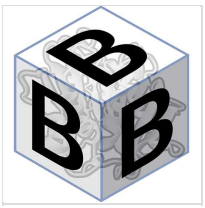

Oct 13, 2021

Recombinant protein expression and purification of Taq DNA polymerase

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This is a slightly modified and simplified version of a protocol by Thomas G.W. Graham et al, which is available at https://gitlab.com/tjian-darzacq-lab/bearmix and has been described in depth in the article 10.1371/journal.pone.0246647, for the recombinant expression of a E602D mutant of Taq DNA polymerase in pET-28a that is available in Addgene (Addgene plasmid # 166944 ; http://n2t.net/addgene:166944 ; RRID:Addgene_166944).

The main goal of this protocol is to eliminate the use of large volumes for dialysis and potential issues with the protein crashing out of the solution due to the use of concentrators for buffer exchange of this enzyme into storage conditions.

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MATERIALS

PMSF **Sigma Aldrich Catalog #P7626**

Imidazole **Sigma Catalog #I5513**

NaCl **Sigma Aldrich Catalog #53014**

HisTrap FF Crude Column **Ge Healthcare Catalog #17528601**

Lysozyme **Thermo Fisher Scientific Catalog #89833**

Glycerol **Merck Millipore Catalog #104092**

DTT **Millipore Sigma Catalog #DTT-RO**

Triton X-100 **Sigma Aldrich Catalog #X100-100ML**

Trizma® base **Merck Millipore Sigma Catalog #93362**

EDTA **Sigma Aldrich Catalog #ED2SS**

Nonidet P40 Substitute **Sigma Aldrich Catalog # 74385**

2-mercaptoethanol **Sigma Aldrich Catalog #M6250**

Buffer A, pH 8.0

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[M]**50 Milimolar (mM)** Tris-HCl pH 8.0

[M]**500 Milimolar (mM)** NaCl

[M]**0.1 % volume** Nonidet P-40

[M]**0.1 % volume** Triton X-100

[M]**10 Milimolar (mM)** Imidazole, pH 8.0

[M]**5 Milimolar (mM)** 2-mercaptoethanol (BME)

[M]**5 % volume** Glycerol

Buffer B, pH 8.0

[M]**50 Milimolar (mM)** Tris-HCl pH 8.0

[M]**500 Milimolar (mM)** NaCl

[M]**0.05 % volume** Nonidet P-40

[M]**10 Milimolar (mM)** Imidazole, pH 8.0

[M]**5 Milimolar (mM)** BME

[M]**5 % volume** Glycerol

Buffer C, pH 8.0

[M]**50 Milimolar (mM)** Tris-HCl pH 8.0

[M]**100 Milimolar (mM)** NaCl

[M]**0.05 % volume** Nonidet P-40

[M]**10 Milimolar (mM)** Imidazole, pH 8.0

[M]**5 Milimolar (mM)** BME

[M]**5 % volume** Glycerol

Buffer D, pH 8.0

[M]**50 Milimolar (mM)** Tris-HCl pH 8.0

[M]**100 Milimolar (mM)** NaCl

[M]**0.05 % volume** Nonidet P-40

[M]**300 Milimolar (mM)** Imidazole, pH 8.0

[M]**5 Milimolar (mM)** BME

[M]**5 % volume** Glycerol

Buffer HA, pH 8.0

[M]**50 Milimolar (mM)** Tris-HCl pH 8.0

[M]**100 Milimolar (mM)** NaCl

[M]**0.05 % volume** Nonidet P-40

[M]**5 Milimolar (mM)** BME

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[M]**5 % volume** Glycerol

Buffer HB, pH 8.0

[M]**50 Milimolar (mM)** Tris-HCl pH 8.0

[M]**1000 Milimolar (mM)** NaCl

[M]**0.05 % volume** Nonidet P-40

[M]**5 Milimolar (mM)** BME

[M]**5 % volume** Glycerol

Storage conditions

[M]**50 Milimolar (mM)** Tris-HCl, pH 8.0

[M]**100 Milimolar (mM)** NaCl

[M]**0.1 Milimolar (mM)** EDTA

[M]**50 % volume** Glycerol

[M]**3 Milimolar (mM)** DTT

DAY 1 – Plasmid transformation 1d

1 Transform **100 ng** of plasmid containing Taq DNA polymerase into *E. coli* C41 competent cells using either heat shock or electroporation. 2h

2 Spread transformed cells in LB Agar plates supplemented with [M]**0.05 mg/mL** Kan. Grow plate overnight at **37 °C** . 12h

DAY 2 – Preinoculum 1d

3 Select a single colony from the LB agar plate to prepare a preinoculum in **10 mL** LB media supplemented with [M]**0.05 mg/mL** Kan. Grow overnight at **200 rpm, 37°C** . 1d

DAY 3 – Protein Overexpression 1d

4 Use the full volume of the preinoculum to inoculate **1 L** of LB (or TB) media supplemented with [M]**0.05 mg/mL** Kan (1% inoculation). Grow at **160 rpm, 37°C** until reaching an optical density at 600 nm (OD_{600}) = 0.8. 4h

5 Upon reaching OD_{600} = 0.8, add IPTG to a final concentration of [M]**0.5 Milimolar (mM)** and incubate **160 rpm, 37°C, 02:00:00** . 16h

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DAY 4A – Protein Purification by IMAC 4h

6 Centrifuge the cell culture **4000 x g, 4°C, 00:20:00** .Then, resuspend the cell pellet in **50 mL** of **Buffer A** freshly supplemented with **1.0 Milimolar (mM)** PMSF and **0.2 mg/mL** lysozyme. 30m

7 Incubate the resuspended cells at **80 rpm, Room temperature , 00:20:00** . 30m

8 Sonicate on ice for **00:08:00** using cycles of **00:00:01** ON and **00:00:06** OFF at 40% amplitude (Qsonica Q125, 125W). 10m

9 On an ultracentrifugation tube, incubate the unclarified lysate at **75 °C** for **00:30:00** to precipitate most of *E. coli* proteins, and then place on ice for **00:05:00** . Centrifuge **20000 x g, 4°C, 00:20:00** and collect the supernatant. You might want to collect a small sample for SDS-PAGE afterwards. 1h

10 On a **1 mL HisTrap column (GE Healthcare)** pre-equilibrated with 10 column volumes (c.v.) (here, 10 mL) of **Buffer A**, load the supernant. Wash with 10-20 c.v. of **Buffer B**. Repeat the wash step with **Buffer C**. Then, elute with 5 c.v. of **Buffer D**, collecting the eluted fractions every **0.5 mL** in 1.5 ml tubes. 1h

11 To quickly pool the fractions containing the protein of interest, prepare a 96-well plate or 1.5 mL tubes with **40 µL** of 5X Bradford reagent and **150 µL** of distilled water. Then, add **10 µL** of each protein fraction and compare against a blank reference sample corresponding to **10 µL** of **Buffer C**. You can determine your protein-containing fractions either by absorbance at 595 nm on a plate reader or visually by comparing the blue coloration of each fraction against the blank reference. Pool your fractions and collect a **10 µL** sample for SDS-PAGE. 5m

DAY 4B – Second purification and buffer exchange by Heparin 2h

12 This method was preferred over protein dyalisis or Amicon protein concentration to avoid using large buffer volumes and proteins crashing out of the solution.

Dilute the pooled fractions 6X in buffer containing 50 mM Tris-HCl pH 8.0 and 100 mM NaCl. 5m

13 Next, load the diluted sample onto a **1 ml HiTrap Heparin column** previously equilibrated with 10 c.v. (here, 10 mL) **Buffer HA**. Then, elute the protein using a 10 c.v. linear gradient against **Buffer HB**, collecting the eluted fractions every **0.5 mL** in 1.5 ml tubes. 30m

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This linear gradient can be achieved by connecting two containers, one with 5 c.v. Buffer HA and the other with 5 c.v. buffer HB, with a syphon or a tube, and withdrawing solution from the Buffer HA container to the column using a cheap peristaltic pump or by gravity.

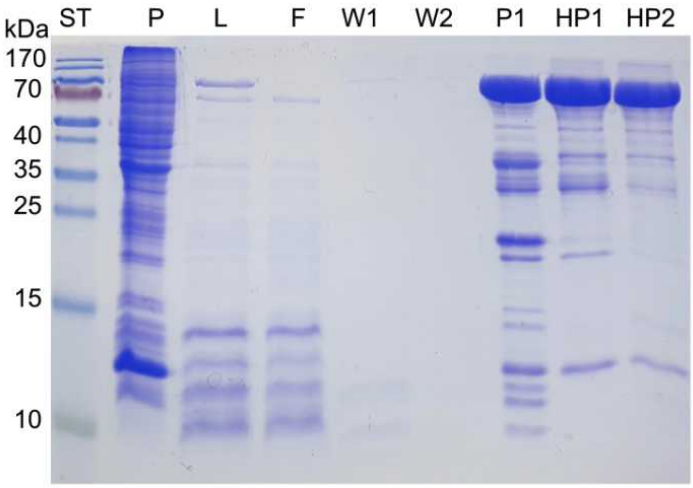
14 Again, determine your protein-containing fractions using the Bradford assay. Pool your fractions and determine its protein concentration using the same method and collect a **10 µL** sample for SDS-PAGE. 5m

15 For storage, supplement your pooled fractions with **100 Milimolar (mM)** Tris-HCl pH 8.0, **0.2 Milimolar (mM)** EDTA and **6 Milimolar (mM)** DTT. Then, dilute the sample by adding glycerol up to **50 % volume** to reach final storage conditions: **50 mM Tris-HCl pH 8.0, ~100 mM NaCl, 0.1 mM EDTA, 3 mM DTT.** 5m

16 Generate **200 µL** aliquots of the enzyme and store it at **-20 °C** until required. 30m

IMAC SDS-PAGE Result

17



SDS-PAGE
12% Acrylamide (29:1 Acryl:BisAcryl)
P: Pellet
L: Clarified Lysate
F: Column flowthrough
W1: Wash 1 with 500 mM NaCl
W2: Wash 2 with 100 mM NaCl
P1: His-trap purified protein Pool
HP1: Heparin purified protein fraction 1
HP2: : Heparin purified protein fraction 2

SDS-PAGE of all purification steps of Taq DNA polymerase. Pooled eluted fractions from IMAC and Heparin correspond to P1, HP1 and HP2.

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