

Expression of selenomethionyl-protein

1. Transform *E. coli* B834(DE3) with your plasmid.
2. Use a single colony from a Luria Bertani (LB) medium agar plate containing 100 $\mu\text{g}\cdot\text{ml}^{-1}$ ampicillin to inoculate a 2ml liquid culture of the same medium.
3. Use 1ml of the over-night culture to inoculate 500ml Erlenmeyer flasks containing 100ml of a **minimal medium** (see below).
4. Grow culture (37°C, ~200RPM) to $\text{OD}_{600}\approx 0.6$, whereas the logarithmic growth curve saturates due to the termination of the methionine in the medium. Note that the minimal medium already contains methionine, which should be exactly sufficient for growth termination at this point, but maybe a further adjustment will be needed.
5. Add selenomethionine (100 $\text{mg}\cdot\text{liter}^{-1}$) 2 min later. ($\text{C}_5\text{H}_{11}\text{NO}_2\text{Se}$; [MP Biomedicals](#))
6. Add IPTG.
7. Incubate cells under your appropriate inducing conditions.

Minimal medium is composed of the following filter-sterilized components: ($\text{g}\cdot\text{liter}^{-1}$, except as noted): alanine, 0.5; arginine, 0.4; aspartic acid, 0.4; asparagine, 0.4; cysteine, 0.05; glutamine, 0.4; glutamic acid, 0.65; glycine, 0.55; histidine, 0.1; isoleucine, 0.23; leucine, 0.23; lysine, 0.42; methionine (8.0 $\text{mg}\cdot\text{liter}^{-1}$); phenylalanine, 0.13; proline, 0.1; serine, 2.1; threonine, 0.23; tryptophan, 0.05; tyrosine, 0.17; valine, 0.23; adenine, 0.5; guanosine, 0.65; thymine, 0.2; uracil, 0.5; cytosine, 0.2; Na_2HPO_4 , 7.0; KH_2PO_4 , 3.0; NH_4Cl , 1; NaCl , 1.0; NaOH , 0.5; D-glucose, 4.0; $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ (7.4 $\text{mg}\cdot\text{liter}^{-1}$); $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ (0.07 $\text{mg}\cdot\text{liter}^{-1}$); $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ (1.1 $\text{mg}\cdot\text{liter}^{-1}$) and ampicillin (100 $\text{mg}\cdot\text{liter}^{-1}$).

References

1. O'Gara M, Adams GM, Gong W, Kobayashi R, Blumenthal RM, Cheng X (1997). Expression, purification, mass spectrometry, crystallization and multi-wavelength anomalous diffraction of selenomethionyl PvuII DNA methyltransferase (cytosine-N4-specific). *Eur J Biochem* 247:1009–1018.
2. Qoronfleh MW, Ho TF, Brake PG, Banks TM, Pulvino TA, Wahl RC, Eshraghi J, Chowdhury SK, Ciccarelli RB, Jones BN (1995). Production of selenomethionine-labeled recombinant human neutrophil collagenase in *Escherichia coli*. *J Biot-echnol* 39:119–128.