Quanzi Li, 02/2012

Expression and purification of GST-fusion proteins

Supplies &Reagent:

PBST buffer: 1XPBS, pH7.4, 5mM EDTA, 1% Triton X-100, 0.1% β-ME.
Glutathione-S-agarose beads: Sigma (G4510-10ml)
Cleavage buffer: 50mM Tris, pH8.0, 150mM NaCl, 2.5mM CaCl2, 0.1% β-ME
Cleavage buffer w/glycrrol: 50mM Tris, pH8.0, 150mM NaCl, 2.5mM CaCl2, 0.1% β-ME,20% glycerol
Thrombin: (Sigma,T6634-250UN) dissolve in 3ml cleavage buffer (contain 20% glycerol), aliquot in 20 tubes, and store at -20.
Storage buffer: 50mM Tris 8.0 (or 7.0, 7.5, which is close to the optimal pH of your protein, if it is an enzyme), 20% glycerol

Protocols

1. Vector construction: Design primers to amplify the coding region, cloned into pGEXKG1 vector. The target gene will be inserted at the MCS, which is behind the *GST* (see attached map).
   Note: The TOP10 cell of pGEXKG1 is stored in the **BOX1** of Quanzi Li's Glycerol Strain Stock (**#17**). Add one or two bases between the restriction site and ATG for the correct reading frame.
2. Transform the construct into *E.coli* BL21/DE3 (Invitrogen). The strain can be stored at -80. (Mix 800μl culture with 400μl of 50% glycerol, freeze in liquid nitrogen, and store at -80).
3. Culture the cell in 5ml LB, containing 100mg/ml Ampicillin, at 37°C overnight.
4. Inoculate 1ml culture of cells into 100ml LB (Amp 50).Culture at 37°C for about 3-4 h, until OD600 reach 0.5-0.8.
   Notes: The culture volume varies from 50ml to 1L, dependending on the gene expression level. Normally 50-100ml is enough.
5. Transfer culture to 28°C, add IPTG to a final concentration of 0.5mM, grow for 8 h.

Notes: Take 2ml out in a eppendorf tube, spin down for 1min, as the control. The concentration of IPGT is from 0.02 to 1mM, and the temperature is from 15 to 37°C. The lower temperature and lower concentration of IPTG, the higher degree of protein solubility. Incubate for ~3 h for 37°C, 8h for 28°C, 12h for 25°C. Normally 25°C and 28°C give good expression.

6. Spin cell down (4°C, 6000Xg, 5m). Resuspend the cells in 20 ml PBST buffer, add PMSF to 1mM.
   Notes: before spin, take 2 ml out for protein expression checking.
7. Sonication in a 50ml Centrifuge tube XXX
8. Centrifuge at 10000g, 4°C, 10min to 1h.
9. Preparation of glutachione-S-agarose beads: The Glutathione-S-agarose beads should be prepared earlier.
   A: Incubate powder agarose beads overnight in Millipore water in a flask. Wash swollen beads twice with Millipore water and equilibrate/wash one with pBST buffer. The beads can be stored at 4°C.
   B: During cell disruption, start to transfer the beads into a column, let beads set down, and reach to 1ml.
10. Use the cell lysate to suspend the bead, transfer to a 50ml tube, incubate ~45min, at 4°C, with gently shaking.
11. Transfer to the column, discard eluent. Note: let the beads set down a bit, then remove the bottom cap.
12. Add PBST buffer (without PMSF), suspend the bead, incubate for 3-5min with gently shaking. Discard eluent.
    Notes: Beads can be washed without suspension.
13. Repeat step 12 at least four times.
14. Incubate (wash) beads once with 15ml thrombin cleavage buffer. Discard eluent.
15. Incubate beads in 3ml thrombin cleavage buffer with a tube of thrombin. Shake gently at room temperature for ~40min.
16. Keep eluent containing the thrombin cleaved GST-fusion protein.
17. Protein Concentration and desalting: use Centrifugal filters (30k), change to storage buffer. 3 times.

    Protein can be stored at -80.

Note: Regeneration of glutathione-S-agarose beads: Beads can be reused many times. Strip Beads four times with 0.5% SDS/1XPBS (pH7.4). Incubate for 10min time with gentle shaking.

Acknowledge:

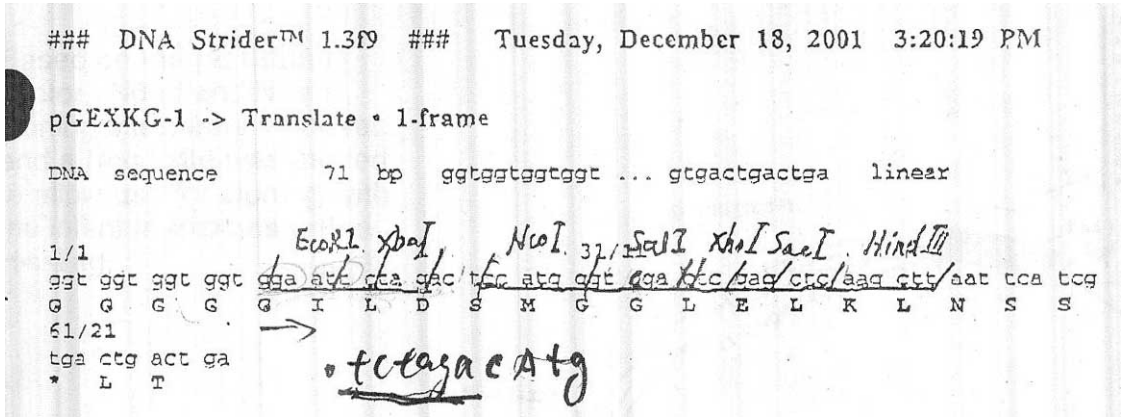
1. The protocol was provided by Dr. Haiqing Yi (Duke University, NC).
2. pGEXKG1 was provided by Dr. Changjun Zhu (The Burnham Institute,CA).

Attached: **To elute the GST-fusion protein:**

-Incubate(wash) beads, once with 15ml of 50mM Tris-pH 7.5, 0.1% β-ME for about 3-5 min, discard eluent

- Incubate beads in 4ml of 50mM Tris pH 7.5, 0.1% β-ME containing 10mM reduced glutathione. Share gently at room temperature (~25°C) for approximately 30 mins. (For higher yields repeat this step twice).

Attached: pGEXKG1 map



DNA Strider™ 1.3f9 ### Tuesday, December 18, 2001 3:20:19 PM

pGEXKG-1 -> Translate • 1-frame

DNA sequence 71 bp ggtggtggtggt ... gtgactgactga linear

EcoRI XbaI, NcoI SalI XhoI SacI HindIII

1/1
ggt ggt ggt ggt gga att cca gac tcc atg ggt cga ctc gag ctc gaa ctt aat tca tcg
G G G G G I L D S M G G L E L K L N S S
61/21
tga ctg act ga
* L T

tctagaCATg