**Taq polymerase purification
Reference** [**1**](http://genetics.med.harvard.edu/~cepko/protocol/mike/T2.html)**,** [**2**](http://stress.tamu.edu/Protocols/Taq%20ref2.html)
Reagents

T-Broth

|  |  |
| --- | --- |
| Tryptone | 12 g/l |
| Yeast extract | 24 g/l |
| separately autoclave |
| Glycerol | 4 ml |
| Phosphate solution(KH2PO4 2.31 g, K2HPO4 12.54 g) | 100 ml |

Buffer A (100 ml)

|  |  |
| --- | --- |
| 1 M Tris-HCl pH 7.9  | 5 ml |
| 0.5 M EDTA | 0.2 ml |
| Glucose | 0.9 g |

Lysozyme  0.17g for 500 ml culture

Other Buffers

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | B (100 ml) | Cload (200ml) | Celu (100 ml) | Dialysis (1 L) | Dilution (50 ml) |
| 1 M HEPES-KOH pH 7.9 | 2 ml | 4 ml | 2 ml | 20 ml | 1 ml |
| 0.5 M EDTA | 0.2 ml | 0.4ml | 0.2 ml | 2 ml | 0.01ml |
| 10% Tween 20 | 5 ml | 10 ml | 5 ml | - | - |
| 10% NP-40 | 5 ml | 10 ml | 5 ml | - | - |
| KCl | 0.372 g | 0.372 g | 1.491 g | 7.45 g | 0.745 g |
| 0.1 M PMSF | 0.5 ml | - | - | - | - |
| Glycerol | - | - | - | 500 ml | 25 ml |
| 1 M DTT (fresh/-80°C) | - | - | - | 1 ml | - |

Day 1   Streak Taq cells on LB + Tic plate
Day 2   Inoculate single colonies to 4x 2.5 ml LB + Tic media

Day 3

1. Inoculate 5 ml overnight cultures to each 500 ml TB + Tic, let it grow on 37 °C shaker till OD600=0.6
2. Add IPTG to final 0.5 mM, keep 10-16 hours (for example, 8 hours) a 37°C shaker

Day 4

1. Harvest cells by centrifugation at 4°C (JA-10, 7000 rpm, 10 min)
2. Resuspend cells with 10 ml/500 ml culture Buffer A, and centrifuge again
3. Discard supernatant, estimate cell volume (for example, 15 ml) and resuspend cells with 40 ml/500 ml culture Buffer A
4. Add 0.17 g lysozyme to suspension, keep at RT for 15 min
5. Add equal volume (for example, 95 ml) of Buffer B, mix well
	* If stopping, freeze in liquid N2, and keep at -80°C

Day 5

1. Treat extract at 75°C for 1 hour, occational mixing
2. Centrifuge, at 4°C (JA-10, 10k rpm, 20 min)
3. Transfer sup to a clean beaker
4. Slowly add 0.164 g/ml Ammonium Sulfate powder, stir for 30 min
5. Centrifuge at at 4°C (JA-17 13K rpm, 30 min)
6. Transfer sup to a clean beaker
7. Slowly add 0.181 g/ml Ammonium Sulfate powder, stir for 30 min
8. Centrifuge at at 4°C (JA-17 13K rpm, 30 min)
9. Discard sup, centrifuge again 1min to remove all the solution
10. Resuspend in 10 ml Buffer Cload
11. Check conductivity
12. Load to DEAE sepharose (10 ml bed volume) equilibrated with Cload
13. Wash the column with 30-50 ml Cload
14. Elute with 50 ml Celu.  Discard the first 5 ml, and collect 5 ml fractions
15. Check protein concentration by Bradford.  Combine protein containing fractions
16. Dialyze in 500 ml dialysis buffer x2 in cold room.  (once for overnight and once for 2-3 hours)

Day 6

1. Recover protein in 15 ml Falcon tube.  Quantify protein by Bradford.
2. Add 4mg/ml gelain (predissolved in dialysis buffer -DTT). Prepare 1 ml aliquat, and keep in -80°C.
	* Make 0.05 ml tube for activity assay
3. Prepare 1/3, 1/10, 1/30, 1/100 dilution, run standard PCR reaction (genome and plasmid) to determine titer.
4. Use the 2nd largest dilution for working dilution.  (If you can see amplification as low as 1/30, use 1/10).
	* Generally, working dilution is ca. 1 mg/ml.
5. **Protocol T.2**
6. Taq Polymerase Purification
7. This is a protocol from Engelke et. al. (1990) which was modified by Dr. Baron. The enzyme has been used for RT-PCR, genotyping and cloning.
8.
9. **Solutions**
10. 1000X IPTG
11. 0.5M IPTG 1.19g IPTG
12. up to 10 ml wih sterile Q
13. filter and store at -20°C
14. Buffer A
15. 50 mM Tris 7.9 25 ml 1M Tris pH 8.5\*
16. 50 mM dextrose 4.5 g dextrose
17. 1 mM EDTA 1 ml 0.5M EDTA
18. up to 500 ml with Q
19. \*Check pH and bring to 7.9 as the dextrose will reduce the pH.
20. Store at room temperature. For some steps add Lysozyme to a
21. final concentration of 4 mg/ml.
22. Buffer B
23. 10 mM Tris 7.9 5 ml Tris pH 7.9
24. 50 mM KCl 1.86 g KCl
25. 1 mM EDTA 1 ml 0.5M EDTA
26. 0.5% Tween 20 5 ml 50% Tween 20
27. 0.5% NP-40 5 ml 50% NP-40
28. up to 500 ml with Q
29. Store at room temperature and add PMSF to a final concentration of 1 mM just prior to use.
30.
31.
32.
33. Buffer C
34. 50 mM Tris 8.0 50 ml 1M Tris pH 8.0
35. 50 mM KCl 3.72 g KCl
36. 1 mM EDTA 2 ml 500 mM EDTA pH 8.0
37. 50% glycerol 500 ml glycerol
38. 0.5% Tween 20 5 ml 50% Tween 20
39. 0.5% NP-40 5 ml 50% NP-40
40. 1 mM DTT 2 ml 0.5M DTT
41. 1 mM PMSF 10 ml 100 mM PMSF
42. up to 1 liter with Q
43.
44. Buffer D
45. 50 mM HEPES 7.9 50 ml 1M HEPES pH 7.9
46. 50 mM KCl 3.72 g KCl
47. 5% glycerol 100 ml 50% glycerol
48. 1 mM EDTA 2 ml 0.5M EDTA 8.0
49. 0.5% Tween 20 10 ml 50% Tween 20
50. 0.5% NP-40 10 ml 50% NP-40
51. 1 mM DTT 2 ml 0.5M DTT
52. 1 mM PMSF 10 ml 100 mM PMSF
53. up to 1 liter with Q
54.
55.
56. **Procedure**
57. • Streak out the *E. coli* strain carrying the Taq polymerase gene on an LB amp plate and innoculate a 5 ml overnight culture with a single colony.
58. • Innoculate a 1 liter culture of LB-amp with the 5 ml of overnight culture and grow to an A600 of 0.2. Add IPTG to a final concentration of 5 mM and culture overnight.
59. • Spin down the culture and resuspend the pellet in 200 ml of Buffer A (ice cold).
60. • Spin down the *E. coli* as before and resuspend in 50 ml Buffer A containing 4 mg/ml lysozyme. Incubate at room temperature for 15 minutes.
61. • Add 50 ml Buffer B and incubate at 75°C for 1 hour.
62. • Chill on ice and spin in the GSA rotor at 8,000 rpm for 15 minutes.
63. • Transfer the supernatant to a beaker on ice. Measure the volume and add pulverized ammonium sulfate slowly to a final concentration of of 0.164 g/ml (30% saturation). Continue to stir for 30 minutes.
64. • Spin down the precipitate by spinning in the SA-600 rotor at 13,000 rpm for 30 minutes at 4°C.
65. • Transfer the supernatant to a clean beaker on ice, measure the volume and add ammonium sulfate to a final concentration of 0.181 g/ml (60% saturation). Stir as before for 30 minutes on ice.
66. • Spin down the precipitate in the SA-600 rotor at 13,000 rpm for 30 minutes at 4°C and resuspend in 10 ml Buffer D.
67. • Equilibrate a DEAE-sephacel column (1.5 cm diameter, 5 diameter height, 9 ml bed volume) in Buffer D.
68. • Load protein and wash with 3-5 column volumes of buffer D.
69. • Elute in 20 ml buffer D containing 0.5M KCl and collect the first six 3 ml fractions.
70. • Dialyze fractions 2,3,4 against 1 liter of Buffer C containing 50% glycerol for 4-8 hours and repeat. This should be done on ice in the cold room.
71. • Freeze in small aliquotes and store at -80°C.
72. •
73. **Home-made Taq Polymerase Purification**
74. I also have the bacteria containing the clone. It appears to produce lots of Taq and is quite stable.
The proceedure takes 4 days start to (15 000 units of Taq) finish.
The Taq also appears very stable and reliable. I made 15 mls a year ago and it still works fine.
75. Reference: Engelke, D. R. et al. Anal. Biochem. 191:396-400 (1990).
Pluthero, F. G. et al. NAR. 21:4850-4851 (1993).
76. **Day 1**
77. 1) Inoculate two 2L flasks of TB/amp (500ml) with 15ml of an overnight of Taq bugs. These volumes may be scaled down.
78. 2) Grow to an OD600 of 0.6 (approx mid log)
79. 3) Add IPTG to 0.5mM (0.119gm/litre), grow o/n but not for more than 16hrs.
80. **Day 2**
81. **N.B:** All the following proceedures should be carried out on ice or at least 4°C.
82. 4) Collect cells by centrifugation (3.5K / 15 mins / 4°C) and resuspend in 40ml buffer A.
83. 5) Add an equal volume of buffer B (45-50ml) and incubate at 75°C for 1hr, with periodic mixing.
84. 6) Centrifuge ( 8K / 15mins / 4°C).
85. 7) Add 1.86mg of KCl / ml of supernatant.
86. 8) Aliquot equal volumes of supernatant into each of 2 x 250ml centrifuge tubes (preferably conical) containing 75ml of washed Sigma DP-1 cation exchange resin (packed volume). This should be washed 2 x with sterile water and 4 x with ice cold Buffer B.
87. 9) Vortex tubes well and incubate on a shaking platform (30mins / 4°C).
88. 10) Centrifuge (approx 3K / 2min / 4°C) to pellet resin and discard supernatant.
89. 11) Wash resin 4 x with 100-200ml of ice cold buffer B, remove supernatant by aspiration.
90. 12) Elute 3 x with one packed bed volume of ice cold buffer C.
91. 13) Add 30gm (NH4)2SO4 / 100ml of eluate while stirring rapidly.
92. **N.B:** At this point it is of great advantage if you use conical or round bottomed tubes. i.e. 50ml tubes for the 8x50 rotor. Prior to this the sample may be handled in 250ml centrifuge bottles for ease of use.
93. 14) Centrifuge at 12-15Krpm for 10mins at 4°C.
94. 15) Resuspend pellet (weakly translucent) in 25-35ml of buffer C.
95. 16) Dialise 2 x against 2L of dialysis buffer (6-18hrs / 4°C).
96. **Day 3**
97. 17) Titre by assaying serial dilutions cf comercial Taq.
98. 18) Aliquot concentrated Taq polymerase and store at -20°C.
99. **Reagents**
100. Terrific broth (TB); per litre
12gm tryptone
24gm yeast extract
4ml glycerol (autoclaved).
100ml 0.17M KH2PO4(2.31gm/100ml) / 0.72M K2HPO4 (12.54gm/100ml); Autoclaved separately.
101. Buffer A (Require 100ml)
50 mM Tris (pH 7.9)
1mM EDTA
50mM Dextrose
102. Buffer B / 100ml (Require 1000ml)
20mM Hepes (pH7.9) 2ml (1M)
1mM EDTA 1ml (0.1M)
0.5% Tween-20 0.5ml
0.5%NP-40 0.5ml
0.5mM PMSF
50mM KCl
103. Buffer C / 500ml (Require 500ml)
20mM Hepes (pH 7.9) 10ml (1M)
1mM EDTA 5ml (0.1M)
0.5% Tween-20 2.5ml
0.5%NP-40 2.5ml
0.5mM PMSF
200mM KCl
104. Dialysis Buffer / 2L (Require 2000ml)
20mM Hepes (pH 7.9) 40ml (1M)
1mM EDTA 4ml (0.5M)
0.5mM PMSF
100mM KCl
50% glycerol 1L
1mM DTT
105. Dilution Buffer Required to dilute Taq
20mM HEPES (pH 7.9)
0.1mM EDTA
100mM KCl
50% glycerol
106. Keywords: Taq, PCR, polymerase
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