Amplify Desire DNA Sequence from Incubated Colony

INTRODUCTION

As manipulate the nucleic acid experiments, using of bacterium, such as *E. coli* or yeast, as vector to enlarge the amount of target DNA by incubation are commonly used. But how to determine which colony have the transfected gene fast and easy? Theoretically, the target sequence can be amplified once it was transfected into the cell no matter with or without the selection marker designed in the constructed plasmid. After the incubation, beside the screening method by selection marker, polymerase chain reaction (PCR) could be a strategy to confirm or amplify the DNA fragments directly from the incubated colonies. Based on few major components that wanted target DNA sequence can be amplified and isolated through PCR from the bacterium without DNA isolation and purification.

MATERIALS

- *E. coli* plates were kindly provides by Dr. Hu's lab in Graduated institute of physiology in National Taiwan University, Taiwan.
- LB broth (Sigma, USA).
- Nutrient Agar (DIFCO, USA).
- SolGent™ 2x Taq PLUS PCR Smart mix (SolGent, Korea).
- 100 bp plus DNA ladder, 100-3000 bp (Genomics, Taiwan).
- Agarose LE (MDBio, Taiwan).
- Ethidium Bromide (Amresco, USA).
- SEDI thermo cycler (Wealtec, Taiwan).
- Agarose Gel Electrophoresis system, GES (Wealtec, Taiwan).
- Dolphin Doc Plus imaging system (Wealtec, Taiwan).

PROCEDURES

1. Prepare stock solution with following recipes:

Reagent	Each reaction (μL)
5'-primer (2.5 or 5 μM)	1
3'-primer (2.5 or 5 μM)	1
2x Taq	4
dd H_2O	13
Total	19

2. Pick one colony with toothpick and mix into one test tube.

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

Step0	95℃	05:00	Off
Step1	95℃	01:00	
Step2	95℃	00:30	
Step3	56℃	00:30	
Step4	72℃	00:30	Go to 2 25 cycles
Step5	72℃	02:00	
Storage	On		

4. After finish with the reaction, loading 10 μL samples along with 5 μL 100 bp ladder in 1.5% agarose gel with 0.5x TAE buffer.
5. Separate samples at 80 voltages for 60 minutes.
6. Stain the gel with 5% EtBr solution for 20 minutes.
7. File with KETA ML imaging system.

Result

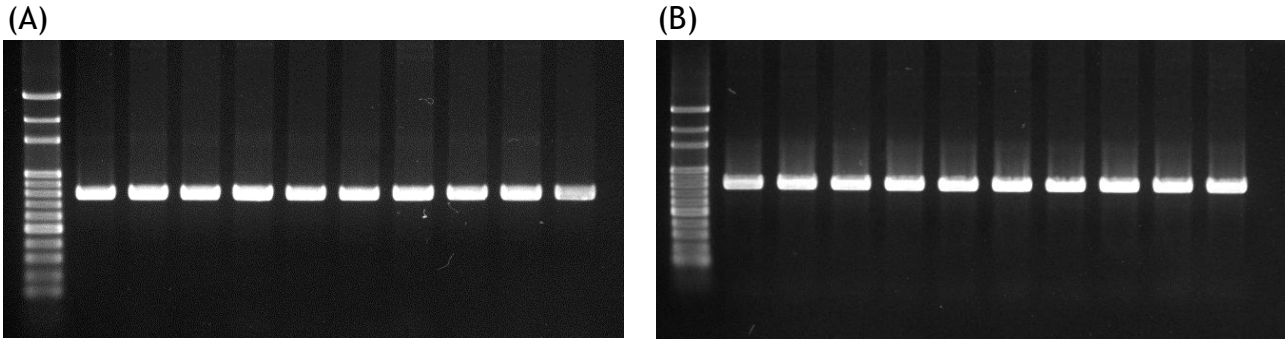

Figure 2. *Desired DNA sequences in E. coli were amplified after incubated for 1 day with (A) 2.5 μM primer and (B) 5 μM primer.*

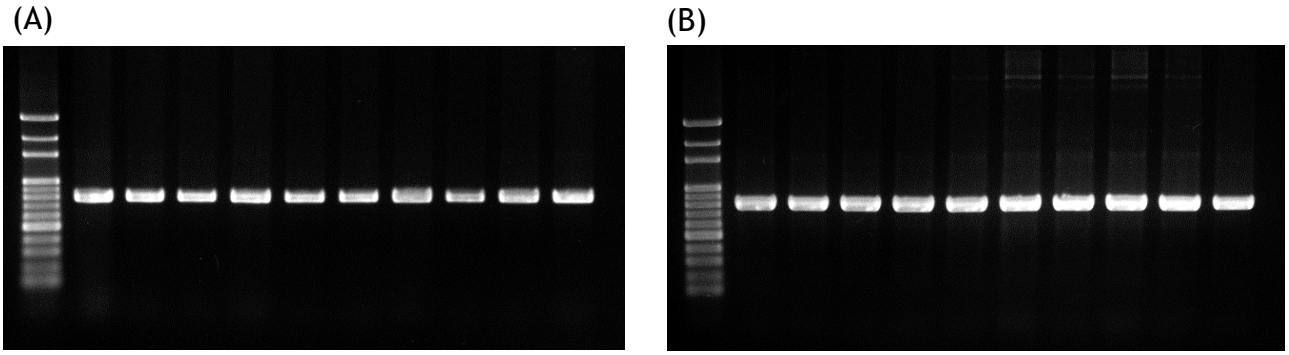

Figure 2. *Desired DNA sequences in E. coli were amplified after stored for 7 days with (A) 2.5 μM primer and (B) 5 μM primer.*

DISCUSSION

Gene transfection plays a major role on studying the gene function, the protein construction, and even gene mutation. Using with selection marker to confirm the transfected cells always taken as standard protocol, but sometimes user wants faster way to move forward. Directly amplify the gene from the incubated colonies is the product introduced due to that idea.

As in the above result, SEDI thermo cycler was proved to have well performance to amplify DNA directly from the incubated colony. With the very precise temperature control in SEDI thermo cycler, the different result can be observed with different stored time colonies. As in the fig. 2 (B), the result was started to appear with more than one band in some colony samples. As performing the amplification from colonies, sample should be as fresh as possible. If the colony plate was stored more than 7 days, it's not recommended to use. It will randomly come out more than one band (data not shown).

Accurate temperature controlled SEDI thermo cycler was proved to perform well with the DNA amplification directly from colony. This article giving out the idea to faster the genetic engineering processing and proved again the SEDI thermo cycler can be widely applied on many aspects that user can imagine.

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