A simple method for the effective cutting of spots from gels stained with LavaPurple.

Key words: epicocconone, fluorescence, electrophoresis, mass spectrometry

Introduction.

LavaPurple™, a total protein stain based on the molecule epicocconone, is one of the most sensitive stains available for electrophoresis. Using a laser scanner it is possible to achieve sensitivity in the low femtomolar range from 1D and 2D gels [1]. In addition the unique reversible nature of this stain make it well suited for subsequent analysis by either MALDI-ToF PMF or ESI peptide sequencing [2, 3]. For analysis by mass spectrometry spots must be excised from the gel, either by hand or with the aid of a spot cutting robot. For many fluorescent stains excision is performed with the aid of a UV light source at either 302 nm or 365 nm [4]. Using trans-illuminators can be hazardous to the health of the researcher as spot cutting can involve long exposure times to UV radiation [5]. When bound to protein the excitation maxima of epicocconone are approximately 390 and 500 nm and the excitation peak at 390 nm is also relatively sharp [6] which results in a substantial loss of brightness when standard trans-illuminators (302 nm) are used to excite the dye [7].

Unlike many other staining protocols LavaPurple does not involve the use of high concentrations of solvents and gels do not shrink or expand during staining. This enables the use of a template, printed directly from a scanned image, to guide spot cutting. Using such a template it is possible to cut even the faintest spots. In addition the method means that spots can be cut on a bench, which prevents photo-bleaching.

Materials and Methods

Reagents.

1. LavaPurple staining kit containing all buffers was obtained from Fluorotechnics (Sydney, Australia).
2. Unless stated below all other reagents and gels were purchased from BioRad (Hercules, CA).

3. Iodoacetamide and EC-1 *E. coli* were purchased from Sigma-Aldrich (St. Louis, MO).

Equipment.

1. Images were captured using a Typhoon 9200™ scanner (GE Healthcare; Little Chalfont, United Kingdom).
2. IEF was performed using an IPGphor 3™ unit (GE Healthcare; Little Chalfont, United Kingdom).
1. Second dimension electrophoresis was performed using a Criterion™ gel tank (BioRad Laboratories; Hercules, CA).

Protocols.

Electrophoresis.

EC-1 strain E coli (100 mg) was dissolved into IEF MSD buffer (3 mL; 5M urea, 2M thiourea, 2% CHAPS, 2% SB 3-10, 40mM Tris). The sample was vortexed and DTT (100 µL of a 1M solution) added. The solution was incubated at 70°C for 20 minutes then allowed to cool to room temperature. Iodoacetamide (120 µL of a 5% solution) was added, the solution was incubated for 2 hrs at RT, and the solution then used to re-hydrate 11cm pH 3-10 IEF strips overnight (250 µL solution per strip). Strips were focused for approximately 50,000Vh then equilibrated in equilibration buffer (6M Urea, 2% SDS, 20% Glycerol, 40 mM Tris-HCl pH 8.8; 25 mL) for 2 × 10 minutes. The strips were then placed onto the top of Criterion 8-16% gels by embedding in 0.5% agarose. The second dimension was run at 200 V for approximately 90 minutes (buffer front run just off the gel).

Staining.

Gels were stained as per the standard protocol supplied with LavaPurple (http://www.fluorotechnics.com/content/documents/LavaPurple_flyer.pdf). Briefly the gels were fixed for 1 hr in 15% ethanol, 1 % citric acid (200 mL), stained for 1 hr in 100 mM sodium borate buffer pH 10.9 containing a 1:200 dilution of LavaPurple concentrate

(50 100 mL buffer, 500 µL stain), de-stained for 30 minutes in 15% ethanol (200 mL), and acidified for 30 minutes in 15% ethanol, 1% citric acid (200 mL).

Visualisation of Gels and Spot Picking.

A match stick was cut into small segments and inserted into the corners of the gel (outside the separation are). These "posts" were used as references for aligning the image with the gel. An image of the gel was generated using a Typhoon 9200 scanner with the following settings: 532 nm laser, 540 PMT, 610BP30 filter, 100 µm resolution and normal sensitivity. The image was exported to tiff format file using ImageQuant 5.2™ (supplied with the scanner) ensuring that the image was not scaled in any way. The image was imported and printed using software that does not automatically modify the picture (see Figure 1). A clean transparency was used to support the gel and the gel was placed over the image in such a way that the outline of the matchstick aligned with the match sticks in the gel. A small amount of acidification buffer was placed onto the gel to prevent excessive drying and the spots were excised using a 200 µL pipette tip that had been cut to an internal diameter of approximately 1 mm. After spot excision the gel was re-imaged on the Typhoon 9200 scanner to confirm accuracy of the cuts.

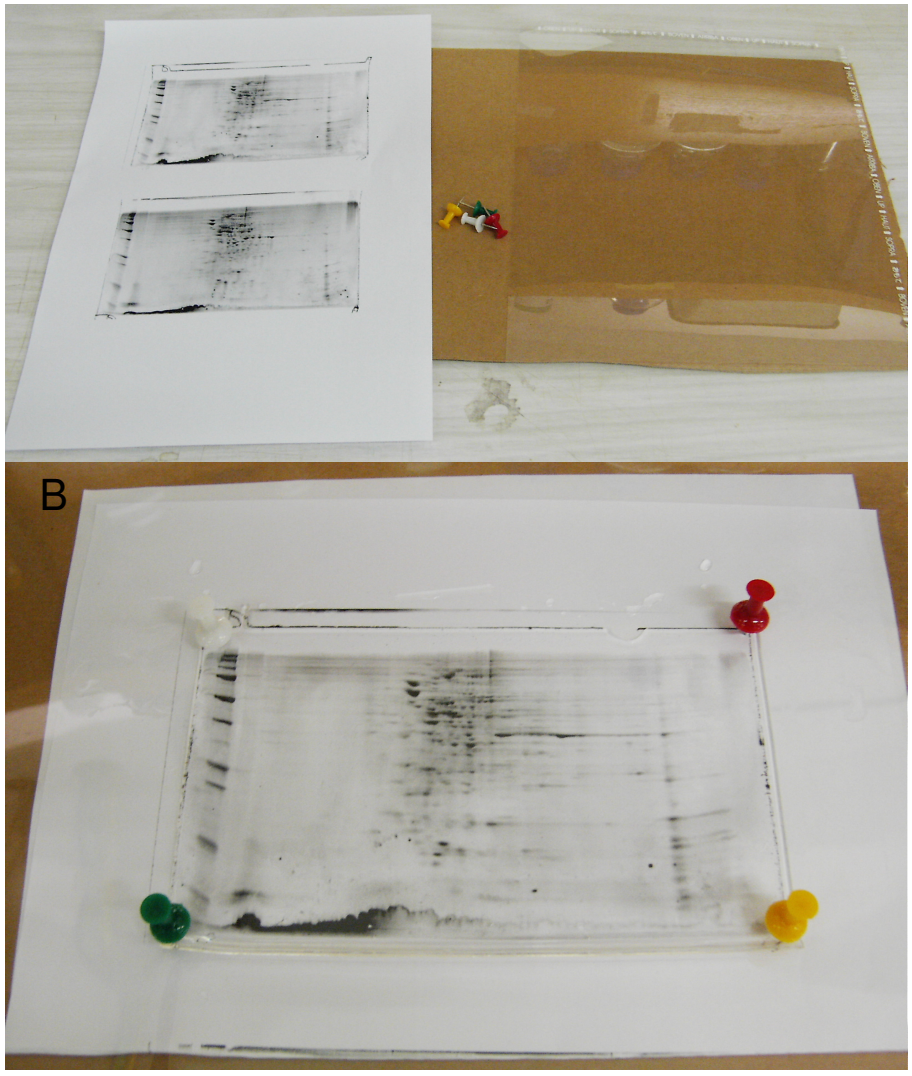

Figure 1: Preparation of the gel for spot cutting showing the equipment required (A) and the gel after it has been aligned with the image and fixed into place (B). By using the pins to fix the gel to a board after alignment it is not necessary to continually re-check the alignment after each cut.

Results and Discussion

Manual spot picking of 2D gels is often the most convenient way to collect samples for MS-analysis, and many labs do not have access to automated spot picking robots. While manual spot picking is often performed using a trans-illuminator this method has a

number of problems associated with it. UV trans-illuminators typically use high energy mercury lamps which are dangerous to the researcher. In order to cut spots protective clothing must be worn and the time taken must be as short as possible. Gels stained with LavaPurple are not well visualized with trans-illuminators fitted with 302nm or 365 nm globes. Sensitivity can be increased by replacing the globes with readily available Black-light Blue or Black-light Actinic globes (Philips lighting cat # TLD15W/08 and F15T8 respectively). The process is simple as the globes in trans-illuminators are standard sizes. Using these globes will not provide the sensitivity of a laser scanner, however.

For high sensitivity manual spot cutting the printed image method is recommended. The method requires simple materials and software available in all labs. Set-up of the gel for spot cutting is easy and takes approximately 5 minutes (Figure 1). By placing a gel over a 1:1 scale printed image from a laser scanner it was possible to accurately cut even the faintest of spots (corresponding to less than 1 femtomole of protein) and 40 spots could be cut within 30 minutes with an accuracy of 97.5 % (see Figure 2). The main issues encountered when using this method of spot cutting were excessive drying of the gel which was overcome by applying small amounts of solution 1 to the gel at approximately 10 minute intervals; and the fact that cutting the spot tends to cause the gel to move out of position. The latter was countered by placing gel and image onto a board and pinning them into place with push pins (thumb tacks/drawing pins). The application of this method was no more difficult than preparing a gel for cutting using a robot and did not involve a UV light box making it safer than traditional UV trans-illuminators. In addition the LavaPurple stain is stable to normal laboratory lights and photo-bleaching was minimal. It is however important to note that if other fixing/acidification solutions are used (such as 30% methanol, 7% acetic acid) it is necessary to first transfer the gel to a 15% ethanol solution to prevent shrinkage during the cutting of the spots.

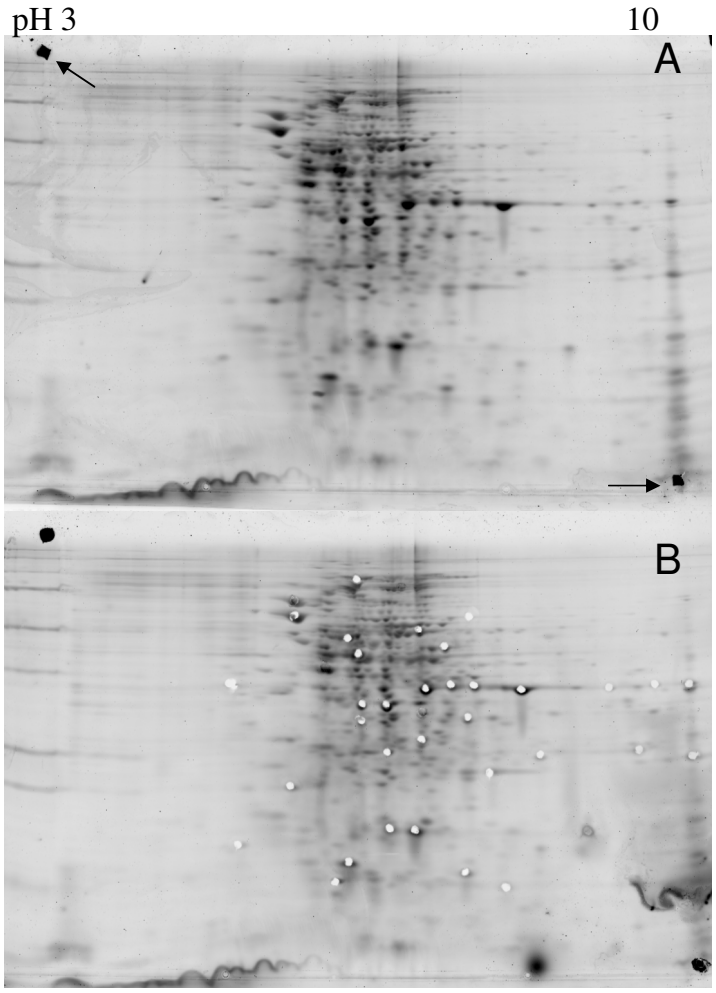

Figure 2: Images of EC-1 cell extract separated by 2D-PAGE, stained with LavaPurple and visualized using a Typhoon 9200 laser scanner both before (A) and after (B) excision of spots. Arrows mark the slices of match stick used as marker positions for gel alignment. The image was printed, aligned with the match stick posts and gel plugs cut using a modified pipette tip. To cut 40 spots with a 97.6% success rate took 18 minutes (5 minutes to align gel and 13 minutes to cut spots).

Conclusion

LavaPurple is a sensitive total protein stain when used with laser based imaging equipment. A simple method for cutting spots involving printing an image and using it as a template is fast and has a high success rate. When this method is used even the faintest spots can be cut in such a way that is safe for the researcher, and photo-bleaching of the gel is also kept to a minimum.

References:

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