Polymerase Chain Reaction (PCR) under low ionic strength

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Polymerase chain reaction (PCR) is a method to amplify a target DNA by several orders of magnitude. PCR is commonly used to detect the existence of the target DNA sequence or estimate the quantity of target DNAs in the sample. It is usual that the amplified dsDNAs are detected by fluorescent dyes, such as SYBR Green. The method looks simple, but as a system, the method needs additional optical measurement system besides electric circuit which is accommodated in a measurement box and control the PCR process, such as temperature cycles and machine-man interfaces. To realize compact PCR monitoring system, exclusion of additional optical system is necessary. We plan to detect the PCR process by electrodes, the charges on which vary as PCR proceed. In this case, electrostatic shield length, Debye length, matters much. Conventionally, PCR is carried out in the solution with high concentration salt, ionic strength higher than 50 mM. The Debye length would be less than 1 nm and only charges in the sub-nm vicinity of electrodes could be detected, which are hard to be discernable. To get clear signals from the electrodes, the PCR solution should have low ionic strength, i.e. long Debye length.

We used polymerase, KOD FX (TOYOBO) as the PCR reaction, which has high fidelity. Conventional PCR condition solution is as follows, 120 mM Tris-HCl (pH 8.0), 10 mM KCl, 1.5 mM MgSO₄. Those ions stabilized the dsDNA structure and worked as polymerase cofactor. We decreased the Tris-HCl from 120 mM to 60 mM, 30 mM and 15 mM and the DNA products were analyzed by electrophoresis. The results showed that the target DNA fragments amplification significantly decreased at 30 mM and could not be detected at 15 mM. Furthermore, the combination of 0, 5, 10 mM KCl and 1.5, 2.5, 4.0 mM MgSO₄ in 30 mM and 15 mM Tris-HCl (pH 8.0) were studied. We found that increase the MgSO₄ concentration 1.5 mM to 2.5 mM could eliminate KCl salt. The minimum ions concentration could decrease to 17.5 mM. We also optimized the conditions employing another polymerase. The band on the PAGE gel is very clear in ions concentration around 11.5 mM, even the amplicons decreased.

It was demonstrated that PCR with KOD FX polymerase can reduce the ionic strength as low as 17.5 mM. In the case of Bio-FET, the charge change could be detected under 1/10 diluted PBS. The ionic strength conditions we showed here was about the same level and it was shown that this low ionic strength condition PCR could be detected by the electrode.

Figure 1. PCR process.