Electrochemical Impedance Spectroscopy (EIS) measurement of PCR products

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Introduction. EIS is a method to detect surface characteristics of electrode (Working electrode; WE) exposed to sample solutions through amperometric measurement. Impedances between WE and counter electrode (CE) placed in the sample solution are measured with frequency scanning. EIS has advantages, high sensitivity, label free and small ions-strength dependence.[1] Especially, EIS allows solutions with a wide range of ionic strength to be used. Therefore, the EIS method can provide a highly sensitive bio-sensors which analyze sample solutions in physiological conditions. We applied this EIS to detect intermediate products of PCR to investigate possibility of real time PCR detection.

Experimental. PCR was carried out using mitochondria DNA as a template and target 108bp DNA, NADH, were amplified. As a negative control, PCR with the same condition except no addition of dNTPs. At 1, 10, 20, 30, each heat cycle, PCR products were taken out and EIS measurement was carried out with 10mM Tris pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 1mM $K_3[Fe(CN)_6]$ /$K_4[Fe(CN)_6]$, 1μM $Ru(bpy)_2DPPZ^{2+}$, frequency with 1k Hz to 0.5 Hz, and applied voltage 100 mVac. measurements were repeated three times. Screen printed carbon electrode were used as WE, CE (Zensor, Taiwan).

Result and Discussion. Figure 1 shows Nyquist plots of the EISs. A semicircles were analyzed by Randles equivalent circuit, parallel combination of a surface charge transfer resistance (Rct) and an electric double layer capacitance.[2] The diameter of the semicircles roughly corresponded to the Rct.[3] Figure 2 shows the Rct increase with heat cycles. The Rct was as small as 50 kΩ at the beginning and increased up to 300 kΩ, indicating PCR products adsorbed onto the WE. Negative control also shows the Rct increase but the increase was less and the difference should correspond to the PCR products. There were small Rct difference, at 20 and 30 cycles. This was consistent with the DNA electrophoresis which showed that the copies of target DNA were saturated around 20 heat cycles. The results indicated that the EIS measurement could be applied PCR detection. More details will be discussed at the presentation.

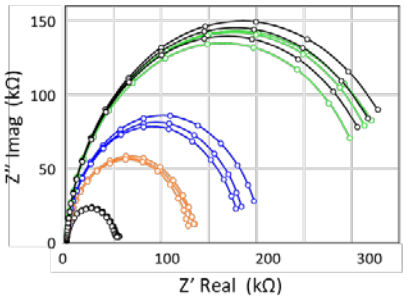
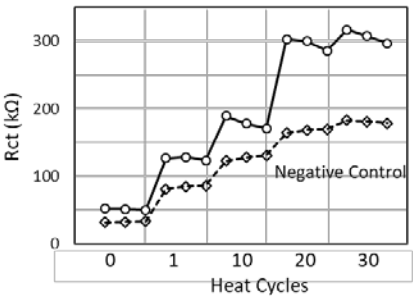


Fig. 1. EIS from PCR products (after 1,10,20,30 heat cycle).



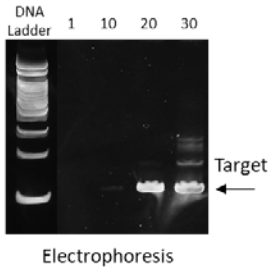


Fig.2. Rct dependence on PCR heat cycle. Real line: PCR products, Broken line: Negative control (without dNTP), Corresponding PCR products electrophoresis.

Reference

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