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[19] Preparation and Characterization of RNA: Overview

By SHELBY L. BERGER

The isolation of bulk RNA and the determination of the properties of specific molecules within that heterogeneous population are the first serious steps in the path toward a cDNA clone. The preparation of messenger RNA also plays a role in the identification and characterization of sequences already cloned, and in some cases, already expressed in cells of a higher organism. Thus, techniques in this section can be used not only at the commencement of cloning but also near its completion. The aim, then, is to explain the organization of this section and to view it within the context of the volume as a whole.

Choice of an RNA Extraction Technique. When investigators not familiar with RNA set out to isolate mRNA, the first question is not which technique to use but whether any technique can succeed. Perhaps more than any other procedure, the preparation of RNA requires near-compulsive elimination of ribonucleases from glassware, pipets, tips, and solutions, indeed, everything that touches RNA. Even implements that do not come into intimate contact with the sample should be scrutinized. Micropipets belong in this category, because they can become contaminated and in turn contaminate solutions. "Finger nucleases" must be barred from the sample by wearing gloves. Hair, dust, sneezes, and coughs are also likely sources of nucleases. When the requirements in chapter [2] have been met, and when the novice is fervently convinced that shortcuts spell failure, only then should a method be selected.

In this section, techniques are presented for isolating RNA from intact cells and tissues [20], nuclei [22], cytoplasm [21], and polysomes [21,23, 24]. The techniques described by MacDonald *et al.* [20] are the most widespread, versatile, and safest in use today. If intact RNA cannot be obtained by blasting whole cells or tissues apart in chaotropic agents, the chances for success with more subtle methods are not great. The main strength of this approach, then, is also the major disadvantage. Subcellular fractionation is not possible under conditions of complete cell disruption.

The methods in chapters [4] and [21] can also be used for preparing RNA from whole cells. Phenol extraction coupled with ethanol precipitation [5] to recover the sample is by far the most common technique of RNA isolation. It is faster and simpler than the guanidine-based methods but may place RNA molecules at slightly greater risk. A second popular

METHODS IN ENZYMOLOGY, VOL. 152, 1987

$$C_0 t_{1/2} \approx N \times 10^{-6} \quad (8)$$

where C_0 is expressed as the molar concentration of nucleotides and $t_{1/2}$ is in seconds. In practical terms, hybridization is virtually complete (75%) in 3 times the number of hours required to reach $C_0 t_{1/2}$. An empirical formula that relates these parameters is

$$t_{1/2} \approx \frac{(F)(N)(\text{ml})}{(\mu\text{g DNA})10{,}000} \quad (9)$$

where the units of $t_{1/2}$ are hours, the units of N are base pairs, and F is the ratio of the rate of hybridization under the conditions used to the rate under standard conditions. (Standard conditions refer to $T_m - 25°$ at 0.18 M cation concentration for a fragment of 500 bp in length.) For example, at a concentration of 0.1 μg/ml (3×10^{-7} mol nucleotides/liter) of a mammalian DNA fragment with a length and complexity equal to 1000 bp and a GC mole fraction equal to 0.4, hybridization at a temperature of 50° in 0.18 M salt will have a $C_0 t_{1/2}$ of 0.001. From either Eq. (8) or (9) we calculate that $t_{1/2} \approx 1$ hr. [From Eq. (8), $C_0 t_{1/2} = 1000 \times 10^{-6} = 10^{-3}$; $t_{1/2} = 10^{-3}/(3 \times 10^{-7}) = 3.3 \times 10^3$ sec = 0.92 hr; from Eq. (9) $(1 \times 1000 \times 1)/(0.1 \times 10{,}000) = 1$ hr.]

At an identical DNA concentration in 50% formamide and 1 M Na^+ at 42° the rate of hybridization will increase 4-fold (~7-fold increase as a result of the increase in salt and ~2-fold decrease as a result of adding formamide) and the reaction will approach the $t_{1/2}$ in 0.25 hr, and completion in ~0.75 hr. Note that these expressions do not include network formation. Therefore, longer hybridization times *may* increase signal strength without affecting noise. Many investigators routinely hybridize for 20 hr when shorter times would seem to be indicated.

[44] Amplification, Storage, and Replication of Libraries

By GABRIEL VOGELI and PAUL S. KAYTES

The successful isolation of clones from cDNA or genomic libraries is dependent in part on the ease of handling the library. For small libraries, such as cDNA libraries in plasmid vectors, methods such as stabbing into microtiter wells or the storage of the initial filters at −80° are feasible.[1]

[1] D. Hanahan and M. Meselson, *Gene* 10, 63 (1980).

height of the flash unit or by adding or deleting filters. The flashed face of the film should face the fluor, in the sample for ^{35}S, in the screen for ^{32}P.

Quantitation of Autoradiographs

Film response to autoradiography (no fluors or screens) is linear up to absorbances of about 1 unit. With ^{35}S autoradiography, it is important that the gel matrix be uniform, because different regions of a gradient gel could quench ^{35}S to different extents. Even though the nonlinear film response in fluorography can be corrected by preflashing the film, linearity should be checked with standards prepared with the same isotope.

[8] Electrophoresis in Agarose and Acrylamide Gels

By RICHARD C. OGDEN and DEBORAH A. ADAMS

The scope of this chapter is to present a range of methods by which DNA and RNA molecules can be fractionated and analyzed by means of gel electrophoresis. This chapter will emphasize those techniques which can be simply and routinely applied in the course of molecular cloning and analysis and, wherever appropriate, reference will be made to more exhaustive practical or theoretical considerations of the techniques.

Gel electrophoresis through agarose or polyacrylamide is a very powerful method for rapidly resolving mixtures of nucleic acid molecules which has found wide application in recombinant DNA research. The resolution afforded far exceeds that generally obtained by other sizing techniques. The fractionated nucleic acids can be directly "viewed" *in situ* in the gel and can be readily recovered by a variety of methods tailored to subsequent steps in an experimental protocol. Because it is such an indispensible technique, a great deal of effort has gone into improving its efficacy for particular applications, with the result that many of the original methods have been simplified, scaled down, and improved. This review will cover the various choices to be made when confronted with experiments requiring gel electrophoresis in the order they would typically arise, starting with the type of gel system, choice of running buffer, and equipment, and continuing through gel running, visualization of the separated molecules, extraction of the material from the gel, and workup of preparative samples. Certain specific applications of electrophoresis, for example gels for sequencing purposes (this volume [56, 57])

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Presence and population dynamics of *Erwinia carotovora* in irrigation water in south central Colorado

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MADDOX, D.A. & HARRISON, M.D. 1988. Presence and population dynamics of *Erwinia carotovora* in irrigation water in south central Colorado. *Journal of Applied Bacteriology* **64**, 169–182.

The presence of *Erwinia carotovora* in surface and underground (well) water was studied using filter concentration and anaerobic enrichment techniques. The organism was found in water samples collected at sites in mountainous (over 80 km from potato-producing regions), transitional (upland) and arable regions every month in 1982 and 1983. Filter concentration and anaerobic enrichment of 3–10 l of water yielded *E. carotovora* from 82·8% of the water samples collected from streams, canals and lakes. The organism was detected by direct enrichment of 50 ml water samples in 56·3% of surface water samples collected. *Erwinia carotovora* subsp. *carotovora* was the predominant subspecies isolated. Of 1029 strains, 999 (97·1%) were identified as *E. carotovora* subsp. *carotovora* and 30 (2·9%) as *E. carotovora* subsp. *atroseptica*. *Erwinia carotovora* subsp. *atroseptica* was found primarily in water samples collected in arable regions during spring months. *Erwinia chrysanthemi* was never isolated. Quantitative bacteriological methods were used in 1982 and 1983 to monitor populations of *E. carotovora* in two streams in south central Colorado. These ranged from undetectable levels to 8·5 cfu/ml of water in Rio Grande River and Saguache Creek. Maximum populations were usually reached by August or September in both streams in both years. *Erwinia carotovora* was isolated from well water samples collected in the San Luis Valley, but only 15·6 and 15·4% of the samples yielded the organism during 1982 and 1983, respectively. *Erwinia carotovora* subsp. *atroseptica* was found only once, and *E. carotovora* subsp. *carotovora* was the predominant subspecies detected. Filter concentration of 3·4–10·0 l of water plus anaerobic enrichment of the samples was usually necessary to detect *E. carotovora* in well water.

Erwinia carotovora subsp. *atroseptica* (van Hall) Dye and *Erwinia carotovora* subsp. *carotovora* (Jones) Dye (Skerman *et al.* 1980), causal agents of potato blackleg and tuber soft rot, are considered to be tuber-borne pathogens (Lazar & Bucur 1964; Perombelon 1974; Lauer 1977) and spread throughout the crop by subsequent contamination of farm machinery, cutting knives or movement of soil water (Perombelon 1974). At present, certified seed programmes utilizing stem-cutting or tissue culture-produced planting stock (Graham & Hardie 1971; Perombelon 1974), along with sanitation and cultural practices (Perombelon & Kelman 1980; Harrison & Brewer 1982), are used to prevent or reduce seed-tuber contamination by erwinias and control the spread of the organisms. Contamination of *Erwinia*-free stocks by *E. carotovora* (especially subsp. *carotovora*) continues to be reported (Perombelon 1974; Graham *et al.* 1976; Perombelon & Kelman 1980). These reports and other evidence (McCarter-Zorner 1981; Jorge 1983; Jorge & Harrison 1986) indicate that the inoculum which contaminates *Erwinia*-free seed potatoes is introduced from external sources.

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New Zealand Journal of Science (1969), vol. 12

A TAXONOMIC STUDY OF THE GENUS *Erwinia*

II. THE "CAROTOVORA" GROUP

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(Received for publication, 6 December 1968)

Summary

A systematic study has been made of the genus *Erwinia* and this paper reports on those organisms considered to be closely related to *Erwinia carotovora* (Jones) Bergey *et al.* It is suggested that the several species should be classified as varieties of *E. carotovora* and the following classification is proposed:
E. carotovora var. *carotovora* (Jones) comb. nov. 1969;
E. carotovora var. *atroseptica* (van Hall) comb. nov. 1969;
E. carotovora var. *chapontici* (Millard) comb. nov. 1969;
E. carotovora var. *chrysanthemi* (Burkholder *et al.*), comb. nov. 1969;
E. carotovora var. *cypripedii* (Hori) comb. nov. 1969.

Introduction

The investigation of white rot disease of hyacinth by Heinz (1889) was the first accurate study of an undoubted soft rot disease of plants and he named the causal organism *Bacillus hyacinthi septicus*, which is now an invalid name. Potter (1901) studied white rot of turnip and named the organism *Pseudomonas destructans* and reported it as having a polar flagellum. Unfortunately Potter's original culture was lost and subsequent isolates sent by him to Harding & Morse (1909) proved to be peritrichous (and pathogenic). The earliest valid, adequately described species of peritrichously flagellated, vegetable-soft-rotting bacterium is therefore *Bacillus carotovorus* Jones (1901 a & b). The organism responsible for potato blackleg was named by van Hall (1902) as *B. atrosepticus* and by Appel (1902) as *B. phytophthorus*. Other early names for soft-rotting bacteria were *B. omnivorus* (van Hall, 1902), *B. aroideae* (Townsend, 1904), *B. oleraceae* (Harrison, 1904) and *B. melonis* (Giddings, 1910). A comparative study by Harding & Morse (1909) led them to suggest that *B. carotovorus*, *B. omnivorus*, *B. oleraceae*, *B. aroideae* and "Potters bacillus" were practically indistinguishable and should be considered as "somewhat variant members of a single botanical species". However, on the basis of differences in fermentative ability, cultural characters and

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Tests for Unit Roots: A Monte Carlo Investigation

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Recent work by Said and Dickey (1984, 1985), Phillips (1987), and Phillips and Perron (1988) examines tests for unit roots in the autoregressive part of mixed autoregressive integrated moving average models (tests for stationarity). Monte Carlo experiments show that these unit-root tests have different finite-sample distributions from the unit-root tests developed by Fuller (1976) and Dickey and Fuller (1979, 1981) for autoregressive processes. In particular, the tests developed by Phillips (1987) and Phillips and Perron (in press) seem more sensitive to model misspecification than the high-order autoregressive approximation suggested by Said and Dickey (1984).

KEY WORDS: ARIMA; Autoregressive; Moving average; Size; Stationarity.

1. INTRODUCTION

Fuller (1976) and Dickey and Fuller (1979, 1981) developed several tests of whether a pth-order autoregressive (AR) process,

$$Y_t = \alpha + \sum_{i=1}^{p} \phi_i Y_{t-i} + u_t, \qquad (1)$$

is stationary. Stationarity implies that the roots of the lag polynomial $\phi(L) = (1 - \phi_1 L - \cdots - \phi_p L^p)$ lie outside the unit circle [see Box and Jenkins (1976) for a discussion of stationarity in the context of AR processes]. The null hypothesis in these tests is that the AR process contains one unit root, so the sum of the autoregressive coefficients in (1) equals 1. Dickey and Fuller estimated the model

$$Y_t = \alpha + \rho_u Y_{t-1} + \sum_{i=1}^{(p-1)} \phi_i' DY_{t-i} + u_t, \qquad (2)$$

where $DY_{t-i} = Y_{t-i} - Y_{t-i-1}$, which is equivalent to the AR model in (1), except that the coefficient ρ_u should equal 1.0 if there is a unit root. Dickey and Fuller used Monte Carlo experiments to tabulate the sampling distribution of the regression t statistic $\tau_u = (\hat{\rho}_u - 1)/s(\hat{\rho}_u)$, where $s(\hat{\rho}_u)$ is the standard error of the estimate $\hat{\rho}_u$ calculated by least squares. The distribution is skewed to the left and has too many large negative values relative to the Student-t distribution. See Dickey, Bell, and Miller (1986) for a recent discussion of autoregressive unit-root tests. Plosser and Schwert (1977) discussed a similar problem that arises when there is a unit root in the moving average (MA) polynomial. This can occur when differencing is used to remove nonstationarity and the true model is a stationary and invertible ARMA model around a time trend.

This article analyzes the sensitivity of the Dickey–Fuller tests to the assumption that the time series is generated by a pure AR process. In particular, when a variable is generated by a mixed autoregressive integrated moving average (ARIMA) process, the critical values implied by the Dickey–Fuller simulations can be misleading. Section 2 describes recent extensions of the Dickey–Fuller test procedure suggested by Said and Dickey (1984, 1985), Phillips (1987), Phillips and Perron (in press), and Perron (1986a,b) that attempt to account for mixed ARIMA processes as well as pure AR processes in performing unit-root tests. Section 3 contains results of a Monte Carlo experiment that calculates the size of the Dickey–Fuller and related test statistics when the true process is ARIMA rather than AR. Section 4 contains concluding remarks.

2. EXTENSIONS OF THE DICKEY–FULLER TESTS

Said and Dickey (1984) argued that an unknown ARIMA(p, 1, q) process can be adequately approximated by an ARIMA(k, 0, 0) process, where $k = o(T^{1/3})$. Given this approximation, the limiting distribution of the unit-root test based on a high-order AR approximation will be the same as the Dickey–Fuller distribution. Of course, for a given application this argument does not indicate the appropriate number of lags k.

To understand why a finite-order AR process may not provide an adequate approximation to a mixed ARIMA process, it is useful to consider the infinite-order AR process implied by an ARIMA(0, 1, 1) process for different values of the MA parameter θ. The autoregressive coefficients are calculated by matching coefficients of the lag operator L in the relations $\pi(L) = (1 - L)/(1 - \theta L) \rightarrow (1 - \theta L) \cdot \pi(L) = (1 - L)$, where π_i is the autoregressive coefficient at lag i. The autoregressive coefficients decay slowly for large absolute values of the MA parameter. The sum of the

ANNUAL PRIMARY PRODUCTION AND NUTRIENT CYCLE IN SOME SCOTS PINE STANDS

EINO MÄLKÖNEN

HELSINKI 1974

TOMATO ROOT DEVELOPMENT ON SAND MULCH, PLASTIC GREENHOUSE IN ALMERIA (SPAIN)

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Abstract

Most of the 11 000 ha of plastic greenhouses in Almeria use beach sand as mulch on a stratified, artificial soil profile. Manure is placed on strips, about 1 m wide and 2 cm deep, between the sand and the 20 cm of loam or clay soil placed on top of the original, rocky, sandy loam soil. Under such conditions, there is great uncertainty on the pattern and extension of root development and on the role that each soil layer plays in supplying water and nutrients to the crop. The use of localized, drip irrigation systems adds an additional level of complexity, increasing the spatial variablity of soil water and nutrients. A three-year study on root development of tomato, under three levels of irrigation and of nitrogen fertilization was conducted at the Experimental Farm of the "Caja Rural de Almeria". A modified trench-profile technique and core sampling using Newman's method to estimate root length densities, gave estimates of root length for each soil layer as affected by the nitrogen and water supply. Root growth was substantial in the interface between the sand layer and the manure, with root length density values between 10 and 100 cm/cm^3. Root length in the sand-manure zone contributed nearly 25 percent of the total measured root length.

1. Introduction

Plastic greenhouses in Almeria use poliethylene (PE) sheet as covering material, placed on a very simple wood or metallic tube structure, between two grids of wire that fix the plastic sheet. Climate in Almeria is mediterranean semiarid subtropical although the microclimate inside these greenhouses is far from optimum for most horticultural crops. Anyhow, this cropping system has been successful and, in the last decade, the surface area under plastic greenhouses has exceeded 11 000 ha.

The original soil profile is normally modified. Once the original rocky sandy loam soil has been levelled, a 20cm-thick layer of loam or clay soil is placed over it, and manure is added on top, in a 2cm-thick layer, under

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Acrosin Activity Assay for the Evaluation of Mammalian Sperm Acrosome Reaction

Yehudit Lax,* Sara Rubinstein,* and Haim Breitbart

1. Introduction

The sperm acrosome reaction (AR) is an exocytotic event that involves multiple fusions between the outer acrosomal membrane and the overlying plasma membrane. It must occur before the spermatozoon penetrates the zona pellucida (ZP) *(1)*. This exocytotic event is mandatory for fertilization because it enables passage of the sperm through the ZP and its subsequent fusion with the egg oolema. Many artificial stimuli are reported to trigger the AR, either by driving extracellular Ca^{2+} into the sperm cell (Ca^{2+} ionophores) or by acting through intracellular second messengers that are involved in the cascade leading to acrosomal exocytosis *(2,3)*.

The acrosomal components, mostly powerful hydrolytic enzymes, are released by the acrosome reaction *(1)*. Acrosin (E.C. 3.4.21.10), an endoprotease with a trypsinlike substrate specificity, is localized in the acrosomal matrix as an enzymatically inactive zymogen, proacrosin, that is converted into the active form as a consequence of the acrosome reaction *(4–6)*. At the acrosome reaction, acrosin serves a role by accelerating the dispersal of acrosomal components and further by assisting the sperm penetration of the ZP *(7)*.

Spermatozoa of many mammals (rat, bull, ram, and human) have very thin or lightly fitted acrosomes; hence, their acrosome reactions are difficult to detect *(1)*. Several methods are currently available for monitoring acrosome reactions:

1. Methods using transmission electron microscopy, allowing ultrastructural examination of the acrosome *(8)*

*Both contributed equally.

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PERSISTENCE OF CHLORINE IN MAIZE PLANTS AND SOIL IN RELATION TO CONTROL OF BACTERIAL STALK ROT OF MAIZE

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ABSTRACT : Chlorine persisted for 10 days in roots and basal stem portions and for 15 days in upper stem portions, leaf sheaths and leaves of maize plants when stable bleaching powder (SBP) solutions containing 1,000 and 5,000 ppm of chlorine were soil drenched. With a few exceptions, the amount of chlorine absorbed and translocated in roots and stem decreased with the increase in time interval between chemical application and chlorine estimation. But it increased in the leaf sheaths and leaves upto first 10 days and decreased on 15th day of application. When three concentrations of chlorine namely, 500, 1,000 and 2,000 ppm were soil drenched, chlorine persisted in soil for 14, 23 and 26 days respectively. On the basis of these data, SBP can be used as a soil drench to control bacterial stalk rot of maize caused by *Erwinia chrysanthemi* pv. *zeae* (Sabet, 1954) Victoria, Arboleda and Munoz, 1975.

Keywords : Maize, Bacterial stalk rot, Chlorine, Persistence

The persistence of a chemical on or in a plant or in the soil is of great importance in plant disease control. This information help in the judicious use of the chemical which in turn results in achieving effective and economical control.

Antibiotics like streptomycin, terramycin and streptocycline and chlorine containing compounds like clorox, bleaching powder and stable bleaching powder (hereafter referred as SBP) have been recommended for the control of maize stalk rot caused by *Erwinia chrysanthemi* pv. *zeae* (Sabet, 1954) Victoria, Arboleda and Munoz, 1975 (Lal and Saxena, 1978 ; Lal *et al.*, 1970 ; Rangarajan and Chakravarti, 1970; Sabet, 1956 ; Thind and Payak, 1972 ; Thompson, 1965).

For lack of information on persistence of chlorine in maize plants and soil resulting after application of chlorine-containing compounds, investigations on these aspects were carried out and the results are reported here.

MATERIALS AND METHODS : SBP (a product of Shri Ram Chemicals Industries, New Delhi) containing 33 per cent chlorine was used as a source of chlorine. Two-month-old plants of maize cv. Vijay composite grown in plastic pots were used. SBP solutions containing 1,000 and 5,000 ppm chlorine were applied in the pots at the rate of 1.5 per pot. Samples of plant parts at following distances above the last point of chemical contact i.e., 0–5, 5–10, 10–15, 15–20, 20–25, 25–30, 30–35, 35–45 and 45 cm to leaf tip were taken after five intervals of application (2, 4, 6, 10 and 15 days) and analysed for chlorine. It was estimated by the method of Association of Official Analytical Chemists (Horwitz, 1975).

To study the persistence of chlorine in soil, SBP solutions containing 500, 1,000 and 2,000 ppm chlorine were added in equal amounts to the finely ground, sieved and thoroughly dried field soil filled in pots. The chlorine content of the soil was determined at periodic intervals for 28 days by the method of Hanna (1964).

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