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Western blot quantification imagej

Western blot results example. Western blot band quantification imagej. Western blotting results. Western blot quantification using imagej. Western blot size. Imagej western blot quantification protocol. How to quantify western blot image lab.

Save and adjust the gel image to be vertically oriented using the box tool to select entire lanes. Use Analyze->Gels->Select First Lane or CTRL-1 to select a lane, then slide the box to the next lane using side arrow keys. Plot the lane by selecting Analyze->Gels->Plot Lanes or pressing CTRL-3. Draw a baseline using the line tool to remove background noise. Connect peaks to the baseline if necessary and isolate individual peaks above the baseline. Calculate the areas of selected peaks in numerical order and transfer the results to an Excel file for analysis. Using Gel Analysis with ImageJ: A Step-by-Step Guide The method outlined here utilizes the Gel Analysis technique described in the ImageJ documentation. This approach is effective and can produce comparable results to other methods. The tutorial was created using ImageJ 1.42q on a Windows 7 64-bit installation. ### Steps for Analysis 1. Open the image file using File>Open. 2. Ensure the image is in gray-scale mode by going to Image>Type>8-bit. 3. Select the Rectangular Selections tool from the toolbar and draw a rectangle around the first lane, aiming for tall and narrow shapes to accurately capture individual lanes. ### Lane Alignment 4. Press the 1 (Command + 1 on Mac) key or go to Analyze>Gels>Select First Lane to set the rectangle in place. 5. Click and hold in the middle of the rectangle and drag it over to the next lane, centering it left-to-right. 6. Repeat steps 4-5 for each subsequent lane. ### Finalizing the Analysis 7. After setting the rectangle in place on the last lane by pressing 2, proceed to step 8. 8. Press 3 (Command + 3 on Mac) or go to Analyze>Gels>Plot Lanes to generate a profile plot of each lane. When analyzing gels or western blots using tools like ImageJ, it's essential to account for background noise that can cause peaks to appear above the baseline. To accurately measure peak size, you may need to close off the peak by drawing a straight line across its base. This requires some subjective judgment to determine where the peak ends and the background noise begins. Once each peak has been closed off, use the Wand tool to highlight it on the profile plot. With all peaks highlighted, go to Analyze>Gels>Label Peaks to label each peak with its size as a percentage of the total size of all highlighted peaks. The resulting values can be copied and pasted into a spreadsheet program for further analysis. Given text is about analyzing data from a western blot image using ImageJ software, specifically calculating relative density of peaks and expressing results in terms of relative density or fold-change values. The process involves selecting peaks of interest, calculating their relative density by dividing their percent value by the percent value of a standard peak, and comparing density values between different samples to test for significant differences in treatment effects. Figure 12 is used as a representative western blot for analysis. Four replicate samples of protein were loaded into separate lanes, with the upper row of bars representing the protein of interest and the lower set representing the loading-control protein. The loading-control protein is expected to ensure equal amounts of total protein were loaded in each lane. However, upon examining Figure 12, it was observed that the size and intensity of the lower bars in each lane vary significantly. While lanes 1 and 2 appear equivalent, lane 3 has half the density compared to lanes 1 and 2, and lane 4 has half the density and half the size compared to lanes 1 and 2. Due to these differences, the density values of the upper set of bands may not be directly comparable. To address this issue, ImageJ's gel analysis routine is used to quantify the density and size of the blots. The results from the loading-controls are then used to scale the values for the protein of interest. When analyzing western blots with ImageJ, it's essential to account for background noise in real-world samples. This involves drawing a line across the base of each peak to enclose it, using the Straight Line selection tool from the ImageJ toolbar. For each lane, choose the Wand tool to highlight the loading-control bands and calculate their relative area/density. Repeat this process for the sample protein peaks separately to ensure areas are not factored into the calculation of density. After labeling each peak with its relative size as a percentage of the total area, copy the results to a spreadsheet for further analysis alongside the data from the loading-control bands. This detailed approach helps ensure accurate and reliable results in western blot analyses. 1. With data loaded into a spreadsheet, relative amounts of protein can be calculated from a western blot using ImageJ. Relative density values are based only on the peaks highlighted on the gel. 2. Calculate Relative Density values for each lane by dividing the Percent value in that lane by the Percent value in the control (Lane 1). This yields a set of density values relative to Lane 1’s loading-control band. 3. For sample protein bands, repeat the calculation as step 2 using the Percent value of the control’s protein band (Lane 1). 4. Due to variations in loading amounts, scale Sample Relative Densities by the Relative Density of corresponding loading-control bands. Having equal amounts of total protein in each lane is ideal, but sometimes it's not possible. If we can't get the same amount of protein into each lane, the densitites should be roughly equivalent (all around 1), which means the ratio of the protein of interest to total protein is similar in each lane. This usually happens when using a process like BCA Protein Assay to measure the total amount of protein in each sample. In this case, we might not need loading-control bands and can just use an standard sample on each western blot for the project. We can buy or make our own standard sample by mixing homogenates, which will have an equivalent amount of total protein (including the protein of interest) on each gel. The goal of the standard sample is to account for variations in antibody binding efficiency, blocking and washing efficiency, chemiluminescent reagent decay rate, and x-ray film exposure time. By using a standard sample on each gel, we can normalize every other band and compare across multiple blots because every band is normalized to the same standard. Normalizing Western Blots for Comparison To compare western blot results between different gels, it is necessary to account for differences in protein loading and exposure. This can be done by analyzing each gel separately using a standard protein sample. The standard sample is used as a reference point, allowing for the normalization of each sample lane to the same standard. The process involves calculating the relative density of the loading-control bands on the lower row of each gel and then adjusting the relative densities of the protein bands of interest (on the upper row) using these values. This ensures that each sample lane is normalized to the same standard sample, allowing for a fair comparison between gels. First, calculate the relative densities of the loading-control bands in each lane, using the standard sample as a reference point. Then, adjust the relative densities of the protein bands of interest by dividing each value by the corresponding loading-control density. This step normalizes the data and allows for accurate comparisons between samples on different gels. By following this protocol, it is possible to accurately compare western blot results between different gels, taking into account differences in protein loading and exposure. To calculate protein expression under treatment conditions, first analyze your standard samples to ensure proper cross-gel comparisons. Then, compute average relative density values for control and treatment samples using the standard sample as normalization. You may need to re-scale these values later. Some details about ImageJ gel analysis: The reported values correspond to a relative measure of band size and density. When altering gray value while maintaining area, ImageJ returns halved values. It also handles equivalent areas with varying shapes or numbers of pixels but maintains the same gray value. For example, in Figure 25, ImageJ reports half the value when you reduce both area and gray value (compare lanes 1+2). Similarly, in Figure 26, it yields consistent results for bands with equal numbers of pixels but differing shapes. In Figure 27, Image-J accurately handles gaps and reports equivalent values for lanes with same number of gray pixels and density, regardless of band size or density changes. Given text here The Importance of Standardization in Gel Image Analysis Standardizing gel images to a common standard is essential for accurate analysis. A thorough understanding of the methods used can help researchers evaluate their own work and identify areas for improvement. For those looking to analyze gel images, it's recommended to consult additional resources such as Gassmann et al. (2009) and Tan & Ng (2008), which provide valuable insights on techniques like densitometry and step wedge calibration to ensure accurate results.