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tains 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-mercaptoethylamine-HCl to the stain and destain and keeping the stained gel away from bright light. The notation "b" and "c" (Figure 1C) refers to two other isoprotein species closely related to H2A1 (a). The notation " γ " (Figure 1C) refers to H2AX phosphorylated on serine residue 139 formed after exposing cells to ionizing radiation. Note that each of these latter forms has a diagonal of " α " forms because these are all independent events.

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 electroblotted 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 electroblotted 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 electroblotted 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.

REFERENCES

1. Bonner, W.M., M.H.P. West and J.D. Stedman. 1980. Two-dimensional gel analysis of histones in acid extracts of nuclei, cells, and tissues. *Eur. J. Biochem.* 109:17-23.
2. Brownell, J.E., J. Zhou, T. Ranalli, R. Kobayashi, D.G. Edmondson, S.Y. Roth and C.D. Allis. 1996. Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell* 84:843-851.
3. Delcuve, G.P. and J.R. Davie. 1992. Western blotting and immunochemical detection of histones electrophoretically resolved on acid-urea-triton- and sodium dodecyl sulfate-polyacrylamide gels. *Anal. Biochem.* 200:339-341.
4. Kohlrausch, F. 1897. Ueber concentrationsverschiebungen durch electrolyse im innern von losungen und losungsgemischen. *Ann. Physik.* 62:209-215.
5. Ornstein, L. 1964. DISC Electrophoresis. *Ann. NY Acad. Sci.* 121:321-349.
6. Panyim, S. and R. Chalkley. 1969. High resolution acrylamide gel electrophoresis of histones. *Arch. Biochem. Biophys.* 30:337-346.
7. Rogakou, E.P., C. Boon, C. Redon and W.M. Bonner. 1999. Megabase chromatin domains involved in DNA double-strand breaks in vivo. *J. Cell Biol.* 146:905-916.
8. Rogakou, E.P., D.R. Pilch, A.H. Orr, V.S. Ivanova and W.M. Bonner. 1998. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J. Biol. Chem.* 273:5858-5868.
9. Sassone-Corsi, P., C.A. Mizzen, P. Cheung, C. Crosio, L. Monaco, S. Jacquot, A. Hanauer and C.D. Allis. 1999. Requirement of Rsk-2 for epidermal growth factor-activated phosphorylation of histone H3. *Science* 285:886-891.
10. Thiriet, C. and P. Albert. 1995. Rapid and effective western blotting of histones from acid-urea-triton and sodium dodecyl sulfate polyacrylamide gels: two different approaches depending on the subsequent qualitative or quantitative analysis. *Electrophoresis* 16:357-361.
11. van Holde, K.E. (1989) Chromatin. Springer-Verlag, New York.
12. Wei, Y., L. Yu, J. Bowen, M.A. Gorovsky and C.D. Allis. 1999. Phosphorylation of histone H3 is required for proper chromosome condensation and segregation. *Cell* 97:99-109.
13. Wu, R.S., H.T. Panusz, C.L. Hatch and W.M. Bonner. 1986. Histones and their modifications. *Crit. Rev. Biochem.* 20:201-263.
14. Zweidler, A. 1978. Resolution of histones by polyacrylamide gel electrophoresis in presence of nonionic detergents. *Methods Cell Biol.* 17:223-233.

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Construction of a Rotisserie Hybridization Apparatus

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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-

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Protocol

1. The project is best started with a base by cutting an approximately 8×26 cm wood board. Place two right-angled shelving braces at one end of the base, with the brace ends facing each other and touching. The braces will serve as supports and the rotisserie motor will be mounted on the braces (Figure 1). Fasten the braces to the base with $3/16 \times 1$ " bolts.
2. From the rotisserie kit, attach the rotisserie brackets to the shelving braces. Adapter pieces supplied with the kit are not needed. Fasten the rotisserie mounting brackets to the shelving braces with the supplied bolts. Ensure the bracket into which the motor inserts is on the side of the base left vacant by the fastened braces (Figure 1). It may be necessary to drill additional holes in the braces to fix and secure the rotisserie mounting brackets in place.
3. Cut the rotisserie rod to a length of 24 cm. Cut a 4 cm length from the 4×4 cm wood beam. With a router, bore a square slot the same width as the rotisserie rod in the wood block. Alternatively, a round hole can be bored with a drill. Fasten the grip clips to the remaining four sides of the wood block.
4. Place the wood block in the center of the rod and secure in place. Silicone or epoxy will serve well to fix the block in position. Attach rubber feet to the bottom of the base.
5. Mount the motor and insert the rod. Insert test 50 mL conical bottom centrifuge tubes on the initial use to ensure the apparatus has a level operation.

cal rpm range is between three and seven, depending on the rotisserie motor kit you choose. Widely available power bars can serve as an on/off switch for motors not equipped with their own.

Membranes are inserted into the 50 mL tubes. The surface carrying the nu-

cleic acids or proteins should face inwards. Membranes should be moist to prevent breakage and air bubble formation from surface tension. To start the operation, reagent solution is added to the tube, which is then capped and inserted into a grip clip for incubation.

Regular procedures for western, northern, northwestern or Southern blotting, including washing, can be used with this apparatus. This apparatus saves time, mess, expensive reagents and can substitute for relatively expensive equipment at room temperature or 65°C inside a dry oven. Results are comparable to those obtained with a commercial rotisserie apparatus.

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Comparison of DNA Polymerases for Quantification of Single Nucleotide Differences by Primer Extension Assays

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Differential termination of primer extension can be used to detect and quantify previously characterized single nucleotide differences in DNA (3) or RNA (1,2). The method relies on the extension of a labeled primer with its 3' end situated just 5' to the site of the divergent nucleotides. Differential termination is effected by selectively limiting the complement of dNTPs (3), possibly by the incorporation of a dideoxy analog at the site of the sequence variation (1,2). Using a standard method (2) on PCR products from genomic DNA, we observed an imperfect termination of the extension products, a phenomenon deleterious to any quanti-

Figure 1. Rotisserie hybridizer. (1) Rotisserie motor; (2) wood block; (3) grip clip; and (4) shelving braces.