

Quantifying expression standards and expression values using GeneTools software

Introduction

To analyze western blots in a quantitative manner a 'standard' such as an internal control i.e. a housekeeping gene (GADPH, β Actin etc) or an external standard such as the purified protein of interest in a known concentration (enables absolute quantification) are required.

In some cases an 'Expression standard' is assigned to one of the bands present on the blot. This band can then be used to calculate the 'Expression Value' of all the other bands present in the same horizontal line.

GeneTools software (4.01 (d)) now enables the user to analyze western blots by identifying Expression Standards and Expression values across a row of bands.

Performing a 'Expression Standard' analysis using GeneTools software

To be able to perform this type of analysis the image captured using GeneSnap software needs to be sent to GeneTools software using the following icon



Once GeneTools software has opened from the 'Samples Properties' dialog box select the area of interest using the red rectangle and automatically locate tracks.

To assign a quantity calibration click on the following icon



This will open up the 'Quantity Calibration' dialog box (Figure 1). Select 'Each track to a separate curve' then select 'Linear through origin (single standard value)' from the drop down menu for the curve type. Click 'Ok' to close this dialog box.

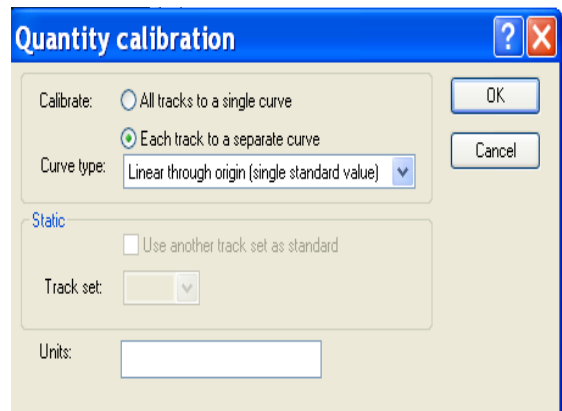


Figure 1- Quantity calibration dialog box

To assign a quantity for all 'standard' (Housekeeping gene or internal standard) bands click on one of the bands peaks to select it then click on the following icon.



In the pop-up window 'Assign calibrated quantities' (Figure 2) enter a value in the calibrated quantity box. Make sure that Propagate by Rf or Peak number is selected. This ensures all bands present in the same horizontal row will be calibrated to this quantity. Click 'Ok' to close dialog box.

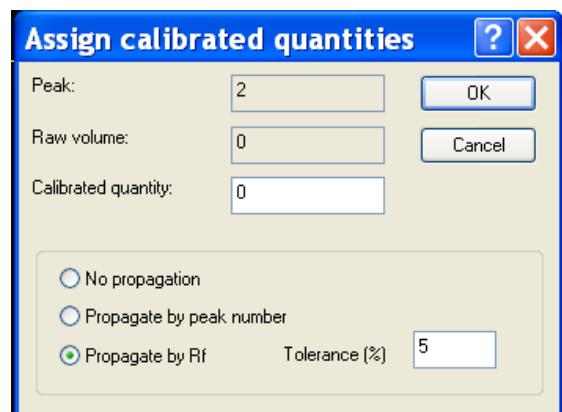


Figure 2- Assign calibrated quantities

To assign an 'Expression value' right click on a band in the row which is not the 'Standard' (housekeeping gene or internal standard) row. From the menu select 'Assign expression standard'. This will automatically assign a quantity of 100 to this band. The software will calculate all the other bands in that row to the 'Expression Standard'.

All calibrated values are shown in the results table which can also be exported to Microsoft Excel or Microsoft word.

Results for selected track				
Track 8				
Number	Height	Raw vol.	Quantity	Express. val
1(m)	40028.434	8309985.00	374.99	100.00
2(m)	4950.911	1108027.75	50.00	0.00

Figure 3- An example of a Results table

The following figure (Figure 4) shows an example analysis of a western blot using Expression standards and Expression values.

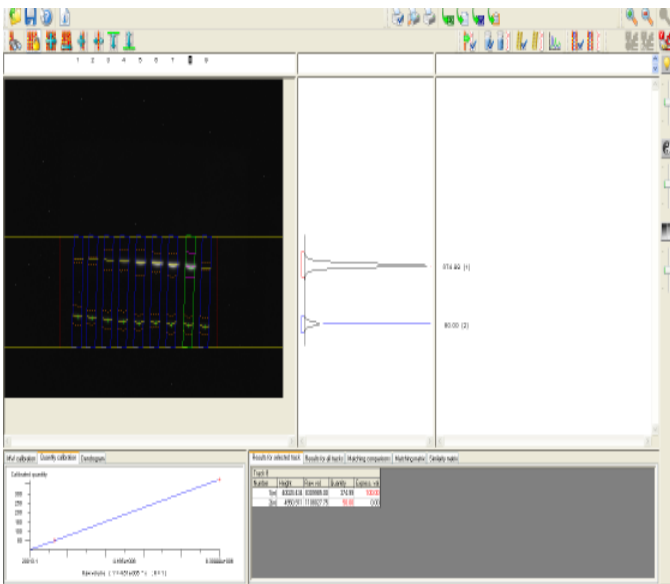


Figure 4- Example of a western blot analyzed using Expression standards and expression Values.

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