

ProPic II: Picking entire lanes of 1-dimensional gels; optimized picking parameters

DIGILAB PROTEOMIC TECHNICAL NOTE

Introduction

Proteomics instrumentation is often used to identify and/or sequence unknown proteins, whether they are binding partners for a known bait protein, or a biomarker for a specific condition. Many times, proteins of interest are of such a low abundance that once resolved by SDS-PAGE, they are not easily visualized using available staining techniques. These proteins may in fact be undetected, residing in the unstained portions of the gel. In the past, researchers have excised gel bands by hand, using a razor blade, and cutting the gel into small pieces prior to protein digestion. By nature, this method is inaccurate, and lends itself to contamination of the samples. This report demonstrates an automated method to excise all of the bands (visible and not) from multiple lanes of a 1-dimensional (1D) SDS-PAGE gel.

The Investigator™ ProPic II gel imaging/picking robot allows automated gel picking and recovery of all the proteins within a sample, maximizing the accurate identification of all of the proteins, including those of low abundance.

Materials & Methods

Sample preparation, separation by SDS-PAGE and protein staining. Samples consisted of a dilution of unstained broad range molecular weight standards (Bio-Rad, Hercules, CA, cat # 161-0317). Specifically, the dilution is 0.5µl standards + 3.75µl 4X sample buffer + 10.75µl ddH₂O. The samples were first boiled for 5 min., followed by centrifugation at 12,000 rpm for 5 min., and finally loaded into every other lane of the gel. The gels (PAGEr® Gold 10% Tris-Glycine precast gels (Cambrex, Walkersville, MD), 1.0mm X 10 well) were run at 200V, 125mA for approximately 75 min. in AccuGENE Tris-Glycine SDS Buffer (Cambrex). For protein visualization, the gels were stained with Coomassie Electrophoresis gel stain (NuSep, Frenchs Forest, Australia) according to standard protocols.

Gel preparation and set up. After staining and destaining to remove background signal, the gel was equilibrated overnight in 20% methanol (v/v) in ddH₂O. To prepare the gel for imaging and picking, the gel was immobilized with black plastic strips laid over top of the gel, both at the edges of the gel, as well as in the blank lanes between the lanes of interest. This gel holder, in turn, was restricted using magnets placed in the corners of the gel holder, both on top and below the gel tray, as shown in Figure 3.

Imaging, lane and band definition and spotlist generation. The gels were imaged using the ProPic II gel imaging/gel picking robot (Genomic Solutions, Ann Arbor, MI). Spots can be directly selected and added to a spotlist using the ProPic II software. However, in this case, Totallab™ TL120 (v2006e) software (Nonlinear Dynamics Ltd., Durham, NC) was used to demonstrate easy integration of this popular 1D image analysis software with the ProPic II software. After opening the image file, the lanes and bands were delineated using manually selected non-overlapping bands which comprise the entire lane. In addition, in the “band picking”

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screen, under options, the setting for number of picks [across lane] should be set to 5 under the conditions used for this set of experiments. This will generate an image depicting the spots to be picked, illustrating that essentially the entire lane will be picked (Figure 1, lanes 1-3). Once this image is acquired, the Total-lab-generated spotlist file is exported to ProPic II by clicking on the “Export to ProPic” button (Figure 1), after which the file can be opened with the ProPic II software. All three entire lanes (as shown) can then be picked using the “Click & Pick” button. This is accomplished by first opening the “Click & Pick” window, opening the image, then opening the spotlist. The exact settings that should be used in the picking parameters of the ProPic II software to maximize harvesting efficiency are given in Table 1, optimal settings.

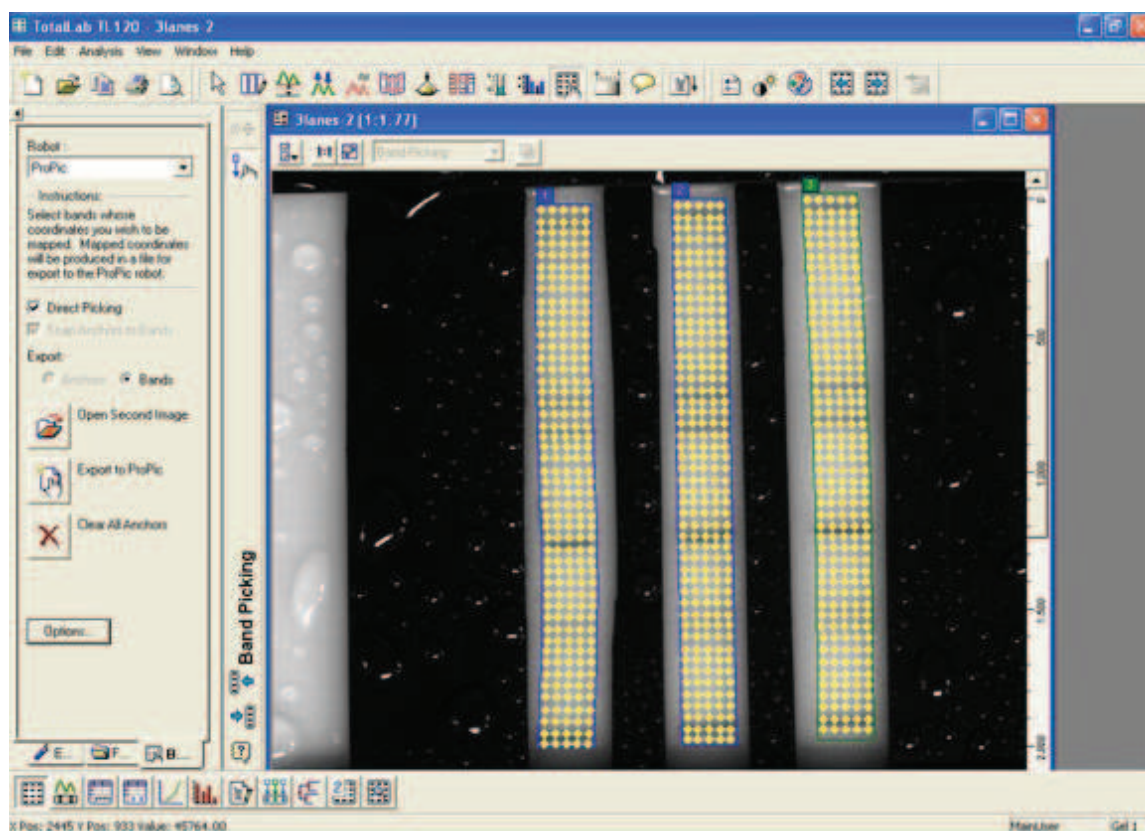


Figure 1. Window generated by TotalLab™, depicting the spots to be picked, with 5 picks across the lane, harvesting the entire lane. The blue or green box represents the lane, as it was defined in the “lane creation” step of the software program. The bands were established in the “detect bands” step of the program. The yellow diamonds indicate the actual site of the spots to be picked. These are chosen in the “band picking” step of the program. Next, the spotlist is exported to the ProPic II, by clicking on the “Export to ProPic” button.

The Investigator™ ProPic II is a third generation gel imaging and picking instrument equipped with a 1.8 mm i.d. picking tip. In the ProPic II software, the following optimal Picking Parameters (accessible via the Set-Up button) should be used:

Table 1. Optimized picking parameters compared to the default settings¹

ProPic II Parameter	Default setting	Optimal setting
Wash volume	400 ml	400 ml
Gel pre-wet volume	40 ml	50 ml
Air gap volume	80 ml	100 ml
Pick volume	120 ml	150 ml
Dispense volume	300 ml	300 ml
Picking shift	0.0 mm	0.3 mm
Totallab™ parameter		
Number of picks/lane	3	5

¹For certain gels, the number of picks across the lane may be set to 4. The optimal picking parameters in this case are likely different, and should be established through experimentation. If the number of picks across the lane is set to 3, the harvesting of the lane is incomplete, therefore this setting should not be used.

The spotlist is then opened in the ProPic II software, as shown in Figure 2.

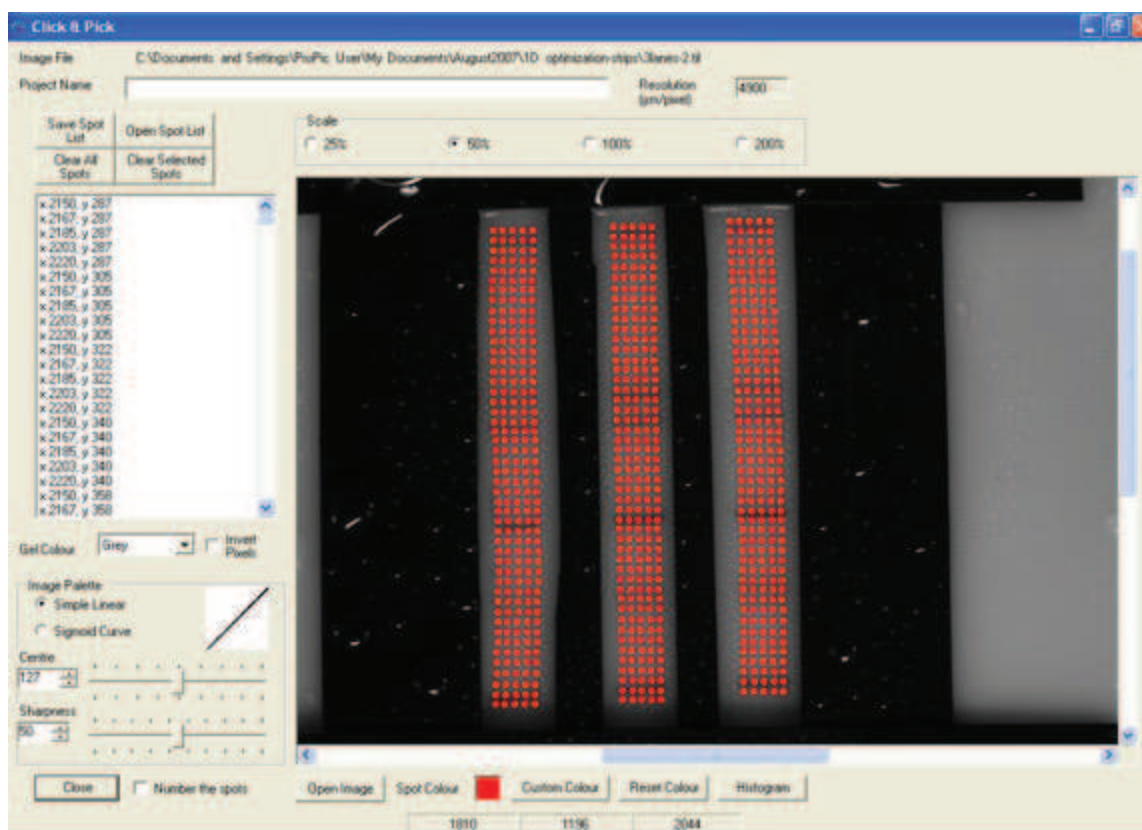


Figure 2. The spotlist generated by Totallab™, exported to ProPic II, picking 5 picks (represented by red circles) across the lane, harvesting the entire lane for each of three lanes. This window is acquired by performing the following series of steps: 1) open the “Click & Pick” window; 2) open the original image of the gel; and 3) open the spotlist file generated with Totallab™ software.

Results

The gel was imaged both before and after picking, in order to verify the harvesting efficiency attained by altering certain picking parameters. As illustrated in Figure 3, these settings allow harvesting of virtually all of the lanes, and therefore all of the proteins contained within each sample. Furthermore, all five of the gel plugs from each band are placed in the same well of a 96 well plate, after which these plates can then be used in conjunction with automated protein digesting and protein spotting equipment. The use of the ProPic II robot for automated gel imaging, picking and depositing multiple gel plugs in the same well will ultimately increase the mass spectral signal, thus enhancing the probability of identification by mass spectrometry of high abundance, visibly stained proteins, as well as low abundance proteins which are not detectable by conventional staining methods.

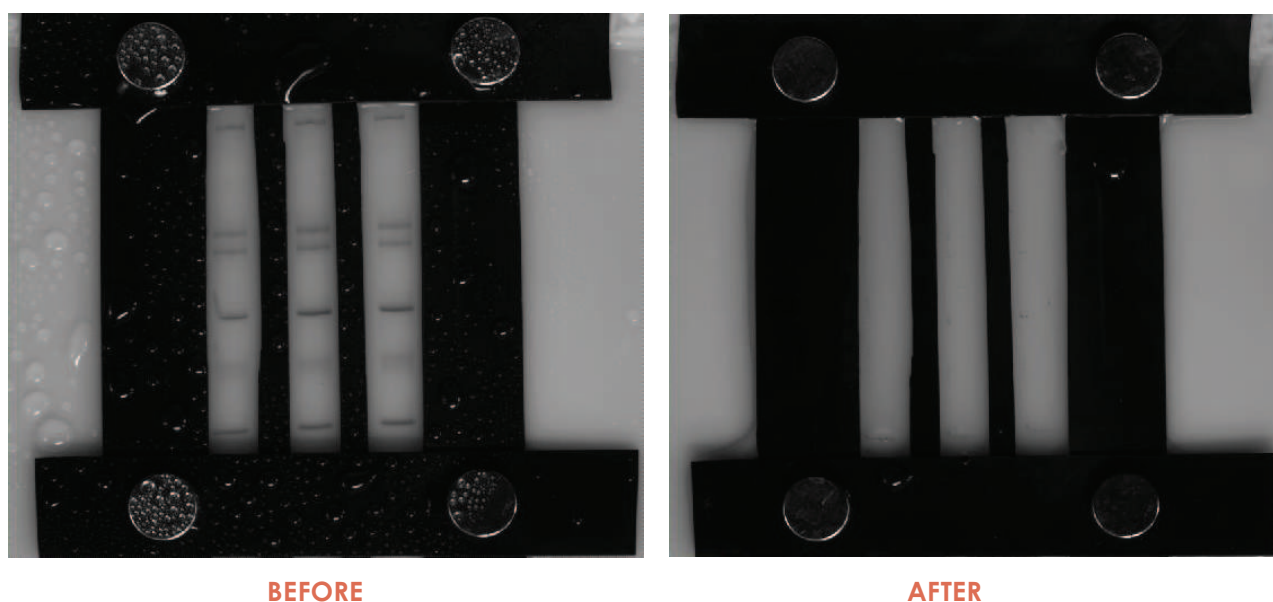


Figure 3. Images taken of the gel before the picking run (left) and after the picking run (right). Note that all three lanes are excised completely, with no residual gel remaining after picking. The gel holder, composed of plastic strips held in place by magnets, aids in keeping the gel hydrated, as it minimizes the exposed surface of the gel, therefore reducing evaporation.

Conclusions

Although researchers have in the past been able to manually excise an entire lane of a 1D SDS-PAGE gel, the ability to precisely cut by hand each lane for protein identification is impractical, time-consuming and lends itself to contamination of the sample. In this report, we outline an automated method to pick and harvest three entire lanes of a 1D gel, with all of the gel plugs from each lane placed in a single well. While the ProPic II software can accomplish direct spot selection and spotlist generation, it also easily integrates with Totallab™. The use of this system will increase the likelihood of identification of all of the proteins within a sample, including those of low abundance. This is a simple, user-friendly, automated method by which to evaluate samples run on 1D gels. A future report will outline optimized picking parameters using a larger, 2.5mm i.d. picking tip.

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