

Dolphin-1D software operation for SDS-PAGE

MATERIAL

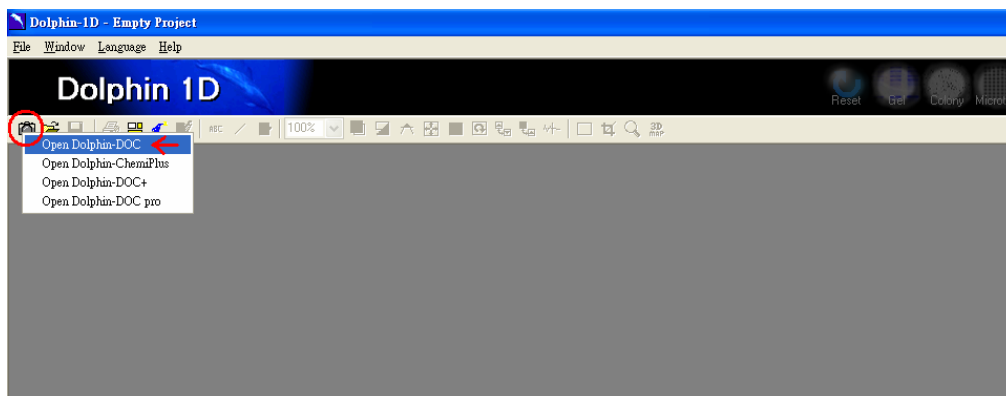
- 12% SDS-PAGE resolving gel (0.75 mm); 2.31 ml ddH₂O, 2.8 ml 30 % Acrylamide/Bis (29:1) (Bio-Rad), 1.75 ml 1.5 M Tris-Cl, pH 8.8 (Sigma), 70 µl 10 % SDS (Bio-Rad), 70 µl 10 % APS (Bio-Rad), 2.8 µl TEMED (Bio-Rad)
- 5 % SDS-PAGE stacking gel (0.75 mm); 1.7 ml ddH₂O, 0.415 ml 30 % Acrylamide/Bis (29:1) (Bio-Rad), 0.315 ml 1 M Tris-Cl, pH 6.8 (Sigma), 25 µl 10 % SDS (Bio-Rad), 25 µl 10 % APS (Bio-Rad), 2.5 µl TEMED (Bio-Rad)
- Coomassie Blue Staining Buffer (0.1 % Coomassie Blue R-250 (Bio-Rad) in Water/Methanol/Acetic acid (45:45:10))
- Destaining Buffer (Water/Methanol/Acetic acid (45:45:10))
- BSA (1 mg/ml), 0.05 g BSA (Sigma-Aldrich Ltd., St Louis, MO; U.S.A.) dissolved in 50 ml ddH₂O, aliquot 1 ml solution per Eppendorf and freeze in -20°C.
- Pre-stained marker (Bio-Rad, Hercules, CA, U.S.A.)
- ProMarker (Wealtec, Taipei, Taiwan)
- CB-1 Block Cooler (Wealtec)
- White Light convert plate (Wealtec)
- Dolphin-Doc image system with Dolphin-1D software (Wealtec)
- V-GES equipment (Wealtec)
- HB-1 Block Heater (Wealtec)

PROCEDURE

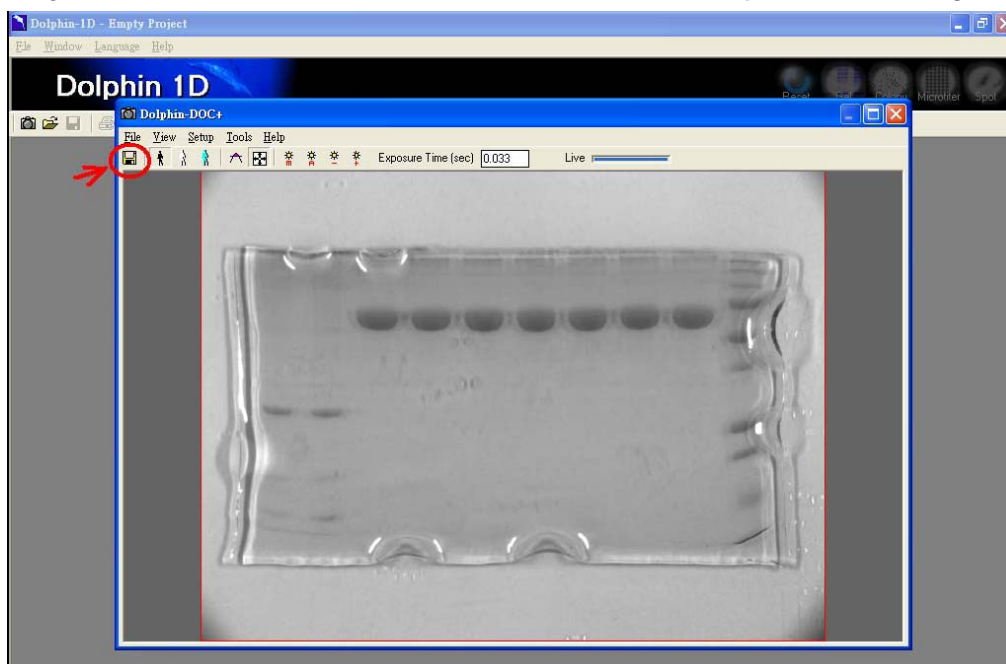
- Frozen BSA (1 mg/ml) was thawed by inserting a tube into the Block Cooler (4 °C). Then 25 µl 4X protein loading dye was added to 75 µl thawed BSA solution.
- The mixed BSA sample, a Pre-stained marker, and ProMarker were all boiled for 5 minutes in 95°C on a block heater.
- 10 µl samples were loaded into each well.
- The electrophoresis was run as follows; 60 minutes at 90 V, and then 60 minutes at 130 V.

- After electrophoresis, the gel was stained in Coomassie blue for 15 minutes.
- The gel was destained in destaining buffer with agitation over night.
- An image of the gel was captured using Dolphin-Doc Image system and the image was analysed using Dolphin-1D software as follows;

1. Open the Dolphin-Doc image system

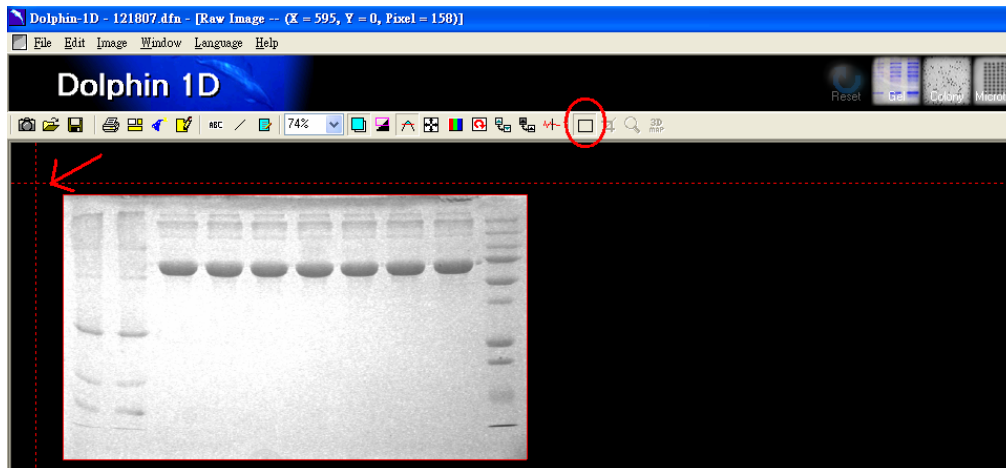


2. Adjust focus, iris and lens and zoom and then Capture the image.

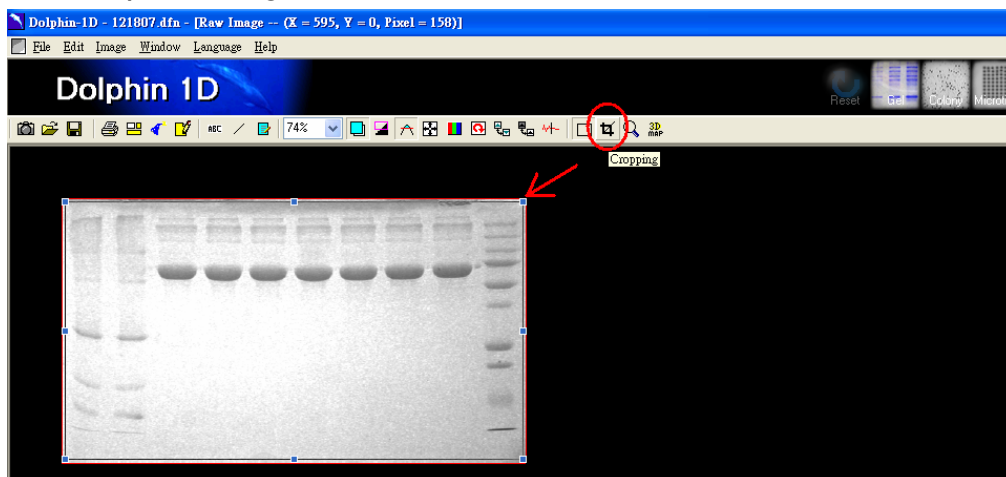


3. Select and Cropping the image to a suitable size.

a. Select the size.

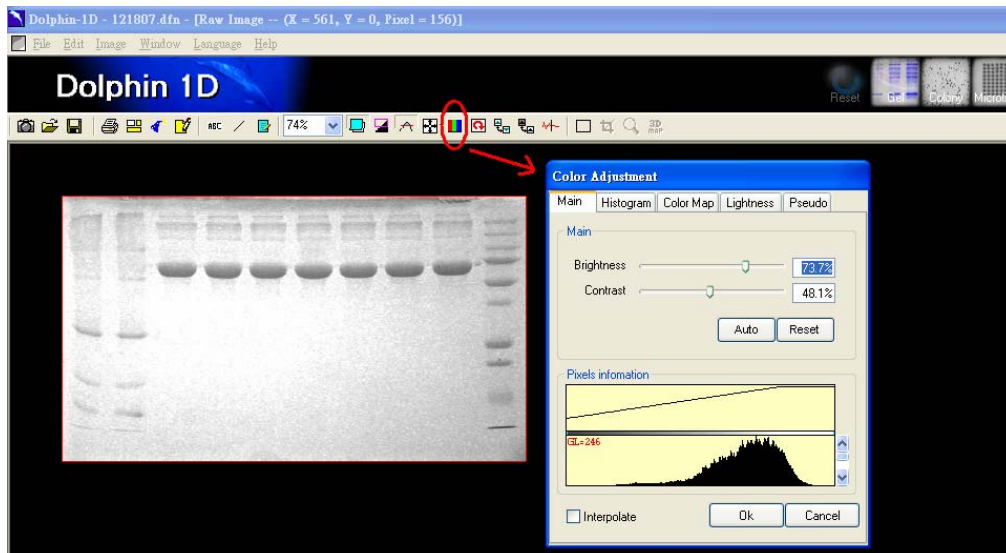


b. Crop the image.

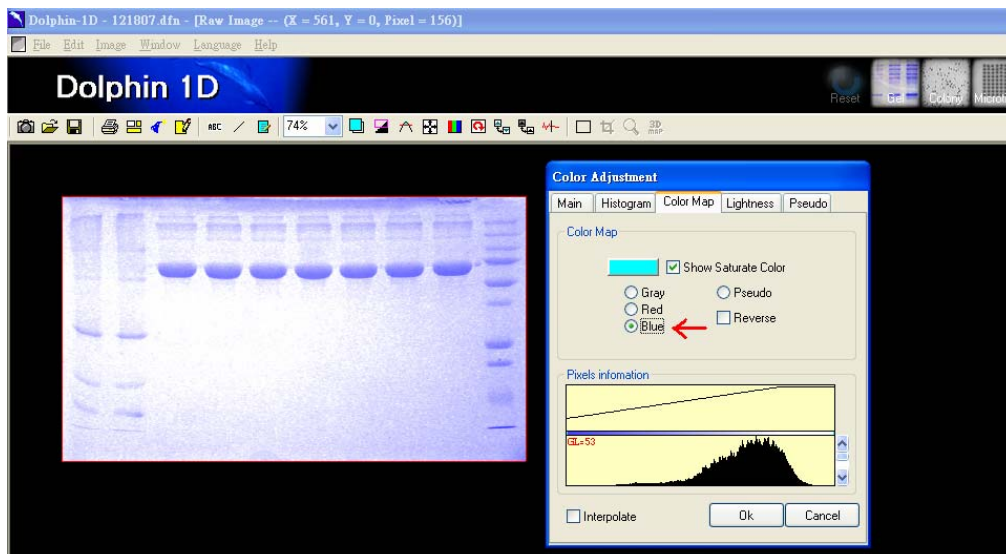


4. Adjust the gel image using “Color Adjustment”-tool.

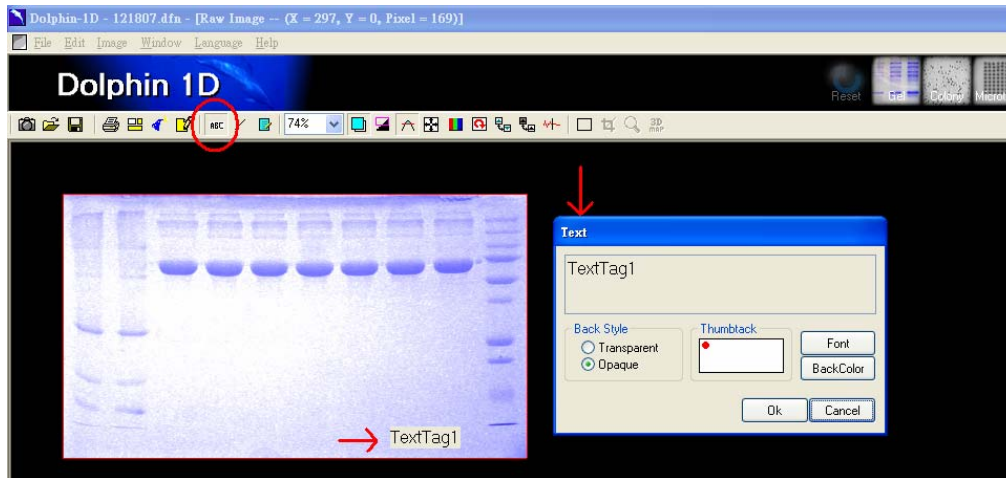
a. Adjust the Brightness and Contrast



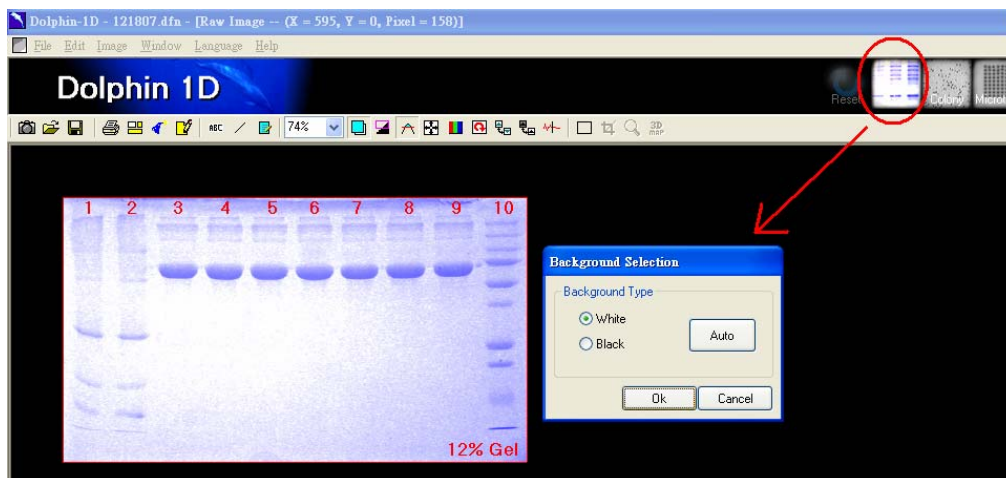
b. Under “Color Map”, change colour from Gray to Blue (optional).



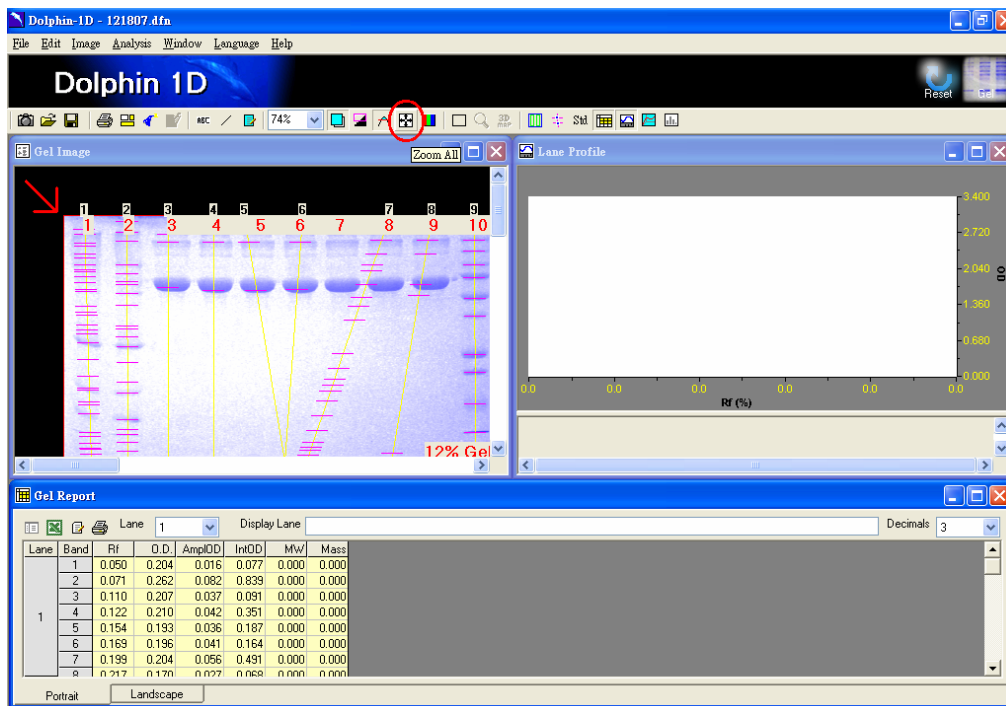
5. Add text to the gel image with the “Text”-tool (optional).



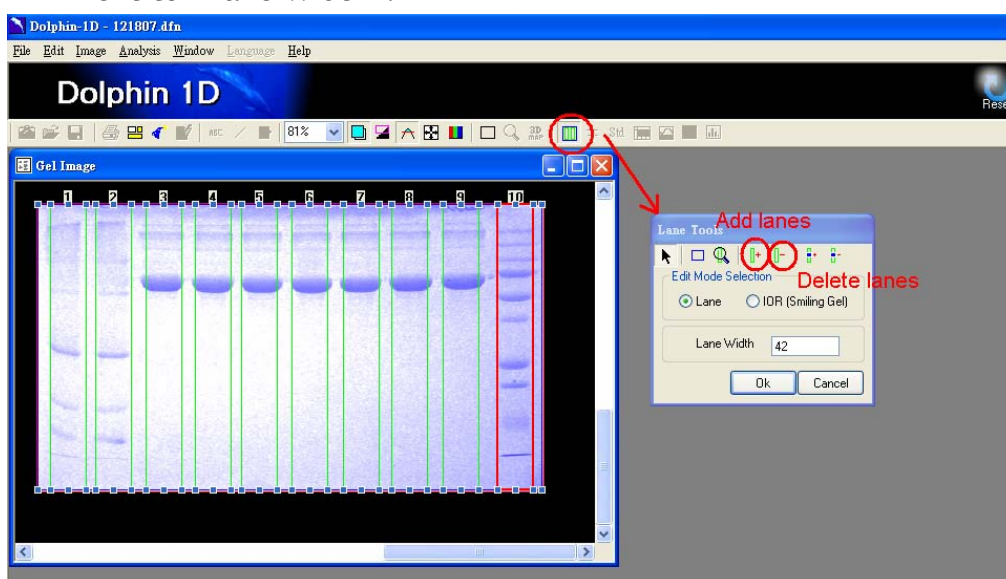
6. Enter the Gel function and use the Band Tools and the Lane Tools to analyze the gel image.
- Enter the Gel Analysis function. Select “white” in the background selection.



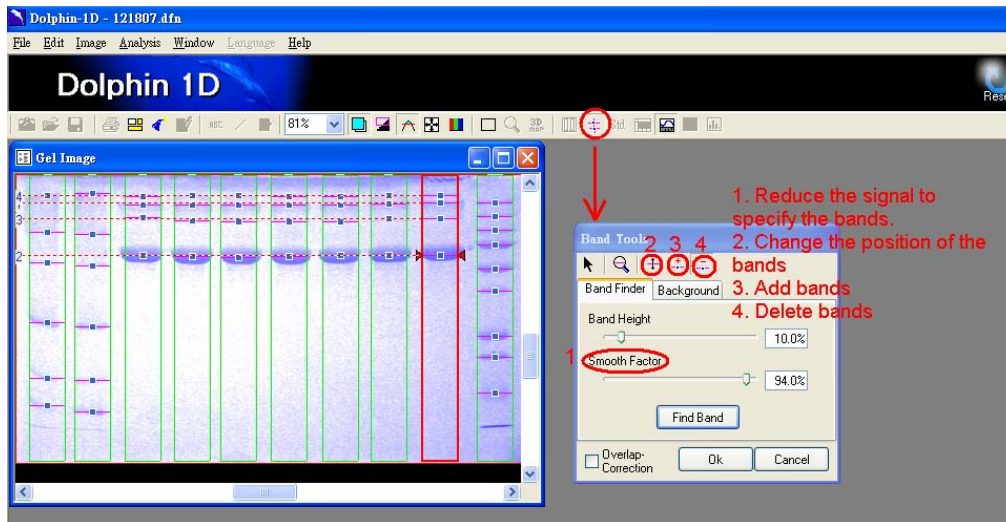
b. Use the Zoom All to auto focus the image



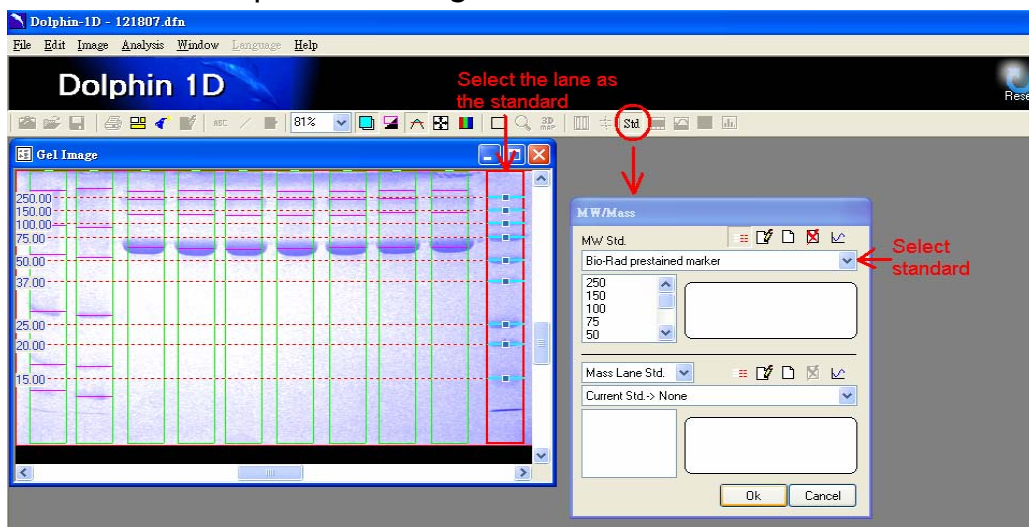
c. Use the Lane Tools to detect designated lanes. If many “unwanted” lanes are detected, use the “delete lane”-tool on all automatically detected lanes. Thereafter, use the “add lane”-tool and mark the lanes you are interested in, in the correct order. Adjust the width of the lanes either directly on one of the lanes, or insert a new number in the empty space next to “Lane Width”.



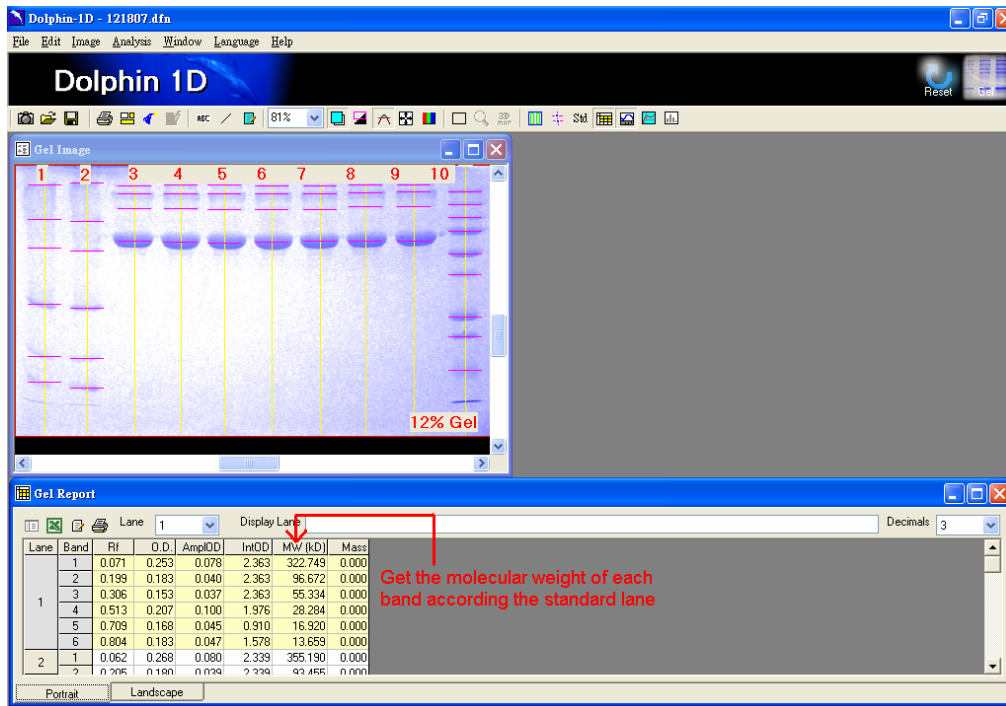
- d. Use the Band Tool to find bands. If too many bands are detected, used the smooth factor (1) to increase decrease sensitivity. If still too many bands are detected, use the “delete bands”-tool (4) and click on each band to remove it. Thereafter, add bands, using the “add bands”-tool (3). The position of a single band can be adjusted using the “adjust band”-tool (2).



7. Get molecular weight of each band based on comparison with size standard.
- Click the “Std”-button and then mark the lane that has the standard by clicking it. If current standard marker is listed, select the correct one. If your standard is not listed, add a new standard to the library by clicking the “new” button (blank sheet). Insert the name of the standard and then insert the molecular weight of the marker with the correct unit, starting from the top with the largest band.

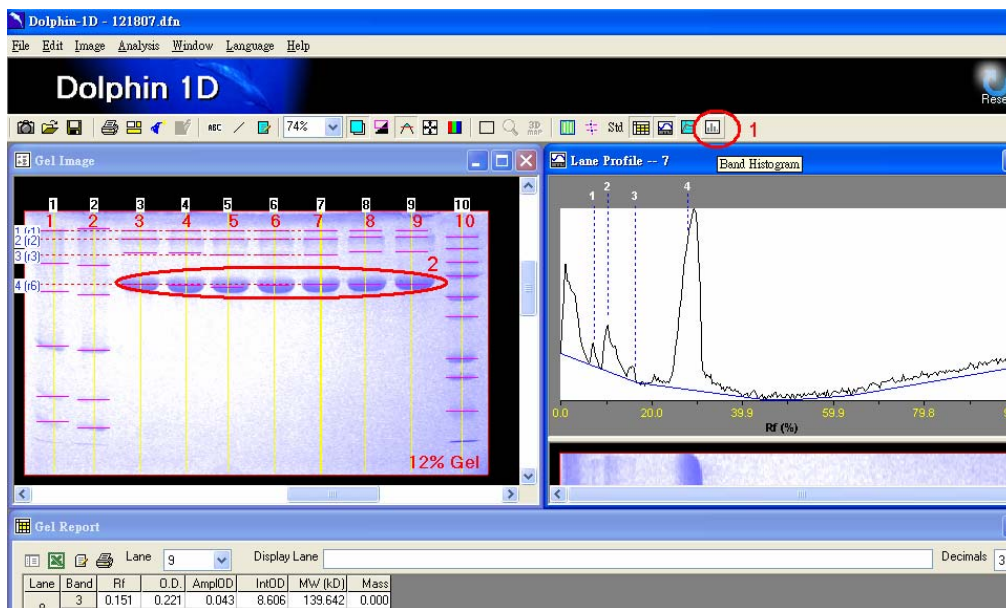


- b. Size information about all bands in the gel, based on size information of the standard bands, becomes updated in the gel report.

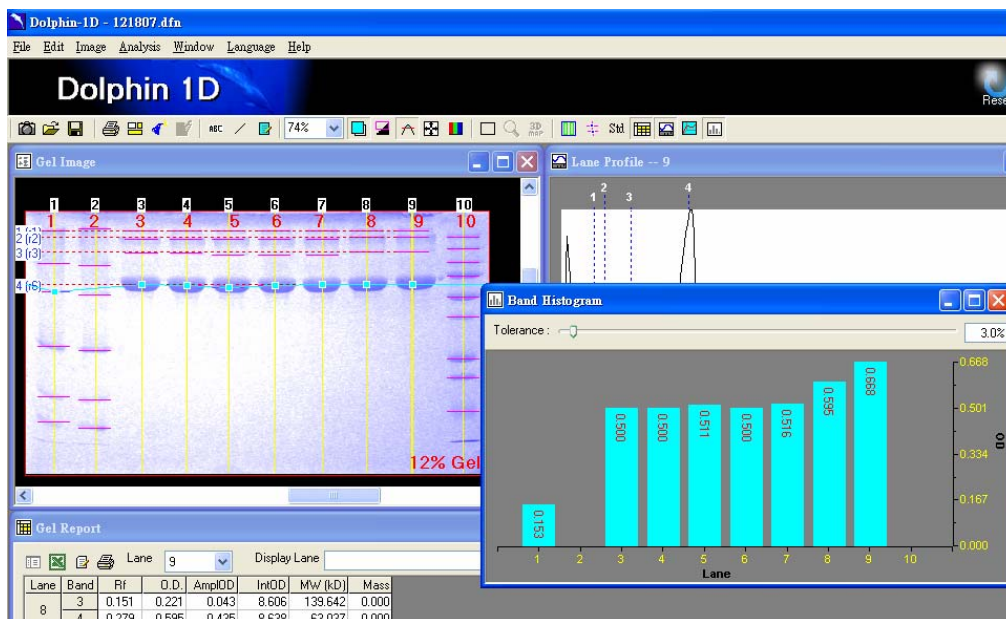


8. Compare bands

- First click the Band Histogram button (1), and then select which bands to compare in the gel image (2). The band histogram to the right of the screen shot represents the banding patterns (vertical) of lane no 7 (not related to the chosen bands in 2). A horizontal version of the seventh lane is shown below the band histogram.



- The histogram with size estimations is presented in a frame next to the gel image



REMARKS

- This bulletin shows how to simply determine molecular weights of bands in a gel based on size standards, using the Gel analysis function of Dolphin-1D. For other applications, the software has other functions, such as Colony, Microtiter, and Spot function. This bulletin shows the basic steps in detecting lanes and bands and how to assess the size of unknown bands based on a standard, however, the software also enables for extended analysis features, e.g. 3D-views of gels.
- In order to enhance the gel image after capture, use background subtraction or any of the available filtering-functions, median, minimum, maximum, average and power mean. For the filtering functions, the filter size is 3 x 3 pixels
 - a. Median: This filter reads the signal in adjacent pixels and averages the differences. In general, this filter smoothes out the noise of the whole image but preserves the data, since more of the edges and contours are preserved.
 - b. Average: The central pixel of a unit of 3 x 3 is replaced by the average of itself and its unit-neighbours. In general, this filter smoothes out the noise of the whole image, it blurs the edges and reduces contrast.
 - c. Maximum: This filter dilutes the greyscale by replacing the value of a pixel by the largest pixel value of that pixel unit (3 x 3). In other words, this filter will increase the lighter pixels and decrease dark ones, making the image brighter.
 - d. Minimum: Replacing each pixel in the image with the smallest pixel value in its unit, this filter darkens the image. In other words, this filter enhances the darker pixels and decreases the lighter ones.
- If too many bands are automatically detected when using the band-tool, the smooth-factor or band-height can be increased in order to increase specificity. Increase smooth-factor for a particular lane or all lanes, and the automatically found bands will usually decrease (unless all the bands are of same thickness).
- After setting a standard molecular weight reference, the software automatically calculates the molecular weight of all bands in the image. The data can be exported to Excel to save and get a further analysis, e.g. draw graphs.



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