

Quantification of Minicells

Executive Summary:

Quantification of the minicells is essential to verifying the yield from formation and purification of minicells. We have looked at multiple methods including: standard plate count, spectrophotometer analysis, and flow cytometry. In determining the best method for quantification of minicells, we considered cost, difficulty, and accuracy.

Standard plate count would normally involve manually counting the number of minicells in a certain amount of dilute solution, but due to the team's proficiency with computers, the program ImageJ will be used to count the minicells present on images of examined cultures. This would avoid the cumbersome process of counting them by hand. Flow cytometry is not as cost effective as spectrophotometric analysis and the materials necessary are not as accessible.

Spectrophotometric analysis provides many benefits, with simplicity and cost effectiveness. This requires using a spectrophotometer to determine the absorbance of light in the medium. We will then use this data and mathematical relationships to determine the total number of cells, both minicells and full *E. coli*. Since we will know the quality of purification, we can determine an accurate quantification of the minicells. From our research, we have found that this method is most commonly used in quantifying minicells.

We have decided to use both spectrophotometric analysis and standard plate count with the ImageJ as our preferred methods. We can then compare our results to ensure accuracy in the quantification of the minicells.

Materials:

- Hemocytometer
- BX51 Olympus Microscope
- Scanning Electron microscope (SEM) or TEM
- Matlab
- Spectrophotometer
- cuvettes (non-acetone soluble)
- Light Microscope
- Kimwipes
- gloves
- p20 and tips
- p200 and tips
- p1000 and tips
- 2 waste beakers
- TE buffer
- Vortex
- *E. coli* Minicell culture

Introduction-Approach:

Confirmation of Purity

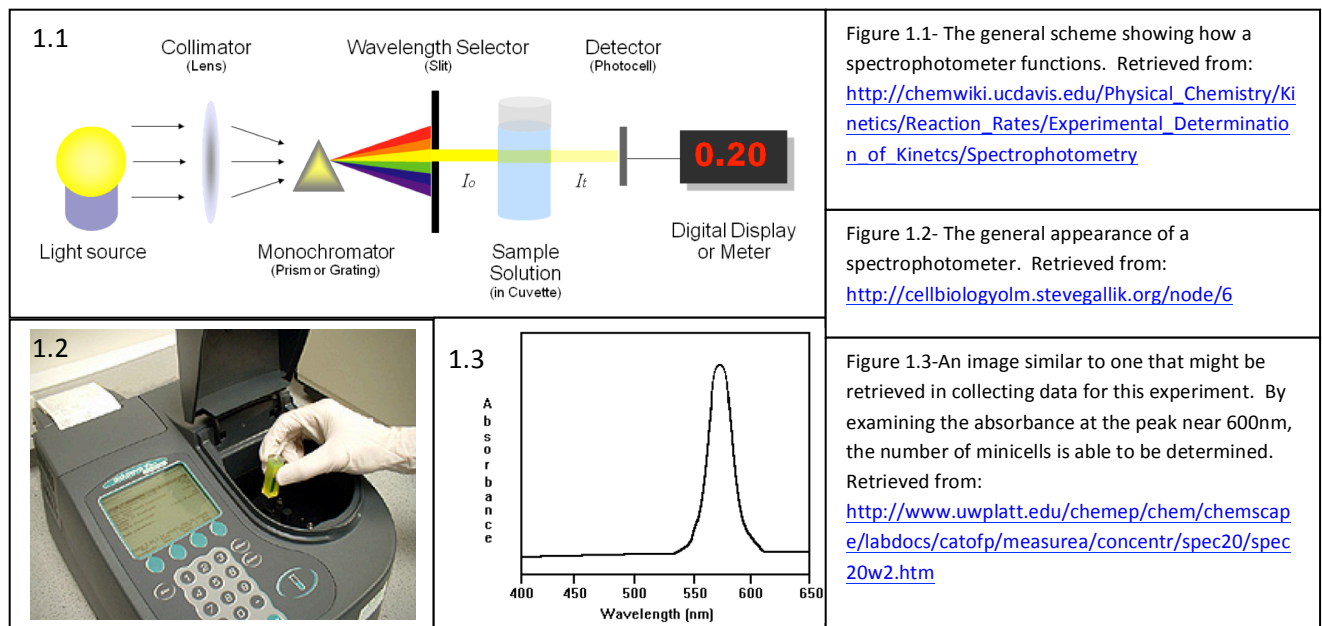
After the purification of the minicells, there will be a preliminary examination of the cell cultures to ensure ample purification has been achieved. This will be done using a high magnification light microscope. The entire minicell cell culture will be placed on said high magnification stereo microscope and examined. The relative number of parent cells present will be determined and the level of purification determined using the ratio of minicells present to parent cells present. If the sample is determined to be adequately pure, then the remainder of the cell culture will proceed to the subsequent phases of analysis.

Combinatorial Approach

The sample culture will then have the number of cells in it determined by one of two methods (protocol for which are discussed in the papers attached to this document). These two methods are spectrophotometry and standard cell counting using an imaging video microscope. The preferred method of the two is the spectrophotometry method, but due to its indirect measurement of the minicells present in the cell cultures, the cell counting method will need to be used initially in order to confirm the viability of the spectrophotometric method.

Spectrophotometry

The elementary principle behind spectrophotometry (1) is that when light of a range of wavelengths is projected onto a sample of material, a certain amount is absorbed or is otherwise reflected. The amount of light that is absorbed at the different wavelengths in the range are then quantitatively recorded. In a series of papers that can be found at the end of this document, there has been an established relationship between the number of minicells present in the culture and absorbance of light at 600nm of the cell culture. By examining the value of the absorbance (probably a peak value) at 600nm an approximation of how many minicells are present in the culture can be made. This relationship can and will be reconfirmed through the cross referencing spectrophotometric data with cell counting data. Data collected by this technique would require a somewhat pure sample as the parent cells exhibit different light absorbance.



normal cells. The minicells created by our IPTG induced FtsZ complex are achromosomal and hence will not be stained and be able to be differentiated from the parent cells.

Confirming protein expression levels

HA tagging for the win. Obviously the answer to our problems. Refer to other document for this information.

Establishing a Baseline

By using the combinatorial approach that has been discussed in previous sections, there will be a baseline established by using a counting method to know how many cells are present and then by using the spectrophotometer on the same sample so as to determine the absorbance of light at 600nm of that known number of minicells. This will be done for multiple minicell samples to establish a mathematical model that relates the number of minicells to the absorbance. The relationship is likely linear, but any higher order behavior will be accounted for by the mathematical model. This mathematical model will then be compared to those found in published minicells papers.

Further Imaging

Seeing the actual shape and form of our minicells will require proper treatment and examination using something like SEM microscopy. Alternatively, TEM use is also another avenue for imaging individual minicells that are produced. Since both of these require significant training/supervision, they will only be used in the latest stages of experimentation. This also means that very few of the researchers will actually need to operate these more expensive instruments.

Reference Articles

- (1)-Spectrophotometer Protocol
- (2)-Counting Bacteria Basics
- (3)-Cell Counting
- (4)-OlympusBX51_Manuel

Analysis:

In order to accurately quantify the minicells we will need to determine the optical density at 600 nm (A_{600}). We will then employ the following equation: # of minicells/ml = $OD_{600} \cdot 5.0 \cdot 10^{10}$ [3].

References

1. Reynolds, J. & Farinha, M. (2005). Counting Bacteria Basics. Richland College.
2. University of Wisconsin-Stout (2013). Spectrophotometer-protocol. Retrieved from <http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CDsQFjAA&url=http%3A%2F%2Fwww.uwstout.edu%2Fchemistry%2Fupload%2FSpectrophotometer>

-Protocol.doc&ei=J9qoUaL7Otah4AO_v4GABQ&usg=AFQjCNFIK9MQk6dSpD4Z2lDWiu-6XIs3hg&bvm=bv.47244034,d.dmg

3. Giacalone, M. J., Gentile, A. M., Lovitt, B. T., Xu, T., Surber, M. W., & Sabbadini, R. A. (2006). The use of bacterial minicells to transfer plasmid DNA to eukaryotic cells. *Cellular microbiology*, 8(10), 1624-1633.