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Full Length Research Paper

A simple apparatus for gel staining used in two-dimensional electrophoresis

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Two-dimensional electrophoresis was widely used in molecular biology. It was required that all gels be in the same condition. The gels can be controlled in the same condition easily during SDS-PAGE. But no paper reported how to ensure the gels were in the same condition during being stained. When the common protein gel staining plates were used, keeping the gels in the same treatment was impossible. A new apparatus was designed in this paper. The apparatus can ensure all of the gels in the same condition during being stained. Furthermore, the problems such as gels being broken, being covered by others can also be prevented when the new apparatus was used.

Key words: Two-dimensional electrophoresis, gel staining, apparatus

INTRODUCTION

As more and more genome DNA had been sequenced, the opportunity to investigate gene expression on a global scale was provided (Phillip, 1998). Proteomics was one way to reach this aim on the level of protein synthesis (Keigo et al., 2005; Adam et al., 2001). Two-dimensional electrophoresis was one essential tool in proteomics (Keigo et al, 2005; Adam et al., 2001; Marie-Christine et al., 2002; Valerie et al., 2004).

In two-dimensional electrophoresis, the protein spots were observed after they were stained in the gel. Generally, there were three major staining methods applied nowadays. Coomass blue was the oldest method. But it was less sensitive (Jung-Kap et al., 1996). Silver staining was the most sensitive (Gopal et al., 2000; Harvey et al., 1997). Many silver staining procedures were compatible with protein identification because of not using glutaraldehyde (Birgitte et al., 2002). This made silver staining widely be used in two-dimensional electrophoresis (Shang-Qing et al., 2007; Chi et al., 2007; Song-Mee et al., 2006; Sacha et al., 2006; Aurélie et al., 2006; Lena et al., 2006; Yoshinari et al., 2004). Although fluenscent staining was also sensitive and compatible with protein identification, the specific visualized devices were required (Gopal et al., 2000). However, although staining methods were studied thoroughly in these reports, the apparatus used for staining were not referred. Furtheremore, when the two-dimensional electrophoresis apparatus were bought, the manufacturer often did not tell about the staining apparatus. The common protein staining plates were always used. It was required that all gels were treated under the same conditions in two-dimensional electrophoresis. The requirement can hardly be met by the common protein staining plates. In this paper, a simple apparatus was designed to stain the two-dimensional electrophoresis gels. All requirements for protein staining in two-dimensional electrophoresis can be met by the designed device. The staining results between the common staining plates and the designed apparatus were also compared.

MATERIALS AND METHODS

Materials

Human bone marrow cell line KG1a was used for two-dimensional electrophoresis in this paper (Shanghai Biosis Biotechnology limited company, Shanghai, China). Cells culture and transfering were mainly according to the method described before (Zhang et al., 2006). KG1a was cultured in IMDM medium.

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Reagents and apparatus

ReadyStrip IPG Strip (1-14), ReadyStrip IPG Strip (5-10), Bio-Lyte Ampholyte, tributylphosphine (TBP), urea and CHAPS were from Bio-Rad (Hercules, CA, USA). Dithiothreitol (DTT). bromophenol blue, mineral oil, Sodium dodecyl sulfate (SDS), Tris-base, iodoacetamide and low melt point agarose were from Sigma (St. Louis, MOU, SA). Acrylamide, N, N'-methylene bisacrylamide, ammonium persulfate, Tetramethylethylenediamine (TEMED), thiourea, Phenylmethanesulfonyl fluoride (PMSF), pepstatin, leupeptin hemisulfate and ethylenediaminetetraacetic acid (EDTA) were from Sangon (Shanghai, China). Protean IEF cell and protean □ xi cell were from Bio-Rad (Hercules, CA, USA). All other chemicals were of analytical grade.

Protein samples preparation

The protein samples were prepared according to the protocol offered by Amersham Bioscience. The cells were cultured at 37℃ untill OD_{600} = 1.0. After the cells were centrifugated at 12000 × g for 10 min in 4℃, the pellet was resuspended with the washing solution (10 mmol/L Tris pH8.0, 5 mmol/L $Mg(Ac)_2$) two times. After being centrifugated once again, the pellet was resuspended with the lysis solution (9 mol/L urea, 4% w/v CHAPS, 200 mg/ml DNase, 1% w/v DTT and a cocktail of protease inhibitors [1 mmol/L PMSF, 1 mmol/L EDTA, 0.7 μg/ml pepstatin, 0.5 μg/ml leupeptin hemisulfate]). The suspension was sonicated (pulse on 10 s, pulse off 5 s; Ampl was 34%) until it was clear. After being centrifugated at 12000 × g for 10 min at 4℃, the supernatant was purified using ReadyPrep™ cleanup kit (Bio-Rad Laboratories). The protein concentration was measured according to the modified Bradford (Kruger, 1996). The standard curve was drawn in the Table 1.

Add 3.5 ml staining solution (every 100 ml staining solution contained 10 mg coomassie brilliant blue G250, 5 ml 95% ethnol and 10 ml 85% phosphoric acid). The protein samples were treated as the standard protein. The concentration was determined using regression analysis.

The first dimensional electrophoresis

The first dimensional electrophoresis was performed as the ReadyStrip IPG Strip Instruction Manual (Catalog# 163-2099, Bio-Rad). 10 mg protein of each sample was dissolved in 1 ml rehydration buffer (9 mol/L urea, 4% w/v CHAPS, 200 mg/mL DNase1, 1% w/v DTT and a cocktail of protease inhibitors [1 mmol/L PMSF, 1 mmol/L EDTA, 0.7 μg/ml pepstatin, 0.5 μg/ml leupeptin hemisulfate]). 300 μl of each sample was pipeted as a line along the edge of a channel in the IEF focusing tray. After being placed in the focusing tray with gel side down, the IPG strips were overlaied with mineral oil and rehydrated under active condition for at least 12 h. The default cell temperature was 20℃ with a maxium current of 50 μA/IPG strip.

When rehydration was over, the ends of each IPG strip were lifted with forceps and a paper wick (catalog#165-4071) gotten wet with deionized water (10 μl per paper wick) was inserted between the IPG strip and the electrode. The start voltage was 0 V with an end voltage of 10000 V. The volt-hours were 40-50000V-hr and Ramp was rapid with a maxium current of 50 μA/IPG strip.

Equilibration

Equilibration was done according to the manual offered Bio-Rad (Catalog# 163-2099). After the first dimensional electrophoresis concluded, the IPG strips were taken out from the focusing tray and transferred into a clean, dry disposable rehydration/equilibration tray with gel side up. The strips were holden vertically with forceps to drain the mineral oil for about 5 seconds. The mineral oil was removed from the IPG strips by placing them (gel side up) onto a piece of dry filter paper and blotting with a second piece of wet filter paper. 6 ml of equilibration buffer I (6 mol/L urea, 0.375 mol/L Tris-HCl, pH8.8, 2% SDS, 20% glycerol, 2%(w/v) DTT) was added to the equilibration/rehydration tray, using one channel per IPG strip. The blotted IPG strips were transferred (gel side up) into the equilibration/rehydration tray. The tray was shaked gently for 10 min. The used equilibration buffer I was discarded and 6 ml equilibration buffer II (6 mol/L urea, 0.375 mol/L Tris-HCl, pH8.8, 2% SDS, 20% glycerol, 2.5% (w/v) iodoacetamide) was added to every IPG strip. After the tray was shaked gently on orbital shaker for 10 min, the strips were rinsed with 1×tris-glycine-SDS (TGS) running buffer and prepared for the second electrophoresis.

Table 1. Protein concentration measured in this paper

Treatment	1	2	3	4	5	6	7	8
μg BSA	5	10	15	20	25	30	40	50
μl volume	1	2	3	4	5	6	8	10
μl lysis solution	9	8	7	6	5	4	2	0
μl 0.1 mol/L HCl	10	10	10	10	10	10	10	10
μl pure water	80	80	80	80	80	80	80	80

The second dimensional electrophoresis

The second dimension was performed in 12% SDS-PAGE gels as the standard method using the protean □ xi cell system (Sambrook et al., 1989). The equilibrated strips were sealed with 0.5% low-melting agarose in stacking buffer (0.1% SDS, 0.25 mol/L Tris, 0.192 mol/L glycine, 0.5% bromophenol blue) at the top of SDS-PAGE gel. The electrophoresis was run at 60 V for 30 min, followed by 250 V until the bromophenol blue front traversed the gel.

Gel staining

Silver staining method compatible with mass spectrometry was performed according to the protocol offered by Amersham Bioscience. After being immersed in the fixed solution (40% methnol, 10% acetic acid) for at least 30 min, the gels were put in the incubate solution (75 ml methnol, 17 g Na-acetate, 0.5 g $Na_2S_2O_3 \cdot 5H_2O$, add pure water to 250 ml) and shaked gently for 30 min. The gels were washed with pure water 3 times and treated with the silvering solution (dissolve 0.5 g $AgNO_3$, add pure water to 250 ml) for 20 min, followed with being washed with pure water 2 times (1 min. for each time). And then, the gels were put in the development solution (6.25 g Na_2CO_3, 100 μl formaldehyde, add pure water to 250 ml) and shaked gently. As soon as the protein spots were stained decently, the staining procedure was stopped by adding stopping solution (3.65 g $EDTA.Na_2.2H_2O$, add pure water to 250 ml).

RESULTS

The commonly-used gel staining plate and the staining result

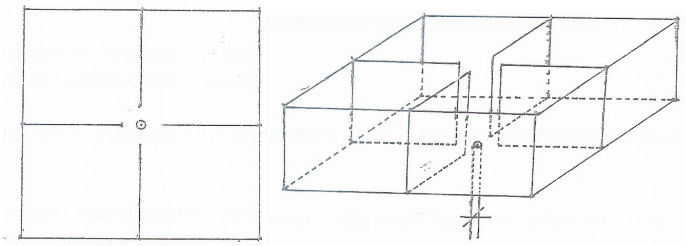
As no specific apparatus for two-dimensional electrophoresis gel staining could be found, the commonly SDS

-PAGE gel staining plate was used, which was single and only one gel can be stained one time (Figure 1). If more gels were put in the plate, some parts of the gels would be covered by others, resulting that the gels were not stained under the same condition. Therefore, when two-dimensional electrophoresis was performed, at least two plates would be used. But the two plates could not be operated by one people at the same time. The alternative approach was that the gels were stained by several people at the same time, one gel by one people. Even if this, it was very difficult for the gels were stained under the same condition and the backgrounds of the gels were different (Figure 2). This increased the difficulties when the differences of the protein spots among the gels were identified (Figure 2). The other problem was that there was no drainage system in the plate. When changing staining solutions was required, the gels would have to be put out from the plate, that easily broke the gels and made further exerperiments difficult (Figure 2B).

A B

Figure 2. the staining result with the commonly used staining plate.

Figure 1. The commonly-used SDS-PAGE gel staining plate.



A **B**

Figure 3. The blueprint of the designed apparatus. A was the horizontal sectional view. B referred to the scenograph.

The new staining apparatus designed

As shown in Figure 3 and 4, a plastic box resistant to acids and bases was compartmented into four identical rooms by clapboards, where one end of the clapboard was fixed with the wall. In the center of the floor, a switch was made to control the staining solution flow out. Five centimeters around the switch, no clapboard was made, resulting that the staining solution can flow freely from different rooms.

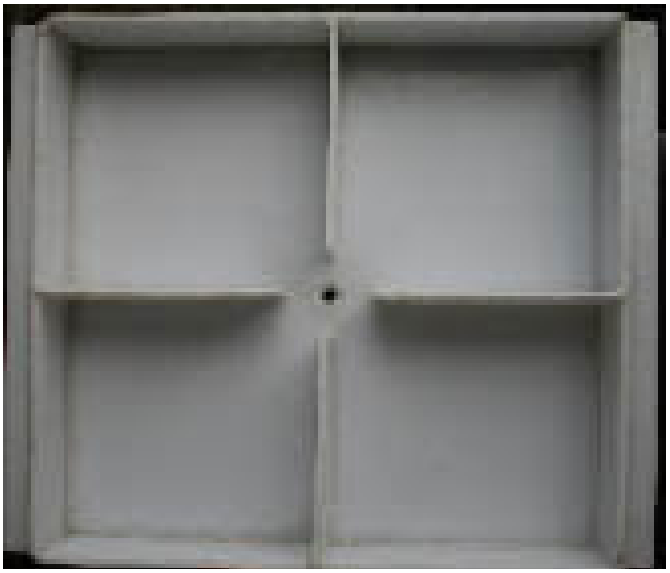


Figure 4. The protein gel staining apparatus designed.

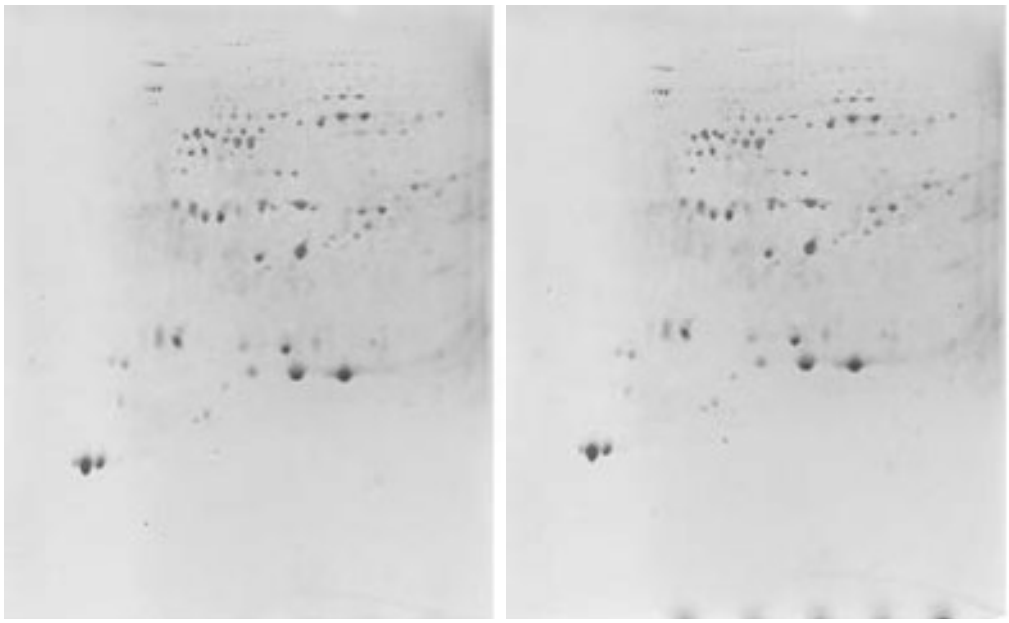


Figure 5. The staining result using the designed staining device.

The gel staining result with the designed apparatus

Using the staining device we designed, the backgrounds among different gels were the same (Figure 5). When the staining solutions were changed, putting gels out from the plates was not prerequisite. It was not necessary to worry about the gels' being broken (Figure 5). The result can be used for further analysis.

DISCUSSION

Two-dimensional electrophoresis was one of the most important tools for proteomics (Keigo et al., 2005; Marie-Christine et al., 2002; Valerie et al., 2004). That all of the gels were treated under the same condition was required except protein samples. The procedures in the two dimensional electrophoresis, such as IPG focuse, SDS-PAGE, rehydration and equilibration can be controlled under the same condition by instruments easily. But it was difficult to stain the protein spots in the two-dimensional electrophoresis gels for no special apparatus could be used.

For the device used in two-dimensional electrophoresis, at least four problems should be considered. The first and the most important was that all gels should be treated under the same condition. Secondly, if all gels were put in one plate to ensure the gels under the same condition, how to prevent the gels from being covered each other? The third was how to shorten the time used for changing solution. During silver staining, it took only several seconds to one minute from the protein spots appearance to the background's becoming dark. How to change the solution as quickly as possible was very important. The fourth problem was how to prevent the gels from being broken. When the common staining plates were used, the gels would have to be put out to change the solution thoroughly. The gels were easily broken, that made the further experiment difficult.

Referring to these problems, a new staining device was designed. The apparatus was comparted into different rooms by clapboards that made the gels not be covered by others. All of the rooms were opened to each other in the center, where the solution can flow freely into every room, which made all gels under the same condition. A switch was made in the floor. When the switch was turned on, the solution in the apparatus would flow out quickly. As the switch was in the center of the apparatus, the solution can flow out at the same speed from all directions.

When the designed apparatus was used, the backgrounds of the different gels were the same and putting gels out of plates was unnecessary. Shortening the experiment time nervously was not necessary either. Just watch the protein spots appearance was enough.

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