Expression of Selenomethionine-labeled Proteins in *E. coli*

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Protocol is a linear combination of the following sources:
Radhakrishan Lab minimal media culture techniques
VanDuyne G. D. et. Al. (1993) *J. Mol. Biol.* Vol. 229 pp. 105-124)
UCLA DOE Lab Protocol (https://www.doe-mbi.ucla.edu/local/protocols/test)

Reagent List:
The following will be required (indicated quantities correspond to 1 L of culture)

anhydrous Na_2HPO_4 (6.8 g)
anhydrous KH_2PO_4 (3 g)
NaCl (0.5 g)
55.509 M UV/UF H_2O (1000 ml) – Do not use anhydrous!
100X Vitamin solution (10 ml)
1 M $CaCl_2$ (100 μl)
2 M $MgCl_2$ (500 μl)
glucose (3 g)
ammonium sulfate (2 g)
1000X Antibiotic stock(s) (1 ml per antibiotic)
lysine (100 mg)
phenylalanine (100 mg)
threonine (100 mg)
isoleucine (50 mg)
leucine (50 mg)
valine (50 mg)
seleno-L-methionine (Sigma-Aldrich, CAS: 3211-76-5) (100 mg)
LB plate (1)
Minimal Media plate (1)
Enthusiasm for cell culture (not quantifiable)

Part 1: Brief Conceptual Overview of Method

-The goal is to produce selenomethionine-labeled protein WITHOUT the use of a methionine auxotroph organism. For example, to use BL21 DE3 cells to produce labeled protein. This is achieved by growing cells in minimal media, and then immediately before induction suppressing amino acid biosynthetic pathways via the addition of selected amino acids. Selenomethionine is also added at this point. Thus, instead of metabolizing glucose and ammonium sulfate in the media in order to build methionine 'from scratch', the bacteria will preferentially import seleno-methionine that was added to the media, and use this to make protein. In order to conserve reagents, and also allow for normal growth of the cells, amino acid biosynthetic pathways are only suppressed after the cells have grown to an OD of 0.6, rather than being suppressed from the beginning of the culture process.

Safety: Selenomethione is toxic

Part 2: Reagent Recipes:

Media:

- *10X M9 solution (1 liter)*
  - To 68 g anhydrous Na_2HPO_4, 30 g anhydrous KH_2PO_4 and 5 g NaCl, add 950 ml UV/UF water. Adjust volume to 1000 ml. Store solution at room temperature.
- *Minimal media (1 liter)*
  - *Solution A*
    - To 100 ml of 10x M9 solution add 850 ml UV/UF water. Autoclave.
    - *Solution B* **Protect from light by covering solution with aluminum foil!**
      - 10 ml 100X Vitamin solution (stored at -20 °C)
      - 100 μl 1 M $CaCl_2$
      - 500 μl 2 M $MgCl_2$
      - 3 g glucose
      - 2 g ammonium sulfate
      - Add 1ml of the appropriate 1000x antibiotic stock solution, bring volume to 50 ml and filter through a 0.22 μm filter. Use sterile technique.
  - Mix 19 parts of Solution A with 1 part of Solution B after Solution A has cooled to room temperature. Use sterile technique

Part 3: Expression Protocol:

1. Transform BL21 DE3 with desired plasmid, plate on LB + appropriate antibiotic(s)
2. Streak minimal media plate with a single colony derived from above LB plate. Allow growth for 24-36 hours (until good-sized, discrete colonies are seen)
3. At ~ 11 pm, inoculate a 50 ml minimal media starter culture with a healthy colony from the above minimal media plate
4. Allow for growth overnight ~10 hours until OD of 50 ml culture is 0.6
5. Transfer entire 50 ml culture to remaining 950 ml of minimal media
6. Allow growth until 1L culture reaches OD of 0.6 (The doubling time in minimal media is roughly 1 hr)

7. Once OD reaches 0.6, add the following compounds as solids to the media. (Specifically, put solid reagents in a sterile 50 ml falcon tube, and gently dissolve the solids with 25 ml of culture using an autopipette-driven 25 ml serological pipette. This will take multiple iterations (3-5) of 25 ml resuspensions. Try not to add many large chunks of solid to the media.)
    a. Solid Mixture (values given for 1L culture)
        i. 100 mg lysine
        ii. 100 mg phenylalanine
        iii. 100 mg threonine
        iv. 50 mg isoleucine
        v. 50 mg leucine
        vi. 50 mg valine
        vii. 100 mg seleno-L-methionine (Sigma-Aldrich, CAS: 3211-76-5)
8. Once these compounds have been added, shake for an additional 15 minutes at 37 C.
9. Transfer culture to optimal expression temperature and allow thermal equilibration for 15 minutes.
10. Take 'Before-Induction' sample for gel.
11. Induce culture with 1 mM IPTG.
12. Allow expression to proceed for 18-20 hrs at 20 C. Adjust this accordingly for various expression temperatures / protocols.
13. Take 'After-Induction' sample for gel and harvest cells

Alternative Culture Technique: 'Hot Start Method'
-Rather than streaking a minimal media plate, and using a minimal media-adapted colony to start the culture, one can use cells from a small liquid culture in LB:

1. 1. Transform BL21 DE3 with desired plasmid, plate on LB + appropriate antibiotic(s)
2. Inoculate 10 ml of LB media with appropriate antibiotic(s)
3. Allow growth for ~4-5 hours
4. Centrifuge 10 min, 6000 rpm
5. Decant supernatant, resuspend cells in 50 ml minimal medial to an OD of 0.1. Grow 50 ml minimal media culture overnight (8 hrs)
6. Once OD is ~0.6, transfer to large culture and proceed as indicated in main protocol.

A note on purification:
-Selenomethionine is more readily oxidized than is methionine. Include reducing agent during purification

Finally, verify selenomethionine incorporation (and assess selenomethionine oxidation state via mass spectrometry)