Development of a Novel Platform of Proteome Profiling Based on an Easy-to-Handle and Informative 2D-DIGE System

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Proteome profiling based on two-dimensional (2D)-DIGE might be a useful tool for investigating drug-like compounds and the mode of action of drugs. However, obtaining data for profiling requires high labor costs, and it is difficult to control the reproducibility of spot positions because 2D-DIGE usually requires large-size glass plates and spot alignments are greatly affected by the quality of DryStrips and polyacrylamide gels (PAGs). Therefore, we have developed a novel platform by employing small size DryStrips and PAGs, and an image analysis strategy based on dual correction of spot alignment and volume. Our system can automatically detect a large number of consistent spots through all images. Cytosol fractions of HeLa cells treated with dimethyl sulfoxide (DMSO) or bortezomib were analyzed, 1697 consistent spots were detected, and 775 of them were significantly changed with the treatment. Deviations between different days and lot sets of DryStrips and PAGs were investigated by calculating the correlation coefficients. The mean values of the correlation between days and lot sets were 0.96 and 0.94, respectively. Clustering analysis of all the treatment data clearly separated the DMSO or bortezomib treated groups beyond day deviations. Thus, we have succeeded in developing an easy-to-handle 2D-DIGE system that can be a novel proteome profiling platform.

Key words two dimensional electrophoresis; profiling; proteome profile; proteomics

INTRODUCTION

Two-dimensional electrophoresis (2DE) and its extended version, 2D-DIGE, have been used in combination with mass spectrometers to identify proteins relating to the phenotype of interest. Although these types of approaches have contributed to the development of life sciences, it is also true that there are several limitations, because a certain level of the purity and the amount are required for protein identification. The candidate spots obtained by the protein identification strategy tend to be restricted to the proteins that account for a relatively large proportion in the specimen. In fact, it was reported that a limited proportion of human gene products can be visualized in 2DE. Due to the development of omics technologies in recent years, various omics methods have been developed, such as transcriptome, proteome, and metabolome. Omics approaches show their stuff when the data is analyzed focusing on the coordinated changes of the variances. Such approaches, referred to as profile data analysis or simply, profiling, have been the mainstream of omics data analysis. One of the most successful projects utilizing such aspects of omics approaches is the connectivity map initiated by the Broad Institute. These are thought to be powerful tools for searching for drugs that lead to improved disease conditions and for classifying compound effects. Considering the central dogma, it is speculated that proteome has a strong relationship with the phenotype. However, the most popular proteome platform, peptide detection with LC-MS/MS, cannot detect the whole length of a protein, only a fragment of it, which may generate gaps between the real and detected protein amounts. This fact turned us to another proteome platform: 2D electrophoresis, which can detect proteins in the intact form.

With regard to profile data, 2D possesses several potential properties: 2D images visualize the posttranslational modification of the proteins, and moreover, 2D profiles reflect the real-world of intact proteins, which is a property not found in other technologies such as proteome with LC-MS/MS. In fact, Muroi et al. developed an innovative proteome profiling method based on 2D-DIGE called ChemProteoBase for predicting the mechanism of action of compounds. They conducted dozens of 2D experiments and obtained the profiles of the cells treated with low-molecular compounds. By comparing these profiles with the focused one, they succeeded in uncovering the mechanism of action of the target compound, such as natural products and some anticancer drugs with unknown mechanisms.

Thus, proteome profiling based on 2D-DIGE has the potential to be a useful tool for exploring drug-like compounds, repositioning drugs, and evaluating the actions of drugs. However, in cases such as the present one, several concerns about 2D-DIGE remain. 2D-DIGE involves high labor costs because of the large-size glass plates used for the second electrophoresis. In addition, it is difficult to control the reproducibility of electrophoretic images because it depends on the quality of IEF DryStrips, which has a high lot-to-lot difference. We consider that room exists for further improvement. Although

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several thousand spots are usually visualized at once in 2DE, many researchers have focused on only limited ones, even in successful ChemProteoBase, analyzing 296 spots that were quantitatively detected and identified with LC-MS/MS. Using many of detected spots could lead to more sensitive analyses of various compounds. Superposition of 2D images by 2D-DIGE is usually performed on the internal standard images for both quantitative value correction and spot alignment (image) correction using two fluorescent dyes. Introducing an additional image of a common specimen stained with another dye would be useful for image alignment and could expand analysis targets on one platform.

In order to address the above concerns and examine the possibilities, in the present study, we established a novel proteome platform based on 2D-DIGE. Using 100 × 100 (mm) size gel and a new DryStrips optimized for size reduction and profiling, we obtained clear 2DE images with high reproducibility. More than 1000 spots can be quantified from these images, which provide sufficient quality as profile data by adding Cy2-labeled and Cy5-labeled internal standards for image alignment and spot intensity correction, respectively, and by using image analysis software optimized for this purpose (Fig. 1). Here, we report the performance of our novel 2D-DIGE system.

MATERIALS AND METHODS

Materials The following reagents were used in this study: Bortezomib and MG132 (Wako, Osaka, Japan), NE-PER Nuclear and Cytoplasmic Extraction Kit (78833, Thermo Fisher Scientific Inc., Tokyo, Japan), and ProteoExtract® Protein Precipitation Kit (539180, Merck, Darmstadt, Germany). DryStrips (Prollex DryStrips, pH 3–10, 7 cm, 1.5 mm, ProMedico, Tokyo, Japan) and polyacrylamide gel (Prollex PAG, 12%, ProMedico) were purchased through WAKEN B TECH Co., Ltd. (Kyoto, Japan). All other chemicals were of analytical grade.

Cell Cultures Sf9 cells were cultured in SF-900 II SFM medium (Thermo Fisher Scientific) under serum-free conditions at 28°C. When the cell density reached 4.0 × 10⁶ cells/mL, Sf9 cells were centrifuged to form pellets and the supernatant was removed. The pellets were then resuspended with phosphate buffered saline (PBS). This centrifugation and subsequent process were repeated three times. Finally, the suspension was centrifuged at 3000 rpm, 15 min at 4°C, and then the supernatant was carefully removed and stored at −80°C until use.

HeLa cells (1 × 10⁶ cells/dish) were seeded on a 100-mm culture dish with 10 mL Dulbecco’s modified Eagle’s medium (DMEM) (10313-021, Life Technologies, Carlsbad, CA, U.S.A.) with 10% Fetal Bovine Serum and 1% MEM non-essential amino acids (11140-050, Life Technologies) and incubated overnight at 37°C under 5% CO₂. Dimethyl sulfoxide (DMSO) or bortezomib (50 µM solubilized with DMSO) was added into the culture medium and incubated for 18 h at a concentration of 50 nM. Cells were rinsed twice with ice-cold PBS, scraped and collected in 1.5 mL tube, and centrifuged at 3000 rpm, 5 min at 4°C. The supernatant was then carefully removed, and the pellets were stored at −80°C until use. Human tumor cell lines HT-1080 (fibrosarcoma; Health Science Research Resources Bank, Osaka, Japan), T24 (bladder carcinoma; American Type Culture Collection, Manassas, VA, U.S.A.), and MDA-MB-231 (breast carcinoma; American Type Culture Collection) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS). Pellets of these cells were collected using the same method described above.

Sample Preparation Pellets of Sf9 cells were resuspended in initial lysis buffer (40 mM Tris [pH 8.8], 1% CHAPS, 5 mM MgCl₂, 1/200× protease inhibitor cocktail [Nacalai Tesque, Kyoto, Japan], 1/100× nuclease mix [GE Healthcare

Fig. 1. Overview of 2DE Image Analysis

First, Cy2 images on each gel are corrected to the Cy2 standard image to determine the deformation field to each gel. Second, the deformation field is applied to Cy3 and Cy5 images to standardize them. Third, a master spot map is calculated from the arbitrary selected Cy5 images and their deformation fields. Fourth, using the master spot map, all spots visualized on Cy3 and Cy5 images are modeled to calculate each spot volume. Finally, the Cy3 spot volume is corrected using the Cy5 spot volume.

(Color figure can be accessed in the online version.)
Japan, Hino, Japan) with 1/20 volume of culture media, and left standing on ice for 30 min. Ultrasonic disruption was carried out for 15 min. Subsequently, an equal volume of secondary lysis buffer (40 mM Tris [pH 8.8], 8 M urea, 2% CHAPS) was added and mixed. Ultrasonic disruption was carried out for 5 min, followed by centrifugation at 20000 × g, 30 min at 20°C. The solvent of the supernatant was replaced with secondary lysis buffer using a desalting column (GE Healthcare) and then stored at −80°C until fluorescent labeling. The lysates of SF9 cells were labeled with Cy2 (CyDye DIGE Fluor minimal dyes; GE Healthcare) according to the manufacturer’s instructions. Cytoplasmic fractions from mammalian cell pellets were prepared using the NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Fisher Scientific) following the manufacturer’s protocol. Protein concentration was estimated using the bicinchoninic acid protein assay using bovine serum albumin (BSA) as standard. Cytosol fractions (100 µg) were cleaned and precipitated using the ProteoExtract® Protein Precipitation Kit (MSD, Tokyo, Japan) following the manufacturer’s protocol. Precipitated pellets were dissolved with 90 µL of labeling buffer (8 M urea, 4% (w/v) CHAPS, 30 mM Tris [pH 8.0]). The specimen (5 µg) was labeled with 1 µL of 2 mM CyDye DIGE Fluor saturated dye (GE Healthcare) according to the manufacturer’s instructions. Additionally, the coupling reaction was terminated by adding 0.5 µL of 8 mM dithiothreitol (DTT). Cy3 and Cy5 labeling were performed for the specimen from the perturbation-treated HeLa cells as quantitative target samples, and for the internal standard specimens that were mixed equivalently with HeLa, HT1080, T24, and MDA-MB-231 cells (reagent-treated cells were equivalently mixed in the same way), respectively.

**Western Blotting** Specimens were loaded into the wells of a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) plate with a 3.75% stacking gel and subjected to Western blotting, as described previously. Anti-ubiquitin antibody (P4D1) was purchased from Santa Cruz Biotechnology (Dallas, TX, U.S.A.). MG132 (5 µM) was employed as a positive control to detect ubiquitinated proteins (Supplementary Fig. 1). Immunoreactivity was detected using an ECL Prime Western blotting Detection Kit (Amersham Biosciences, Piscataway, NJ, U.S.A.).

**2D-DIGE** Cy3-labeled proteins (3 µg), Cy5-labeled proteins (3 µg), Cy2-labeled proteins (6 µg), 0.43 µL immobilized pH gradient (IPG) buffer (pH 3–11 NL, GE Healthcare), and 1.1 µL hydroxyethylidisulphide (Merck) were mixed and diluted with rehydration buffer (8 M urea, 4% (w/v) CHAPS, trace Bromophenol Blue) up to 90 µL. The mixture was applied onto immobilized pH gradient strips (Prollex DryStrips, ProMedico) overnight with cover fluid (GE Healthcare) to be rehydrated and then subjected to IEF using the IPGphor IEF system (GE Healthcare). IEF voltage was as follows: 250V, 0.5 h; 250–1000 V gradient, 0.5 h; 1000–8000 V gradient, 1.5 h; 8000 V, 2.0 h. Strips were incubated in equilibration buffer (6 M urea, 30% glycerol, 2% SDS, 50 mM Tris–HCl [pH 8.8]) containing 1% DTT for 20 min and then incubated in the same buffer containing 2.5% iodoacetamide for 20 min. The strips were transferred onto the top of 12% polyacrylamide gels (Prollex PAG, ProMedico) and electrophoresed. After electrophoresis, the gels were scanned using a Typhoon 9100 imager (GE Healthcare) at 50 µm/pixel resolution. Here, we define “day variations” as the variations between 2D-DIGE experiments. Thus, to evaluate day variations, 2D-DIGE of the same lot sample was performed three times on different days (technical replicates across days).

**Image Analysis** We used an image analyzing service by ProMedico (currently provided by WAKEN B TECH). The backgrounds of all images were removed by the rolling ball method (radius: 30 pixels). For determining the deformation field for each gel, which corrects the distortion of all gels, all Cy2 images obtained from each gel were matched to the arbitrarily selected Cy2 image as the standard image. We hypothesize that spot shape follows a Gaussian distribution. To catch low intensity spots, a spot detected as a mixture of several Gaussian distributions after deformation is separated into the number corresponding to a fitting parameter (the Gaussian separation parameter). We set the Gaussian separation parameter at “2” (default setting) in this study. The master spot map, which defines the positions and shapes of all spots on the image, was calculated from five arbitrarily selected Cy5 images and their deformation fields using our own algorithm. The master spot map was applied to all images to calculate the temporal volume of all spots. The temporary volume of all spots was normalized so that the total amount became 10^6 for each image and the normalized volume was taken as the raw spot volume. The corrected spot volume on each Cy3 image is obtained using the following formula:

\[
\log CV_{\text{Cy3}} = \log V_{\text{Cy3}} - \log V_{\text{Cy5}} + \log \left( \sum_{i=1}^{N} V_{\text{Cy5},i} \right) / N
\]

where \( CV_{\text{Cy3}} \), \( V \), \( I \), \( R \), and \( N \) denote the corrected spot volume, the raw spot volume on each image, the gel number of all samples, the arbitrarily selected Cy5 images, and the number of arbitrarily selected Cy5 images, respectively. As a reference analysis, we utilized ImageMaster 2D Platinum 7 (GE Healthcare) for the same image data according to the manufacturer’s protocol.

**Statistical Analysis** Various statistical analyses were performed with Python 3.6.6 using the sklearn and scipy libraries. A comparison of cosine distances and determination of differentially changed spots (DCSSs) were conducted using Welch’s t test considering the reliability of a normality test (such as the Shapiro–Wilk test) for small sample size data. \( p \) values of cosine distances and DCS were corrected by a familywise error rate using Bonferroni method and by a false discovery rate using the Benjamini–Hochberg method, respectively (alpha < 0.05). Among the statistically significant spots, those whose absolute values are more than 1 are defined as DCSSs. Unsupervised clustering analysis was performed using Ward’s method.

With regard to comparing day variations with drug effects, we have conducted multivariate ANOVA (MANOVA) with following model:

\[
Y = W\beta + E,
\]

where \( Y \) is the observed matrix, \( W \) is the design matrix, \( \beta \) is the matrix representing parameters, and \( E \) is the matrix of random errors. Firstly, principal component analysis was carried out to contract the dimensions of spot data. The scores of the obtained top 2 features (PC1 and PC2) and days and treatments were set as dependent variables and independent variables, respectively. Then, MANOVA was conducted with
Pillai’s trace considering its relative robustness in small sample size.

RESULTS

Concept of Novel Proteome Platform Based on 2D-DIGE

The concept of our 2D-DIGE system is shown in Fig. 1. First, 2D-DIGE images are obtained by the combination of 100 × 100 (mm) “mini”-size gel and a new DryStrips developed by ProMedico. The obtained electrophoretic images are corrected based on Cy2 images (image correction), which enables the images to overcome the effects of deformation on spot detection in electrophoresis. The master spot map generated from several Cy5 images after image correction determines the positions and shapes of the spots, corrects the volume of the spots in Cy3 images (quantitative value correction), and provides a profile data matrix consisting of the quantitative values of the spots. We hypothesize that the spot shape in the master spot map is a Gaussian distribution. The position, height, and SD of both the lateral and vertical directions on the Cy5 images are employed for determining the parameters of the distribution.

Acquisition of 2D-DIGE Profile Data

The cytosolic fraction obtained from HeLa cells treated with DMSO or bortezomib (50 nM) for 18 h was analyzed using our 2D-DIGE system. We determined the concentration of bortezomib because it induced the accumulation of ubiquitinated proteins, but did not cause cell death (Supplementary Fig. 1). Bortezomib is a proteasome inhibitor and has been approved as an anticancer drug against multiple myeloma. Considering the mode of action, its effects on cellular machineries are considered to be relatively high, which was the reason we chose this drug as a test compound in the present study. In order to test the reproducibility of the platform, 2D-DIGE was conducted on 3 separate days with two sets of DryStrips and PAGs with different lots. For each day or set, 2D-DIGE was conducted twice ($N = 2$). Typical images of raw data obtained from one 2D-DIGE result are shown in Fig. 2A. The master spot map was generated from the sets of 2D-DIGE results. Figure 2B shows an artificial image reconstituted from the data based on the master spot map. At that time, 1697 spots were detected as “master spots” in the master spot map generated by co-detecting spots on five arbitrarily selected Cy5 images, which means that we can obtain profile data consisting of 1697 consistent spots from any number of gels. On the other hand, ImageMaster, a relatively old but well used spot-detecting software, detected 719 consistent spots (Supplementary Fig. 2). To obtain insight into the performance of image correction, we compared the degree of overlapping before and after image correction. In Fig. 2C, Cy5 images from two independent gels are superposed and overlapped well after image correction (right) compared with the one before correction (left). The spot volume data were transformed by taking the logarithm, and is available as a matrix in Supplementary Materials.

Data Analysis

First, we investigated day variations by comparing 12 sets of data obtained on 3 different days and analyzing the HeLa cells treated with DMSO or bortezomib. Figure 3A shows a scatterplot of a pair of Days 1 and 2 data.
for a DMSO- or bortezomib-treated sample as an example. The correlation coefficients of these representatives were 0.962 and 0.961 for the data derived from the DMSO- and bortezomib-treated cells, respectively. On the other hand, the correlation coefficient between the data for the DMSO- and bortezomib-treated sample obtained on Day 1 was 0.927, less than those between days (Fig. 3B). To obtain insight into the effect of day variations and drug treatment, the correlation coefficients of all pairs of both groups were calculated, and are summarized in Supplementary Materials. Figure 3C shows a scatterplot of principal components of all pairs of day and treatment variation data. Open and closed circles represent DMSO and bortezomib treated samples obtained different days, respectively. (D) A representative scatterplot and correlation coefficient of the data obtained from different lots of IEF DryStrips and PAGs (lot set). R, correlation coefficient. (E) Plot of the cosine distance of all pairs of lot sets. Multiple hypothesis testing was corrected with Bonferroni adjustment. *p < 0.05; **p < 0.01. (F) The histogram of the CV value in the case of using raw spot volume (red) or corrected spot volume (blue). (G) The result of clustering (ward method) using spot volumes obtained from 12 images of HeLa cells treated with or without bortezomib from 3 different days. (Color figure can be accessed in the online version.)

Table 1. Result of MANOVA with Scores of Top Two Principal Components as Dependent Variables, and Days and Treatments as Independent Variables

| Value    | Num DF | Den DF | F Value  | Corrected p |
|----------|--------|--------|----------|-------------|
| (Intercept) | 0.9854  | 2.0000 | 7.0000   | 236.0943    |
| Days     | 0.8275  | 4.0000 | 16.0000  | 2.8227      |
| Treatment| 0.9957  | 2.0000 | 7.0000   | 807.8524    |

Analysis was conducted with statsmodels module from sklearn, Python. Multiple testing was corrected with Bonferroni adjustment. Value, Pillai’s trace; Num DF, degree of freedom of numerator of F value; Den DF, degree of freedom of denominator of F value; F value, F value of each factor; Corrected p, corresponding p values.

All pairs showed that the correlation coefficients between days were significantly larger than those between treatments. This is consistent with the correlation matrix visualized as a heat map, in which the data is clearly separated by the treatment (Supplementary Fig. 3). Lot-to-lot differences in DryStrips are a critical problem for 2DE reproducibility. We have compared different lots of DryStrips and PAGs. Figure 3D shows a scatterplot of a pair of lot sets A and B with high correlation coefficient (R = 0.946). In addition, the cosine distance between all pairs of the two sets of DryStrips and PAGs of different lots were calculated, and are visualized in Fig.

(A), (B) A representative scatterplot and correlation coefficients of spot volumes obtained from HeLa cells treated with DMSO or bortezomib on different days. R, correlation coefficient. (C) Scatterplot visualizing the scores of principal components of all pairs of day and treatment variation data. Open and closed circles represent DMSO and bortezomib treated samples obtained different days, respectively. (D) A representative scatterplot and correlation coefficient of the data obtained from different lots of IEF DryStrips and PAGs (lot set). R, correlation coefficient. (E) Plot of the cosine distance of all pairs of lot sets. Multiple hypothesis testing was corrected with Bonferroni adjustment. *p < 0.05; **p < 0.01. (F) The histogram of the CV value in the case of using raw spot volume (red) or corrected spot volume (blue). (G) The result of clustering (ward method) using spot volumes obtained from 12 images of HeLa cells treated with or without bortezomib from 3 different days. (Color figure can be accessed in the online version.)
first electrophoresis enables a PAG size reduction to 100 × 100 (mm) without a decrease in resolution in the final 2DE images. We consider that the PAG size reduction has greatly improved handling of the gels in such cases where lots of 2D-DIGE are performed at once.

With regard to a spot detection strategy, we have introduced a novel system. In the conventional system, it is necessary to select the landmark spots by hand to obtain consistent spots through all images. This process is time consuming and virtually impossible for profile data analysis, which handles dozens of images. Strong spots, for instance, those derived from housekeeping proteins, tend to be prioritized compared with the weak spots. Considering the property of profiling, weak spots are of significance as well. The reproducibility of the positions of spots is essential, while extracting only the consistent spots reduces the number of available spots in correlation with the number of images for superposing. In this study, we found one of the answers to these problems by utilizing two different internal standards: a Cy2-stained specimen, which is common in many experiments (image alignment), and a Cy5-stained specimen containing similar proteins to the target to be analyzed (quantitative value correction). Separating the internal standards for both corrections generates a master spot map, which is well corrected against image deformation, and a large number of spots are stably detected based on the master spot map. In addition, the dual correction system can evaluate various samples on one platform regardless of sample origin, such as specimens from cultured cells and human tissue, because the spot positions are aligned by the images of the common specimen, such as Sf9 lysate in the present study. Strong signal spots obtained from Sf9 lysate spread over the whole regions of gels and is observed in different locations from those of the specimens derived from humans. Therefore, improvement in the overlapping accuracy of even weak signal spots derived from humans can be expected. Notably, all processes are automated after determining the field to be evaluated in images, and the spots are derived from exactly the same positions regardless of the number of images increases. These two properties in spot detection, positional consistency and automatic detection, are indispensable for profile data analysis.

In this study, our system detected 1697 consistent spots. This number is larger than those obtained by ImageMaster or employed in ChemProteoBase, which supports the usefulness of the dual internal standards system (Supplementary Fig. 2).

It should be noted that we have not precisely tested whether the spots reflect biologically meaningful changes in this study, although it is partially supported by the results of the clustering analysis in Fig. 3G. Bortezomib is a proteasome inhibitor that is approved for treating patients with several types of cancer, such as multiple myeloma. Considering the mode of action, this anti-neoplastic agent affects relatively large parts of cellular machineries, which may be reflected by the clear separation in the clustering analysis. The proportion of significantly changed spots and DCSs of DMSO vs. bortezomib to the total was 0.46 and 0.14 in this study, respectively.

We should know whether this value is high or low. It is essential to collect 2D-DIGE data analyzing the cells treated with several types of drugs or compounds in the range of weak-to-strong or narrow-to-broad effects. Profile data analysis of such series is an essential future task to grasp the prop-

### Table 2. Statistics of CV Values of Spot Volume

|         | Cy3  | Cy3/Cy5 |
|---------|------|---------|
| Minimum | 0.037| 0.010   |
| 1st Quartile | 0.26 | 0.13    |
| Median  | 0.40 | 0.23    |
| 3rd Quartile | 0.63 | 0.41    |
| Maximum | 2.2  | 2.2     |

Spot volume was calculated from six images obtained from the DMSO-treated cells. CV values were calculated and summarized. The number of spots was 1697. Cy3 and Cy3/Cy5 indicate the spot volume of Cy3 without or with normalization by Cy5 volume.
erties of profiling with our 2D-DIGE system.

Compared with proteome platforms using LC-MS/MS, 2DE is inferior in terms of variance annotation because this platform lacks information of annotation by itself; however, annotation is not absolutely necessary for profile data analysis. In addition, 2DE profiling can utilize rich information from the real world of intact proteins. Although proteome platforms using LC-MS/MS are promising for profile data analysis, they detect peptides generated by trypsinization of the proteins in specimens. Focusing on these differences, a comparison of two proteome platforms would shed light on each property; this remains to be investigated.

In general, high-dimensional data such as profile data is almost impossible to perceive by researchers (curse of dimensionality). Therefore, to comprehend profile data, dimension reduction is necessary, and many mathematical methods exist. It is important to choose an appropriate method that matches the goal of each researcher. Recently, we developed an unsupervised analysis method for dimension reduction: orthogonal linear separation analysis (OLSA). OLSA is based on factor analysis and a linear analysis, which means that the method makes it possible to come back to the original variances after dimension reduction, which is difficult using nonlinear analyses. To our knowledge, no studies have investigated dimension reduction methods appropriate for 2DE profile data because there has been little information of the data properties to date. These tasks remain to be elucidated after accumulating 2DE profile data.

Through this study, we have developed a proteome profiling platform using 2D-DIGE that is expected to expand the potential of 2DE and to be more versatile and convenient than existing methods. We hope that this platform will be useful for various studies and help contribute to drug discovery.

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Conflict of Interest Setsuo Kinoshita and Megumi Hori are employees of ProMedico Co., Ltd. Michiaki Kohno is a scientific advisor of WAKEN B TECH Co., Ltd.

Supplementary Materials The online version of this article contains supplementary materials.

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