Preparation of Taq DNA Polymerase
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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. (NH₄)₂SO₄
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. MgCl₂ solution (see Recipes)

Equipment

1. 2 L flask
2. Centrifuges
3. Oakridge tubes

**Procedure**

Day 1

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 OD600 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, centrifuge at 15,000 rpm for 10 min at 4 °C.
6. Transfer supernate to clean flask, add 15 g of (NH₄)₂SO₄, 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**
2. Lysis buffer

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount/Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mM Tris - HCl (pH 7.9)</td>
<td>1 ml of 1 M Tris-HCl (pH 7.9)</td>
</tr>
<tr>
<td>50 mM KCl</td>
<td>5 ml of 1 M KCl</td>
</tr>
<tr>
<td>1 mM EDTA (pH 8.0)</td>
<td>0.2 ml of 0.5 M EDTA (pH 8.0)</td>
</tr>
<tr>
<td>1 mM PMSF*</td>
<td>2 ml of 50 mM PMSF</td>
</tr>
<tr>
<td>0.5% Tween 20</td>
<td>0.5 ml Tween 20</td>
</tr>
<tr>
<td>0.5% NP40</td>
<td>0.5 ml NP40</td>
</tr>
</tbody>
</table>

q.s. to 100 ml with ddH2O, 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

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount/Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mM Tris - HCl (pH 7.9)</td>
<td>150 ml of 1 M Tris-HCl (pH 7.9)</td>
</tr>
<tr>
<td>50 mM KCl</td>
<td>150 ml of 1 M KCl</td>
</tr>
<tr>
<td>0.1 mM EDTA (pH 8.0)</td>
<td>0.6 ml of 0.5 M EDTA (pH 8.0)</td>
</tr>
<tr>
<td>1 mM DTT</td>
<td>3 ml of 1 M DTT</td>
</tr>
<tr>
<td>0.5 mM PMSF*</td>
<td>30 ml of 0.5 mM PMSF</td>
</tr>
<tr>
<td>50% glycerol</td>
<td>1.5 L of glycerol</td>
</tr>
</tbody>
</table>

q.s. to 3 L w/ D.D. H2O, autoclave, store at RT.

5. Taq buffer

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount/Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 mM KCl</td>
<td>5 ml of 1 M KCl</td>
</tr>
<tr>
<td>100 mM Tris</td>
<td>1 ml of 1 M Tris (pH 9.0)</td>
</tr>
<tr>
<td>1% Triton</td>
<td>100 µl of Triton-X 100</td>
</tr>
</tbody>
</table>

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

6. MgCl2 solution

Add 0.0238 g of MgCl2 to 10 ml of ddH2O. Autoclave and divide into 1.5 ml tubes, store at -20 °C.

References