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

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1 Works for me dx.doi.org/10.17504/protocols.io.bksrkwd6

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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

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PROTOCOL CITATION

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KEYWORDS

RT-LAMP, isothermal amplification, COVID-19, SARS-CoV-2

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MATERIALS

NAME	CATALOG #	VENDOR
Amicon Ultra-15 Centrifugal Filter Unit	UFC910024	Emd Millipore
Sodium phosphate monobasic monohydrate	S9638	Sigma Aldrich
PMSF	P7626	Sigma Aldrich
Sodium phosphate dibasic	7558-79-4	Sigma Aldrich
Imidazole	I5513	Sigma
NaCl	53014	Sigma Aldrich
HisTrap FF Crude Column	17528601	Ge Healthcare
Lysozyme	89833	Thermo Fisher Scientific
Glycerol	104092	Merck Millipore
DTT	DTT-RO	Millipore Sigma
Triton X-100	X100-100ML	Sigma Aldrich
Trizma® base	93362	Merck Millipore Sigma
EDTA	ED2SS	Sigma Aldrich
KCl	P9541	Sigma

MATERIALS TEXT

Buffer A, pH 8.0

[M] **50 Milimolar (mM)** NaPO4, pH 8.0

[M] **300 Milimolar (mM)** NaCl

[M] **30 Milimolar (mM)** Imidazole, pH 8.0

Buffer B, pH 8.0

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

[M] **200 Milimolar (mM)** KCl

[M] **30 Milimolar (mM)** Imidazole, pH 8.0

Buffer C, pH 8.0

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

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

[M] **150 Milimolar (mM)** Imidazole, pH 8.0

Buffer D, pH 8.0

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

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

Storage conditions

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

[M] **50 Milimolar (mM)** KCl

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

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

[M] **50 % volume** Glycerol

[M] **1 Milimolar (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 2h

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cells using either heat shock or electroporation.

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 Milimolar (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 Milimolar (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 **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. 1h

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 1h

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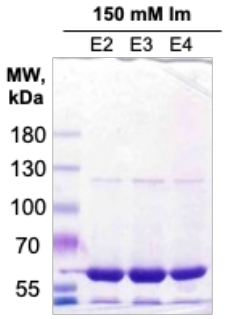
Millipore). Centrifuge **3000 x g, 10°C, 00:10:00** , discard the flowthrough, add **Buffer D** to decrease the imidazole concentration and repeat this step, until the imidazole concentration reaches < 30 mM.

13 Recover the concentrated protein and determine its concentration using the Bradford assay. Then, supplement with 5m **[M]0.2 Milimolar (mM)** EDTA, **[M]0.2 % volume** Triton X-100, **[M]2 Milimolar (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.

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).

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