

Electronic supplementary information

**Universally applicable, quantitative PCR method utilizing fluorescent
nucleobase analogs**

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Table S1 Oligonucleotide sequences employed in this study.

| Experiment | Oligonucleotide name | Sequence (5'→3') | |
|-------------------------|---|---|---|
| Optimization | 1 PdC-incorporated DNA | TCC TCA GAA GTT TAT GCA X ^(a) T | |
| | 2 PdCs-incorporated DNA | TCC TXA GAA GTT TAT GCA XT | |
| | 3 PdCs-incorporated DNA | TCX TXA GAA GTT TAT GCA XT | |
| PdC-based qPCR | Signal-off | F primer | CTA GGC GTT TGT ACT CCG TGA |
| | | R primer | TCX TXA GAA GTT TAT GCA XT |
| | Signal-on | F primer | AGA GAG AGA GAG AGA GAG AG ^(b) C AAT GGC TAA TGC CGG ATA CGC |
| | | R primer | GGT ACC GTC AGT CTG CAA T |
| | | UFB probe | CTC TCT CTC TCT XTX TXT CT |
| TaqMan probe-based qPCR | F primer for CT | CTA GGC GTT TGT ACT CCG TCA | |
| | R primer for CT | TGG TGG GGT TAA GGC AAA TCG | |
| | TaqMan probe for CT | [FAM] ^(c) CCG CAC GTT CTC TCA AGC AGG ACT ACA [BHQ] | |
| | F primer for MH | GCC ACA TTG GGA CTG AGA TAC G | |
| | R primer for MH | ACA GCA CTT TAC AAT CCG AAG ACC | |
| TaqMan probe for MH | [FAM] TGC ACG CTG TGT CGC TCC ATC AAG CT [BHQ] | | |

(a) The bold X indicates PdC.

(b) The red-colored sequences indicate the 5'-overhang sequences where the UFB probe binds.

(c) The bracket indicates the labeling of fluorophore (FAM) and quencher (BHQ).

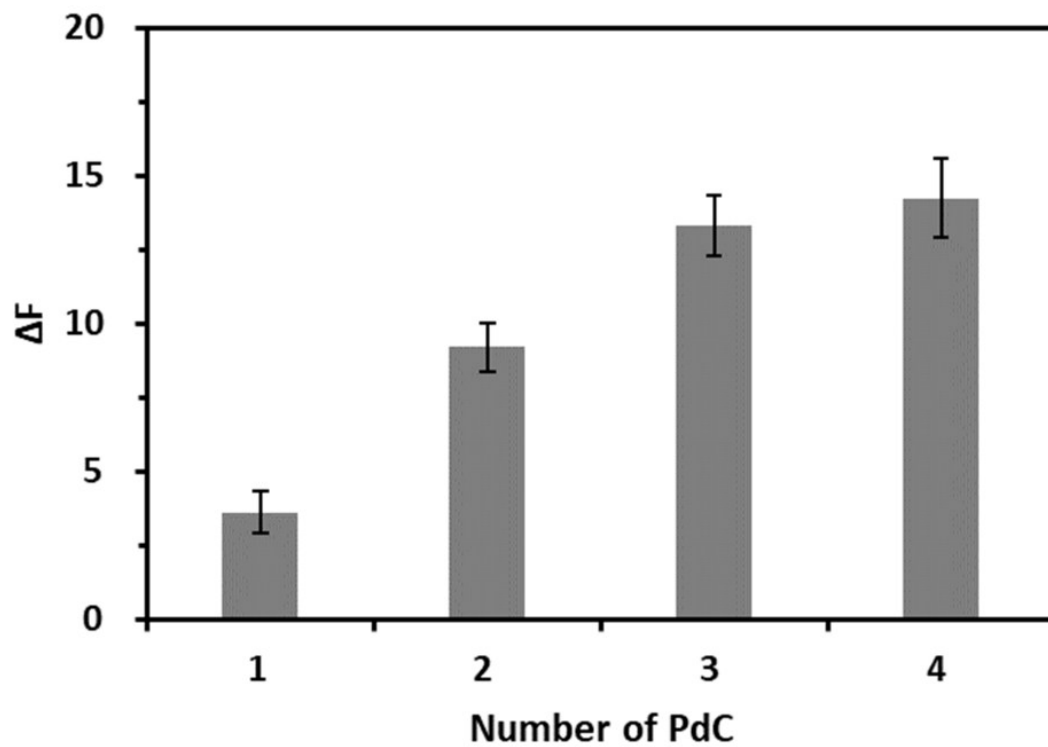


Fig. S1 The effect of the number of PdC on hybridization-induced fluorescence signal change. ΔF is defined as $F_0 - F$ where F_0 and F are fluorescence signal intensities measured before and after addition of complementary DNA strand, respectively.

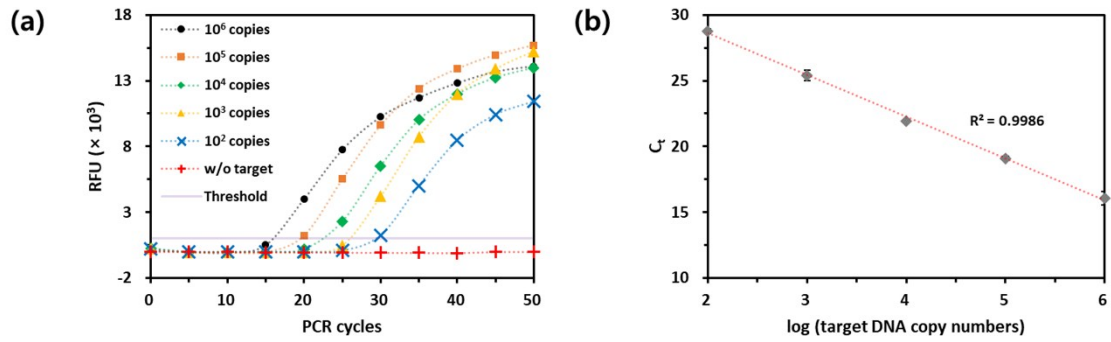


Fig. S2 Quantitative analysis of target nucleic acids from CT using TaqMan probe-based qPCR method. (a) Real-time fluorescence signals from the reaction solutions with varying initial copy numbers of target nucleic acids. (b) Linear relationship between C_t and logarithm of initial copy number of target nucleic acids ($10^2 - 10^6$ copies).

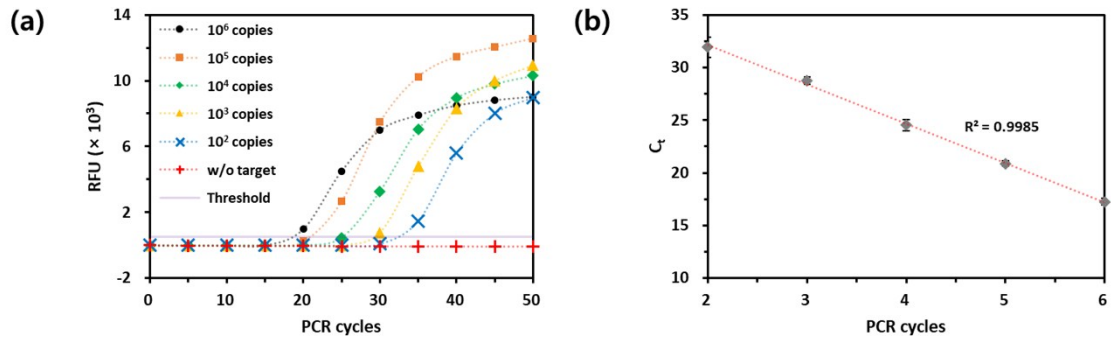


Fig. S3 Quantitative analysis of target nucleic acids from MH using TaqMan probe-based qPCR method. (a) Real-time fluorescence signals from the reaction solutions with varying initial copy numbers of target nucleic acid. (b) Linear relationship between C_t and logarithm of initial copy number of target nucleic acids ($10^2 - 10^6$ copies).