attained, the development was stopped with 5% acetic acid and, after 10 min, the gel was finally washed in distilled water and air-dried onto the glass support. Air-drying allows the long and thin gel to be easily submitted to photographic and/or digital recording using a sequencing gel-sized scanner, such as the HP ScanJet 4C/T (Hewlett-Packard, Palo Alto, CA, USA). This avoids wet-handling or the need of an apparatus for gel-drying between cellophane sheets. Once examined and/or recorded, the dried gel can be detached from the glass plate after overnight rinsing with warm 5 mol/L NaOH and the latter reused after extensive washing with detergent, ethanol and distilled water.

In our laboratory, we have applied the full-length electrophoresis technique several times to the study of the pattern of proteins synthesized by MDA-MB231 tumor cells in response to microenvironmental stimuli (data not reported). Figure 1 shows that the protein gels submitted to full-length electrophoresis exhibit a sensitive staining of protein bands (from 30–60, depending on acrylamide concentration) and a low level of background, which make them suitable for documentation and for software-assisted qualitative and quantitative analysis of the electrophoretic pattern. The number of resolved proteins cannot be compared to that obtained by 2-D electrophoresis (see the 2-D protein map from MDA-MB231 cells available online at http://www.anl.gov/BIO/PMG/projects/index_hbreast.html). Nevertheless, full-length 1-D electrophoresis is a faster method that (i) does not require special consumables and expensive apparatus (such as ampholytes and IPG gel systems), (ii) improves the resolution of the pattern of polypeptides submitted to monodimensional electrophoresis and (iii) expands the applications of the DNA sequencing apparatus for protein studies.

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Claudio Luparello
University of Palermo
Palermo, Italy

Rapid Histone Extraction for Electrophoretic Analysis


Histones, the class of basic proteins that complex with DNA to form chromatin (11), consist of multiple post-translationally modified forms and iso-protein species (13). Several of these have recently been specifically implicated in such cellular functions as transcription (2), detection of DNA damage (7,8) and mitosis (12), as well as a human genetic disease, Coffin-Lowry syndrome (9). While the major histone protein families can usually be resolved on SDS gels, these gels are not capable of resolving either the histone isoprotein species or the post-translationally modified forms. On the other hand, much of the complexity of histone species and forms can be resolved on acidic gels with or without Triton® X-100 (1,6,14). However, these gels have not been as widely used as the SDS gels because sample preparation is more laborious, often requiring dialysis or precipitation steps that can lead to selective loss, degradation and oxidation of histone forms.

Histones can be extracted in 0.5 mol/L HCl or 0.25 mol/L H2SO4 solutions and analyzed on gels containing acetic acid. Bonner et al. (1) had shown that it is possible to neutralize the extraction acid, add urea and acetic acid and, without further preparation, load the samples onto a type of acetic acid gel that incorporates a discontinuous buffer system. Discontinuous buffer systems permit the concentration of protein components from varied ionic solutions (4,5). Because both the extraction and gel buffers for histones are acidic, we decided to examine whether these gel systems are compatible with a strongly acidic sample. If they were found to be so, then the histone extraction buffer could also serve as the gel loading buffer, thus greatly simplifying histone sample preparation. In this report, we describe procedures by which histone extracts of cells and tissues can be transferred directly from nuclear pellets onto these types of gels. In addi-
tion to a variety of mammalian cell types, these procedures have been used with *Xenopus laevis* cells, *Drosophila melanogaster* cells and *Saccharomyces cerevisiae* spheroplasts.

For attached mammalian cell cultures containing $10^6-10^7$ cells, the growth medium is removed, a 0.5–1.0 mL aliquot of lysis buffer (0.01 mol/L Tris-Cl, pH 7.5, 0.001 mol/L MgCl$_2$, 0.5% NP40) is added to the monolayer and the cells are scraped off the bottom of the dish. For unattached cells, an aliquot of culture medium containing $10^6-10^7$ cells is centrifuged at 2000×g for 5 min, and the cells are resuspended in 0.5–1.0 mL of lysis buffer. These cells are transferred to a 1.5 mL tube that is centrifuged for 3 s in a microcentrifuge capable of 10,000×g. The centrifuge is not advised to go top speed; the purpose is to pack the nuclei tightly enough so that the supernatant can be removed, but at the same time, loosely enough so that the pellet can be easily resuspended. The supernatant fraction is aspirated away leaving a pellet containing 5–50 µL. The nuclear pellet is resuspended in 3 vol (15–150 µL) of extraction solution (0.5 mol/L HCl or 0.25 mol/L H$_2$SO$_4$, with 10% glycerol and 0.1 mol/L 2-mercaptoethanol-HCl) and centrifuged at 10,000×g for 5 min. After centrifugation, an aliquot of the supernatant fraction is carefully pipetted off the nuclear pellet and directly into a well of an acetic acid gel prepared with discontinuous buffer system (4,8). Methylene blue may be added to the extraction solution as a visual aid for sample loading and for marking the buffer discontinuity during electrophoresis.

After appropriate adjustments for cellular histone content, the above-described procedure can presumably be used with any cell type that can be lysed with nonionic detergents. For *D. melanogaster* cells with 5% and haploid *S. cerevisiae* spheroplasts with 0.3% the DNA content of mammalian cells, a sample contains 20 and 300 times, respectively, as many cells as a sample from mammalian cells. For Coomassie® Brilliant Blue R-250 (Sigma, St. Louis, MO, USA) staining of bands on two-dimensional (2-D) gels, at least 10$^6$ mammalian cells, 2 × 10$^7$ *D. melanogaster* cells and 3 × 10$^8$ *S. cerevisiae* spheroplasts are needed, but smaller amounts are usable with more sensitive methods of detection.

The Bradford assay can be used to determine protein concentration if desired because there is little interference from sulphydryl compounds. In some experiments with yeast, we have used protease inhibitors in the extraction solutions, but have not noticed any difference in the resultant gel patterns. When protease inhibitors were added to the extraction solution, the final concentrations were 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L 4-[(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride], 5 µg/mL each of aprotonin, leupeptin and pepstatin A.

Histones have been prepared from tissues, including mouse liver, heart, brain, testes and ovary in a similar manner after using a homogenizer to disrupt the tissue. The tissue of interest is removed, diced, suspended in cold homogenization buffer (0.01 mol/L Tris-HCl, pH 7.5, 0.001 mol/L MgCl$_2$) at a ratio of 0.5 g wet weight per 3 mL and homogenized until no tissue fragments are visible, usually approximately 10 s. The homogenizer speed is adjusted to just below the point at which foaming occurs. NP40 is added to the suspension to a final concentration of 0.5%, and homogenization is continued for another 10 s at slower speeds, again just below the point at which foaming occurs. With a Polyclot® Model PCU2, a 12 mm generator (Kinematica, Luzern, Switzerland), and a 3 mL sample volume, power settings up to the maximum of 10, could be used before adding NP40 and settings up to 4 after adding NP40. A 1 mL aliquot of the suspension is transferred to a 1.5 mL tube, and the nuclei pelleted with a 3 s centrifugation at 10,000×g. After the supernatant is removed, the nuclear pellet is resuspended in 3 vol of extraction solution and processed as described above.

Histones were found to be extracted without a specific incubation period. When nuclei were extracted 3× in succession with no incubation except for the 5 min centrifugation, all the histone was found in the first extract except for traces in the second, and none was detected in the third. Similarly, when the nuclear pellet remaining after the three extractions was dissolved by boiling in SDS gel sample buffer and analyzed by SDS gel electrophoresis, no histones were detected. If a more concentrated histone extract is needed, 2 vol of 0.6 mol/L or 1 vol of 0.8 mol/L HCl with 10% glycerol and 0.1 mol/L 2-mercaptoethanol-HCl can be used. These latter modifications may be useful for *D. melanogaster* cells, *S. cerevisiae* spheroplasts and other cell types that have smaller cellular DNA contents than do mammalian cells.

Samples may be stored at 4°C in extraction buffer for several days or analyzed immediately. For long-term storage, samples should be kept at -20°C or below. Upon thawing, the samples are centrifuged to pellet the nuclear residue just before loading the gels. Extraction solutions without reducing agents are usable as long as the pH remains <1. Those containing H$_2$SO$_4$ are more stable than those containing HCl because of the volatility of the latter. Reducing agents such as 2-mercaptoethanol-HCl are added to the extraction solution just before use to prevent disulfide formation between cystine residues in H3 and oxidation of methionine residues.

Rogakou et al. (8) described the conditions for 2-D gel analysis of histones on acetic acid gels containing a discontinuous buffer system. Histone gels comprise a first acetic acid-urea-Triton X-100 (AUT) dimension followed by a second acetic acid-urea-cetyltrimethylammonium bromide (AUC) dimension. All gel solutions contain the common components acetic acid (1 mol/L), ammonia (0.03 mol/L), TEMED (0.5%) and riboflavin (0.0004%). The various gel solutions, which differ in their concentrations of urea, acrylamide, bisacrylamide and whether or not they contain Triton X-100, are all polymerized by placing the filled shells between two vertical fluorescent light boxes.

We use two recipes for first-dimension resolving gels. The first, 18.5% polyacrylamide, resolves all the histones including H4, but the H2A region is crowded. The second, 12% polyacrylamide, resolves the H2A region well. In addition to the common components listed above, the 18.5% first-dimension resolving gel contains urea (8 mol/L), acrylamide (18.5% from a 40% stock), bis-acrylamide (0.1% from a 2% stock) and Triton X-100 (0.5% from a 25% stock). The 12% resolving gel differs...
The resolving gel solution is poured into a shell and polymerized, leaving space at the top for the stacking gel and an equal space for the sample comb. The stacking gel solution is poured in, the comb inserted and the solution polymerized. The one-dimensional (1-D) running buffer contains acetic acid (1 mol/L) and glycine (0.1 mol/L). When finished, the gel is stained in a solution containing Coomassie Blue R-250 dye (0.1%), acetic acid (6%), ethanol (40%) and destained in a solution containing acetic acid (6%) and ethanol (20%). A few crystals of mercaptoethylamine-HCl are added to the stain and destain just before use to decrease in-gel oxidation of H3 and H2A2 if the 1-D gel samples are destined for 2-D analysis.

In addition to the common components listed above, the 2-D resolving gel contains urea (5 mol/L), acrylamide (20%) and bis-acrylamide (0.1%). The 2-D stacking gel contains urea (5 mol/L), acrylamide (5%), bis-acrylamide (0.16%) in addition to the common components. The resolving gel solution is poured into a shell thick enough to receive a 1-D gel inside and polymerized, leaving space at the top for 1-D gel samples and an equal space for the stacking gel. The stacking gel solution is poured in and polymerized. The lane or region of interest of the 1-D gel is incubated in soaking solution containing acetic acid (1 mol/L), ammonia (0.03 mol/L), and mercaptoethylamine-HCl (1%) for 30 min, then slide down the opening of the 2-D gel shell until it lies on top of the stacking gel. It is embedded in place with a melted agarose solution (1%), to which acetic acid (1 mol/L) and ammonia (0.03 mol/L) are added after melting. After the agarose has gelled, 2-D running buffer containing acetic acid (1 mol/L), glycine (0.1 mol/L) and cetyltrimethylammonium bromide (CTAB, 0.15%) are added, and electrophoresis is begun. When finished, the gel is stained and destained as before.

The miniformat, 10 x 10 cm, commercially available gel apparatus can be used for most histone analyses except for those requiring the highest resolution. The 1-D gel is prepared in a 1 mm thick minigel shell and the second in a 1.5 mm thick shell (Novex Novel Experimental Technologies, San Diego, CA, USA). For the 1-D gel, a 1 mm thick shell contains 12% or 18.5% resolving gel, 1 cm of stacking gel and 1 cm of sample wells. After the samples are applied and the running buffer added, electrophoresis is performed at 100 V until the methylene blue dye in the samples nears the bottom, 3–4 h.

For the 2-D gel, a 1.5 mm thick shell contains resolving gel, 1 cm of stacking gel and 1 cm of space for the 1-D gel sample. The stained 1-D gel samples, incubated as described above are embedded on top of a 2-D stacking gel. After running buffer is added, electrophoresis is performed at 100 V until the Coomassie Blue dye from the 1-D gel samples near the bottom, 3–4 h.

If higher resolution is needed, gels are prepared in shells in which the methylene blue sample dye migrates at least 15 cm in the first dimension (Figure 1A), and the Coomassie Blue dye from the sample gel migrates at least 20 cm in the second (Figure 1B). For the 1-D gel that is prepared in a shell 36 cm wide, 45 cm long and 0.4 mm thick as used for DNA sequencing (8), the shell contains 12% or 18.5% resolving gel, 2 cm of stacking gel above and 2 cm of sample wells on top.

After the samples are applied and the running buffer added, electrophoresis is performed at 10 W overnight. For the 2-D gel that is prepared in a shell 36 cm wide, 30 cm long and 1 mm thick, the shell contains resolving gel, 3 cm of stacking gel above and 3 cm of space for the 1-D gel sample on top. The stained 1-D gel samples, incubated as described above, are embedded on top of the second dimension stacking gels. After the running buffer is added, electrophoresis is started at 67 mA constant current per gel, approximately 12 W per gel. When the wattage reaches 26 W per gel, the setting is switched to constant wattage at 26 W until the Coomassie Blue migrates 20–25 cm. The total time of electrophoresis is approximately 7 h.
contains less polyacrylamide, 12% (Figure 1C) compared to 18.5% (Figure 1B). Figure 1C also demonstrates how similar spot patterns can result from different causes. Three unmodified parental forms of histone H2A are identified by the vertical dotted lines. The dashed lines refer to the N-terminally modified forms, the first (α) being H2A molecules with either a phosphate on serine residue 1 or an acetate on lysine residue 5, the second with both modifications (not visible in Figure 1C). The origins of the other forms differ in each case. The notation “ox” (Figure 1C) refers to a form of H2A2 with a methionine oxidized by the combination of Coomassie Blue stain and bright light while in the 1-D gel. These oxidized forms can be eliminated by adding a crystal of 2-mercaptoethanol-HCl to the stain and destain and keeping the stained gel soaked in a solution containing Tris base and 0.9% acetic acid. One- and two-dimensional gels stained with Coomassie Blue may be electroblotted onto PVDF membranes for Southern, northern, western and northwestern analysis.

Histones may be immunoblotted by several different procedures (3,11). Since histones are highly cationic proteins while their SDS complexes are anionic, polarity is important. One dimensional gels containing Triton X-100 may be immediately electrobotted at 25 V for 1 h onto PVDF membranes placed between the gel and the negative electrodes in a transfer buffer containing acetic acid (0.9%). Two-dimensional AUT-AUC gels containing the detergent cetyltrimethylammonium bromide may be electrobotted using the same parameters after being soaked for 1 h in 0.9% acetic acid. One- and two-dimensional gels stained with Coomassie Blue may be electrobotted onto PVDF membranes placed between the gel and the positive electrodes after being soaked in a solution containing Tris base (0.012 mol/L) and SDS (0.1%) for 1 h. The transfer is performed for 1 h at 25 V in a solution containing Tris base (0.01 mol/L), glycine (0.1 M), SDS (0.1%) and methanol (20%). The Coomassie Blue stain will also transfer to the membrane under these conditions, giving a replica of the stained gel. Although the staining pattern remains through the blotting procedure and permits ready localization of components, the stain may quench luminescent emissions.

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Construction of a Rotisserie Hybridization Apparatus


The hybridizer described in this report is an inexpensive alternative to plastic bags, dishes and commercial rotisserie hybridization systems. It can be used for incubating and washing membranes for Southern, northern, western and northwestern analysis.

This system uses a retail barbecue rotisserie motor, standard 50 mL conical bottom centrifuge tubes and common hardware items.

For materials we used: an electric universal rotisserie kit (Waye-mar Products, Pickering, ON, Canada); a wood board approximately 8 × 26 cm, 0.5 cm thick for the base; four vinyl feet (19 mm vinyl bumpers; Sheppard Hardware Products, Three Oaks, MI, USA); four nuts and bolts—3/16 × 1″; two right angle shelving braces—4″; four 1/4″ × 1/8″ grip clips (Crawford Products, Whittier, CA, USA); and a 4 × 4 cm wood beam of any length.

Fifty-milliliter conical bottom centrifuge tubes can hold membrane blots from mini-gels up to 8 × 8 cm. Thorough coverage of the membrane surface can be achieved with 5 mL and even 3.5 mL of reagent volume. A typi-