Optimize Cycling Numbers in DNA Amplification

INTRODUCTION

Routine polymerase chain reaction experiment mainly composed with 3 steps: denaturation, annealing and extension. As the DNA products duplicate cycle by cycle, assuming having the 100% efficiency, the DNA can amplified exponentially. React with sufficient material condition, the cycling number is the main factor that affects the yield of amplification. Other factors that also affect the experiment result such as the reaction slows down as long as the DNA polymerase loses activity after extreme temperature change for many cycles, so as decrease of consumption reagents such as dNTPs and primers. The optimal cycling number depends on the template concentration of the starting sample and primer.

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. 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

2. Run the SEDI thermo cycler with following cycling program:

   Step 0: 95°C, 05:00, Off
   Step 1: 95°C, 01:00
   Step 2: 95°C, 00:30
   Step 3: 56°C, 00:30
   Step 4: 72°C, 00:30, GoTo Step 2, with different cycling numbers.
   Step 5: 72°C, 02:00
   Storage: ON

3. 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.
4. Stain the gel with EtBr solution for 10 minutes.
5. Filed with KETA ML imaging system.

RESULT

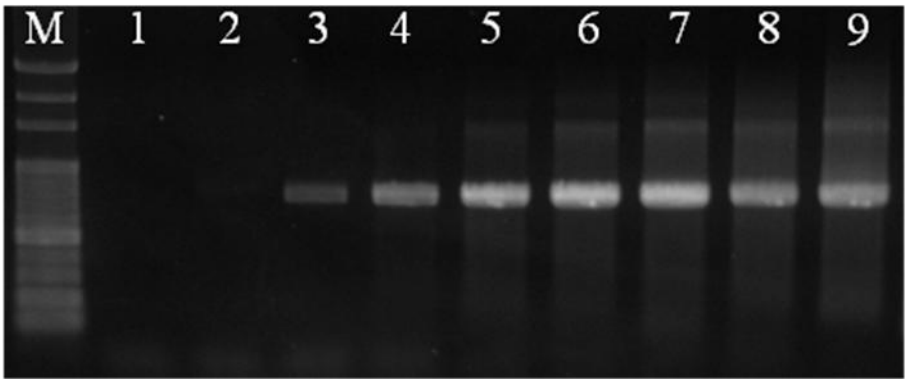

Figure 1. *Different cycling number of PCR result. Lane 1: 10 cycle, Lane 2: 15 cycle, Lane 3: 20 cycle, Lane 4: 25 cycle, Lane 5: 30 cycle, Lane 6: 35 cycle, Lane 7: 40 cycle, Lane 8: 45 cycle, Lane 9: 50 cycle. M: DNA marker.*

DISCUSSION

When the amount of template DNA is low, increase of cycling numbers can improve the detection ability. While using with unknown amount of DNA template, as the figure 1 shown, there is no product detection in lane 1 and 2 until cycling number increased over than 20 cycles in lane 3. After optimized the cycling regimes, a clear single band was

observed and the yield increased by more cycling numbers (lane 3, 4 and 5). However, increasing in 5 cycling numbers which amplified the DNA amount for 5 logarithmic times caused the non-specific band and background signal were both detected as in lane 5. As long as reaction compounds have being consumed and inhibitors accumulate, the reaction efficiency decreased. In other words, the reaction no longer follows exponential kinetics and the amplification stops in the plateau phase. Excess cycle does not lead to a higher yield of PCR product, instead it increase secondary product and lower down the specific product resulting in a diffused smear in lane 8 and 9.

Optimizing the cycling numbers is very important while amplifying the DNA under working with stable thermo cycler, using of same cycling numbers in routine amplifications can distinguish the amount difference between each target DNA samples. Even small amount difference will be enlarged in times.

Other factors, such as annealing temperature, DNA concentration, activity of polymerase, that involved with different effects will be taken together to discuss in the coming article.

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