## *ImageJ*

Revised on September 23, 2011

1. Go to http://rsb.info.nih.gov/ij/.
2. Click “Applets/WebStart”.
3. Click “Run ImageJ using Java WebStart”.

### *Determination of Recombinant Protein Purity*

1. Select *Process>Subtract Background>Light Background>OK*. Try rolling ball radius of 50.
   **Note:** This might remove some of specific signal with the background from your image.
2. Use the rectangular selection tool to outline the first lane.
   **Note:** This should be the left most lane if the lanes are vertical or the top lane if the lanes are horizontal. Note that lanes are assumed to be vertical unless the width of the initial selection is at least twice its height.
3. Select *Analyze>Gels>Select First Lane* (or press "1").
4. Move the rectangular selection right to the next lane (or down if the lanes are horizontal) and select *Analyze>Gels>Select Next Lane* (or press "2").
5. Repeat the previous step for each remaining lane.
6. Select *Analyze>Gels>Plot Lanes* (or press "3") to generate the lane profile plots.
7. Use the straight line selection tool to draw base lines and/or drop lines so that each peak of interest defines a closed area.
   **Note:** To get to all the lanes, it may be necessary to scroll the image vertically using the "Hand" tool. (Hold down the space bar to temporarily switch to this tool).
8. For each peak, measure the size by clicking inside with the wand tool. If necessary, scroll the image vertically by holding down the space bar and dragging.
9. Select *Analyze>Gels>Label Peaks* to label each measured peak with its size as a percent of the total size of the measured peaks.

***Quantifying Western blots***

1. Open an existing file by choosing *File>Open*.
2. Select *Image>Type* and click on 8-bit to convert the image to grayscale.
3. Select *Analyze>Set Measurements*, and click the boxes for *Area*, *Mean Gray Value*, and *Integrated Density*.
4. Select *Analyze>Set Scale*, and enter “pixels” in the box net to *Unit of length*.
5. Select Edit>Invert to invert the colors on the image. Now the dark bands are light, and the light areas are dark.
6. Choose the freehand selection tool from the tool palette.
7. Draw a line around the boundary of your first band.
8. Hit the “m” key to take a measurement of the enclose area that you selected. The results window should pop up.

   **Note:** The *Integrated Density* (*IntDen*) column is simply the *Area* and *Mean Gray Value* (*Mean*) columns multiplied together.
9. Use the freehand selection tool to select the next band, and press “m” to take the measurement. Repeat this for each of your band.