Supporting Information

Digital analysis of the expression levels of multiple colorectal cancer-related genes by multiplexed digital-PCR coupled with hydrogel bead-array

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Table of contents

Table S-1. The effect of electrophoresis on the specificity of probe hybridization by using various mismatched probes to hybridize β-actin amplicons immobilized on bead's surface.

Probes	Number of bases complementary to template	Number of red beads [a]	Number of yellow beads [b]	Ratio of yellow beads to red beads
control	-	49197	4	0.000102
COX-2	8	49064	5	0.000102
MMP7	4	52191	5	0.000096
DPEP	7	56844	6	0.000106
c-myc	7	58866	6	0.000102
probe-mix [c]	-	51410	5	0.000103

[a)] Red beads were decoded by the hybridization of Cy5-labeled β-actin-specific probe.

[b)] Yellow beads were decoded by hybridization of Cy5-labeled β-actin-specific probe and then Cy3-dUTP based primer extension reaction.

[c)] Probe-mix means the hybridization was performed using a mixture of the four gene-specific probes (Cox-2, MMP7, DPEP, c-myc).

Table S-2. Copy numbers of templates used to evaluate the template concentration suitable for single-molecule bead-based emPCR.

Templates	Copy numbers	
	emPCR-1	emPCR-2
house-keeping gene [a]	5×10^6	5×10^7
CRC-related genes [b]	5×10^6	5×10^7

[a)] House-keeping gene refers to human β-actin gene.

[b)] CRC-related genes contain equal amounts of c-myc , COX-2, MMP7, and DPEP1.

Table S-3. Copy numbers of the artificially prepared templates used for evaluating the quantitative performance of MDHB.

Templates	Copy numbers						
	emPCR-1	emPCR-2	emPCR-3	emPCR-4	emPCR-5	emPCR-6	emPCR-7
house-keeping gene[a]	5×10^6	5×10^6	5×10^6	5×10^6	5×10^6	5×10^6	5×10^6
CRC-related genes[b]	5×10^6	1×10^6	2×10^5	0.4×10^5	5×10^3	1.7×10^3	0
ratio[c]	1:1	5: 1	25: 1	125: 1	1000: 1	3000: 1	0

[a)] House-keeping gene refers to human β-actin gene.

[b)] CRC-related genes contain equal amounts of c-myc , COX-2, MMP7, and DPEP1.

[c)] Ratio refers to the ratio of the copy numbers of house-keeping gene to that of all the four CRC-related genes in artificially prepared template.

Table S-4. Characteristics of CRC patients

Patient No.	Age	Sex	Dukes' stage	Tumor location[a]	Tumor size[b]	Sample for MDHB
1	52	male	C	R	M	tissue & feces
2	43	female	B	S	M	tissue
3	51	male	C	R	L	tissue & feces
4	63	male	B	R	M	tissue
5	39	male	D	R	L	tissue & feces
6	75	female	B	S	S	tissue
7	74	male	A	A	L	tissue& feces
8	70	female	C	S	L	tissue & feces
9	65	male	B	T	S	feces
10	54	male	B	R	L	feces
11	48	male	D	R	L	feces
12	68	female	C	S	L	feces
13	60	female	B	R	M	feces
14	58	female	A	A	M	feces
15	45	female	B	R	S	feces
16	67	male	B	S	M	feces

[a] A, ascending colon; T, transverse colon; S, sigmoid colon; R, rectum.

[b] L, large (≥50 mm); M, medium (30 mm-49 mm); S, small (10 mm-29 mm).

Table S-5. Characteristics of CRC patients and healthy volunteers.

	CRC patients	Healthy volunteers
n	16	10
Sex (M/F)	9/7	5/5
Age (years)	58 (39-75)	39 (23-55)

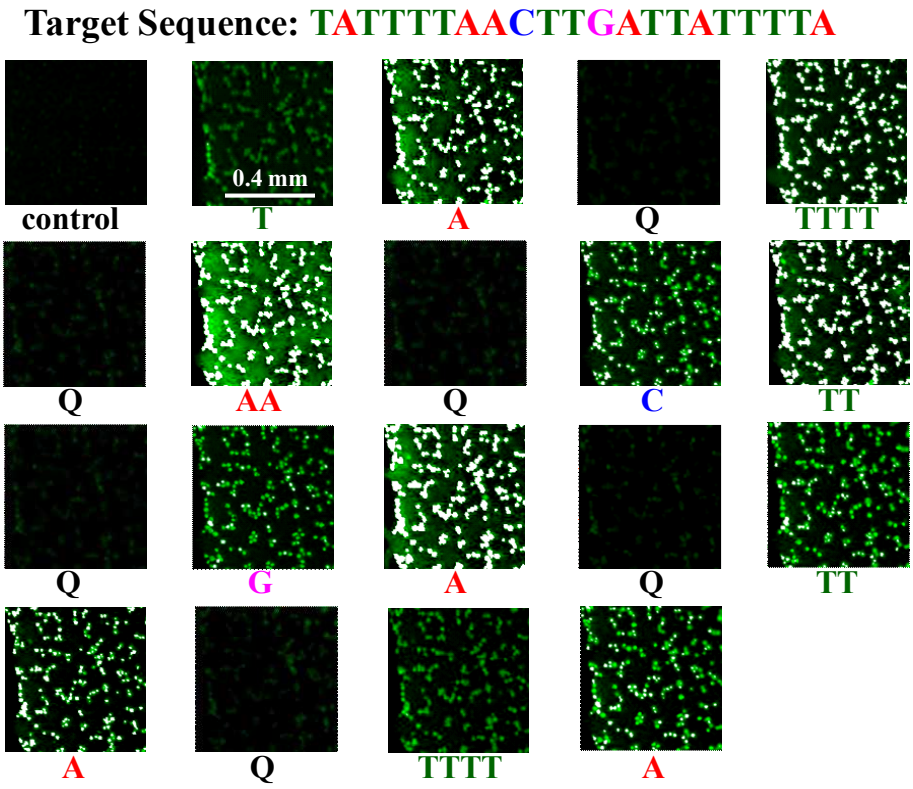

Figure S-1. Evaluation of the extension reaction on amplicon-coated beads immobilized with hydrogel on a chip.

The Cy3-dNTP (including dATP, dCTP, dGTP, dTTP) was individually added to perform the extension reaction with Therminator DNA polymerase. The fluorescence is deactivated using hydrogen peroxide, and the sequence was read by determining the intensity of fluorescence. A same area of hydrogel bead-array was scanned at the same laser power and PMT gain. The target sequence was shown on the top of the figure. The first image was a negative control and the letter "Q" in the figure means the "quenching" step.

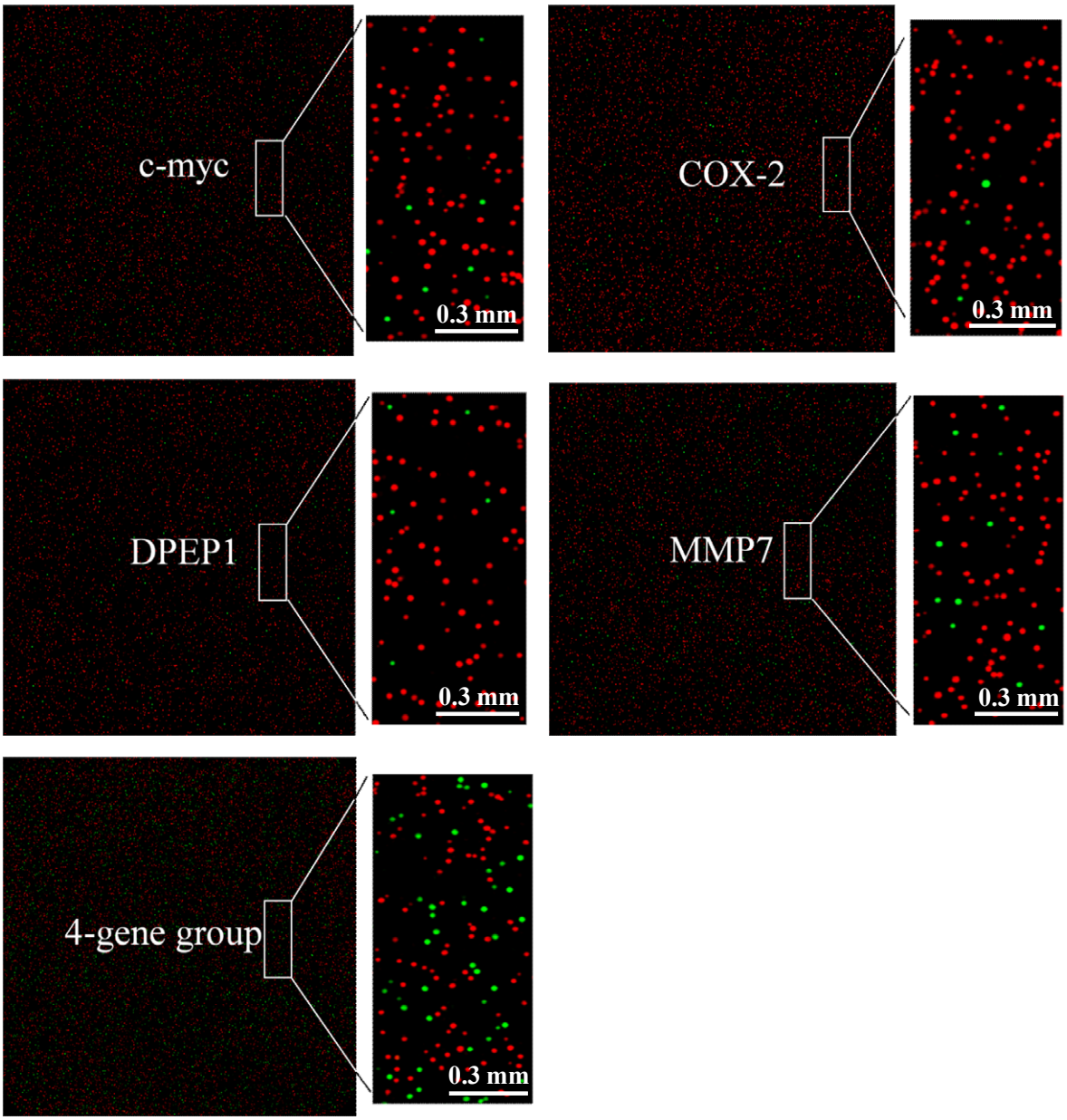

Figure S-2. The scan-images of MDHB for analyzing the relative expression level of each of the four CRC-related genes (c-myc, COX-2, MMP7, and DPEP1) as well as a 4-gene group in the tumor tissue from a CRC patient.

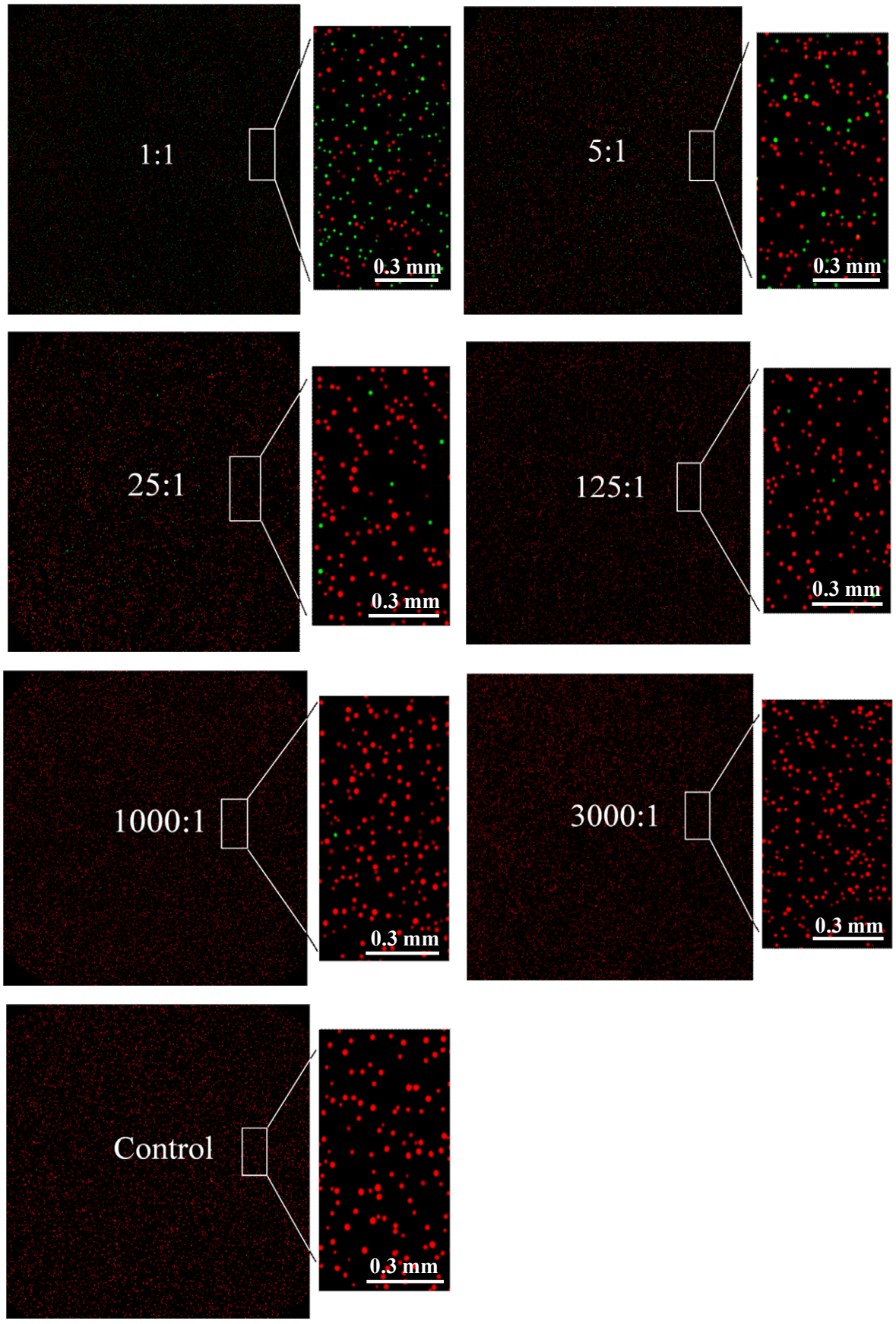

Figure S-3. The scan-images of MDHB with different templates in Table S-3 for emPCR to illustrate the discrimination power of the method.

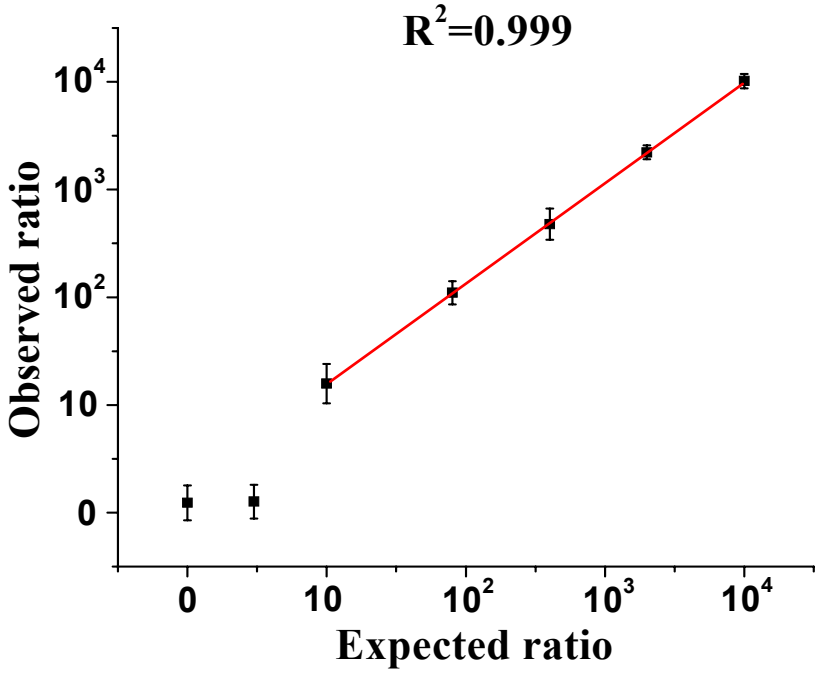

Figure S-4. Quantitative dynamic range of MDHB by using a serial dilution series of ssDNA listed in Table S-3 (n=3). The relation between the expected ratios and the observed ratios is correlated as a red line.

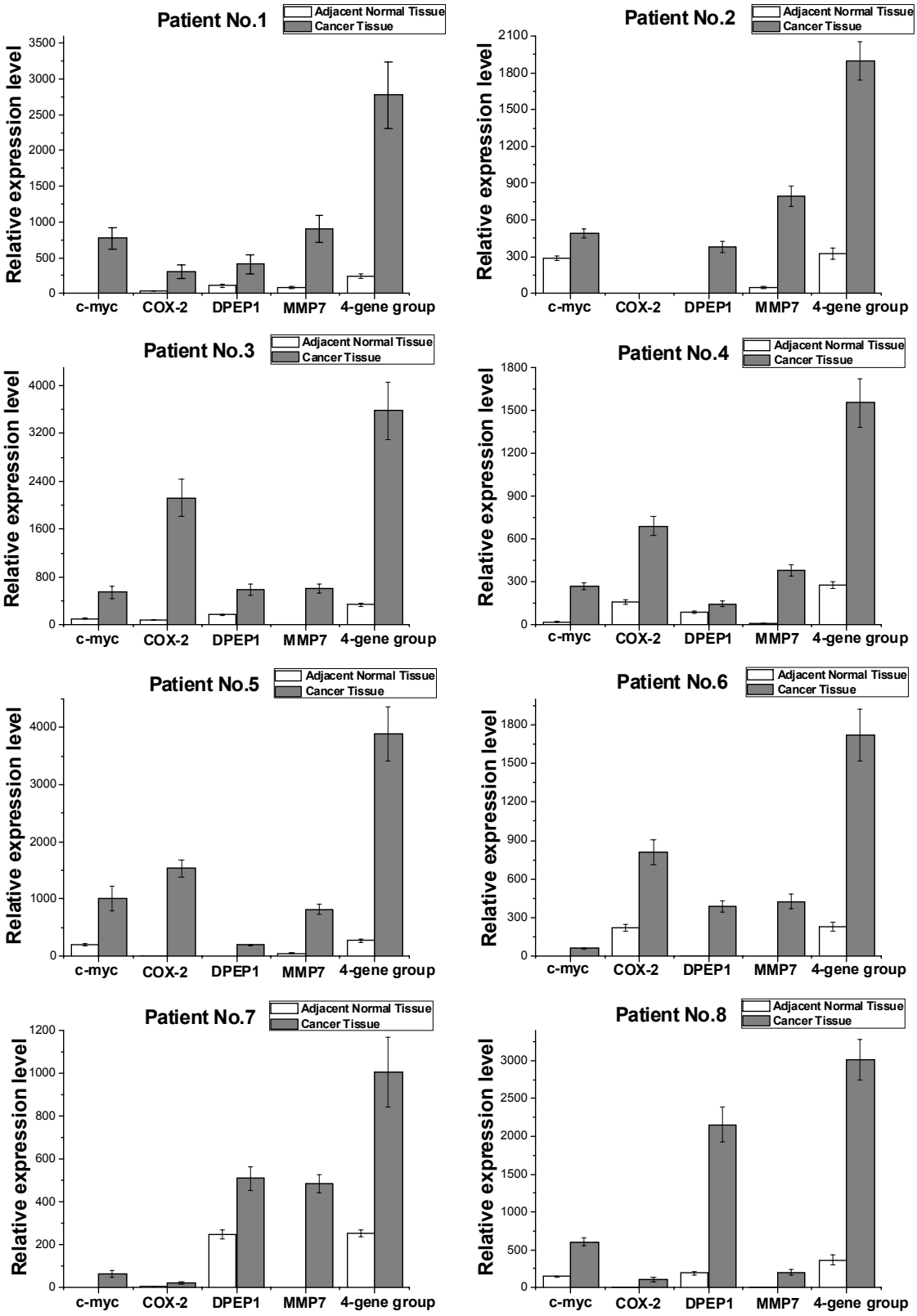

Figure S-5. Multiplexed gene expression analysis in the tissues from 8 CRC patients with MDHB (n=3). The 4-gene group means a panel of four CRC-related genes (c-myc, COX-2, MMP7, and DPEP1) as a single diagnostic biomarker.

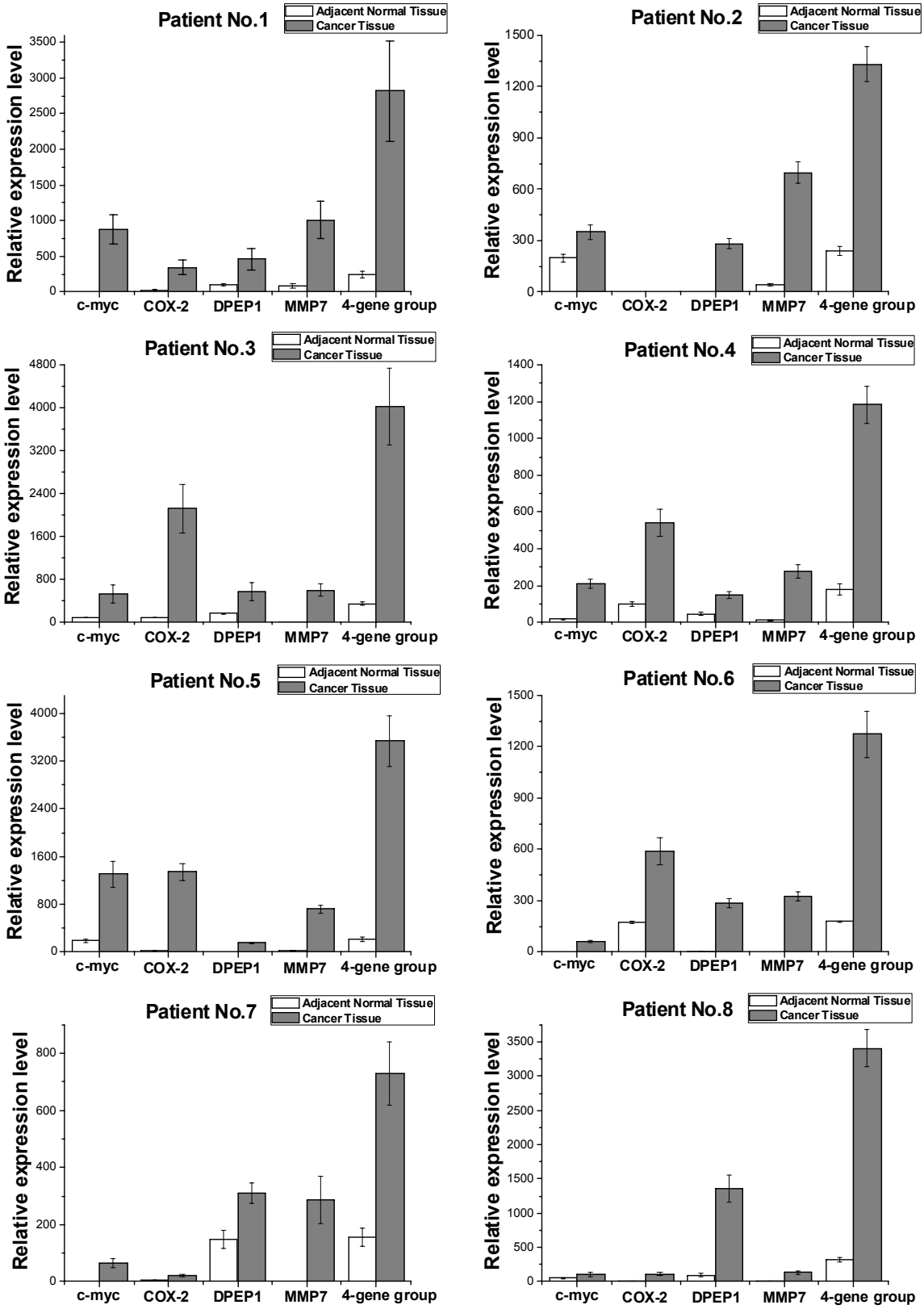

Figure S-6. Gene expression analysis in the tissues from 8 CRC patients with qPCR (n=3). The results from qPCR were calculated by dividing the copy number of the CRC-related gene with that of the house-keeping gene, and then were multiplied by 10^4. The 4-gene group means a panel of four CRC-related genes (c-myc, COX-2, MMP7, and DPEP1) as a single diagnostic biomarker.