# Influence Factor in Polymerase Chain Reaction

## INTRODUCTION

The polymerase chain reaction now is the one of the powerful biotechnical tools. According the experimental principle, the throughput of the reaction is closely related to the template DNA concentration throughout the same procedures. More amount of target DNA can reach certain copies after amplified with less cycles of temperature regimes, which less amount of target DNA gets more cycles while applied with a stable thermo cycler system for the test. In the previous technical bulletin, we show the temperature uniformity of SEDI thermo cycler. Here, the effect of the template DNA quantity was performed on this section. Besides, the series dilutions of the primers were also tested to optimize the proper primer concentration.

## MATERIALS

- SEDI thermo cycler (Wealtec)
- Target DNA, 5'-Primer, and 3'-Primer samples were kindly provided by Dr. Hu's lab in Graduated Institute of Physiology in National Taiwan University, Taiwan.
- 2X Ready to Load PCR Master Mix (MDBio)

## PROCEDURES

1. The series dilution of the template DNA as following:
   1/20X, 1/100X, 1/500X, 1/2500X/ 1/12500X, 1/62500X
2. The series dilution of the primer concentration as following:
   1, 0.5, 0.25, 0.125, 0.0675 μM
3. Prepare stock solution with following recipes for series dilution of template DNA

| Reagent | Each Rex. (µL) |
|---|---|
| DNA Template | 1 |
| 5'-Primer | 1 |
| 3'-Primer | 1 |
| 2X Master Mix | 10 |
| $ddH_2O$ | 7 |
| Total | 20 |

4. Run the SEDI thermo cycler with following cycling program:
   Step 0: 95°C, 05:00, ON
   Step 1: 95°C, 00:30
   Step 2: 56°C, 00:30
   Step 3: 72°C, 00:30, Go To Step 1, 25 cycles
   Step 4: 72°C, 02:00
   Storage: ON
5. After finish with the reaction, separate 5 µl samples along with 5 µl 100 bp ladder in 1.5% agarose gel with 0.5x TAE buffer.
6. Stain the gel with 5% EtBr solution for 10 minutes.
7. Document with KETA ML imaging system.

## RESULT

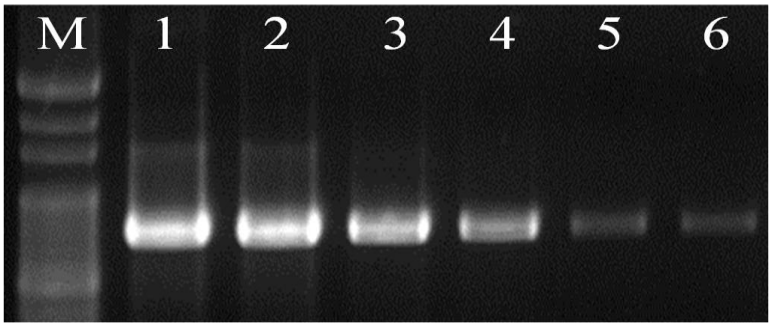


**Figure 1.** *Amplification result of Target DNA series dilution. Lane 1: 1/20X, Lane 2: 1/100X, Lane 3: 1/5000X, Lane 4: 1/25000X, Lane 5: 1/125000X, Lane 5: 1/625000X template concentration. M: DNA marker.*

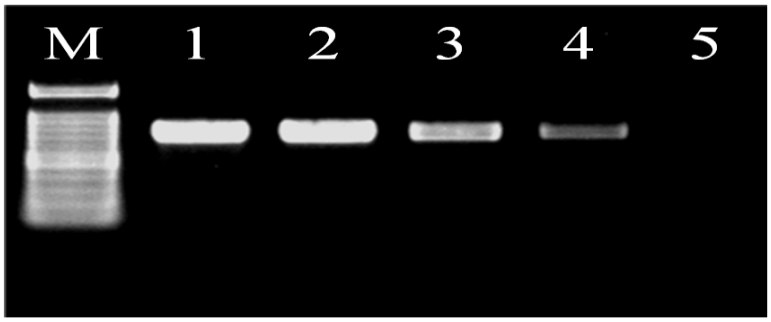


**Figure 2.** *Electrophoresis confirmation of amplification result. Lane 1: primer 1* μM*, Lane 2: primer 0.5* μM*, Lane 3: primer 0.25* μM*, Lane 4: primer 0.125* μM*, Lane 5: primer 0.0675* μM*. M: DNA marker.*

## DISCUSSION

While manipulating the DNA amplify experiment, more DNA template can reach target copies within less temperature cycles. There still have some limitation on sample preparation. Such as in the figure 1 lane 1 to 3, the non-specific band are observated as the amount of target DNA increased. Although the product yields increased along with the amount of DNA template, too much template will lead to the non-specific amplification. Besides, if the amount of primers are not enough, there will not have significant difference as increasing the amount of target DNA.

On the other hand, when using of unknown DNA target samples, primer DNA will be another factor to control the whole reaction. As demonstrated in the figure 2, under the same cycling regimes with same machine, the primer concentration significantly affects the product yields. Higher primer concentration promote higher production, but there is no significant different when primer concentration is higher than 1 μM (figure 2, lane 1 and 2). Interestingly, the high level primer concentration doesn't lead to the non-specific amplication. In this case, the 0.5 μM will be sufficient.

Experiments focus on concnetration of both target DNA and primers prove again that SEDI thermo cycler provides very stable and reliable temperature controlling regime.

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