A Rapid Method for Collecting and Assaying Gradient Fractions for Radioactive Macromolecules

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A rapid method is described for collecting gradients after centrifugation, and assaying them for acid-precipitable radioactivity. An entire gradient is collected and processed on a single glass-fibre filter disc. This method allows one easily to assay six gradients for acid-precipitable radioactivity in less than 2 h.

Rate-zonal and equilibrium centrifugation are two of the most widely used techniques in molecular biology today. In general, fractions are sequentially collected from the centrifuge tube and often are then assayed for acid-precipitable radioactivity. The collection and assay procedures have been simplified by the use of the filter-paper-disc technique (Bollum, 1959) in gradient collection (McGrath & Williams, 1966). In this method, fractions are collected directly on to small filter discs, which are then treated as desired and assayed for radioactivity. The handling of many small filters required for gradient analysis has proven to be quite tedious, however. Methods to eliminate this problem have been reported by Carrier & Setlow (1971) and by Furano (1971). In the former, a long strip of Whatman 3MM paper is used for gradient collection. In the latter, samples (e.g. portions of gradient fractions) are applied to the centres of the squares of a gridded 12.5cm-diam. disc of Whatman no. 3 paper, thus simplifying washing of the samples by allowing use of a Buchner funnel.

We have now developed a method which enables one person easily to process six gradients, containing 35–40 fractions each, for measurement of acid-precipitable radioactivity in less than 2 h. It involves the use of large segmented glass filter discs to simplify both the collection and washing of the fractions. Gradients of volumes up to 12–13 ml are collected directly on to single discs; thus all fractions of an individual gradient can be processed in one operation.

Experimental

For the collection of gradients, Whatman GF/A and GF/B glass-fibre filter discs (22.8cm diam., available commercially) are prepared by making 40 equidistant radial cuts [each approx. 5cm (2in) long] to produce 40 segments. A filter, supported by an aluminium-foil-covered cardboard disc, is placed on a Rubbermaid plastic turntable [27cm (10½in) diam.] and drops are collected on to the

segments of the filter. The turntable is rotated to change from one segment to the next. Gradients of small volume (3ml) are collected on GF/A discs, which have a capacity of about 0.1 ml per segment, and larger gradients are collected on GF/B discs (capacity about 0.4 ml per segment). After the gradient is collected, the disc is air-dried before being removed from its foil-covered support. The dried disc is then placed in a large [27cm (10½in) inside diam.] polyethylene Buchner funnel (Bel Art) and washed with 1 litre of 6% (w/v) trichloroacetic acid, followed by 500 ml of 95% (v/v) ethanol. The wet segments are pulled off with forceps, placed in scintillation vials, and dried either under heat lamps or at room temperature. Radioactivity is assayed, by using a toluene–Liquifluor scintillation mixture [containing 4 g of 2,5-diphenyloxazole/l and 50 mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene/l]. Whenever less than 40 fractions are collected from a gradient some of the remaining segments are counted for radioactivity as blanks.

Results and discussion

The first experiment demonstrates the applicability of the large-filter-disc technique to band centrifugation of labelled simian virus 40 (SV40) DNA. Fig. 1 shows that collection of this 3 ml gradient on segments of a 22.8cm Whatman GF/A disc produces essentially the same profile as with the technique using individual filter discs. Fig. 2 shows the use of the large-filter-disc technique for collection of a larger gradient. Labelled SV40 DNA was centrifuged through 12 ml sucrose gradients. One gradient (Fig. 2a) was collected directly on to a 22.8cm Whatman GF/B disc. A parallel gradient was collected in test tubes, and 50 µl samples were transferred to segments of a GF/A disc (Fig. 2b) or a GF/B disc (Fig. 2c). The three patterns are virtually identical. Thus at least 0.33 ml can be collected on each segment of a GF/B filter without loss of radioactivity or spreading of the gradient fractions from segment to segment.

Fig. 1. Comparison of gradient collection of a large Whatman GF/A filter disc with collection on individual filters

SV40-infected Vero cells (a continuous line of African-green-monkey kidney cells) were labelled from 48 to 72 h after infection with [*methyl*-³H]thymidine (Schwarz-Mann, Orangeburg, N.Y., U.S.A.; 14.1 Ci/mmol; 5 μ Ci/ml final concn.). Viral DNA was extracted by the method of Hirt (1967) and samples were sedimented through 3 ml of 46% (w/w) CsCl solution [$\rho = 1.50$, containing 0.01 M-Tris-HCl (pH 7.4)–0.001 M-EDTA] for 110 min at 44000 rev./min at 15°C in the Spinco SW50L rotor (Vinograd *et al.*, 1963). The tubes were pierced, and fractions of volume approx. 0.11 ml [11 drops from a 2.5 cm (1 in) 20-gauge Monoject blood-collecting needle installed in a Buchler Universal Piercing Unit] were collected from one gradient on to segments of a single 22.8 cm Whatman GF/A filter (●), and fractions from a parallel gradient were collected on 2.4 cm GF/A discs (○). The 22.8 cm filter was dried and treated as described in the text. The 2.4 cm discs were dried, then washed individually with 6% trichloroacetic acid and 95% ethanol while supported on the fritted glass base of a Millipore Pyrex filter holder, and counted for radioactivity.

We have also successfully used the present method to collect and assay fractions from gradients containing other types of samples, such as polyribosomes or multiple RNA species (not shown). We find that the resolution attainable is just as good as with the other methods of fraction collection we have used.

Fig. 2. Collection of a gradient on a large Whatman GF/B filter disc

Viral DNA from SV40-infected Vero cells was extracted by the method of Hirt (1967). Samples were sedimented through 12 ml 5–30% (w/v) sucrose gradients [containing 0.05 M-Tris-HCl (pH 7.5)–0.1 M-NaCl–0.025 M-EDTA] for 4 h at 40000 rev./min in the Spinco SW41Ti rotor at 10°C. Fractions of volume approx. 0.33 ml (15 drops from the 18-gauge piercing needle supplied with the Buchler Universal Piercing Unit) were collected on to segments of a 22.8 cm Whatman GF/B filter (a). Fractions from a parallel gradient were collected in test tubes, and 50 μ l samples were transferred to segments of a 22.8 cm GF/A filter (b) or a 22.8 cm GF/B filter (c). Filters were dried and treated as described in the text.

In the course of developing the present method we have found alternative techniques that, although not preferable, can also be used. For example, washing the filters can be done by soaking them in three changes of 6% trichloroacetic acid followed by two changes of ethanol. This procedure is

somewhat longer, as each soak should take at least 5 min. In this regard, we tried to wash the filters in a Millipore 239mm filter holder, but found it unacceptable since the acid-soluble radioactivity had not been completely washed out, and the blank segments had picked up radioactivity.

We have also collected 3ml gradients on 24.0cm discs of Whatman no. 3 paper. The radioactivity-counting efficiency is almost as high as with the GF/A filters. However, the Whatman no. 3 paper is not as absorbent as the GF/A filters, and is harder to work with because of the stiffness of the segments. Conceivably, larger gradients could be collected on discs of Whatman no. 17 paper, as Carrier & Setlow (1971) have done with paper strips.

Of course, when more than 40 fractions are desired from an individual gradient, more than one filter can be used. We find this useful for the collection of 0.4ml fractions from gradients larger than 13ml, as well as for increasing the resolution of smaller gradients by collecting more fractions. (Another method for the collection of more fractions would involve the use of even larger glass filters,

turntable and Buchner funnel.) In other experiments, in which recovery of the sample is desired, fractions can be collected in test tubes and small portions transferred to the segmented discs for processing. Finally, this filter method can be used to process efficiently samples from large numbers of reaction mixtures or other samples which must be assayed for acid-precipitable radioactivity.

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Bollum, F. J. (1959) *J. Biol. Chem.* **234**, 2733-2734

Carrier, W. L. & Setlow, R. B. (1971) *Anal. Biochem.* **43**, 427-432

Furano, A. V. (1971) *Anal. Biochem.* **43**, 639-640

Hirt, B. (1967) *J. Mol. Biol.* **26**, 365-369

McGrath, R. A. & Williams, R. W. (1966) *Nature (London)* **212**, 534-535

Vinograd, J., Bruner, R., Kent, R. & Weigle, J. (1963) *Proc. Nat. Acad. Sci. U.S.* **49**, 902-910