Use of dimethyl pimelimidate with microfluidic system for nucleic acids extraction without electricity

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Supporting Information:

Supplementary Figures 1-4

Supplementary Table 1

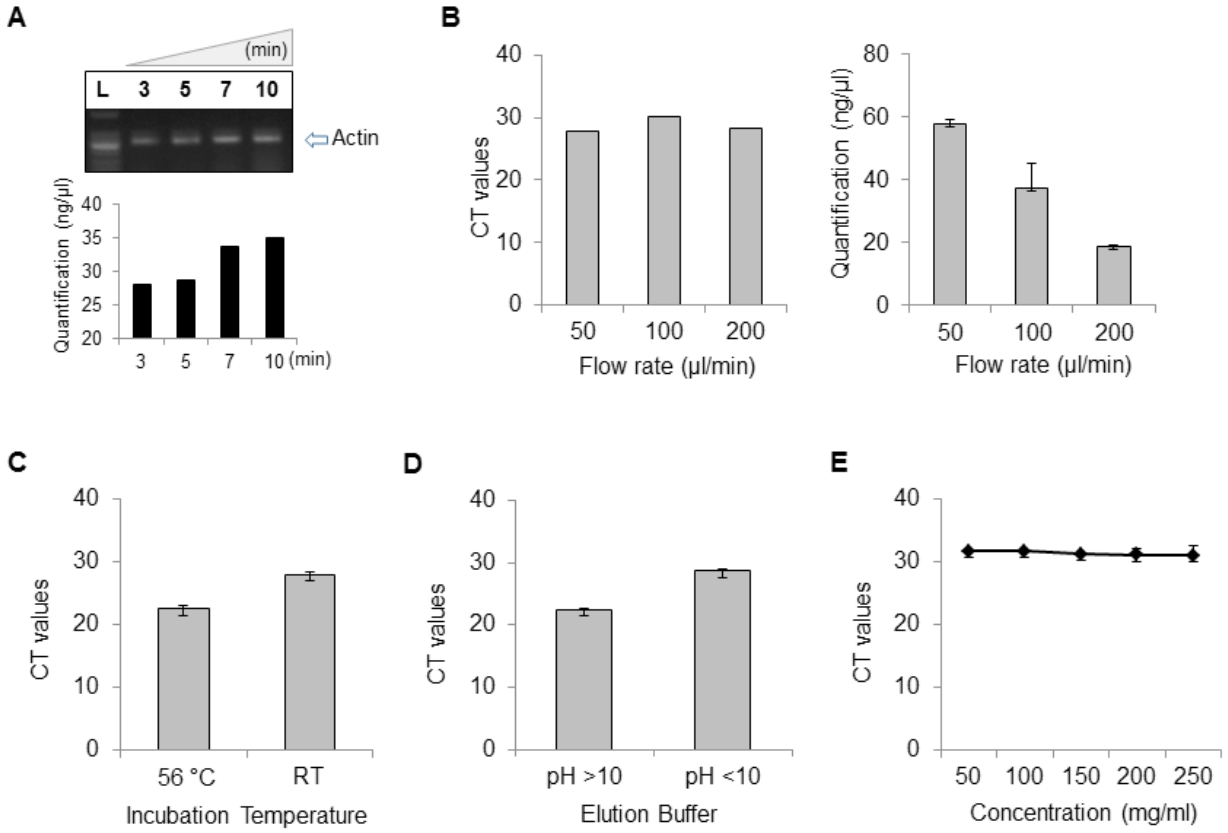

Fig. S1. Fundamental characterization of the DMP system for nucleic acid extraction. (A) The DNA amplification efficiency with DNA extracted from the DMP system by using PCR depends on the plasma oxidation time (3, 5, 7, and 10 min) for the surface modification of the thin film. (B) The efficiency (real-time PCR and Quantification) of DNA extraction depends on the flow rate of the elution step (50, 100, and 200 μl/min) using a syringe pump. (C) The efficiency of DNA extraction depends on incubation temperature [56 °C and room temperature (RT)]. The efficiency of the DNA extraction in 56 °C is better than that of RT. (D) The efficiency of DNA extraction depends on elution buffer (pH>10 and pH<10). The efficiency of the elution buffer (sodium bicarbonate (pH >10)) is better than that of the buffer (pH <10). (E) Downstream analysis for DNA genetic testing with the *Actin* gene using the DNAs extracted from the cancer cells (1×10^3 cells) with the DMP system, was performed using real-time PCR with different concentrations (50, 100, 150, 200, and 250 mg/ml) of HI. All error bars indicate the standard deviation of the mean based on at least 3 independent experiments.

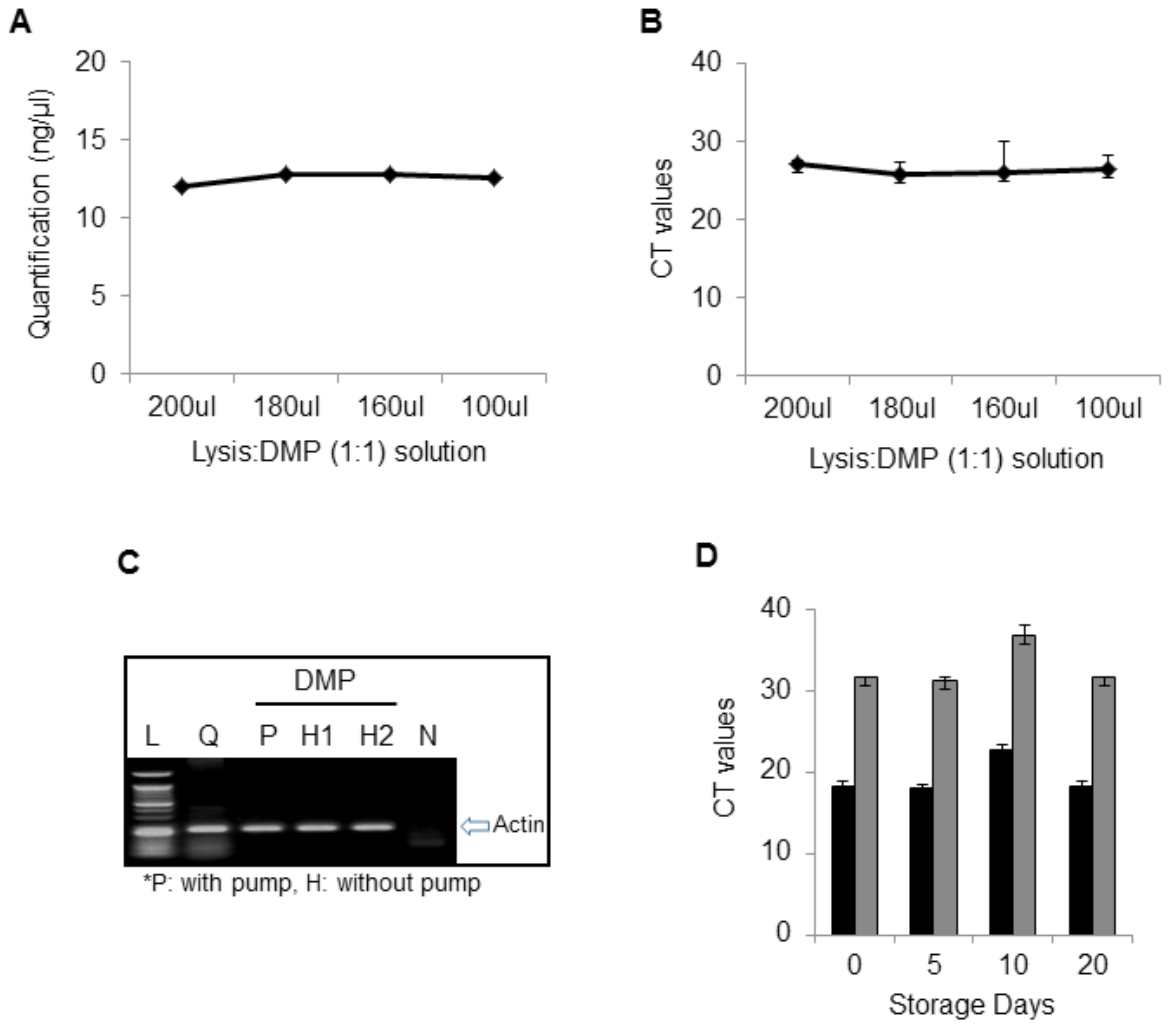

Fig. S2. Characterization of the DMP system. (A) DNA quantification depends on the assay solution (Ratio 1: 1 of lysis and DMP). (B) Amplification efficiency of the DNA extracted from the assay solutions. (C) Capacity testing of the DMP system without the syringe pump (no electricity). Gel electrophoresis of the *Actin* products using end-point PCR. (L: DNA size marker; Q: Qiagen kit; P: with the pump; H1&H2: without the pump; N: negative control). (D) DNA amplification testing depends on the storage periods (0 day to 20 days) using qRT-PCR. The colors represent the amount of the cells: black (1 x10^6 cells/mL) and gray (1 x 10^3 cells/mL).

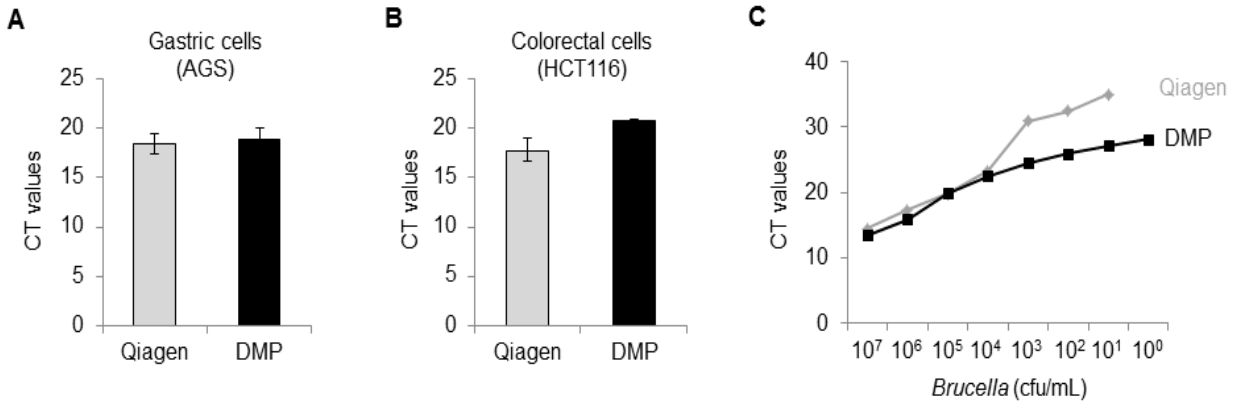

Fig. S3. Application of the DMP system for DNA extraction with cancer cell lines. (A-B) The capacity of the DMP system with (A) AGS (gastric cancer cell line) and (B) HCT116 (colorectal cancer cell line) for DNA extraction using real-time PCR. (Qiagen: white, DMP: black). All error bars indicate the standard deviation of the mean based on at least 3 independent experiments. (C) Capacity of the DMP system with *Brucella* in concentrations ranging from 1×10^0 to 1×10^7 colony forming units by using real-time PCR. (Qiagen: white, DMP: black).

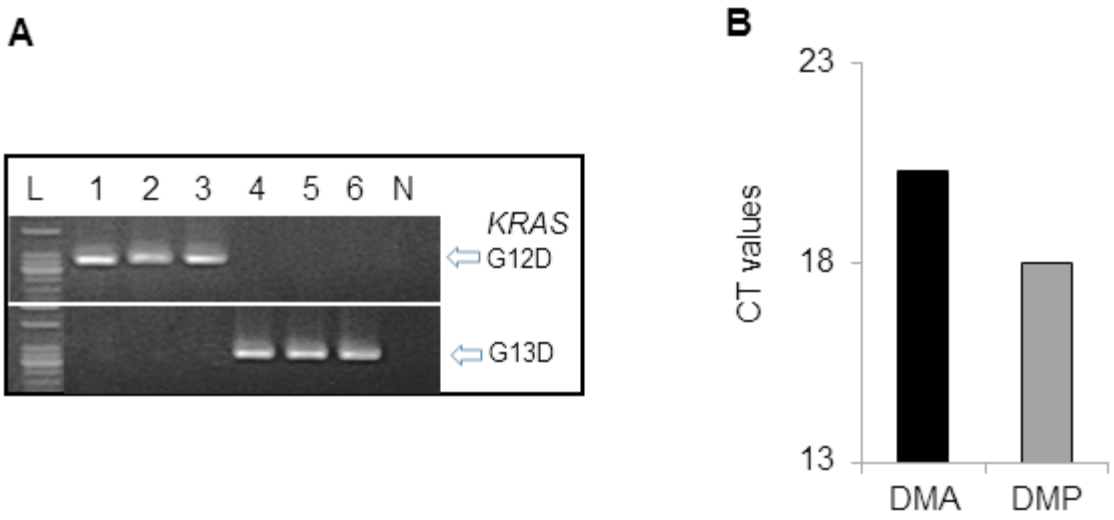

Fig. S4. Validation of the DMP system for *KRAS* mutation analysis. (A) Genetic analysis with *KRAS* gene point mutations (codon 12 [G12D] and codon 13 [G13D]) using the DNAs extracted from the DMP system. DNA amplification with mutation primer pairs in AGS cells containing the G12D mutation and HCT116 cells containing the G13D mutation. When a codon 12 mutation primer set (G12D) was used, the amplification only occurred in the AGS cells with the Qiagen, and DMP assays, and not in the HCT116 cells. In contrast, when the codon 13 mutation primer set (G13D) was used, the amplification only occurred in the HCT116 cells with the Qiagen, and DMP assays, and not in the AGS cells. (L: DNA size marker; 1: AGS cells with the Qiagen kit; 2: AGS cells with the DMP assay-I; 3: AGS cells with the DMP assay-II; 4: HCT116 cells with the Qiagen kit; 5: HCT116 cells with the DMP assay-I; 6: HCT116 cells with the DMP assay-II; N: negative control). (B) Comparison of the DNA amplification efficiency with the DNA extracted from DMA, and DMP by using qRT-PCR.

Table S1. Primer Sequences of DNA and RNA amplification

Nucleic Acids	Targets	Type	Sequence
DNA	*Actin*	Forward	5′-ATGGTGGGCATGGGTCAGA-3′
		Reverse	5′-GCCACACGCAGCTCATTG-3′
	ST (Orientia tsutsugamushi)	Forward	5′-GCAGCAGCTGTTAGGCTTTT-3′
		Reverse	5′- TTGCAGTCACCTTCACCTTG-3′
	E. coli	Forward	5′- CAACTCTGGCTCCGTCTCTG -3′
		Reverse	5′- CATCATGCAAGCGGCCTCTG -3′
	KRAS (G12D)	Forward	5'-TGTGGTAGTTGGAGCTGA-3'
		Reverse	5'-TCATGAAAATGGTCAGAGAAACC-3'
	KRAS (G13D)	Forward	5'-TGTGGTAGTTGGAGCTGGTGAG-3'
		Reverse	5'-TCATGAAAATGGTCAGAGAAACC-3'
RNA	*18S*	Forward	5′-GCTTAATTTGACTCAACACGGGA-3′
		Reverse	5′-AGCTATCAATCTGTCAATCCTGTC-3′
	SFTS (Huaiyangshan virus)	Forward	5′-CAGCCACTTTACCCGAACAT-3′
		Reverse	5′-GGCCTACTCTCTGTGGCAAG-3′