Excellent PCR performance delivered by SEDI Thermal Cycler, GES Gel Electrophoresis System and ELITE Power Supply

Introduction

Temperature accuracy, stability, and temperature uniformity are the most important points when customers evaluate an excellent thermal cycler system. Temperature instability and differences both can affect the experimental results. The main purpose of the horizontal electrophoresis system and power supply is to provide a good electrophoresis environment and a uniform and stable electric field. The Wealtec products can support the operators with an outstanding and high quality experimental equipment from the Polymerase Chain Reaction (PCR) to the electrophoresis system to fulfill most laboratories' needs.

The experiment uses three kinds of reaction volumes and temperature gradient modes to test the temperature uniformity and accuracy of SEDI Thermal Cycler, and further discusses the resolution of the PCR products by electrophoresis system. The aim of this article is to confirm the results from gene amplification to electrophoresis analysis by SEDI Thermal Cycler, horizontal electrophoresis system and ELITE 600 power supply, which developed by Wealtec.

Materials and Methods

Reagents

1. 0.4 ng/µl DNA template (pFLAG-mLRH-1 provided by Dr. Hu from NTU)
2. 2.5 µM primer

5'- rLRH-cF984	CTC GCC AGA GTC AAT GAT G
3'-mLRH-cR1683	GAG GAT CCT TAG GCT CTT TT

3. 2x Master Mix (Solgent)
4. Ultrapure water
5. 1.5 ml microcentrifuge tubes
6. Sterilized PCR tubes
7. 100 bp DNA ladder (EBL)

Instruments

1. SEDI Thermal Cycler
2. GES Agarose Gel Electrophoresis System
3. ELITE 600 Power Supply
4. KETA G Gel Documentation System

Agarose Gel Preparation

1. To make 1.5% agarose gel, wight 1.5g of agarose powder into 100mL 1x TAE buffer.
2. Microwave the solution 1 minute each time, until the agarose powder dissolve completely.
3. While waiting for the molten agarose gel cool to about 60 °C assemble the gel trays.
4. Pour the molten agarose gel into the trays, 100 ml for each tray, then insert the well comb.

5. Wait at least 50 minutes to let the gel completely solidified.

Polymerase Chain Reaction

PCR mix recipe:

Reaction volume	**1 reaction**	**20 µl**	**50 µl**	**80 µl**
Reagent	**µl**	**µl**	**µl**	**µl**
Ultrapure water	7	210	525	840
2X PCR master mix	10	300	750	1200
0.4 ng/µl DNA Template	1	30	75	120
2.5 µM of 5'-rLRH-cF984	1	30	75	120
2.5 µM of 3'-mLRH-cR1683	1	30	75	120
Total volume	**20**	**600**	**1500**	**2400**

1. Follow the table above. add DI water, 2x Master Mix, DNA template and primer into 1.5 ml Eppendorf in sequence.
2. Vortex the PCR mixture and spin down.
3. According to the experimental requirements dispense PCR mix into PCR tubes.
4. Cap each tube tight and spin down the PCR tubes

5. Turn on the power of SEDI thermal cycler, adjust the temperature of the top lid to 99 °C and reaction volume, and then set up the PCR method.

20 ul				20 ul Gradient ±6℃			
Step 0	95℃	Off		Step 0	95℃	Off	
Step 1	95℃	2:00		Step 1	95℃	2:00	
Step 2	95℃	0:30		Step 2	95℃	0:30	
Step 3	56℃	0:30		Step 3	56℃ ±6℃	0:30	
Step 4	72℃	1:00	Go to step2 Cycle 25	Step 4	72℃	1:00	Go to step2 Cycle 25
Step 5	72℃	5:00		Step 5	72℃	5:00	
Storage	6℃	On		Storage	6℃	On	
50 ul				80 ul			
Step 0	95℃	Off		Step 0	95℃	Off	
Step 1	95℃	2:00		Step 1	95℃	2:00	
Step 2	95℃	0:30		Step 2	95℃	0:30	
Step 3	56℃	0:45		Step 3	56℃	1:00	
Step 4	72℃	1:00	Go to step2 Cycle 25	Step 4	72℃	1:00	Go to step2 Cycle 25
Step 5	72℃	5:00		Step 5	72℃	5:00	
Storage	6℃	On		Storage	6℃	On	

6. The PCR tube place in 96-well plate with following arrangement:

11				12				13			14
	1	2	3	4	5	6	7	8	9	10	
	15	16	17	18	19	20	21	22	23	24	
25				26				27			28

DNA Electrophoresis

1. Load 5 ul of 100 bp DNA ladder in the first well of the upper row and the lower row.
2. Load 10 ul PCR product in the following well of the upper row and the lower row by the number labelled on the tubes.
3. Connect to power supply and run the gel at 110 V for 50minutes.

Visualization

1. Stain each gel in 250 ul EtBr + 1000 ul 1×TAE tank for 30 minutes.
2. Visualize the gel under UV transilluminator and capture the image by KETA G.

Results

20ul reaction

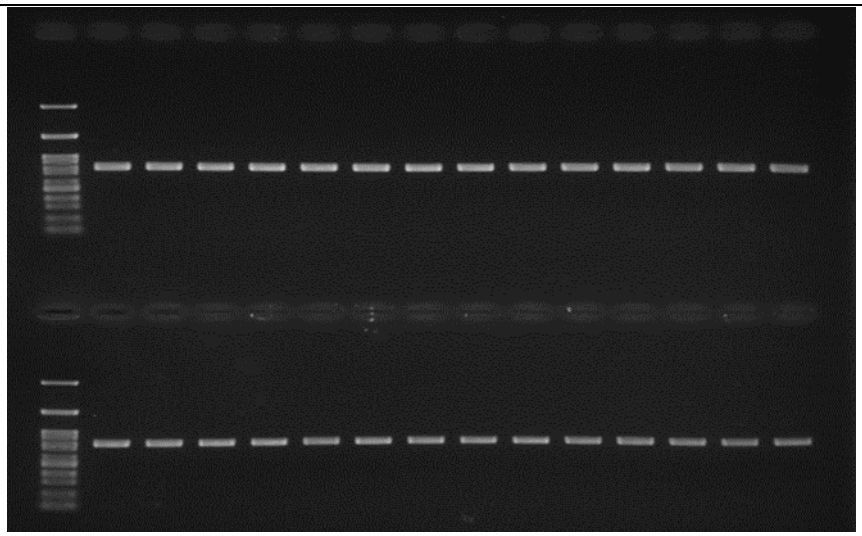

Figure 1 Electrophoresis result of 1.5% agarose gel running 20ul PCR product with 110V 50mins, and then stained with EtBr 30mins.

20ul Gradient±6℃ reaction

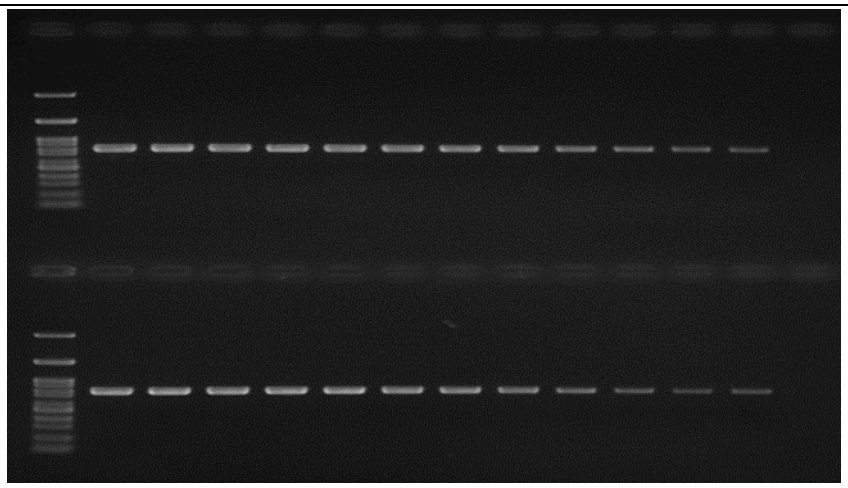

Figure 2 Electrophoresis result of 1.5% agarose gel running 20ul Gradient±6℃ PCR product with 110V 50mins, and then stained with EtBr 30mins.

50ul reaction

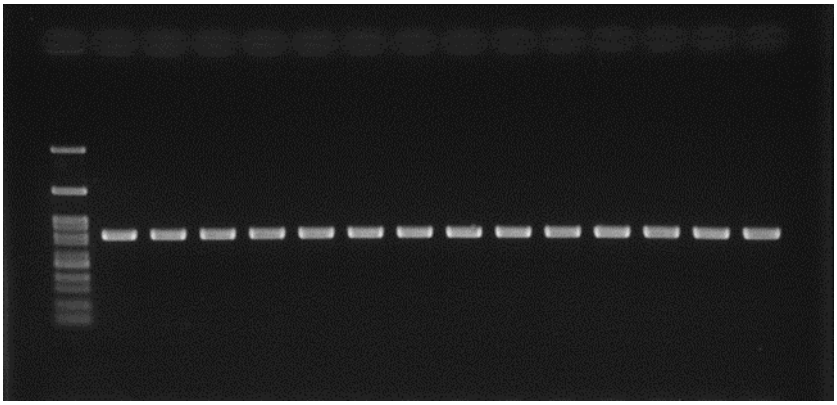

Figure 3 Electrophoresis result of 1.5% agarose gel running 50ul PCR product with 110V 50mins, and then stained with EtBr 30mins.

80ul reaction

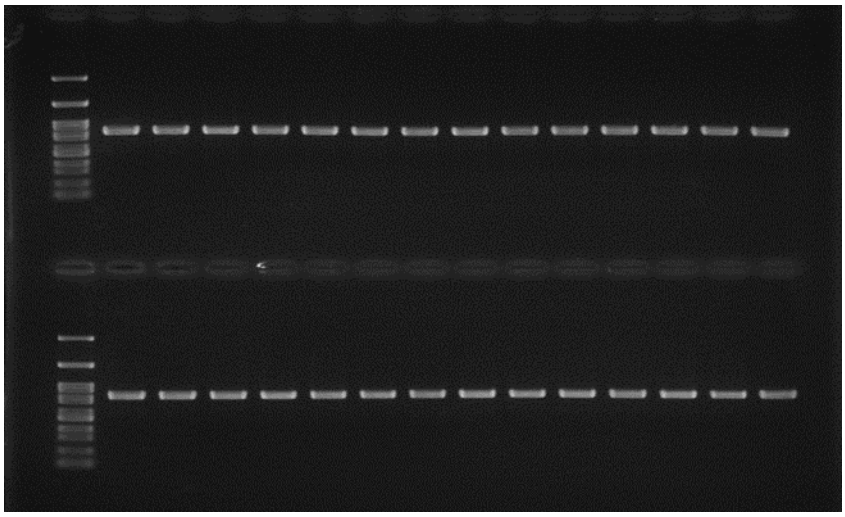

Figure 4 Electrophoresis result of 1.5% agarose gel running 80ul PCR product with 110V 50mins, and then stained with EtBr 30mins.

Discussion

1. No matter where samples were placed in the 96-well reaction block in the SEDI Thermal Cycler and how much volume PCR reaction were run at, the intensity of each band was the same. The results showed that SEDI thermal cycler can provide the most even temperature distribution (Fig.1.3-4).
2. In temperature gradient mode, the decreasing trend of signal with temperature change shows high temperature accuracy of the SEDI thermal cycler (Fig.2).
3. The flat band also indicate that the ELITE 600 offer an uniform electric field which let the sample migrate smoothly from negative charge to positive charge。

Notes

1. Shaking the molten agarose solution allow it to cool down evenly after heating by microwave.
2. Both the PCR tubes and DDW must be sterilized before experiment.
3. Prepare fresh agarose gel for every experiment as much as possible. And do not store it in buffer more than two days.
4. Pay attention to the EtBr tank before staining the gel. Any residue will likely affect the background in the gel image.

Conclusion

A series of instruments which manufactured by Wealtec are used to accomplish the process from Polymerase Chain Reaction to DNA electrophoresis. Different kind of reaction volumes and temperature gradient modes can obtain uniform, stable and repeatable experimental results.

Ordering Information

Catalog no.	SEDI Thermal Cycler
1310001	SEDI B Thermal Cycler, gold-plated 96-well reaction module, 100-240V, 50-60 Hz
1310006	SEDI G Thermal Cycler, gradient unit, gold-plated 96-well reaction module, 100-240V, 50-60 Hz
1310026	SEDI X Thermal Cycler, gradient unit, gold-plated 384-well reaction module, 100-240V, 50-60 Hz

Catalog no.	ELITE Power Supplies
1001007	Mini ELITE Power Supply, 100-240V, 50-60Hz
1001021	ELITE HC 2.0 Power Supply, 5-200V, 0.01-2.0A, 100-240V, 50/60Hz
1001023	ELITE HC 2.5 Power Supply, 2-250V 10mA-2500mA, 100-240V, 50/60Hz
1001024	ELITE HC 3.0 Power Supply, 2-300V 0.01A-3.0A, 100-240V, 50/60Hz
1001026	ELITE 300U Power Supply, 1-300V, 1-500mA, 100-240V, 50/60Hz
1001031	ELITE 600U Power Supply, 5-600V, 1-750mA, 120-240V, 50/60Hz

Catalog no.	GES Agarose Gel Systems
1001002	GES Agarose Gel Electrophoresis System with 10 x 15cm tray, two 1.0 mm thick fixed height combs: 15-well and 20-well
1001003	GES Agarose Gel Electrophoresis System with 7 x 15cm tray, two 1.0 mm thick fixed height combs: 15-well and 20-well
1001004	GES Agarose Gel Electrophoresis System with 15 x 15cm tray, two 1.0 mm thick fixed height combs: 15-well and 20-well

Catalog no.	GES with Power Supply
1011033	GES Agarose Gel Electrophoresis System with 7 x 15cm tray and ELITE 300U Power Supply
1011034	GES Agarose Gel Electrophoresis System with 10 x 15cm tray and ELITE 300U Power Supply
1011035	GES Agarose Gel Electrophoresis System with 15 x 15cm tray and ELITE 300U Power Supply

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sales@wealtec.com / www.wealtec.com
Follow us at www.facebook.com/wealtec

Hsin Huang
Product Division
Wealtec Bioscience Co., Ltd.
Tel: +886-2-8809-8587 / Fax: +8886-2-8809-8589