Bi1X, Spring 2011
Assignment 7: Gene Expression

Daniel Jones

May 18, 2011

1 Things to turn in:

1. Start with the HG104::NoFluo strain (Pad 2). Choose one snapshot of your choice, print out
   (a) the original phase image with scale bar
   (b) the raw phase mask obtained by binary thresholding (before applying area selection criteria)
   (c) a histogram of area
   (d) a filtered phase mask after applying area selection criteria
   (e) an overlay of the original phase image with your final, filtered phase mask
   (f) your own Matlab code.

   Be sure to include a caption, a title and labeling the axes when applicable. What is the mean fluorescence per pixel (in units of fluorescent counts per pixel) for this strain? Remember for this strain, the autofluo is zero.

2. For each of the other 5 strains, choose one snapshot of your choice, print out
   (a) the original phase image with scale bar
   (b) the raw phase mask obtained by binary thresholding (before applying area selection criteria)
   (c) a histogram of area
   (d) a filtered phase mask after applying area selection criteria
   (e) an overlay of the original phase image with your final, filtered phase mask.
   (f) your own Matlab code

Be sure to include a caption, a title and labeling the axes when applicable. Report the mean fluorescence per pixel (in units of fluorescent counts per pixel) for each of these 5 strains in a table format. Remember for these strains, the autofluo is the mean fluorescence per pixel you got from the previous problem.

3. For each of the four experimental strains, compute the repression as defined by

$$\begin{aligned}\text{Repression} &= \frac{\text{<Gene expression in the absence of repressor>}}{\text{<Gene expression in the presence of repressor>}}. \\ &= \frac{\text{Mean YFP Fluorescence in the absence of repressor}}{\text{Mean YFP Fluorescence in the presence of repressor}}\end{aligned}$$

Remeber the strain with no LacI repressor is the HG105 strain. Report the values you obtain in data table format. Show your calculation step for at least one strain.

4. Now that you have computed the repression for each strain, use the expression discussed in class to compute the repressor-DNA binding energy $\Delta\epsilon_{rd}$ **in units of kT** for each strain:

$$\text{Repression} = 1 + \frac{R}{N_{NS}} e^{-\Delta\epsilon_{rd}/kT}. \tag{1}$$

N_{NS}, the number of possible nonspecific binding binding sites for LacI, is well approximated by the length of the *E. coli* genome, 4.6×10^6 base pairs. R, the number of LacI molecules in the cell, is about 10 for the strains we're using. Be sure to report your answer in data table format and show your calculation step for at least one strain.

5. What is absolute value of the difference in binding energy (in units of kT) between your **most** repressed and **least** repressed strain? Call this difference ΔE.

6. 1 calorie is defined as the amount of heat energy required to raise the temperature of 1 ml of water by 1 degree Celsius. How many milliliters of water could ΔE raise by 1 degree Celsius (i.e., what is ΔE in units of calories)? Be sure to show your work.

7. Consider your **least** repressed strain. Holding its binding energy constant, how many repressors would have to be present in the cell to acheive the same repression as your **most** repressed strain? What can you conclude about the effect of changes in LacI-DNA binding energy?