

**Differential Expression Proteomic Analysis between Selenomethionyl and *wt E. coli***

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## **Introduction and Background**

Proteins carry out a myriad of cellular functions that make life possible, and the key to how these molecular workers function lies in structure. Although there are many tools and methods used to determine structure, multi-wavelength anomalous diffraction has proven to be especially useful since lighter atoms can be used for phasing. In this technique, uncommon or unnatural amino acids are biosynthetically incorporated into the protein in place of their natural counterparts which contain sulfur. Selenomethionine (SeMet) and telluromethionine (TeMet) are the most frequently used amino acids in this technique. Although this method works well once the unnatural amino acids are incorporated, the process of incorporation possesses its own challenges. While growing bacterial cultures in SeMet and TeMet containing media, growth slows dramatically, suggesting a toxic effect introduced by the unnatural amino acids.

The field of proteomics offers a unique way to examine the inhibitory effects of artificial amino acids on growing bacterial cultures. Proteomic methods usually involve the separation, isolation and mass spectral analysis of proteins of an organism cultured under two or more different sets of conditions. Once the proteins are separated, they are assessed for differential expression (concentration). Those differentially expressed are then isolated, digested and subjected to mass spectral analysis to obtain protein identity. Protein identity can then be used to propose molecular level mechanisms as to the cause of the toxicity. This information could then be used to propose either altered culturing conditions (media component alterations) or metabolic engineering to reduce toxicity in future applications.

## **Objective**

This project aims to 1) separate (resolve), 2) isolate, 3) analyze, and 4) identify the primary differentially expressed proteins generated in the following two cultures: Selenomethionyl DL41 (DE3) pCock *E. Coli* and L-Methionyl DL41 (DE3) pCock *E. Coli*.

## **Methodology**

Two cultures will be grown; one where the sole source of authentic methionine is Selenomethionine, the other containing methionine (control). Proteins will be extracted and separated by two-dimensional gel electrophoresis (2-DE), differentially expressed proteins will be identified, excised from the gel, digested with trypsin (enzyme) to break the proteins into peptides, subjected to mass spectral analysis and identified by peptide mass fingerprinting.

## **Research Plan**

Two 1 L cultures of chemically defined media will be prepared, identical in every aspect with the exception of methionine (both devoid of methionine but containing all of the other 19 amino acids, salts and a carbohydrate source for energy). The DL41 *E. Coli* culture is a methionine auxotroph, meaning that it must acquire methionine from the culture environment. *E. Coli* cultures will be grown separately in the presence of 20 µg/mL SeMet or L-Met. Once the cultures have reached the appropriate stage of growth (late log phase), the cells in each culture will be spun down in a centrifuge and lysed (extraction of proteins occurs simultaneously with selection of a commercial lysis buffer, otherwise, the cell pellet will be subjected to sonication in 20 mL of a buffered sonication buffer). Protein concentrations will be determined by the Bradford Dye Binding Assay

(Sigma) by comparison with a standard curve constructed with Bovine Serum Albumin (BSA) as a standard protein. Obtained protein solutions will be mixed with the Bradford reagent, subjected to visible spectroscopy (595 nm) and the protein concentration will be determined with the equation of the line (linear least squares fit) generated with the standard BSA solutions. Identical amounts of each protein set will then be used to rehydrate IPG strips containing a linear pH gradient and subjected to isoelectric focusing. During isoelectric focusing, the proteins will migrate along the strip until they reach their isoelectric point (the pH where the protein is neutral based on its amino acid composition). Isoelectric focusing is the first dimension of separation in 2-DE. Then, the strips with the focused proteins will be applied to an SDS-polyacrylamide gel (in house prepared by a standard procedure) where they will be separated in the second dimension by molecular weight under standard electrophoresis conditions. Once the electrophoresis migration is complete, the gels will be stained with a commercial dye, destained and then subjected to visual and digital analysis for differentially expressed proteins using RedFin Software.

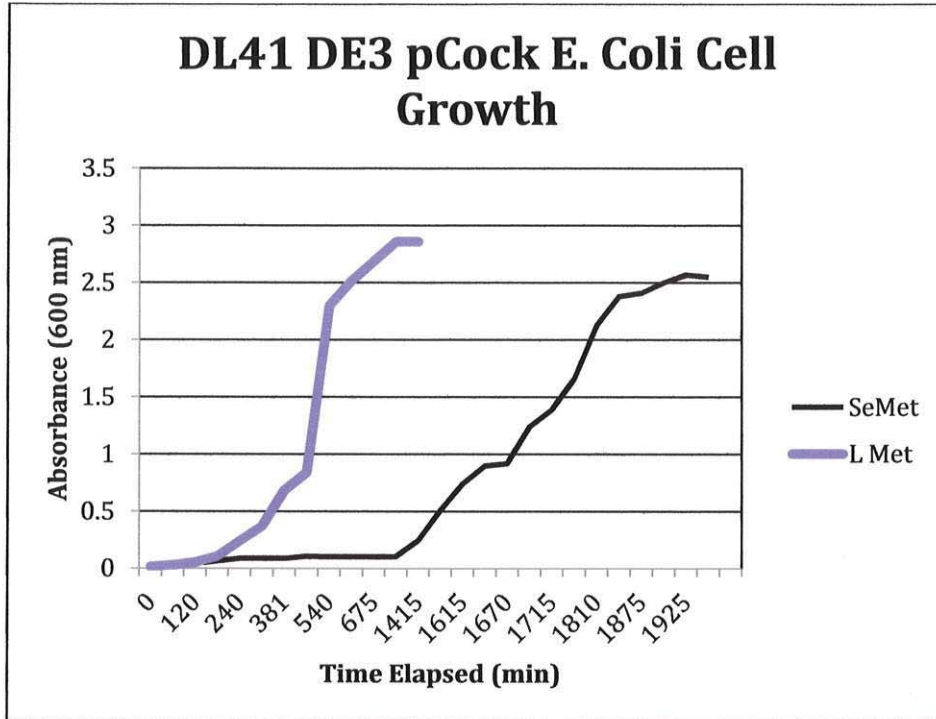
Proteins will be compared based on the size and intensity of the spot on the gel. If a gel spot is smaller on one gel compared to the other, the average cell in that culture was producing less of that particular protein than a cell in the comparison culture. Once the gel is scanned with a digital scanner, image analysis software (RedFin) will be used to distinguish the differential expression, also providing the quantitative difference. Once the differentially expressed proteins have been selected, peptide mass fingerprinting will be used to determine the proteins' identity. Briefly, the gels spots will be excised and subjected to in-gel tryptic digestion in an extraction solvent (this hydrolyzes the proteins

into smaller peptide fragments by breaking the bonds on the carboxy side of Arg and Lys residues). The peptide fragments from each protein will then be analyzed for mass using nano-ESI/qTOF mass spectrometry with the assistance of experienced lab personnel. Once determined, the peptide masses will be uploaded into Protein Global Surveyor for comparison to hypothetically cleaved *E. coli* proteins *in silico* for protein identification.

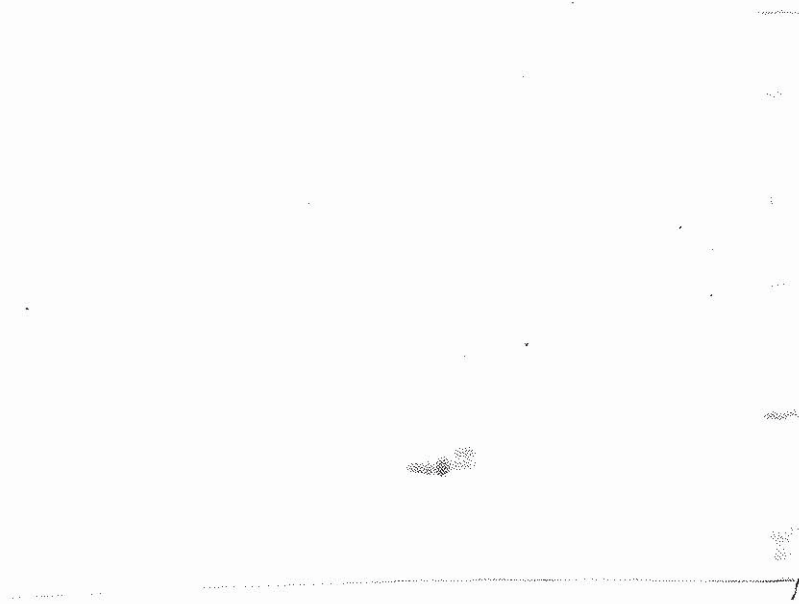
### **Previous Research**

Previous research has been performed to test the reproducibility of the 2-DE process with proteins obtained using two different cell lysis conditions. Cultures of SeMet and L-Met *E. Coli* have been grown. (Figure 1) Two samples from the SeMet culture were prepared in an identical fashion and run simultaneously under the same conditions. This was performed twice, once by using a lysis buffer to lyse the cells and separate the proteins from the other cell components (Figure 2), and once with sonication and precipitation (Figure 3). Although other groups performed the lysis buffer technique with success (Cash et al., 2009), the lysis buffer attempt yielded poor results with little to no protein showing up on the final gel. The precipitation process provided better results, but although that method is more predictable, it is time-consuming and more research will be carried out to determine which method best conserves proteins while providing sufficient resolution on the gel.

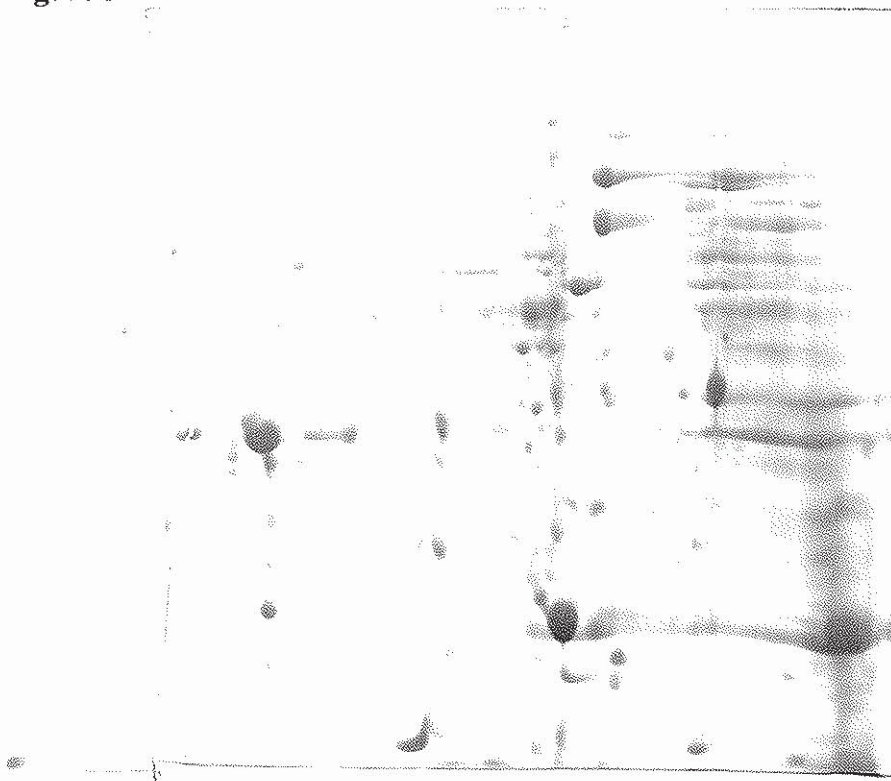
Figure 1



**Figure 2**



**Figure 3**



## References

Cash, Philip, and Evelyn Argo. "Analysis of Bacterial Proteins by 2DE." Trans. *Array Methods in Molecular Biology, Two-Dimensional Electrophoresis Protocols*. Humana Press, 2009. 131-144. Print.



## Student Research Grant Budget Form

**Student name:** ...Savannah J. Taylor.....

**Proposal title:** .Differential Expression Proteomic Analysis between Selenomethionyl and *wt E. coli*

**Amount requested:** .....\$1137.14.....

**Itemize as specific as possible below:**

IPG strips pH 4-7 , 17 cm	\$98.00
Carrier ampholyte (pH 3-10)	\$61.69
DTT (Dithiothitol)	\$82.14
Selenomethionine	\$210.00
Promega* Trypsin Gold, Mass Spectrometry Grade	\$106.09
ZipTips/C18 Media (mass spect analysis)	\$226.17
IPG Box kit	\$72.00
Acrylamide: Bis-Acrylamide 37.5:1 (40% Solution)	\$278.05
<b>Total:</b>	<b>\$1137.14</b>