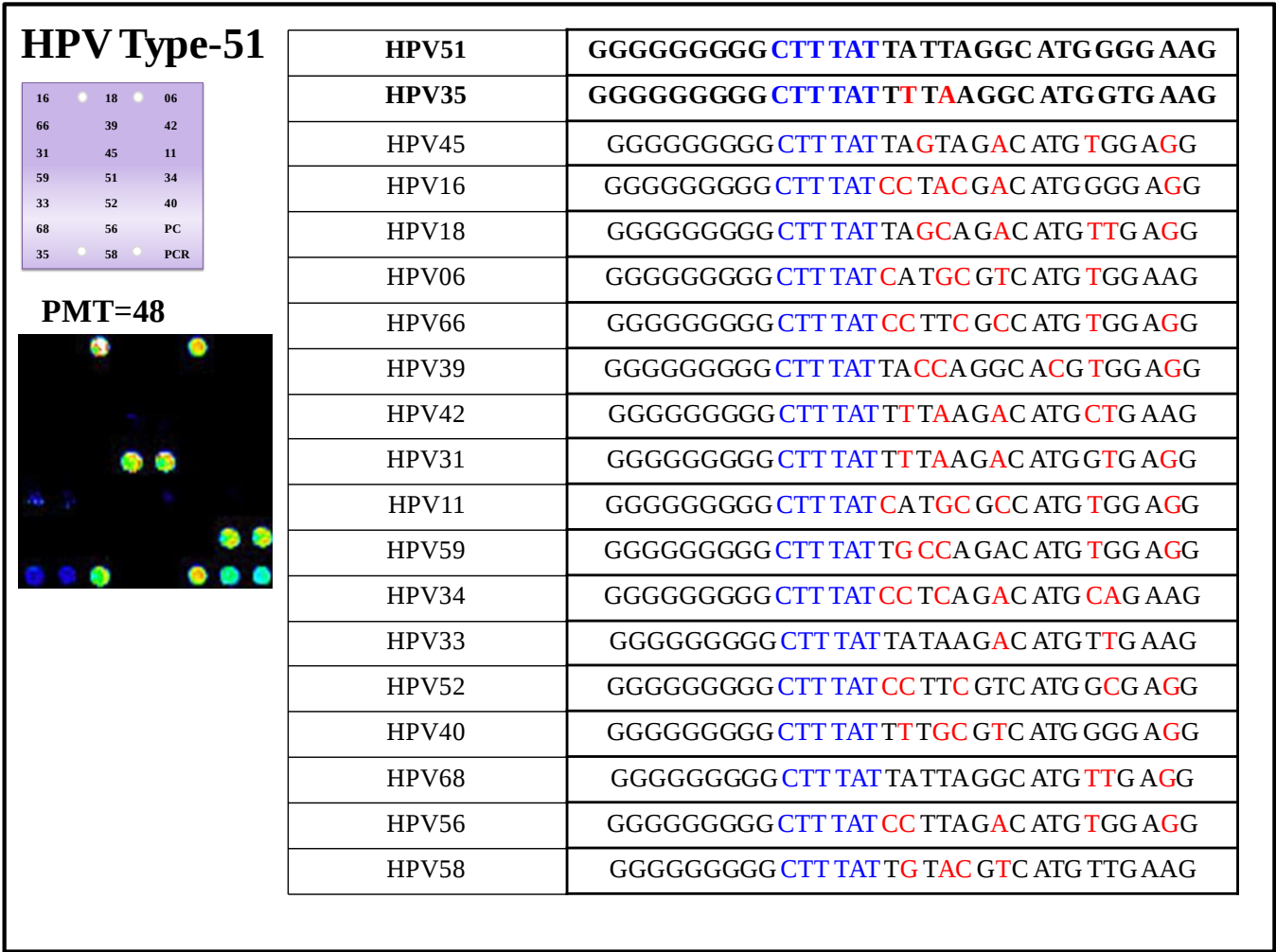


Figure S12: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV51

8g. Hybridization with Cy5 labeled PCR product of HPV06

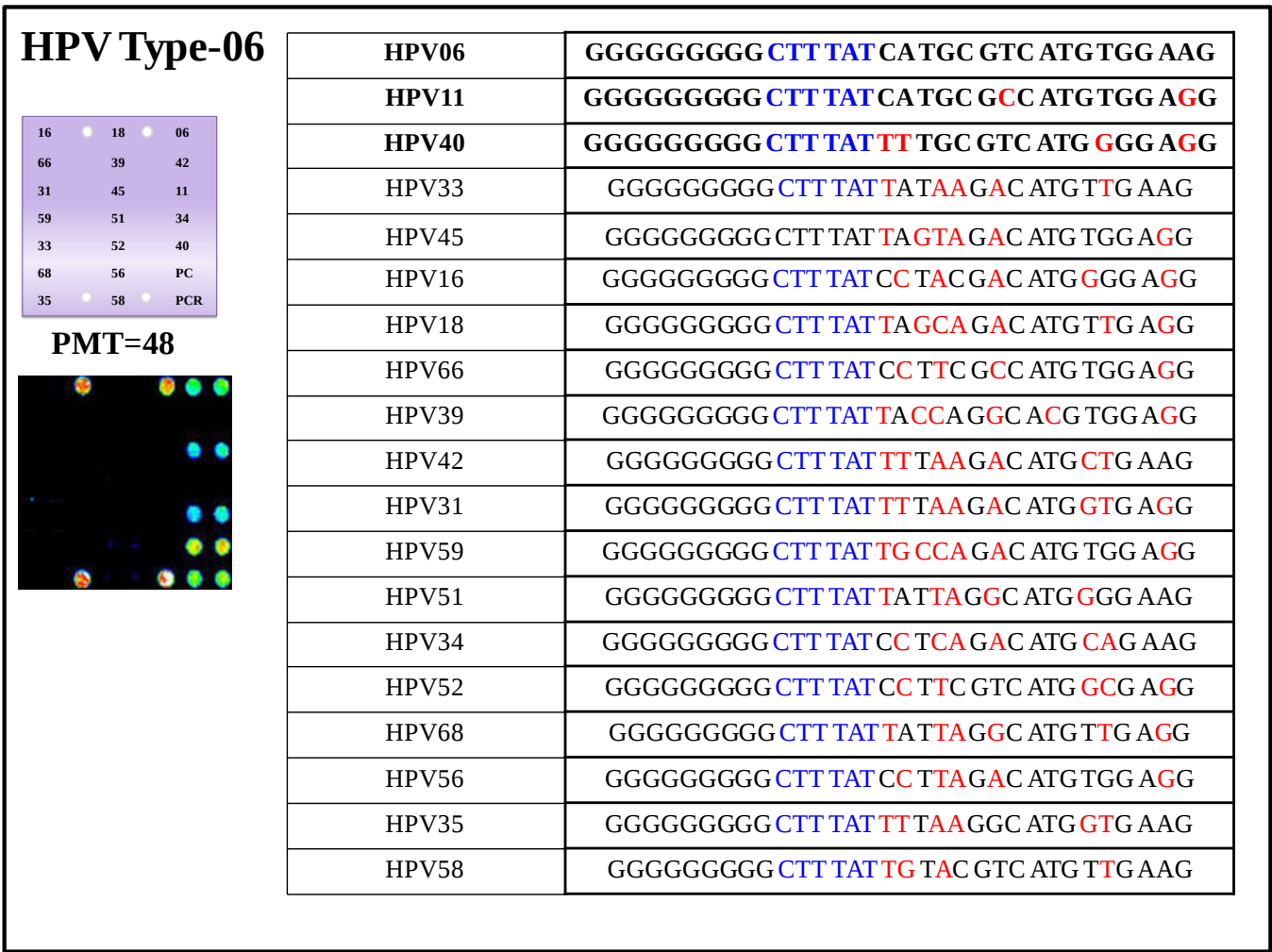


Figure S13: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV06

8h. Hybridization with Cy5 labeled PCR product of HPV58

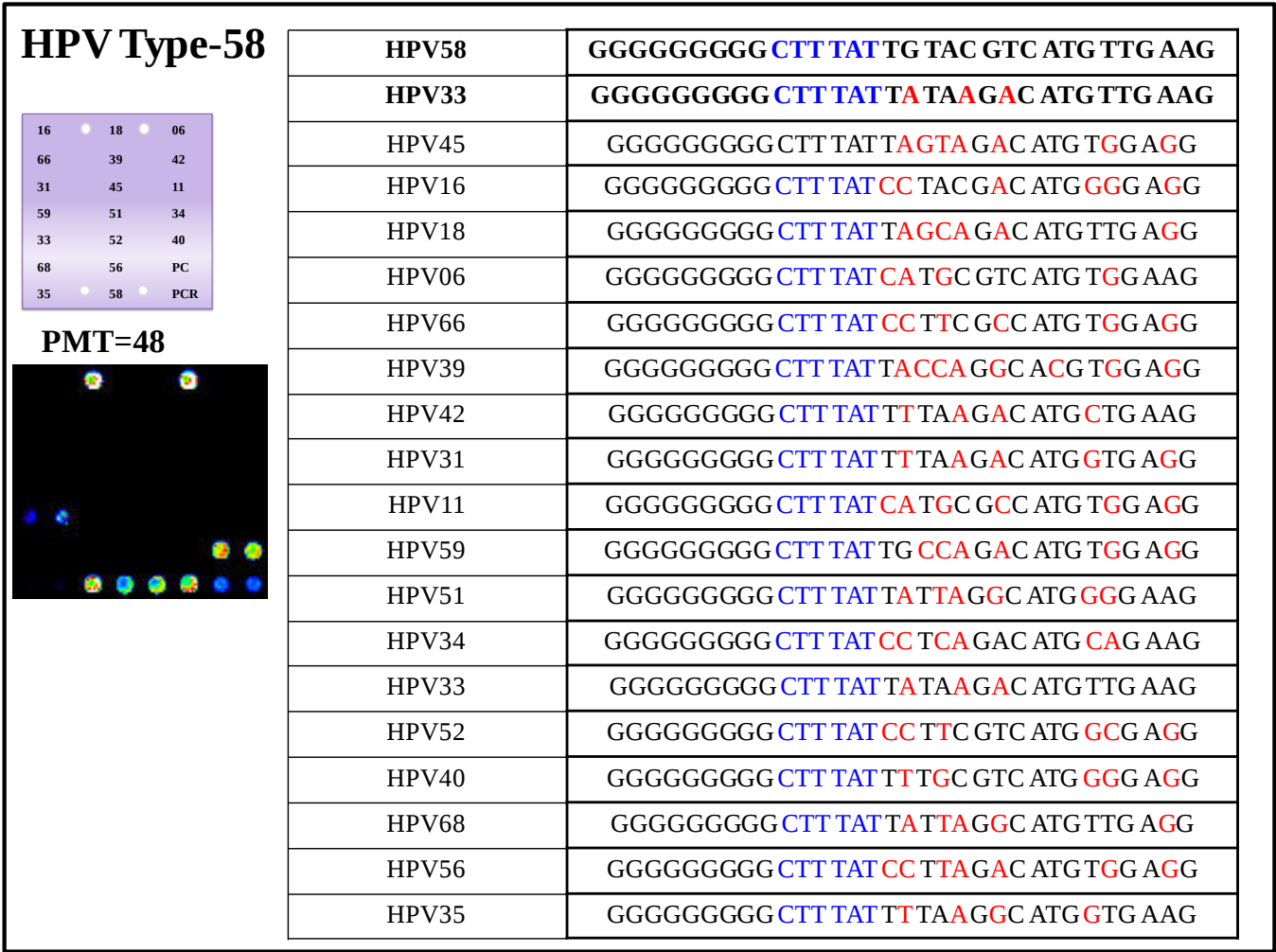


Figure S14: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV58

8i. Hybridization with Cy5 labeled PCR product of HPV56

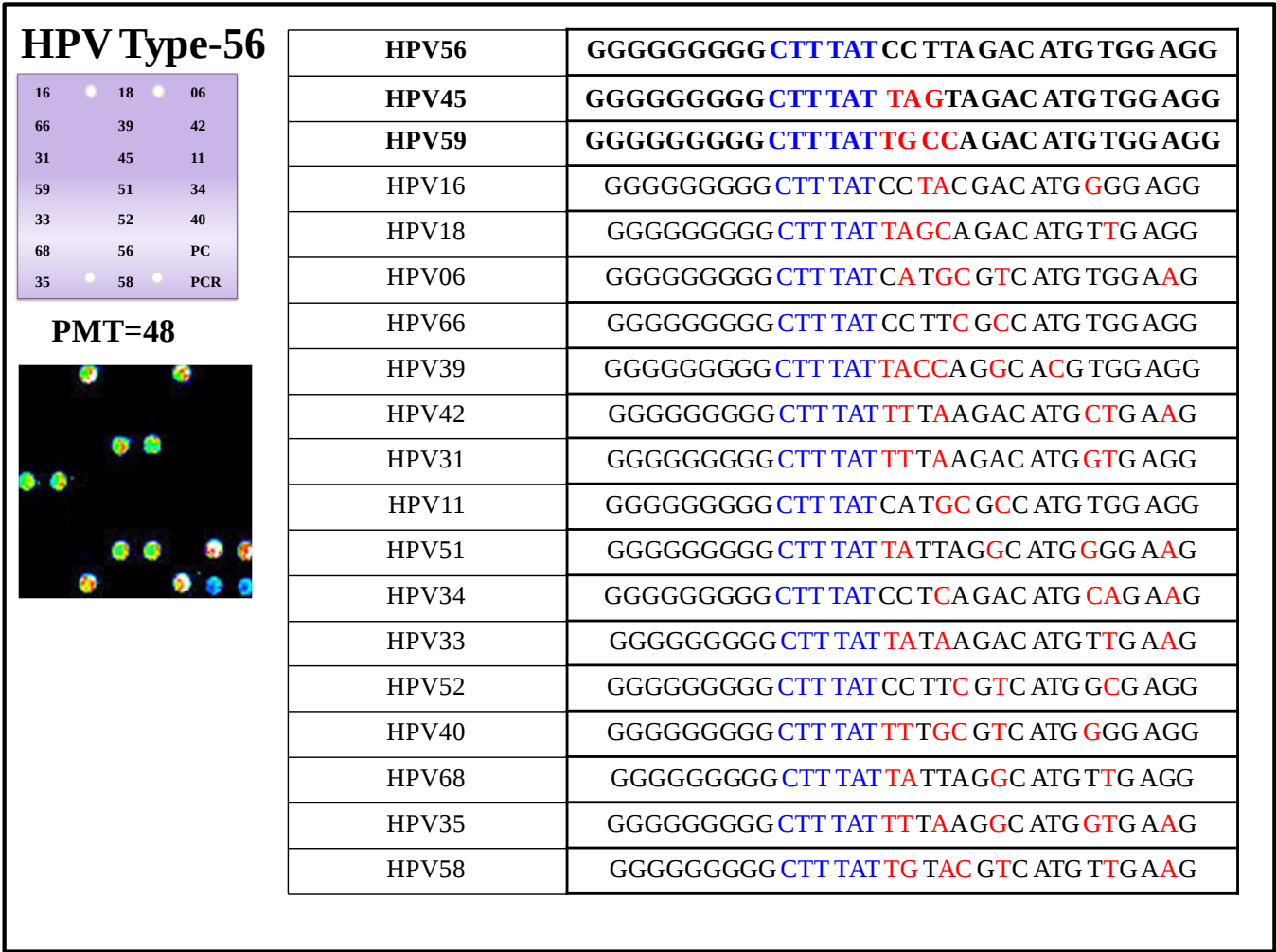


Figure S15: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV56

8j. Hybridization with Cy5 labeled PCR product of HPV18

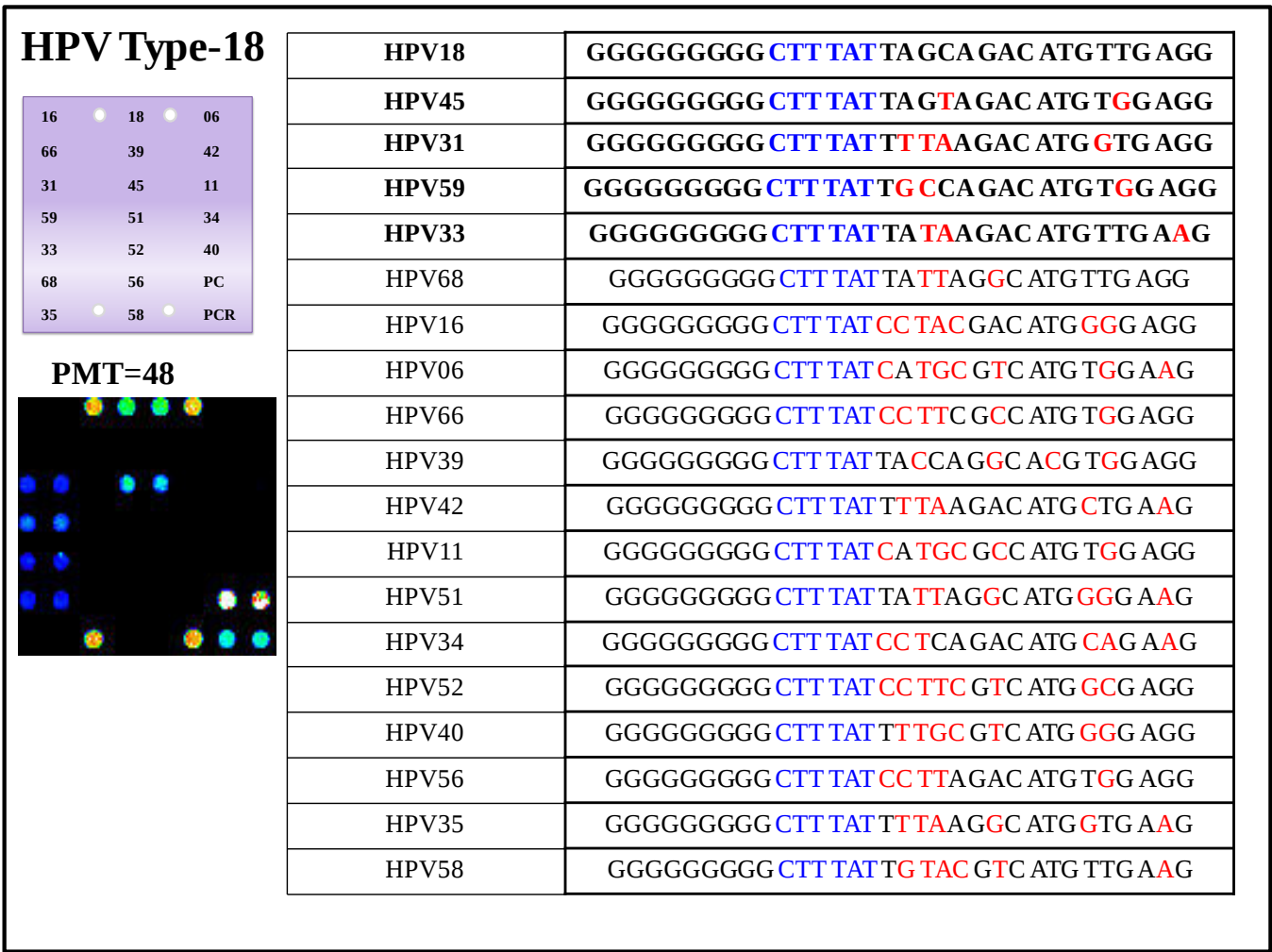


Figure S16: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV18

8k. Hybridization with Cy5 labeled PCR product of HPV66

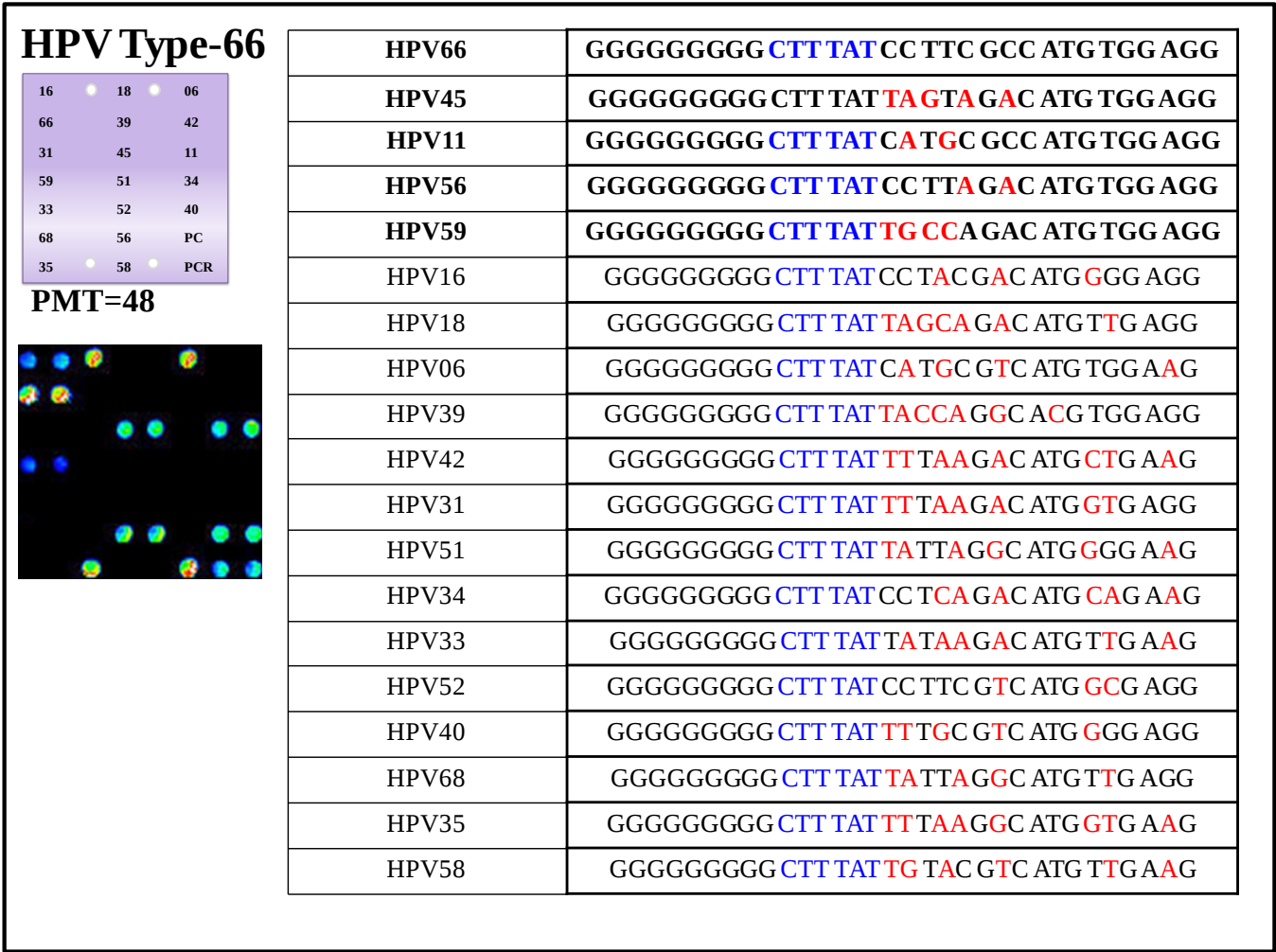


Figure S17: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV66

8l. Hybridization with Cy5 labeled PCR product of HPV40

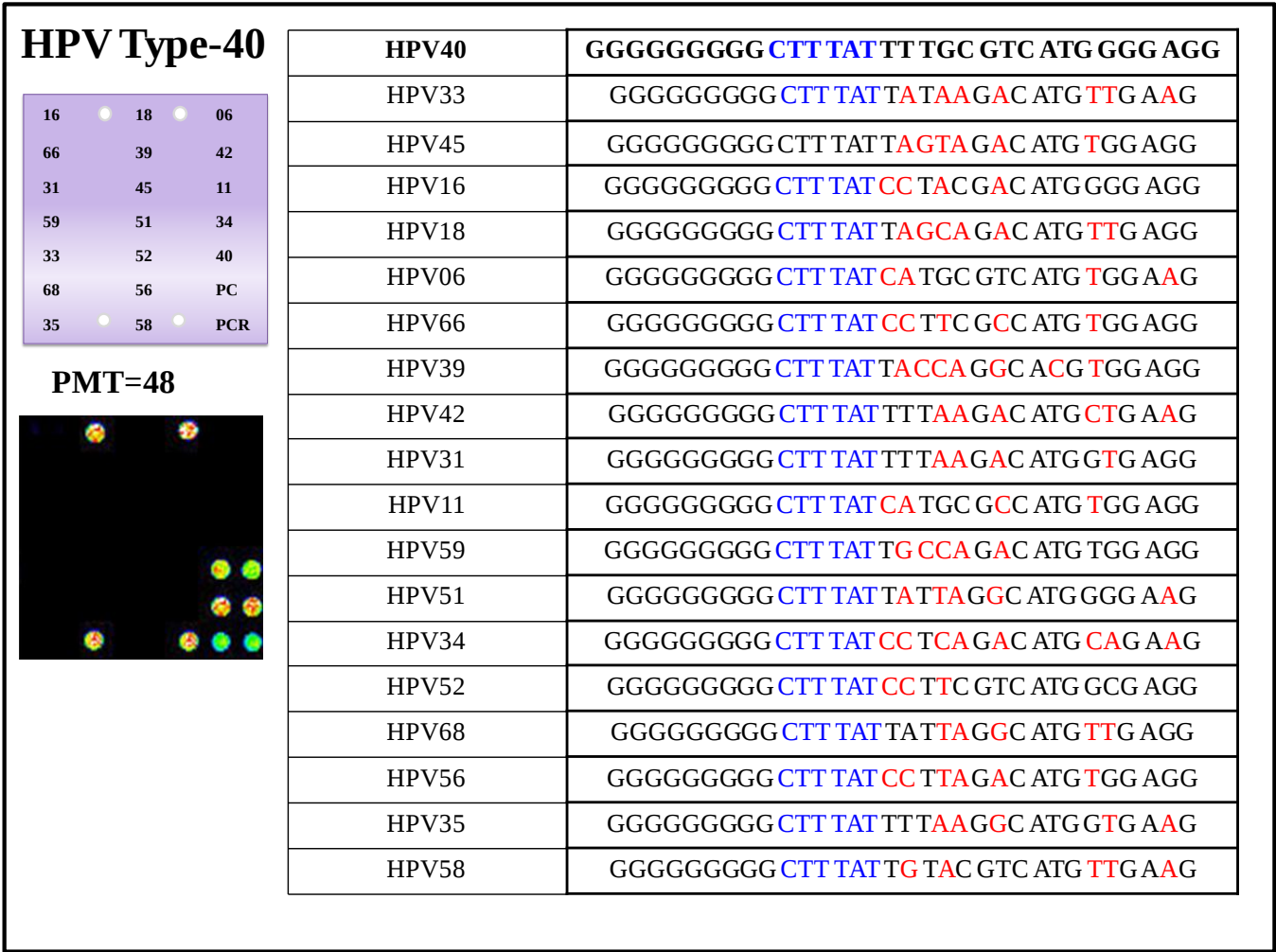


Figure S18: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV40

8m. Hybridization with Cy5 labeled PCR product of HPV16

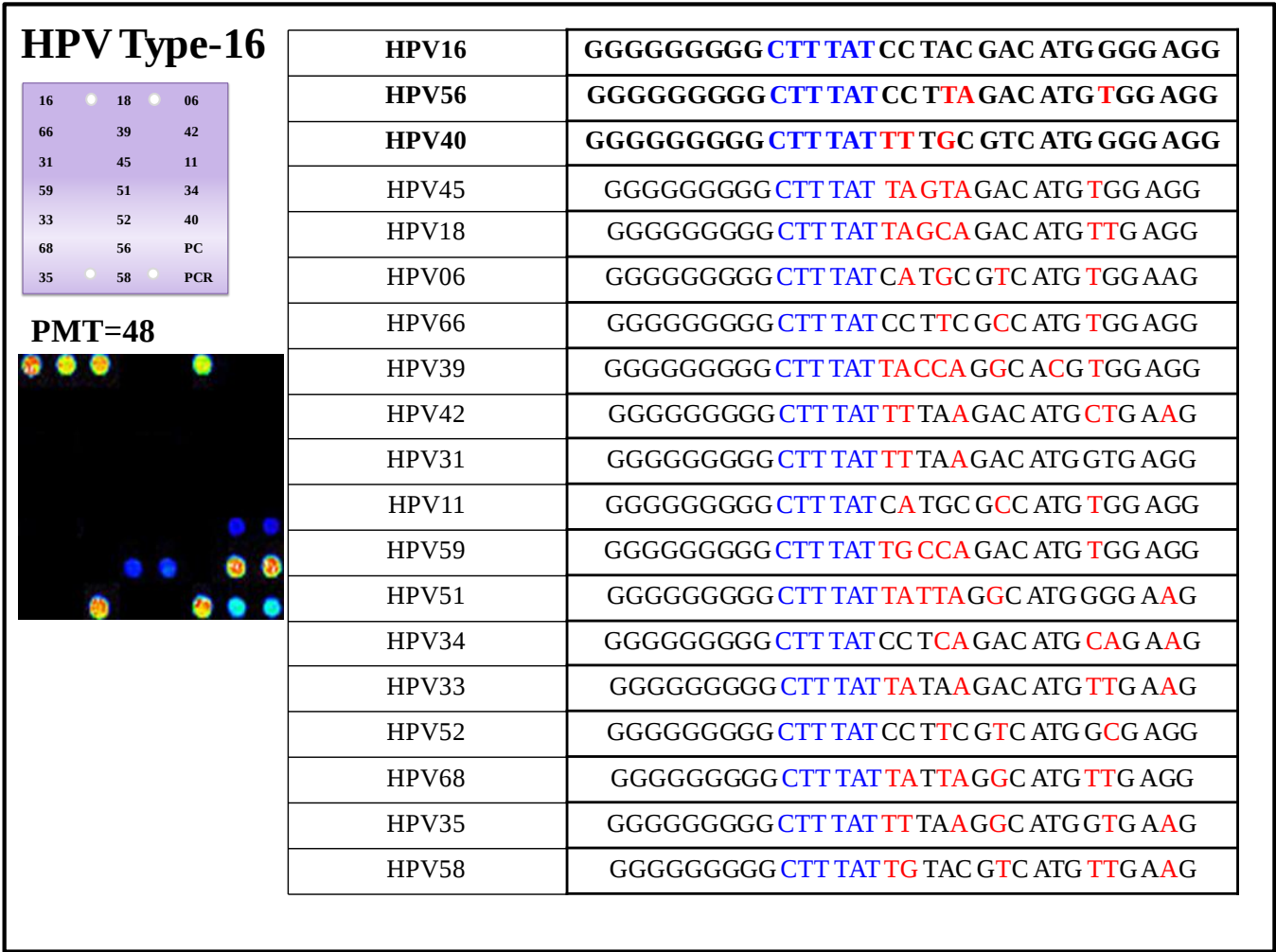


Figure S19: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV16

8n. Hybridization with Cy5 labeled PCR product of HPV35

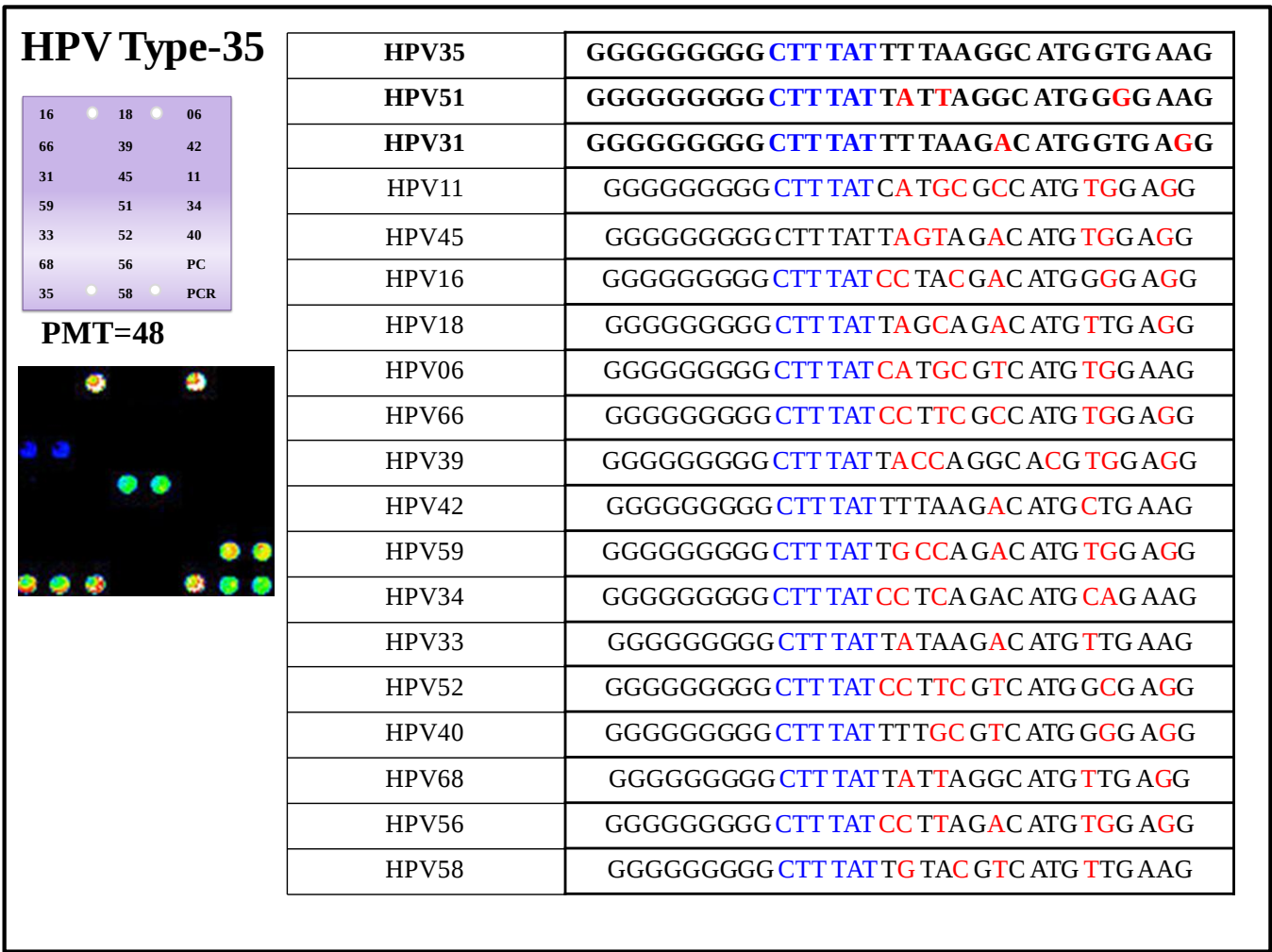


Figure S20: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV35

8o. Hybridization with Cy5 labeled PCR product of HPV52

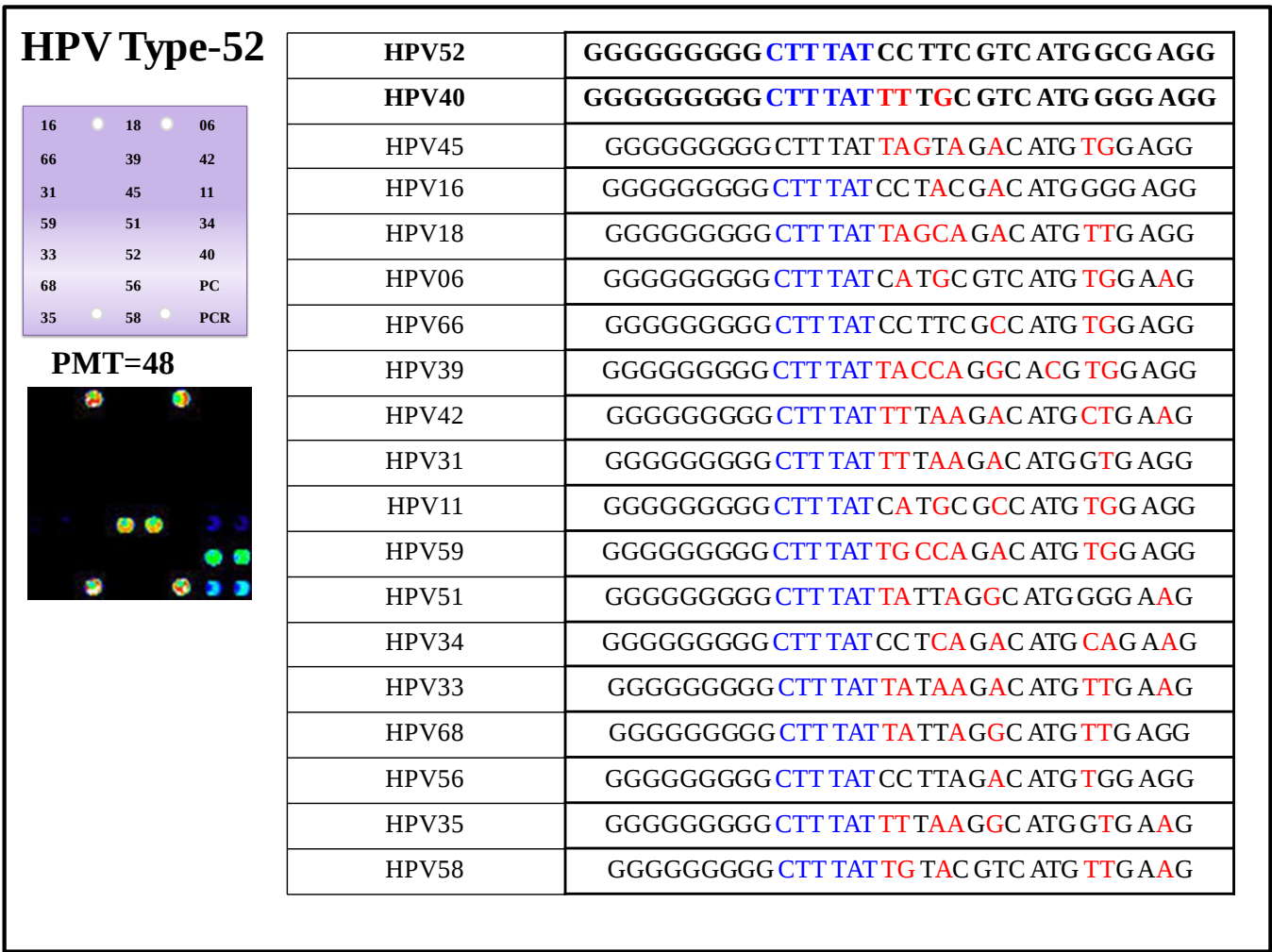


Figure S21: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV52

8p. Hybridization with Cy5 labeled PCR product of HPV11

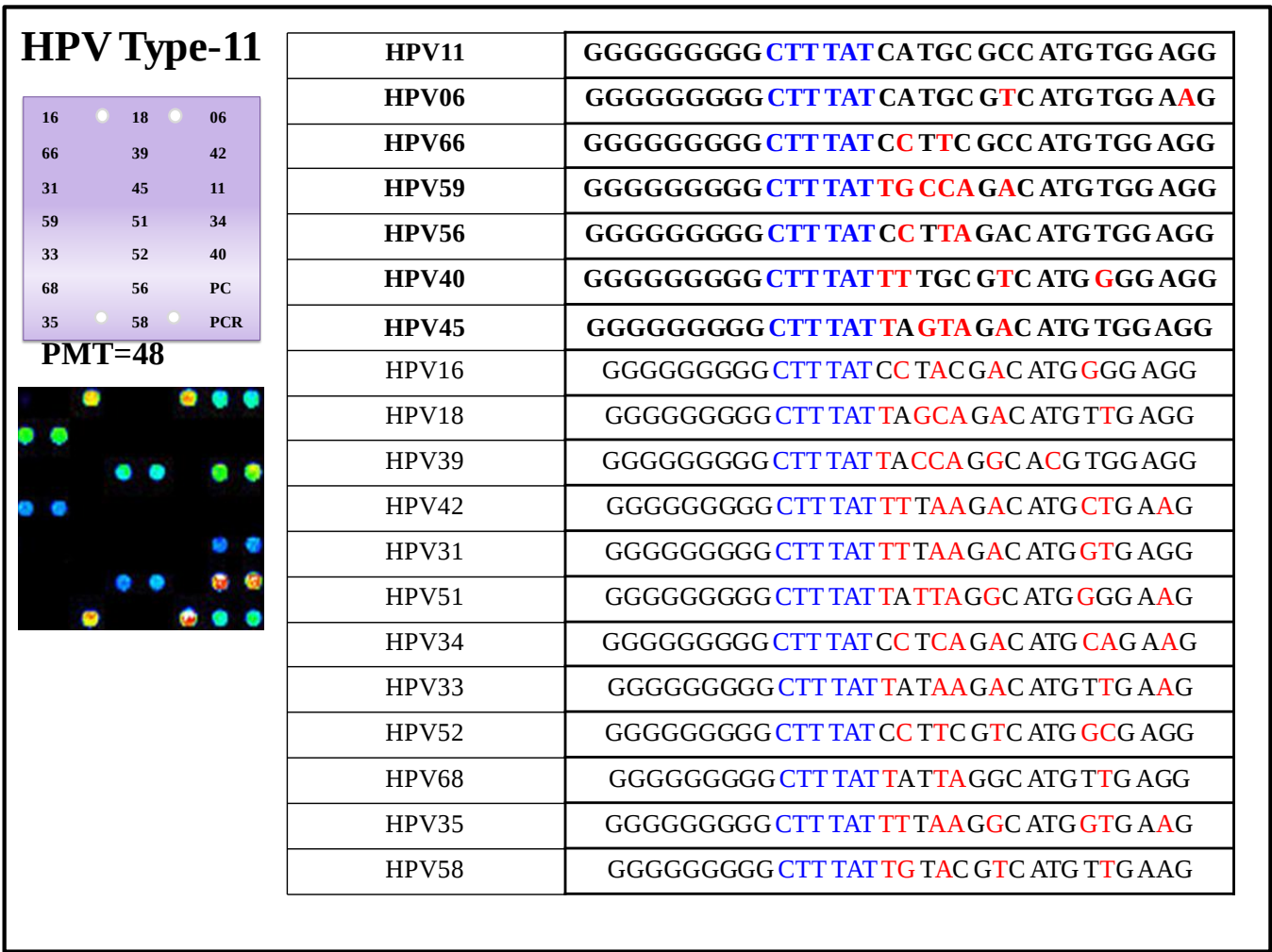


Figure S22: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV11

8q. Hybridization with Cy5 labeled PCR product of HPV34

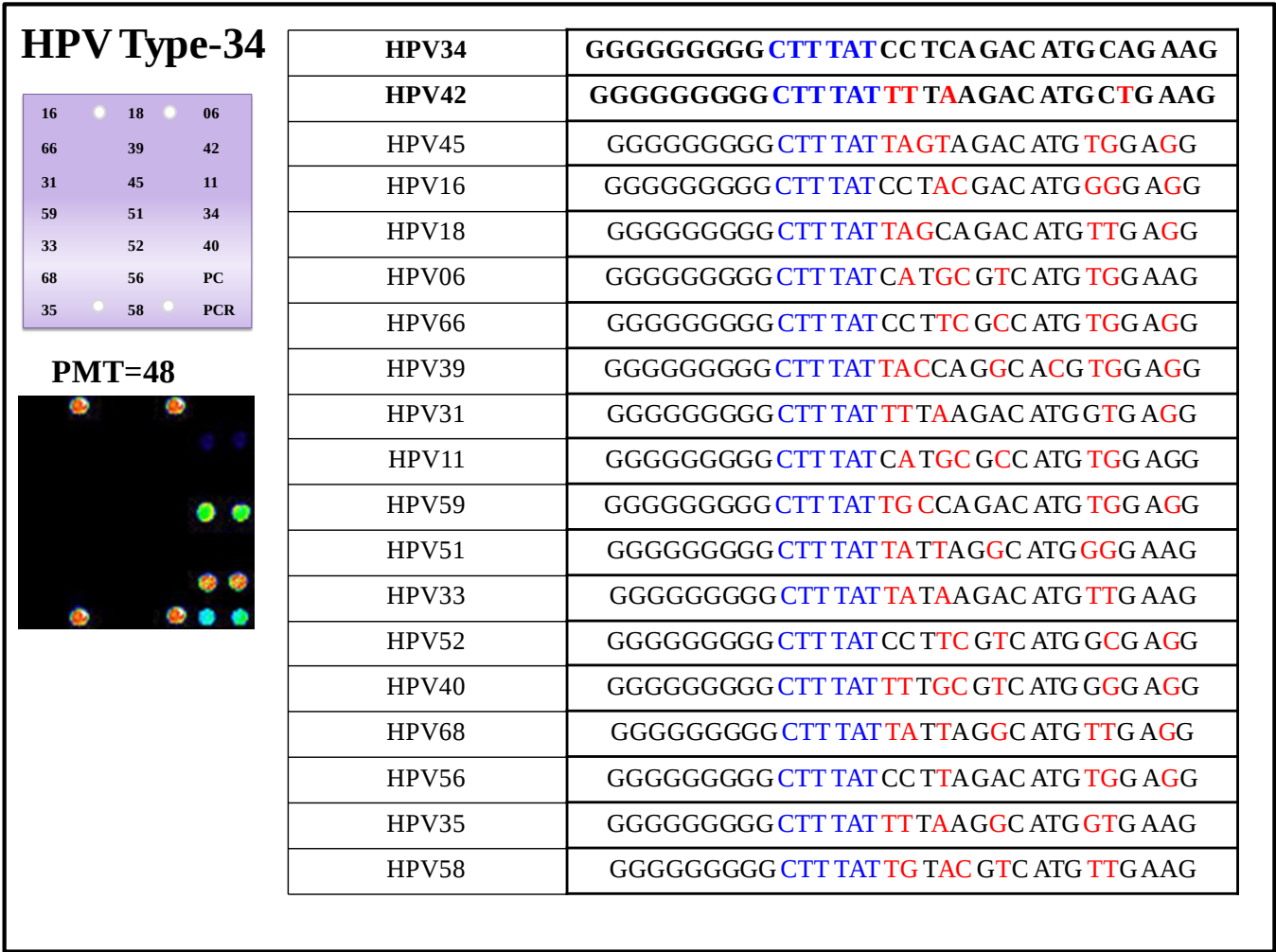


Figure S23: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV34

8r. Hybridization with Cy5 labeled PCR product of HPV39

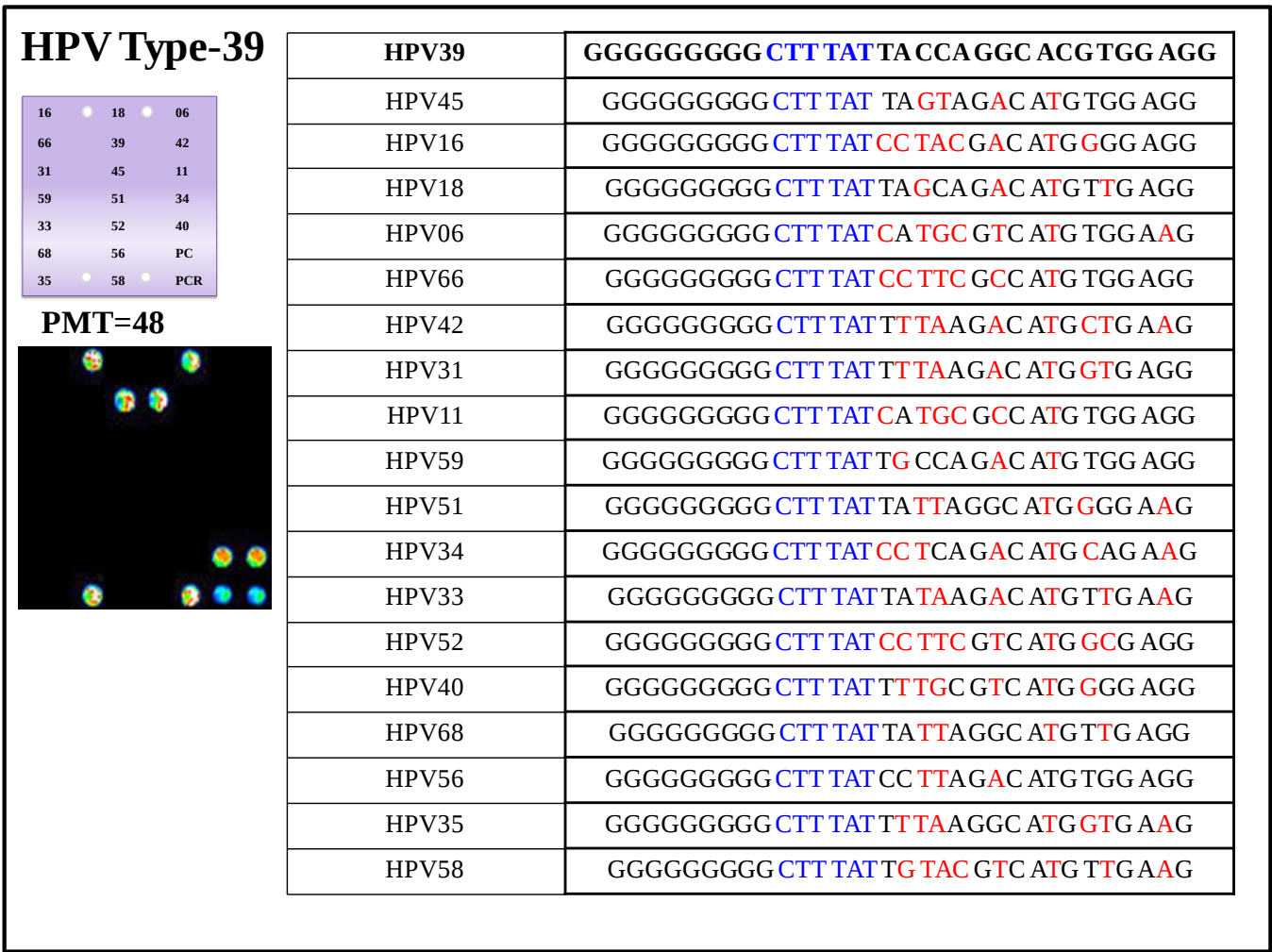


Figure S24: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV39

8s. Hybridization with Cy5 labeled PCR product of HPV68

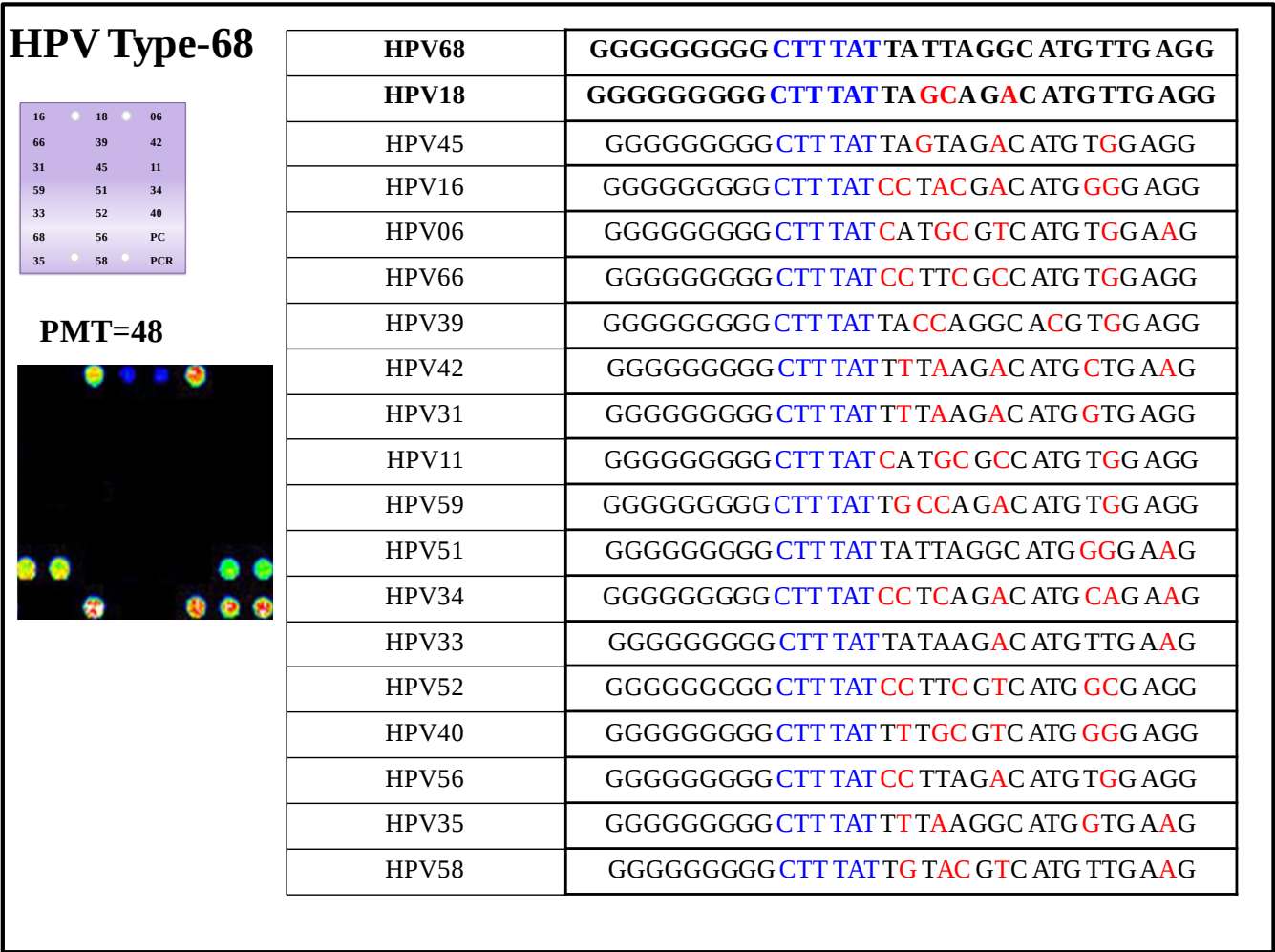


Figure S25: Fluorescence image after hybridization of Pre-HPV 9G DNAChip with Cy5 labeled PCR product of HPV68

9. Artificial mutation(s) in the probes and its effect on the hybridization with target

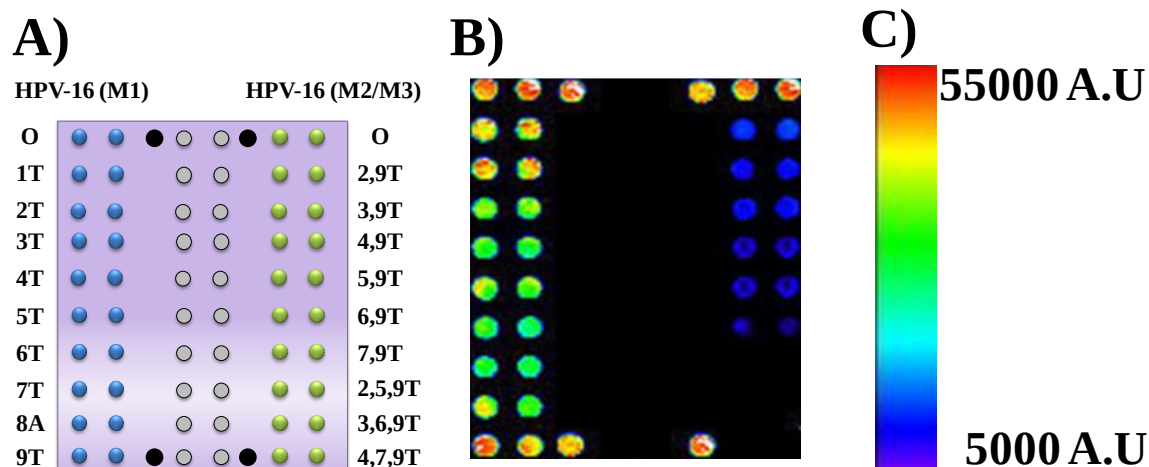


Figure S26: A) Depicts the scheme for the immobilization of the probes (Probe42 – Probe59) on the AMCA slide. B) Fluorescence image after the hybridization of immobilized probes (Probe42 – Probe59) with Cy5 labeled PCR product of HPV16, C) Fluorescence scale depicting lowest to highest fluorescence, PMT gain = 48.

10. Comparison of the results after hybridization of the original probes (without mutation) and final probes (with one artificial mutation)

10a. Hybridization with Cy5 labeled PCR products of HPV type 31, 42, 33, 35

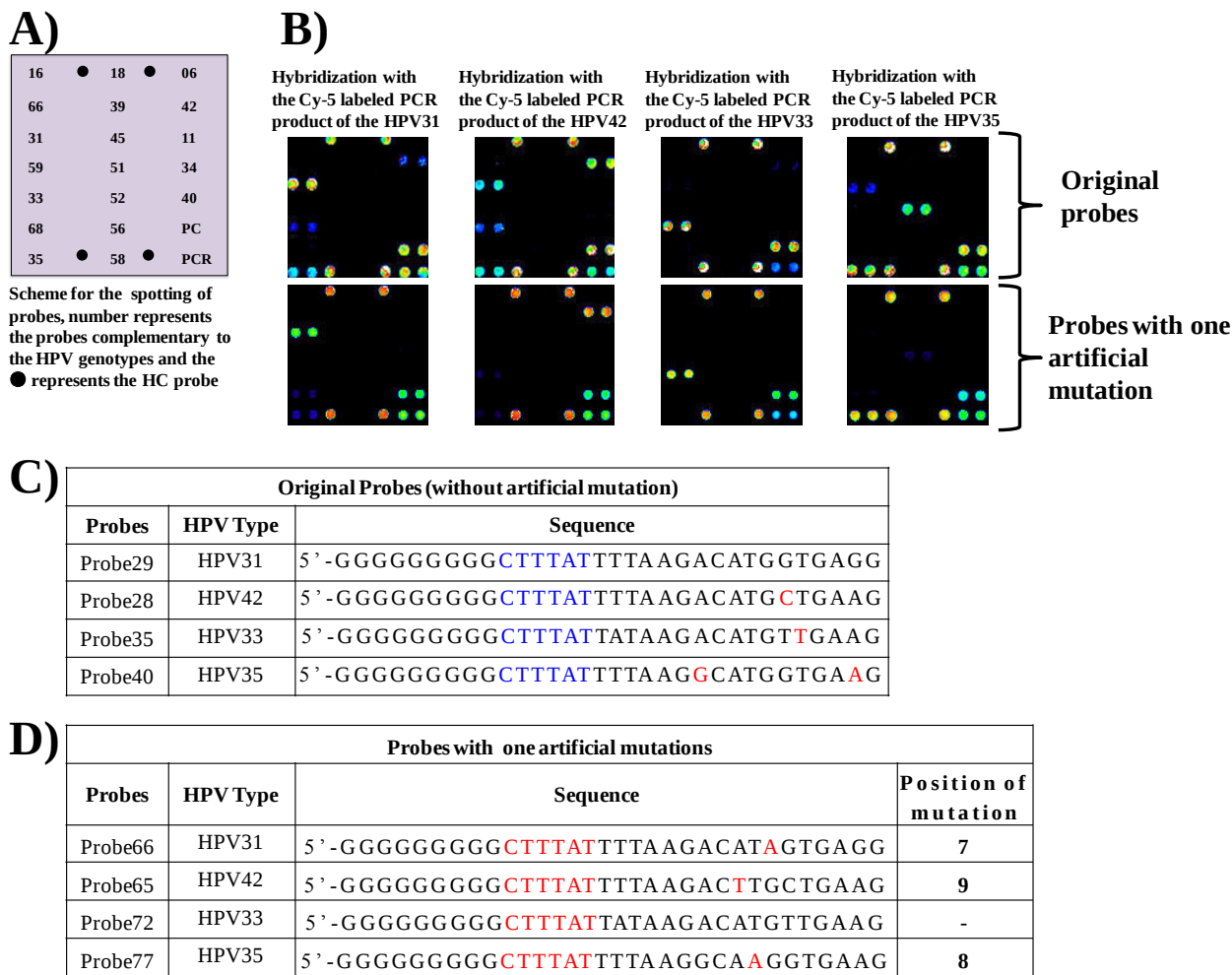


Figure S27: Fluorescence image after hybridization of HPV 9G DNAChip with Cy5 labeled PCR product of HPV31, HPV42, HPV33, and HPV35

10b. Hybridization with Cy5 labeled PCR products of HPV type 42, 35, 33, 31

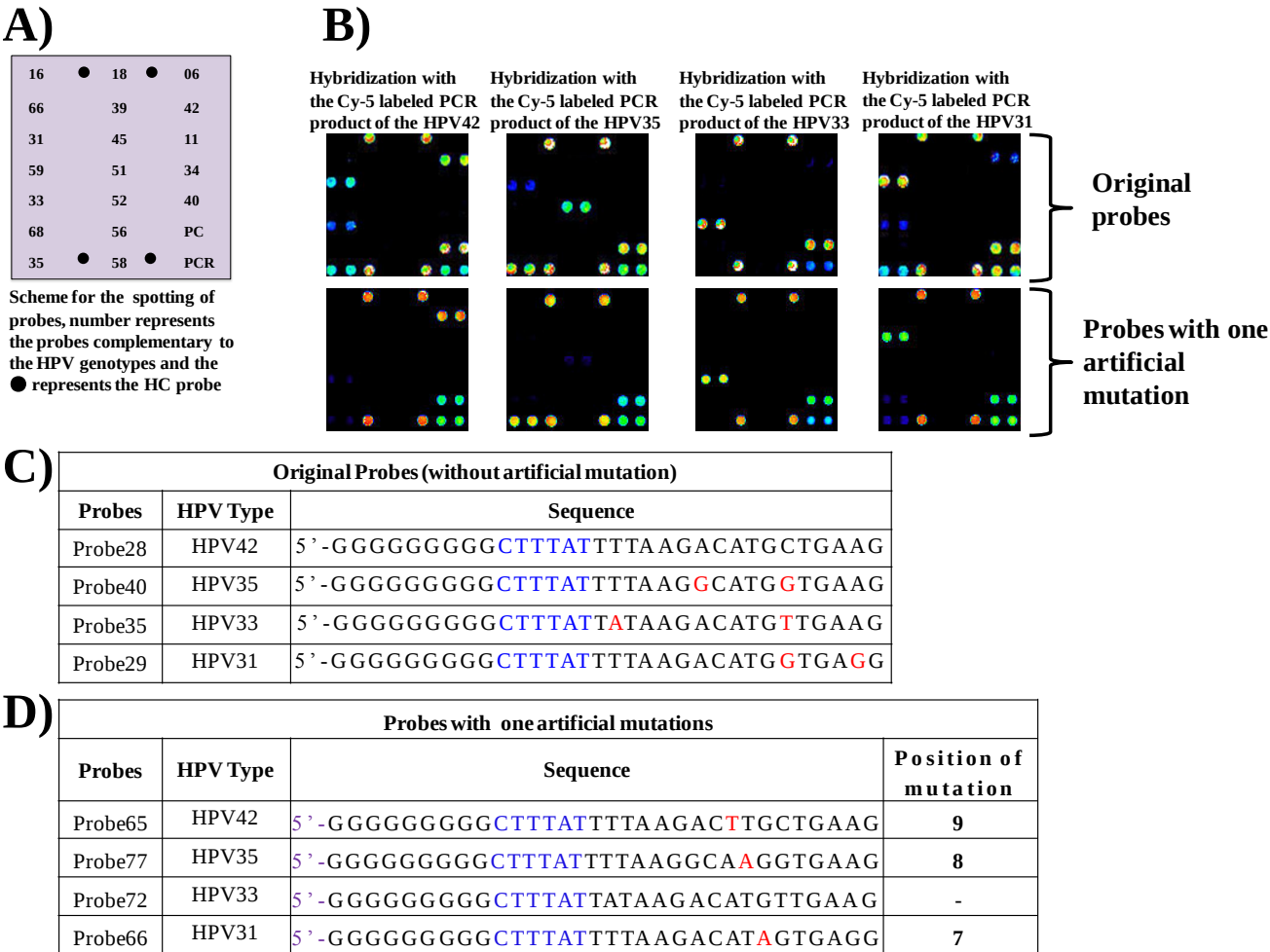


Figure S28: Fluorescence image after hybridization of HPV 9G DNAChip with Cy5 labeled PCR product of HPV42, HPV35, HPV33, and HPV31

10c. Hybridization with Cy5 labeled PCR products of HPV type 06, 11, 40

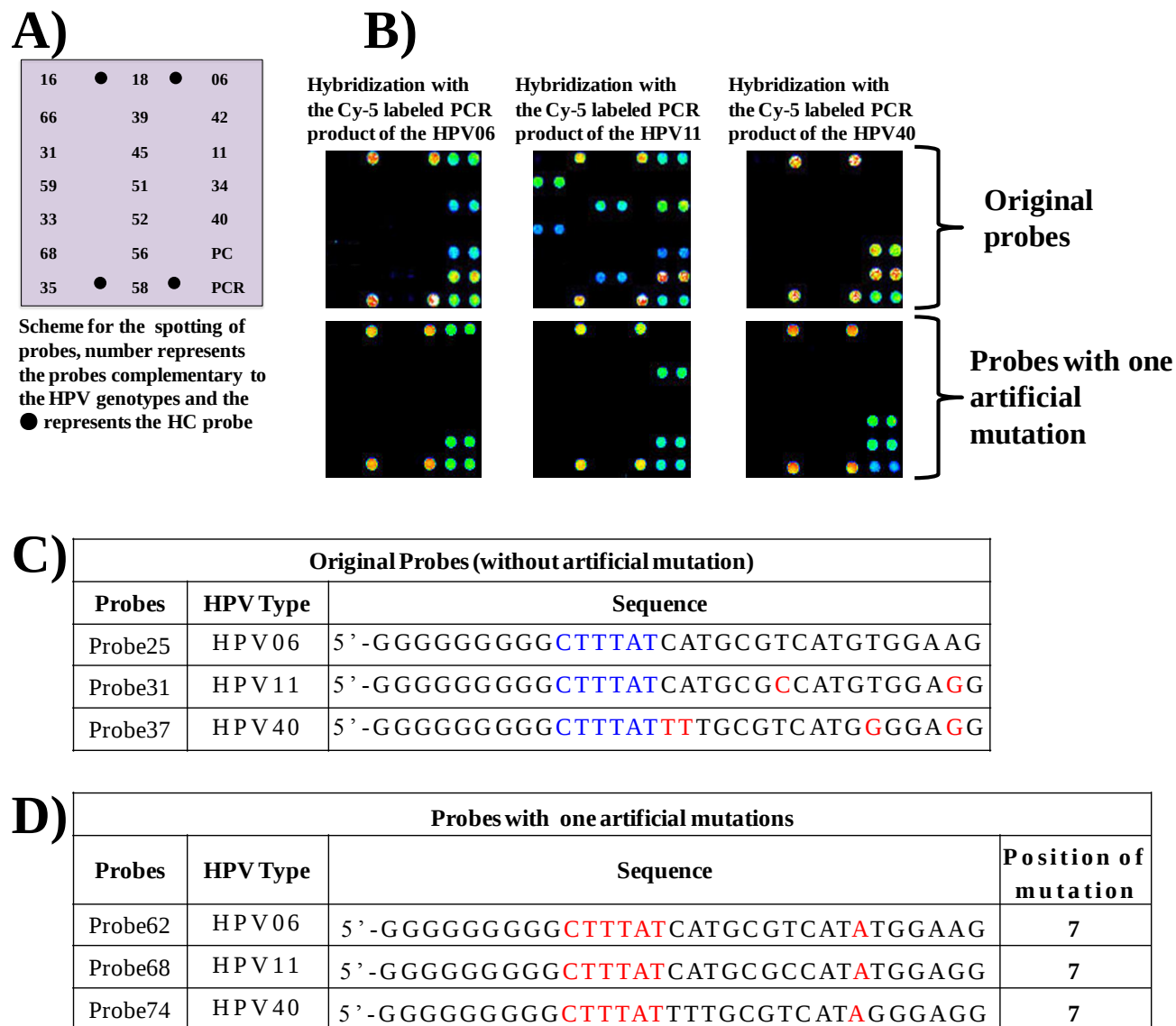


Figure S29: Fluorescence image after hybridization of HPV 9G DNAChip with Cy5 labeled PCR product of HPV06, HPV11, and HPV40

10d. Hybridization with Cy5 labeled PCR products of HPV type 18, 45, 31, 59, 33

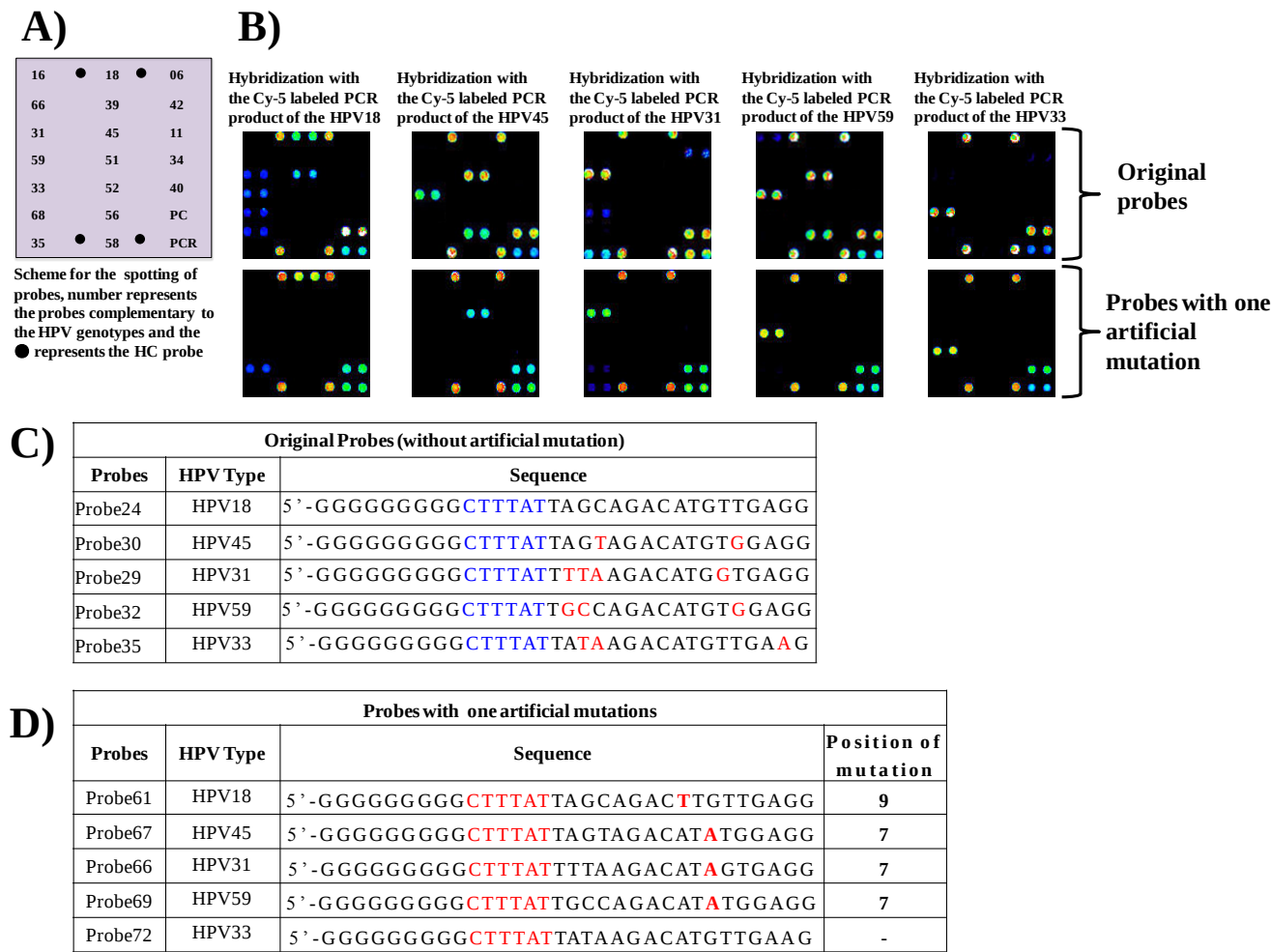


Figure S30: Fluorescence image after hybridization of HPV 9G DNAChip with Cy5 labeled PCR product of HPV18, HPV45, HPV31, HPV59, and HPV33

10e. Hybridization with Cy5 labeled PCR products of HPV type 66, 45, 11, 56, 59

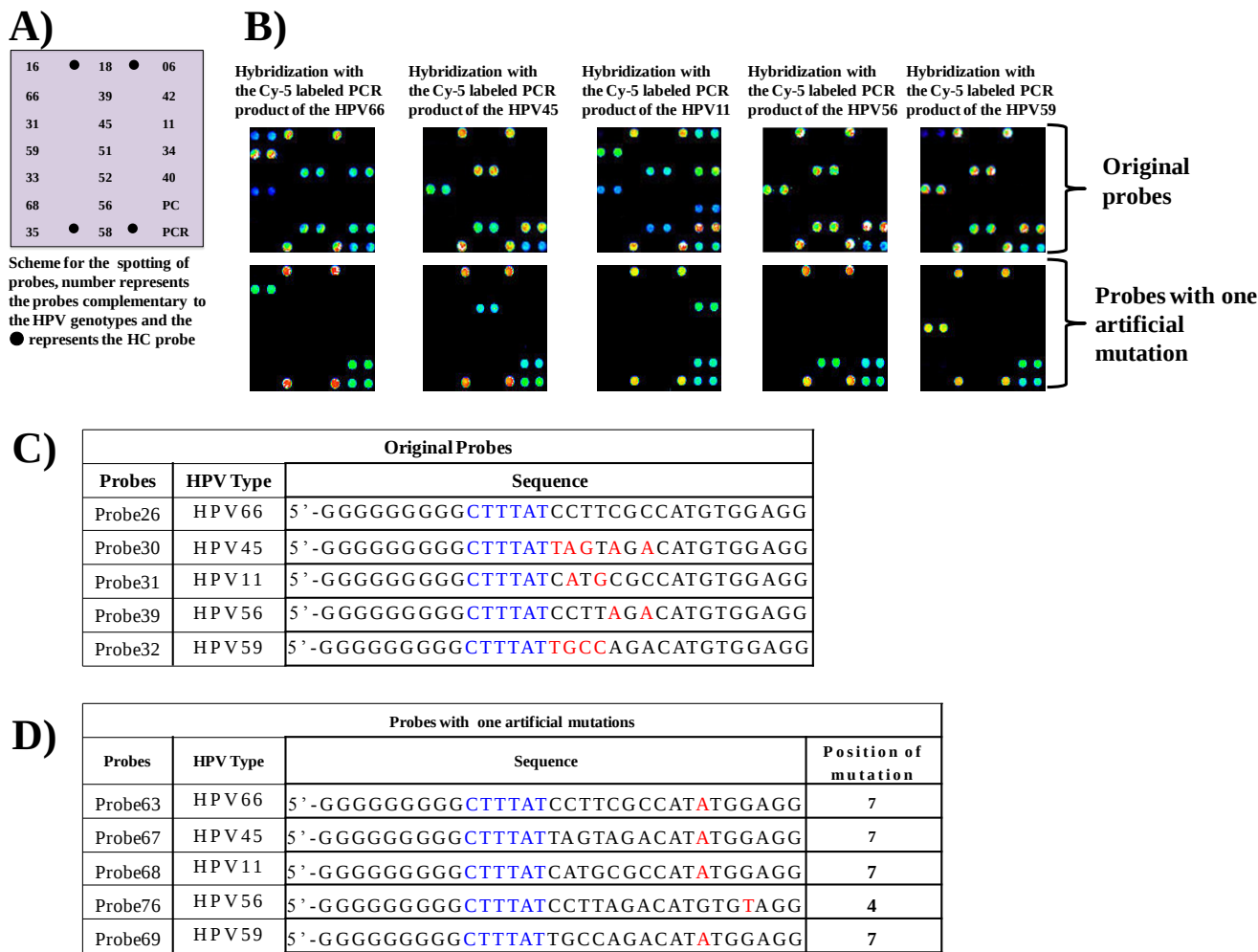


Figure S31: Fluorescence image after hybridization of HPV 9G DNAChip with Cy5 labeled PCR product of HPV66, HPV45, HPV11, HPV56, and HPV59

10f. Hybridization with Cy5 labeled PCR products of HPV type 16, 56, 40

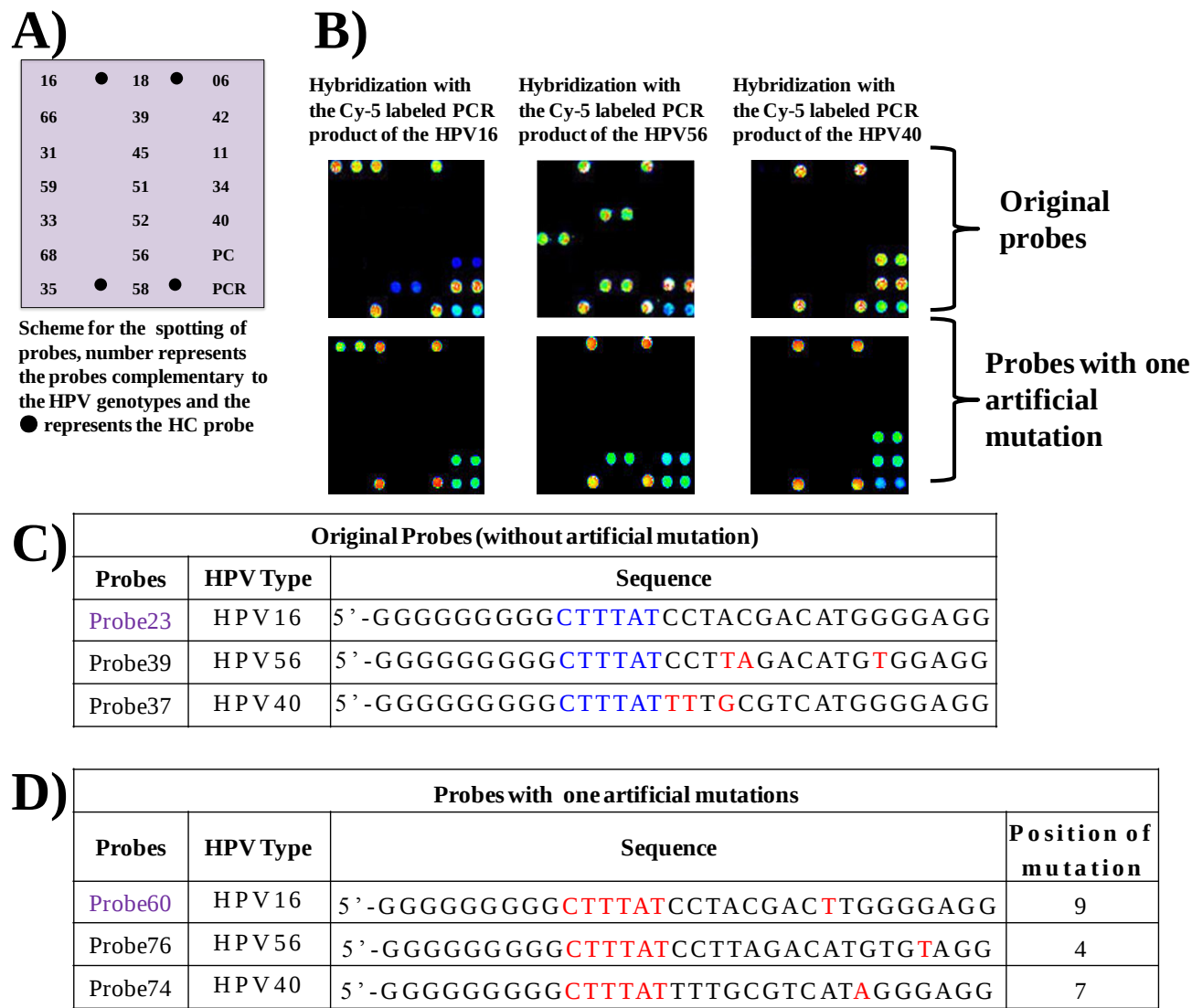


Figure S32: Fluorescence image after hybridization of HPV 9G DNAChip with Cy5 labeled PCR product of HPV16, HPV56, and HPV40

10g. Hybridization with Cy5 labeled PCR products of HPV type 35, 51, 31

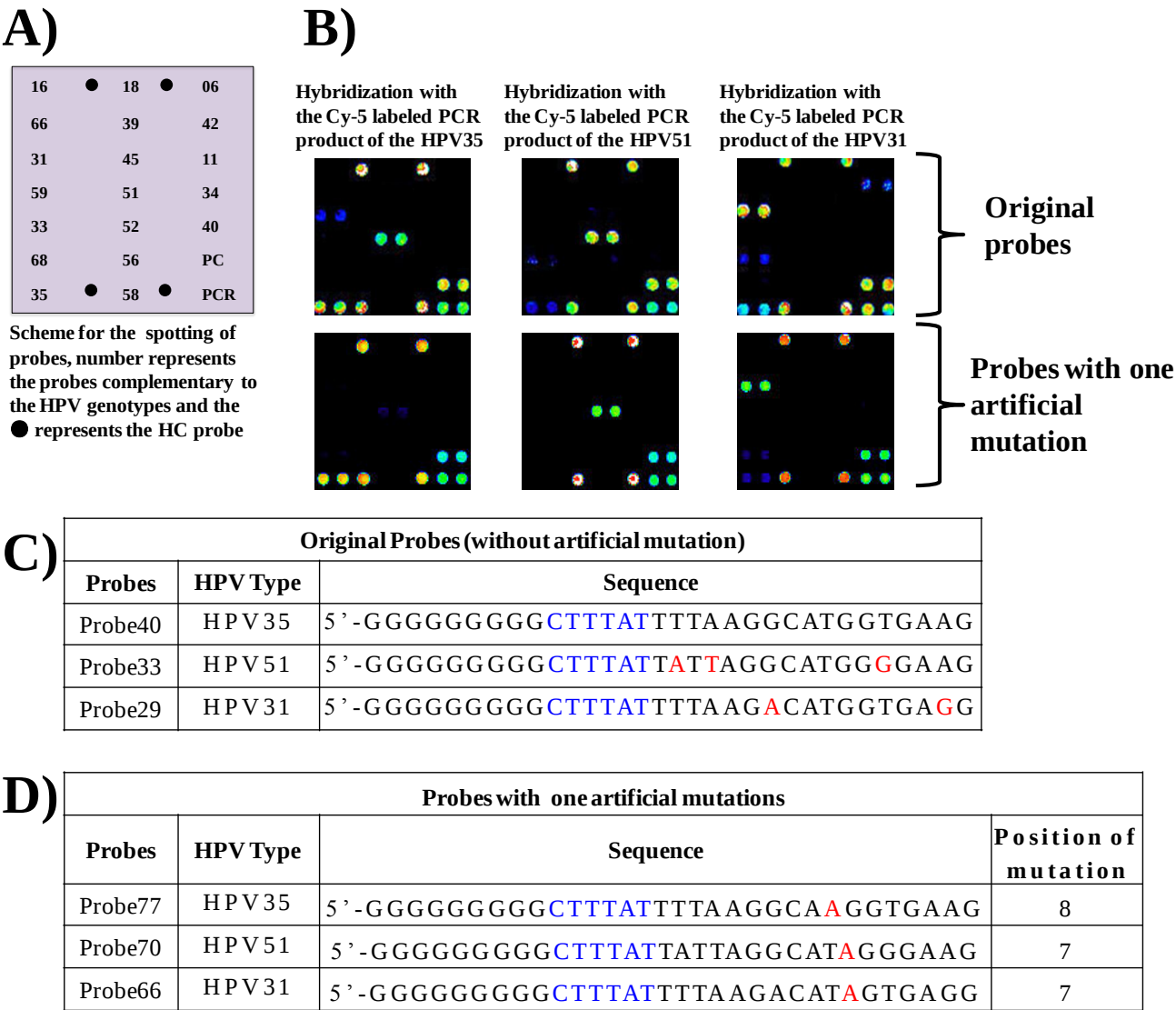


Figure S33: Fluorescence image after hybridization of HPV 9G DNAChip with Cy5 labeled PCR product of HPV35, HPV51, and HPV31

10h. Hybridization with Cy5 labeled PCR products of HPV type 11, 06, 66, 59, 56, 40, 45

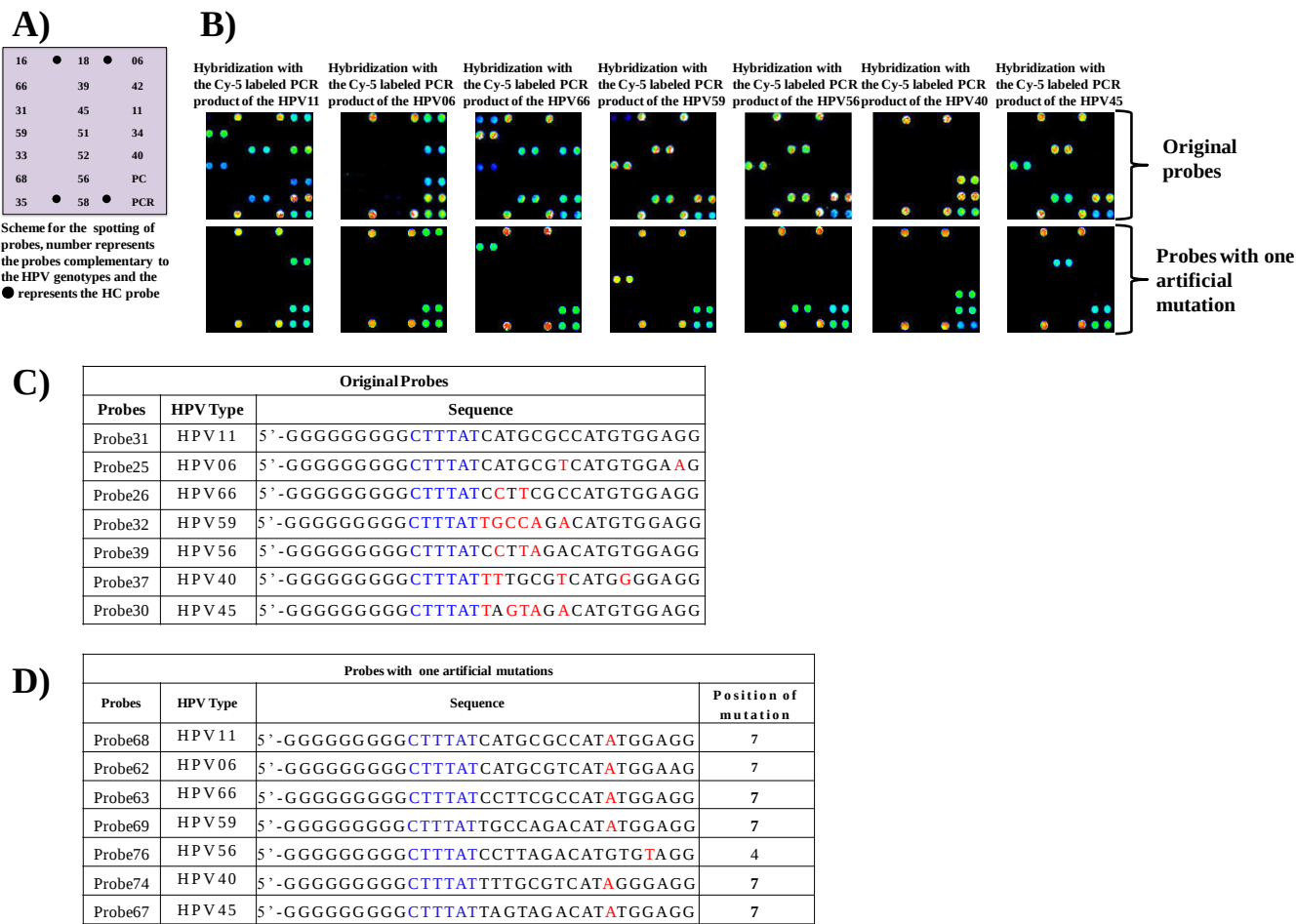


Figure S34: Fluorescence image after hybridization of HPV 9G DNAChip with Cy5 labeled PCR product of HPV11, HPV06, HPV66, HPV59, HPV56, HPV40, and HPV45

11. Comparison of the probes with and without artificial mutations:

The probes immobilized on the Pre-HPV 9G DNACChip (original probes without mutations) and HPV 9G DNACChip (probes with one artificial mutation) were hybridized with the Cy5 labeled PCR products of the HPV45, HPV56, HPV59. After washing and drying the respective chips were scanned and the results are presented in the **Figure S35**.

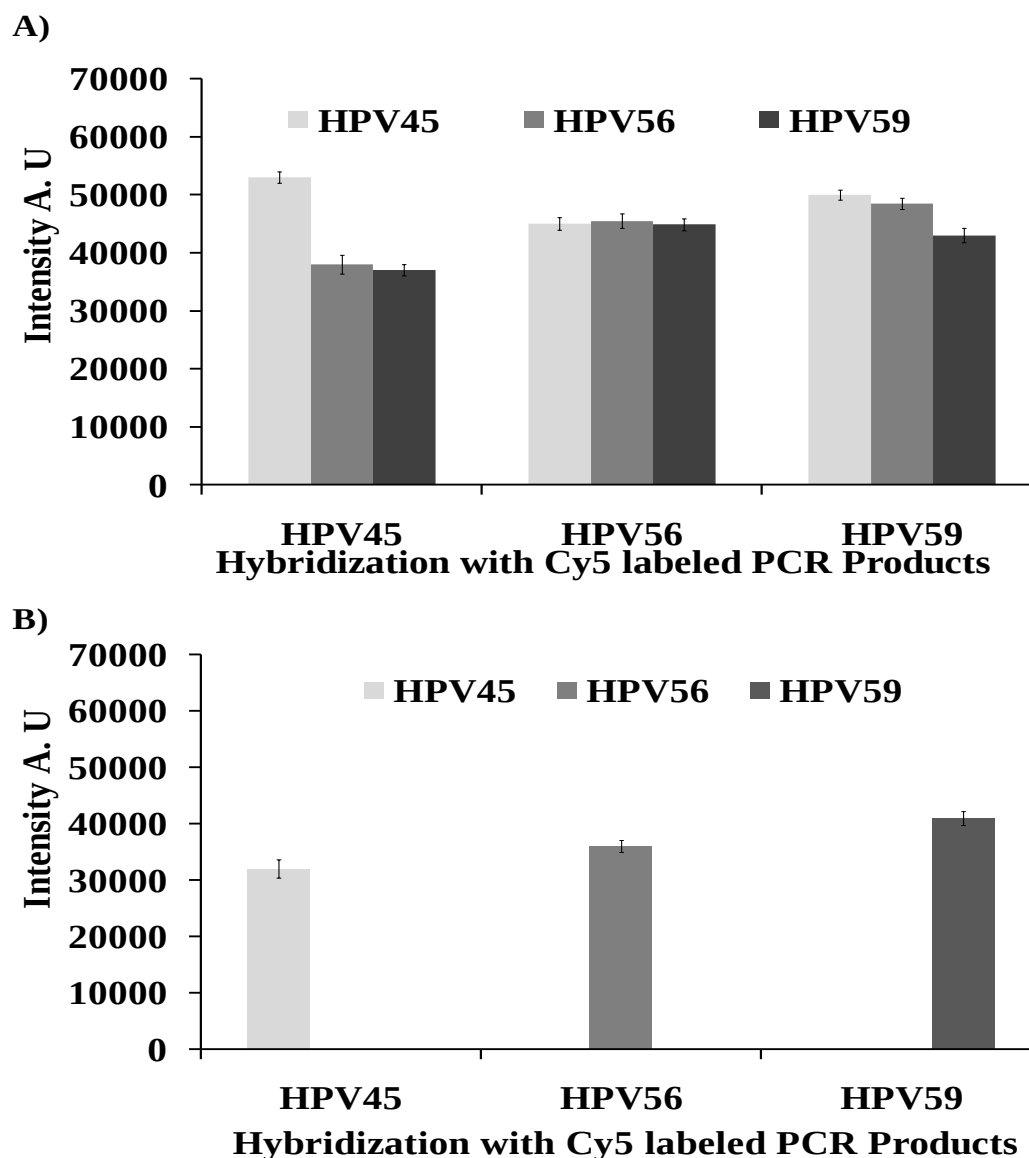


Figure S35: Fluorescence intensities after the hybridization with the Cy5 labeled PCR product of HPV45, HPV56, and HPV59, A) Graph of fluorescence intensities after the hybridization of the probes without mutation with the Cy5 labeled PCR product of HPV45, HPV56, and HPV59, B) Graph of fluorescence intensities after the hybridization of the probes containing one artificial mutation with the Cy5 labeled PCR product of HPV45, HPV56, and HPV59. PMT gain = 48.

12: Application of Proposed method for detection and discrimination HPV genotypes

Table S2: Results of the 1041 clinical samples for the diagnosis of HPV infections by using HPV 9G DNACChip

HPV Type	Total	Normal	ASC-US	ASC-H	LISL	HSIL
16	139	54	31	16	3	35
18	101	64	23	3	6	5
45	45	29	8	2	2	4
31	18	8	4			6
6	13	6	4		3	
11	24	19	3		2	
34	26	16	6		3	1
35	50	19	18	1	4	8
39	29	19	7		2	1
40	66	55	9		1	1
42	18	10	8			
51	51	18	14	2	13	4
56	70	31	16		23	
59	27	19	5		2	1
58	103	45	24	7	14	13
66	24	9	11		4	
68	52	36	11	1	4	
52	58	23	16	9	5	5
33	31	17	1	3	3	7
11/52	1		1			
16/18	1		1			
16/31	1		1			
16/52	1			1		
16/66	1					1
18/34	1		1			
18/39	1		1			
18/45	1	1				
31/35	1	1				
35/52	1		1			
39/56	1		1			
40/52	1	1				
51/6	1		1			
58/45	1		1			
Normal	82	79	3			
Total	1041	579	231	45	94	92

AGUS, atypical glandular cells undetermined significance; ASCUS, atypical squamous cells undetermined significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; WNL, within normal limit.

Table S3: Results of the 439 clinical samples for the diagnosis of HPV infections by using HPV 9G DNAChip cross checked by the gene sequencing analysis

	Total	Sequencing		HPV 9G DNAChip		Total for each type (Sequencing , BMT)
		+	-	+	-	
Normal	361	75 (20.8%)	286	75 (20.8%)	286	361
ASC-US	47	39 (83.0%)	8	39 (83.0%)	8	47
ASC-H	9	7 (77.8%)	2	7 (77.8%)	2	9
LSIL	9	8 (88.9%)	1	8 (88.9%)	1	9
HSIL	13	13 (100%)		13 (100%)		13
Total	439	439		439		439

AGUS, atypical glandular cells undetermined significance; ASCUS, atypical squamous cells undetermined significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

(Note: The details of the Clinical sample analysis by using the proposed method and gene sequencing will be reported in our next communication).

13. References:

-
1. K. Song, S. B. Nimse, J. Kim, J. Kim, V. Ta, V. Nguyen, T. Kim, *Chem. Comm.* 2011, 47, 7101;