"Supporting Information" of

Real-time and visual detection of viable *Salmonella* in milk by a competitive annealing mediated isothermal amplification (CAMP) combined with propidium monoazide (PMA)

Xu Chen,*[a] Wei Li [b] and Yue Ma[a]

[a] Food Science College, Shenyang Agricultural University, Shenyang, China, 110866

[b] Department of Publication, Shenyang Agricultural University, Shenyang, China, 110866

Optimization of CAMP reaction

To improve the amplification efficiency of the PMA-CAMP assay, CAMP primers concentration (25-50 μM), loop primers concentration (5-30 μM), out primers concentration (5-30 μM), betaine concentration (10-50 M), Mg^{2+} concentration (0.1-0.14 M), and dNTPs concentration (5-25 mM) were optimized in this study. As shown in Fig. S1a to c, the concentrations of 45 μM for the NF and NR primers each, 15 μM for the Lin and Rin primers each, and 10 μM for the F2 and R2 primers each, were found to be optimum. In addition, when the concentration of betaine, Mg^{2+} and dNTPs in the reaction solution was 20 M, 0.13 M, and 10 mM respectively, the Ct value was the minimum (Fig. S1d to f). For the CAMP reaction, the key is to establish a dynamic reaction environment under a constant temperature. In this study, the reaction temperature (61-65°C) was also optimized. As shown in Fig. S1g, the Ct value was the minimum at 65°C. Thus, the reaction temperature was finally set at 65°C.

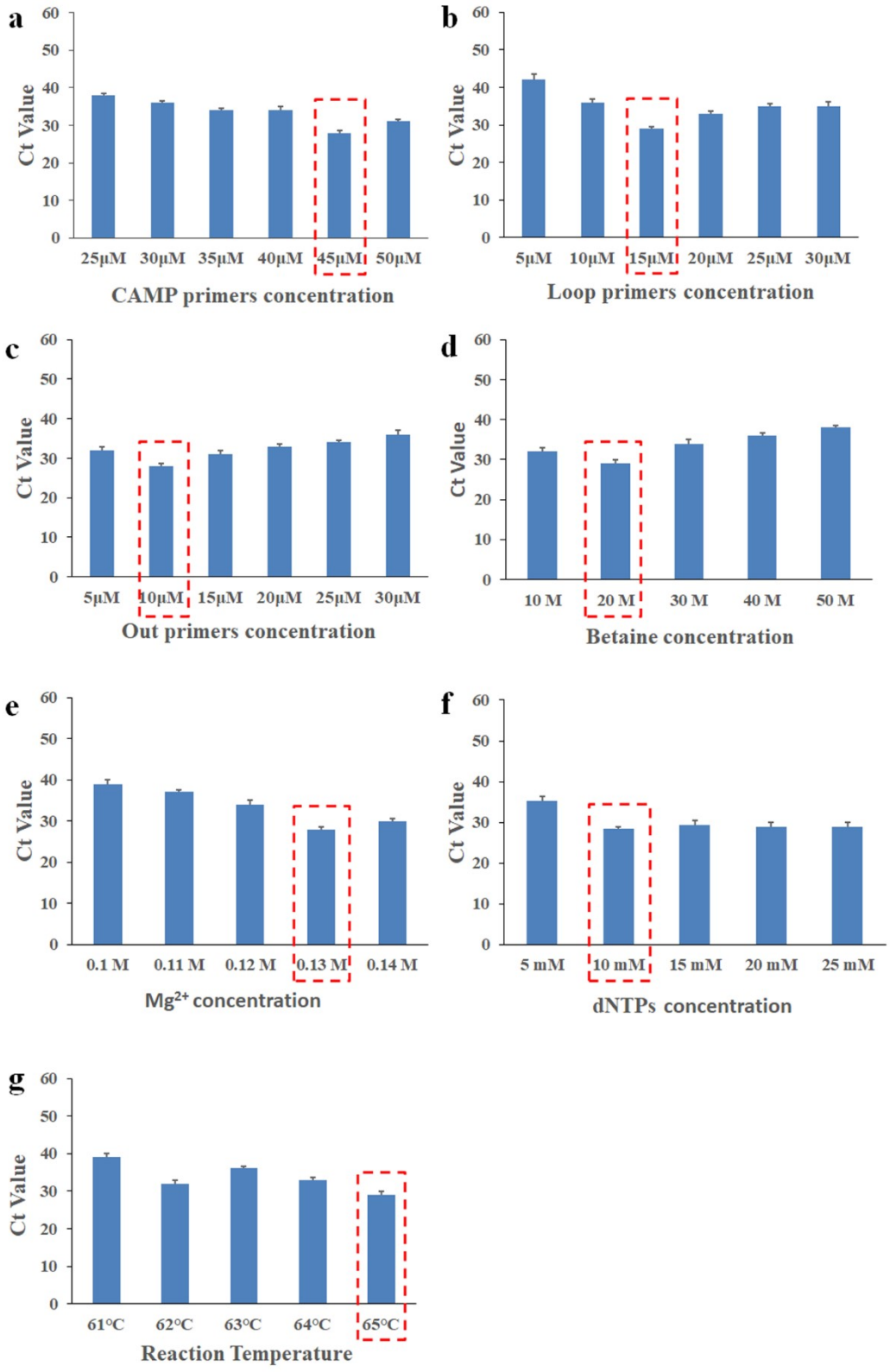

Fig. S1 a: Effect of CAMP primers concentration on the Ct value; b: Effect of loop primers concentration on the Ct value; c: Effect of out primers concentration on the Ct value; d: Effect of betaine concentration on the Ct value; e: Effect of Mg^{2+} concentration on the Ct value; f: Effect of dNTPs concentration on the Ct value; g: Effect of reaction temperature on the Ct value.