Temperature Uniformity for SEDI Thermo Cycler

INTRODUCTION

Ramping rate, temperature accuracy, stability, and temperature uniformity are most important points when customer evaluates an excellent thermo cycler system. Although the polymerase chain reaction itself has wide range tolerance on temperature differences, one degree difference may cause false interpret in the differential gene expression experiment. In this situation, the temperature uniformity of thermo cycler is considered as the most important issue for the result. Wealtec SEDI thermo cycler provides the most outstanding temperature uniformity to fulfill all laboratories' needs.

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.
- ExcelSure 5X PCR Master Dye Mix (Premier)

PROCEDURES

- Prepare stock solution with following recipes for 28 reactions:

Reagent	Each Rex (μL)	28 Rex (μL)
DNA Template	1	29
5'-Primer 50 nM	1	29
3'-Primer 50 nM	1	29
Master Mix	4	116
ddH_2O	13	377
Total	20	580

- Aliquot 28 samples with 20 µL stock solution each into 96 wells plate with following arrangement:

	1	2	3	4	5	6	7	8	9	10	11	12
A	11				12				13			14
B												
C		1	2	3	4	5	6	7	8	9	10	
D												
E												
F		15	16	17	18	19	20	21	22	23	24	
G												
H	25				26				27			28

- 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, GoTo Step 1, 30 cycles
  - Step 4: 72°C, 07:00
  - Storage: ON
- After finish with the reaction, separate 10 µL samples along with 5 µL 100 bp ladder in 1.5% agarose gel with 0.5x TAE buffer.
- Stain the gel with EtBr solution for 30 minutes.
- File with KETA ML imaging system.

RESULT

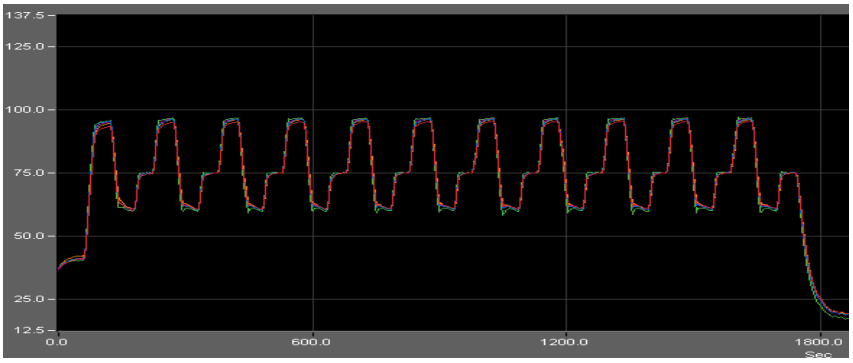

Figure 1. *Temperature Distribution of Reaction zone in the SEDI thermo cycler*

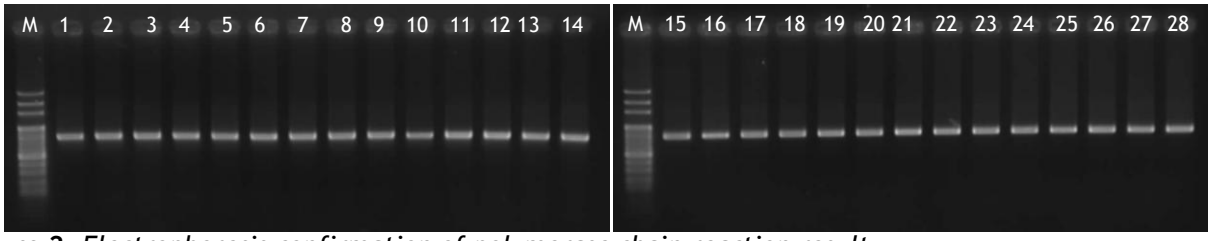

Figure 2. *Electrophoresis confirmation of polymerase chain reaction result*

DISCUSSION

Due to the temperature accuracy and uniformity of the thermo cycler is the most crucial point for polymerase chain reaction experiment, SEDI thermo cycler was tested as in figure 1. Cycling programs was set with 95°C, 56°C, and 72°C, and the temperature profile was recorded with six different positions on the reaction zone. As in the result, temperature differences within each well were all located less than ±0.5°C no matter what temperature were set.

As plasmid DNA was the most common target that used for manipulate the gene cloning or genomic research, DNA used for the temperature uniformity test here is circle form plasmid DNA which carried with specific sequence that was cloned from mammalian cells. Since the amount of amplified DNA fragments will significantly affected by tiny temperature difference along with the amplification, same polymerase chain reaction master solution can be used to evaluate the temperature uniformity in 96 wells. Amplified through SEDI thermo cycler, same volume of 28 reactions can get the same intensity on each band as seen in figure 2. No matter samples are placed at the surrounded part or the central part of the 96 wells, the intensity of each band was the same. It is the strongest evidence of that SEDI thermo cycler can provide the most even temperature distribution when randomly place the samples within 96 wells.

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