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Recombinant protein expression and purification of fuGFP

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol has been optimized for the recombinant expression of fuGFP encoded in an open pTi vector. The plasmid encoding fuGFP used here can be found on reclone.org. The purified protein can be used for teaching about the properties of fluorescent proteins.

Materials

MATERIALS

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

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) NaCl

[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) NaCl

[M] 300 millimolar (mM) Imidazole, pH 8.0

Troubleshooting

DAY 1 – Plasmid transformation

1d

1 Transform 100 ng of the open pTi plasmid containing fuGFP into *E. coli* BL21 (DE3) 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 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] 0.05 mg/mL Kan (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 180 rpm, 18°C 16h

DAY 4 – Protein Purification by IMAC

6h

6 Centrifuge the cell culture 4000 x g, 4°C, 00:20:00 .Then, resuspend the cell pellet in 40 mL of **Buffer A** freshly supplemented with [M] 0.5 millimolar (mM) PMSF and [M] 0.2 mg/mL lysozyme. 20m

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 00:00:01 OFF at 40% amplitude (Qsonica Q125, 125W). 8m 2s

9 Centrifuge the unclarified lysate 20000 x g, 4°C, 00:20:00 and collect the supernatant. You might want to collect a small sample for SDS-PAGE afterwards. 20m

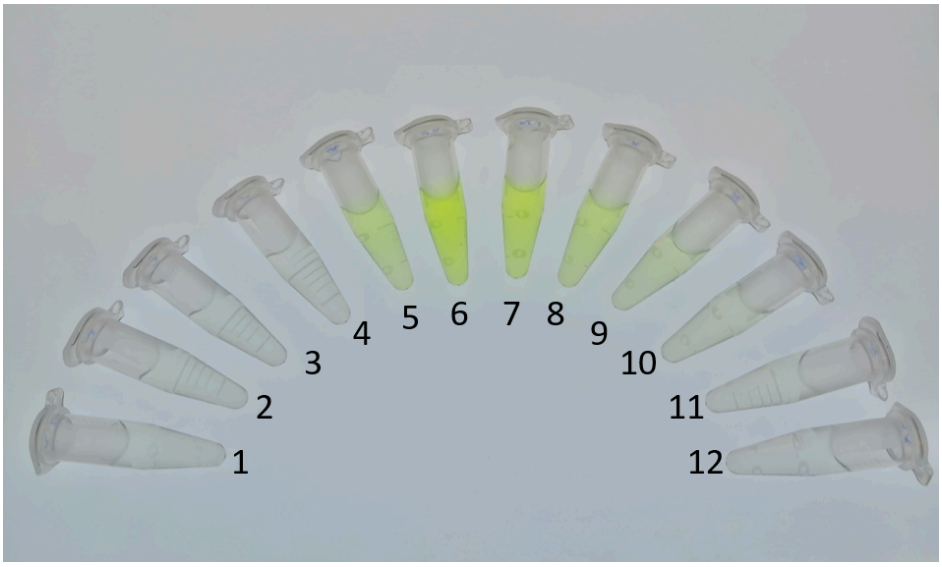
10 On a **1 mL HisTrap column (GE Healthcare)** pre-equilibrated with 10 column volumes (c.v.) (here, 10 mL) of **Buffer A**, load the supernant. Wash with 20 c.v. of **Buffer B**. Then, elute with 5 c.v. of **Buffer C**, collecting the eluted fractions every 1 mL in 1.5 ml tubes. 1h

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 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

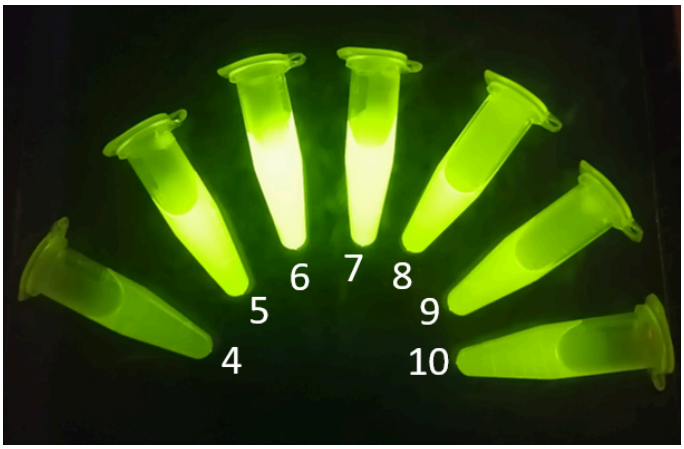
12 For storage, we suggest to do a dialysis against **Buffer A**, and store at 4º C.

IMAC SDS-PAGE Result

13



Eluted fractions from immobilized metal affinity chromatography (IMAC) after recombinant protein purification of fuGFP using open pTi vector.



Pooled fractions from immobilized metal affinity chromatography (IMAC) after recombinant protein purification of fuGFP using open pTi vector under blue light.