Signaling mechanism investigation and performance verification of electrochemical real-time PCR based on methylene blue-DNA interaction

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We demonstrate the electrochemical (EC) real-time PCR based on the intercalative binding of methylene blue (MB) with dsDNA. We confirmed that the MB-DNA binding had a high enough association constant to decrease the electrochemical current signal generated from MB. And we found that this signal decrease is mainly due to lower apparent diffusion rate of MB-DNA complex than that of free MB. With this signaling mechanism, we performed the PCR in the presence of 10 μM MB in a PCR mixture solution on a fabricated electrode-patterned glass chip having 20 μL reaction volume. With the square wave voltammogram (SWV) recorded at the end of every extension step, the decreased cathodic current peak was observed according to the increasing PCR cycle number as expected. Finally, we successfully performed EC real-time PCR with various initial copy numbers of C. trachomatis DNA templates, and the threshold cycle (Ct) value showed a reliable linearity depending on the input template quantity providing a good PCR efficiency (106%), which were comparable to the conventional TaqMan probe-based real time PCR.