Key Success Factors in DNA Amplification

INTRODUCTION

Polymerase chain reaction, so-called DNA amplification, composed with 3 major steps: denaturation, annealing and extension. Assuming having the 100% efficiency, the DNA can exponentially amplified after every cycle. According to the experimental principle, the yield of DNA amplification will be definitely affected by 4 key success factors as specified below.

1. Annealing temperature.
2. Cycling numbers setting.
3. Concentration of integrants.
4. Activity of DNA polymerase.

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.
- PCR Master Mix from MDBio, Promega, and Premier.

PROCEDURES

- Prepare stock solution with following recipes

Reagent	Each Rex (μl)
DNA Template	1
5'-Primer 250 nM	1
3'-Primer 250 nM	1
Master Mix	4
ddH_2O	13
Total	20

- Run the SEDI thermo cycler with following cycling program:

	Normal Amplification	Gradient Program
Step 0	95°C, 05:00, Off	95°C, 05:00, Off
Step 1	95°C, 01:00	95°C, 01:00
Step 2	95°C, 00:30	95°C, 00:30
Step 3	56°C, 00:30	56±6°C, 00:30
Step 4	72°C, 00:30, GoTo Step 2, With different cycling numbers.	72°C, 00:30, GoTo Step 2, 25 cycles
Step 5	72°C, 02:00	72°C, 02:00
Storage	ON	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 10 minutes.
- Filed with KETA ML imaging system.

RESULT

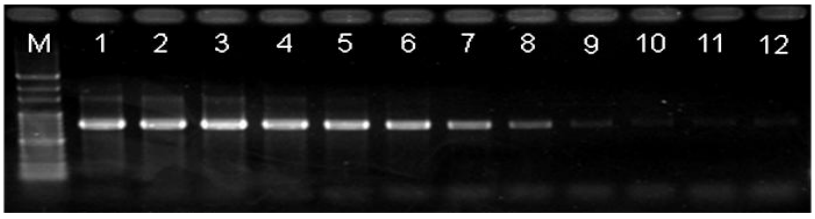

Figure 1. *Effect of gradient program.*

Setting the annealing temperature with 56°C at lane 6, +6°C for lane 12, and -5°C for lane 1.

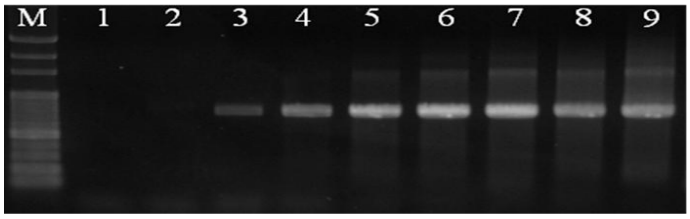

Figure 2. *Effect of cycling numbers.*

Lane 1: 10 cycle, Lane 2: 15 cycle, Lane 3: 20 cycle, Lane 4: 25 cycle, Lane 5: 30 cycle, Lane 6: 35 cycle, Lane 7: 40 cycle, Lane 8: 45 cycle, Lane 9: 50 cycle. M: DNA marker.

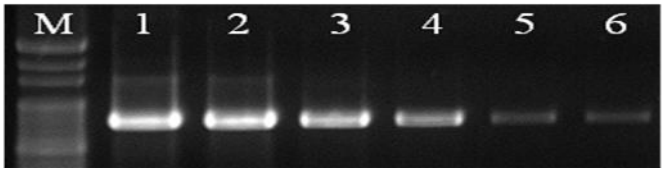

Figure 3. *Effect of Target DNA series dilution.*

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.

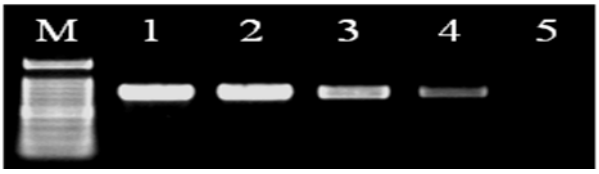

Figure 4. *Effect of primer concentration.*

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.

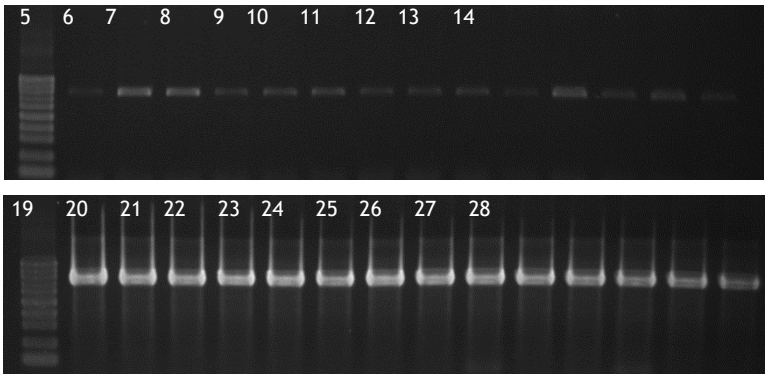

Figure 5. *Effect of Polymerase.*

Lane 1~6: Brand A Batch 1 Polymerase, Lane 7~12: Brand A Batch 2 polymerase, Lane 13, 14: Brand B Polymerase. Lane 15~20: Brand C Batch 1 polymerase, 21~28: Brand C Batch 2 polymerase. M: DNA Marker.

DISCUSSION

Factors that affect the amplification result are various. First of all is the annealing temperature setting. DNA amplification is the temperature depending reaction that the reaction specificity and yields are all related to the temperature. Refer to the figure 1, as increasing of the annealing temperature in SEDI Thermo cycler which the accuracy is ±0.3°C, the specificity gets restrict and the yield decrease, vice versa. As lower down the annealing temperature, it also caused the non-specific amplification. The other parameters that affect are the cycling numbers and the concentration of template and primer DNA. As decrease all these factors, the yields also decrease as in figure 2, 3 and 4, vice versa.

Besides, as long as the DNA amplification experiment gets more common used, there have more and more commercialized products coming out. It's recommended to test the activity of the commercialized polymerase prior to start the experiment with important sample. As in the figure 5, under the same amplify condition, different brands and even different batches of enzyme will affect the amplify result. Moreover, the components of buffer also take an important part in the reaction. However, as it is a commercial product that special paired to DNA polymerase now, salt content is not really an issue anymore.

As mentioned in the previous article, SEDI thermo cycler is the most trustable system for daily DNA amplification. Once users end up with different result in the same program, all factors that had been mentioned in this article should be all taken in considered, especially the reaction ingredients.

REFERENCE

- Sambrook J and Russell DW, *Molecular Cloning: A laboratory manual*, 3rd ed.,Vol: 2, Cold Spring Harbor, N.Y., 2001, Chapter 8.
- Bartlett JMS, Stirling D (2003) A Short History of the Polymerase Chain Reaction, *PCR Protocols,* Vol. 226, U.K. p 3-6.
- 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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