Preparation of *Taq* DNA Polymerase

Wei Zheng*

Keck Biotech Services, Yale University, New Haven, USA

*For correspondence: wei.zheng.madison@gmail.com

Materials and Reagents

1. Dialysis tubing cellulose membrane avg. flat width 33 mm (Sigma-Aldrich, catalog number: D9652)
2. *E.coli* BL21 strain
3. Ammonium sulfate (Sigma-Aldrich, catalog number: A4418)
4. IPTG
5. Ampicillin
6. $(NH_4)_2SO_4$
7. 1 M dextrose
8. 1 M Tris - HCl (pH 7.9)
9. 0.5 M EDTA (pH 8.0)
10. KCl
11. PMSF
12. Tween 20
13. NP40
14. Lysozyme
15. Glycerol
16. Triton-X 100
17. Lysis buffer (see Recipes)
18. Buffer A (see Recipes)
19. Pre - lysis buffer (see Recipes)
20. Storage buffer (see Recipes)
21. TAQ buffer (see Recipes)
22. $MgCl_2$ solution (see Recipes)

Equipment

1. 2 L flask
2. Centrifuges

3. Oakridge tubes

Procedure

Day1

1. Inoculate a single colony of *E.coli* BL21 strain with *Taq* plasmid driven by IPTG inducible promoter into 5 ml of LB broth containing 20 µl of ampicillin (100 mg/ml). Incubate at 37 °C overnight with shaking.

Day 2

1. 7:00 A.M. prepare 500 ml of LB broth containing 40 mg of ampicillin in a 2 L flask, add 500 µl of overnight culture. Incubate at 37 °C for about 11 h with shaking. Grow these cultures to an OD_{600} of approx. 0.8.
2. 8:00 P.M., add 62.5 mg of IPTG, incubate at 37 °C for 12 h with shaking.

Day 3

1. Transfer overnight culture to 500 ml plastic bottle, centrifuge at 4.5 K for 10 min at 4 °C. Decant supernatant, and resuspend the prep in 50 ml of Buffer A.
2. Centrifuge at 4.5 K for 10 min at 4 °C, decant supernate, and resuspend the prep in 25 ml of pre-lysis buffer.
3. Incubate at room temperature (RT) for 15 min.
4. Add 25 ml of lysis buffer, mix well, transfer prep to Pyrex flask, and incubate at 75 °C for 1 h.
5. Divide the prep into two Oakridge tubes, cetrifuge at 15,000 rpm for 10 min at 4 °C.
6. Transfer supernate to clean flask, add 15 g of $(NH_4)_2SO_4$, stir rapidly at RT for 1/2 h.
7. Centrifuge at 15,000 rpm for 10 min at 4 °C.
8. Decant supernate, resuspend prep in 10 ml of Buffer A.

Day 3 & 4

1. The resuspended protein was dialyzed against 3 changes of storage buffer at 4 °C. (One 7 h, one 12 h and one 7 h, each with> 800 ml of storage buffer. The volume of the protein was reduced to about 5 ml after the dialysis.)
2. Protein was diluted 1:1 with sterilized storage buffer, and store at -80 °C.

Recipes

1. Buffer A

50 mM Tris - HCl (pH 7.9)	25 ml of 1 M Tris - HCl (pH 7.9)
50 mM dextrose	25 ml of 1 M dextrose
1 mM EDTA (pH 8.0)	1 ml of 0.5 M EDTA (pH 8.0)

q.s. to 500 ml with ddH_2O, autoclave, store at RT.

2. Lysis buffer

10 mM Tris - HCl (pH 7.9)	1 ml of 1 M Tris-HCl (pH 7.9)
50 mM KCl	5 ml of 1 M KCl
1 mM EDTA (pH 8.0)	0.2 ml of 0.5 M EDTA (pH 8.0)
1 mM PMSF*	2 ml of 50 mM PMSF
0.5% Tween 20	0.5 ml Tween 20
0.5% NP40	0.5 ml NP40

q.s. to 100 ml with ddH_2O, autoclave, store at RT.

* Toxic material, always wear a mask and gloves when dealing with it!

PMSF can only be dissolved in 100% EtOH.

3. Pre - lysis buffer

Buffer A plus 4 mg/ml lysozyme.

4. Storage buffer

50 mM Tris - HCl (pH 7.9)	150 ml of 1 M Tris- HCl (pH 7.9)
50 mM KCl	150 ml of 1 M KCl
0.1 mM EDTA (pH 8.0)	0.6 ml of 0.5 M EDTA (pH 8.0)
1 mM DTT	3 ml of 1 M DTT
0.5 mM PMSF*	30 ml of 0.5 mM PMSF
50% glycerol	1.5 L of glycerol

q.s. to 3 L w/ D.D. H_2O, autoclave, store at RT.

5. TAQ buffer

500 mM KCl	5 ml of 1 M KCl
100 mM Tris	1 ml of of 1 M Tris (pH 9.0)
1% Triton	100 µl of Triton-X 100

Bring to a final volume of 10 ml. Autoclave and aliquot into 1.5 ml tubes. Store at -20°C.

6. $MgCl_2$ solution

Add 0.0238 g of $MgCl_2$ to 10 ml of ddH2O. Autoclave and divide into 1.5 ml tubes, store at -20 °C.

References

1. Pluthero, F. G. (1993). Rapid purification of high-activity *Taq* DNA polymerase. *Nucleic Acids Res* 21(20): 4850-4851.