Sep 05, 2020

Recombinant protein expression and purification of codon-optimized Bst-LF polymerase

DOI
https://dx.doi.org/10.17504/protocols.io.bksrkwd6

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Protocol Citation: Maira Rivera, Severine Cazaux, Ariel Cerda, Anibal Arce Medina, Isaac Núñez, Tamara Matute, Alex Brown, Javier Gasulla, Fernan Federici, Cesar A Ramirez-Sarmiento 2020. Recombinant protein expression and purification of codon-optimized Bst-LF polymerase. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.bksrkwd6**

Protocol status: Working
We use this protocol in our workspace and it is working. Typical protein yield is about 20 mg per liter of cell culture. Functionally tested in a LAMP assay using both commercial and in-house buffers equivalent to Bst2.0 from New England Biolabs and an initial protein concentration of 0.25 ug/ml (8-fold dilution from the initial 2 mg/ml stock). LAMP and RT-LAMP Assay protocols soon to be published.

Created: September 04, 2020

Last Modified: September 05, 2020

Protocol Integer ID: 41521

Keywords: RT-LAMP, isothermal amplification, COVID-19, SARS-CoV-2, If polymerase this protocol, If polymerase, purification of codon, If enzyme, recombinant protein expression, recombinant expression, purification, optimized bst, plasmid, codon, dyalisis through the use,

Abstract

This protocol has been optimized for the recombinant expression of a codon-optimized Bst-LF polymerase.

The goal of this protocol was to eliminate the use of large volumes for dyalisis through the use of concentrators for buffer exchange before storage conditions.

The plasmid encoding the codon-optimized Bst-LF enzyme used here can be found on reclone.org

Materials

MATERIALS

Amicon Ultra-15 Centrifugal Filter Unit **Merck Millipore (EMD Millipore) Catalog #**UFC910024

Sodium phosphate monobasic monohydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S9638

PMSF **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P7626

Sodium phosphate dibasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**7558-79-4

Imidazole **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**I5513

NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**53014

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

Lysozyme **Thermo Fisher Scientific Catalog #**89833

Glycerol **Merck Millipore (EMD Millipore) Catalog #**104092

DTT **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**DTT-RO

Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**X100-100ML

Trizma® base **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**93362

EDTA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**ED2SS

KCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P9541

Buffer A, pH 8.0

[M] 50 millimolar (mM) NaPO4, pH 8.0

[M] 300 millimolar (mM) NaCl

[M] 30 millimolar (mM) Imidazole, pH 8.0

Buffer B, pH 8.0

[M] 25 millimolar (mM) Tris-HCl, pH 8.0

[M] 200 millimolar (mM) KCl

[M] 30 millimolar (mM) Imidazole, pH 8.0

Buffer C, pH 8.0

[M] 25 millimolar (mM) Tris-HCl, pH 8.0

[M] 100 millimolar (mM) KCl

[M] 150 millimolar (mM) Imidazole, pH 8.0

Buffer D, pH 8.0

[M] 25 millimolar (mM) Tris-HCl, pH 8.0

[M] 100 millimolar (mM) KCl

Storage conditions

[M] 12.5 millimolar (mM) Tris-HCl, pH 8.0

[M] 50 millimolar (mM) KCl

[M] 0.1 millimolar (mM) EDTA

[M] 0.1 % volume Triton X-100

[M] 50 % volume Glycerol

[M] 1 millimolar (mM) DTT

DAY 1 – Plasmid transformation

1d

1 Transform 100 ng of pET15b plasmid containing codon-optimized Bst-LF 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] 0.1 mg/mL Amp. 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] 0.1 mg/mL Amp. Grow overnight at 250 rpm, 37°C. 1d

DAY 3 – Protein Overexpression

1d

4 Use the full volume of the preinoculum to inoculate 1 L of LB media supplemented with [M] 0.1 mg/mL Amp (1% inoculation). Grow at 200 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 millimolar (mM) and incubate overnight at 200 rpm, 18°C. 16h

DAY 4 – Protein Purification and Storage

6h

6 Centrifuge the cell culture 4000 x g, 4°C, 00:30:00 .Then, resuspend the cell pellet in 40 mL of **Buffer A** freshly supplemented with [M] 1.0 millimolar (mM) PMSF and [M] 0.2 mg/mL lysozyme. 30m

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

8 Sonicate on ice for 00:08:00 using cycles of 00:00:01 ON and 00:00:04 OFF at 40% amplitude (Qsonica Q125, 125W). 30m

9 On an ultracentrifugation tube, incubate the unclarified lysate at 65 °C for 00:25:00 to precipitate most of *E. coli* proteins, and then place on ice for 1h

00:05:00 . Centrifuge 20000 x g, 4°C, 00:30:00 and collect the supernatant. You might want to collect a small sample for SDS-PAGE afterwards.

10 On a **5 mL HisTrap column (GE Healthcare)** pre-equilibrated with 10 column volumes (c.v.) (here, 50 mL) of **Buffer A**, load the supernant. Wash with 10-20 c.v. of **Buffer B**. Then, elute with 5 c.v. of **Buffer C**, collecting the eluted fractions every 1 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 160 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 B**. 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

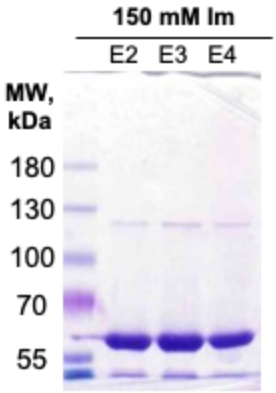
12 To decrease the imidazole concentration, perform a buffer exchange step with an Amicon Ultra-15 concentrator (Merck Millipore). Centrifuge 3000 x g, 10°C, 00:10:00 , discard the flowthrough, add **Buffer D** to decrease the imidazole concentration and repeat this step, until the imidazole concentration reaches < 30 mM. 1h

13 Recover the concentrated protein and determine its concentration using the Bradford assay. Then, supplement with [M] 0.2 millimolar (mM) EDTA, [M] 0.2 % volume Triton X-100, [M] 2 millimolar (mM) DTT and add glycerol up to [M] 50 % volume to reach **Storage Conditions**. Do consider that a final protein concentration ≤ [M] 2 mg/mL is appropriate for subsequent experiments. 5m

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

IMAC SDS-PAGE Result

15



SDS-PAGE at 10% PA of eluted fractions from IMAC purification of Bst-LF. The high MW contaminant is due to disulfide bond formation and eliminated upon addition of DTT (data not shown).