Temperature Differential Application in SEDI Thermo Cycler

INTRODUCTION

Thermo cycler is now one of the most widely used tools in molecular biology, provides a fast and simple way to amplify trace DNA which needs only a few ingredients. Thermo cycler was widely used in various fields such as criminal investigations, food science, ecological field studies, and diagnostic medicine [1].

Condition optimization of temperature cycling regimes is the most important procedure to amplify the correct products. There are several parameters that determine the success of amplification such as annealing temperature, number of cycling, the concentration of ingredients, and activity of DNA polymerase. The annealing temperature is a crucial point among success factors. The purity and yield of reaction products are based on the optimized annealing condition. Lower temperature will cause the formation of non-specific bands and dimers. As if the temperature is too high, the efficiency and yield of desired products will decrease. In this bulletin, four ranges of temperature differential modes were designed as a reference for researchers to optimize the amplification conditions.

MATERIALS

- Target DNA, 5'-primer and 3'-primer samples were kindly provides by Dr. Hu's lab in Graduated institute of physiology in National Taiwan University, Taiwan.
- SolGentTM 2x Taq PLUS PCR Smart mix (SolGent).
- 100 bp plus DNA ladder (Genomics).
- Agarose LE (MDBio).
- Ethidium Bromide (Amresco)
- SEDI thermo cycler (Wealtec).
- Agarose Gel Electrophoresis system, GES (Wealtec).
- KETA ML image system (Wealtec).
- Digital thermometer Midi Logger GL220 (Graphtech)

PROCEDURES

1. Prepare stock solution with following recipes for 28 reactions:

Reagent	Each reaction (μL)	28 reactions (μL)
DNA template solution	1	29
5'-primer (2.5 μM)	1	29
3'-primer (2.5 μM)	1	29
2x Taq mix (μL)	4	116
dd H2O (μL)	13	377
Total (μL)	20	580

2. Run the SEDI thermo cycler with following cycling program and with different setting in temperature differential step:

Step0	95°C	05:00	Off
Step1	95°C	01:00	
Step2	95°C	00:30	
Step3	56°C	00:30	(Temperate Differential Step)
Step4	72°C	00:30	Go to 2 25 cycles
Step5	72°C	02:00	
Storage	On		

3. After finish with the reaction, loading 10 μL samples along with 5 μL of 100 bp ladder in 1.5% agarose gel with 0.5x TAE buffer.
4. Separate samples with 80 voltages for 60 minutes.
5. Stain the gel with 5% EtBr solution in 0.5x TAE buffer for 20 minutes.
6. File with KETA ML imaging system.
7. Run the same cycling regimes without sample and measure the temperature distribution with digital thermometer.

Result

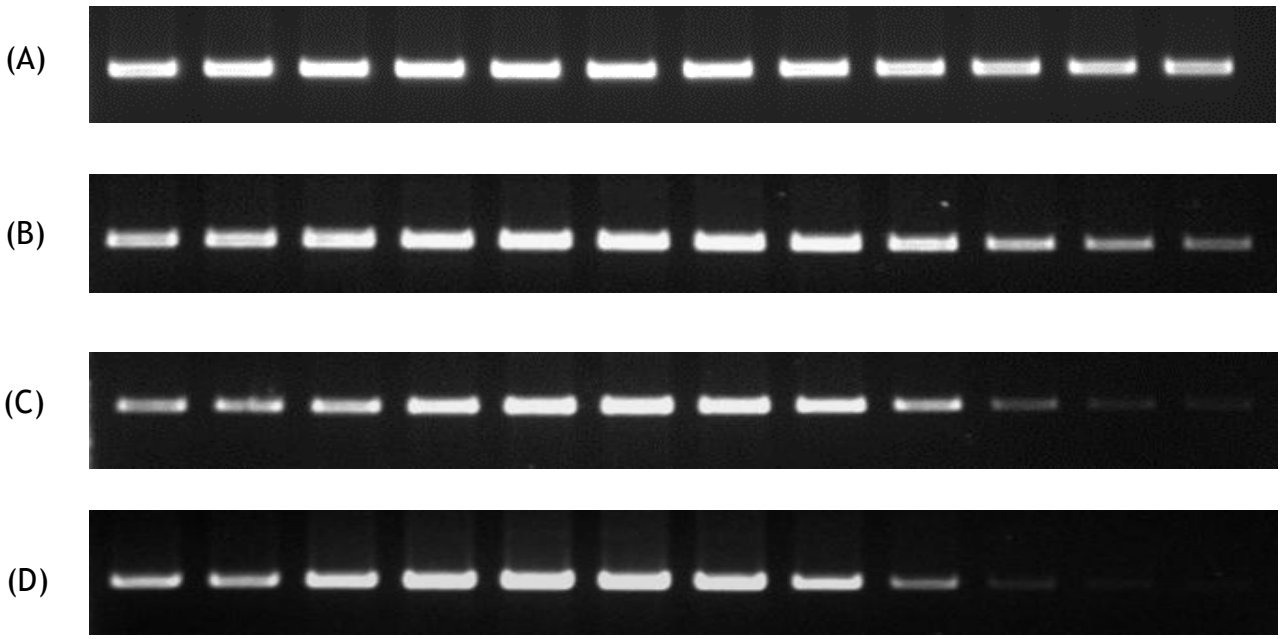

Figure 1. *Temperature differential performance comparison with (A) 56 ± 3°C, (B) 56 ± 6°C, (C) 56 ± 9°C and (D) 56 ± 12°C.*

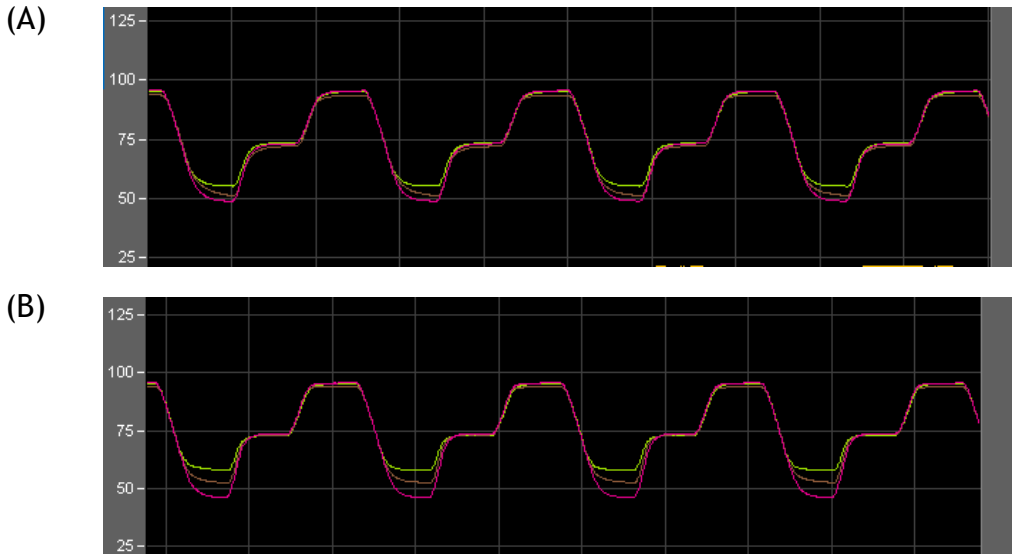

Figure 2. *The actual temperature of temperature differential mode comparison with (A) 56 ± 3°C, (B) 56 ± 6°C*

DISCUSSION

Successful nucleic acid amplification is depending on the uniformity and accuracy of temperature distribution in the system. When deal with an unknown sample, researchers would optimize the conditions and methods after designed primers and ingredients, especially the annealing temperature. As mentioned in the previous paragraph, determine the optimal annealing temperature is one of the crucial points for amplification.

Beside the annealing temperature, the purity and yield of the amplification product are also closely related to primer that used, including the concentrations of primer, the GC content ratio, and primer size. As shown in figure 1, the different settings of temperature differential step were performed with the SEDI thermo cycler. Regardless of setting with various ranges of temperature differential steps, SEDI thermo cycler showed with excellent reproducibility, even through the comparison with 56°C ± 3, ± 6, ± 9, and ± 12°C. It was found that yield of desired products was similar at 56°C and no appearance of nonspecific bands with each condition. Wealtec SEDI thermo cycler performed with ultra excellent accuracy and precision that was the crucial factor for amplification. Figure 2 was shown with the results of actual temperature distribution of temperature differential steps as set with 56°C ± 3 and ± 6°C. The temperature repeatability of each cycling steps were all the same.

The SEDI thermo cycler provides the ultra outstanding temperature uniformity meet all laboratories' needs. As noted previously, running with the temperature differential step for new primer allows researchers to optimize the annealing temperature, and also the purity and yield of desired products that related with the annealing step. Therefore, the SEDI thermo cycler is the most trustable system for amplification experiment.

REFERENCE

1. Bartlett J. M. S. and Stirling D. PCR Protocols. 2nd ed. Vol: 226. Humana Press. 2003.
2. Rychlik W, Spencer WJ, Rhoads RE (1990) Optimization of the annealing temperature for DNA amplification in vitro. *Nucl Acids Res* **18** (21): 6409–6412.

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