Smart Protein Layers

Processing and Evaluation of SPL-Data with LabImage

NH DyeAGNOSTICS

1 Required Data

1. Band volume of SMA basic detected in GLO for normalization of gel load
2. Band volume of SMA label detected in GTO for normalization of labeling efficiency
3. Lane volume (excl. band volume of SMA label) detected in BTO for normalization of the target
4. Band volume of the targets detected in BTA
5. For experiment to experiment comparisons: band volume of Cal B (50 kDa band) detected in BTA

2 Preparation and nomenclature of the single images

For the purpose of analysis you require raw date files of your images, including all meta data and highest amount of grey values (ORCA and Octoplus users have to choose the „SAVE"-option).

1. **GTO** = Gel Total Protein (incl. SMA label) after electrophoresis (cut of the dye front before imaging to avoid disturbing irradiation)
2. **GLO** = Gel Loading Control after electrophorsis
3. **BTO** = Blot Total Protein at target detection
4. **BTA** = Blot Target Protein

Nomenclature: Abbreviation_Distinct ExperimentHint_MoreExperimentDetails
Bsp.: GTO_WB180514_red12sec_repition1

DO NOT USE SPACES.

Note:
This analysis does not have to be done with LabImage software. The required date (1.) can be collected with any 1D analysis software. The calculation can be done with Excel or similar programs. For an appropriate manual please ask our service team.

3 Detection of lanes and bands

1. Load every image as a single LabImage project.
2. Optimize your display image (optional; raw data will not be changed).
3. Define ROI (Region Of Interest) in GTO.
4. Detect lanes in GTO.
5. Detect and subtract the background.*
   (recommended setting: „Rolling Disk"; radius: 45 pixels)
6. Transfer ROI, lanes and background from GTO to GLO.
7. Detect SMA basic bands on GLO.
8. Transfer SMA basic GLO to GTO.
9. Define ROI, background and lanes, SMA label band in BTO.
10. Transfer ROI, lanes and background from BTO to BTA (only possible when the same imaging device was used) or detect seperately in BTA and detect target bands.
11. Load the name templates for each project (download available at www.dyeagnostics.com/site/products/protein-analysis-software/).
12. Save all projects.

4 Perform SPL normalization by starting the „Project Comperator"

(„extensions" in the menu bar)

5 Results and data export

* Please take care that the ROI is selected not a certain lane. Otherwise the action is not for all lanes and the name templates can not be assigned correctly.