

tinued till we get another Nobel Prize. C. V. Raman continues to be the only Indian scientist to have been awarded the Nobel Prize way back in 1930 for work done in India, viz. the 'Raman Effect'. However, to honour this great discovery, since 1987, 28 February is being celebrated as the National Science Day.

The presentation of research papers at the Congress needs to be minimised if not scraped altogether in the week-long session as there is every conceivable opportunity round the year to do this both in India and abroad.

The annual stock-taking of the Indian Science Congress will hopefully convince

the taxpayers that their money is being spent on S & T in a meaningful manner.

N. C. JAIN

B/11 6/6 New Minto Road Apartments
New Delhi 110 002, India

SCIENTIFIC CORRESPONDENCE

A modified alkaline lysis procedure for isolation of plasmid from *Escherichia coli*

IN recent years several methods have been developed for isolation of plasmids from *E. coli*¹⁻⁶. But the widely used method is the alkaline lysis procedure of Birnboim and Doly¹. This method makes use of sugar solutions such as glucose or sucrose in the initial buffer. Under laboratory conditions, the sugar solutions are easily contaminated and hence it becomes necessary to prepare fresh sterile solutions each time. To circumvent this problem, an alternative method has been standardized using sodium chloride solution instead of sugar solutions which can be stored for prolonged periods and needs neither aseptic transfer nor refrigeration as is required for sugar solutions. The modified procedure was tested on small-scale and large-scale isolation of plasmids. DNAs thus obtained were tested for their digestibility with restriction enzymes and for their transformation efficiency.

For small scale isolation of plasmids, the bacterial strain harbouring the plasmid pUC18 was streaked on a LB plate containing ampicillin (50 µg/ml) and incubated for about 12 h at 37°C. A single large, well-isolated colony was transferred into 10 ml LB medium containing ampicillin (50 µg/ml) and was grown on a shaker for about 10 h at 37°C at 200 rpm. The flask was placed on ice for about 15 min and 1.5 ml of the culture was centrifuged for 3 min in a microcentrifuge at room temperature (30° ± 3°C). The cell pellets were suspended by vortexing in 100 µl of either TEN (Tris 50 mM, pH 8.0; EDTA 12.5 mM, pH 8.0; NaCl 0 to 500 mM) or TES (Tris 50 mM, pH 8.0; EDTA 12.5 mM, pH 8.0, glucose 0 to 5% or sucrose 8%). To the suspended cells, 50 µl of

freshly prepared solution II (0.2% SDS and 0.2 N NaOH) was added and mixed gently by inverting the tubes a few times. The tubes were placed on ice. Once the suspension became viscous indicating cell lysis (in about 5–10 min), 75 µl of ice-cold 3 M potassium acetate (pH 4.8) was added and mixed gently by inverting the tubes. The tubes were placed on ice for 15 min and then centrifuged for 10 min at room temperature. About 175–200 µl of the supernatant was carefully removed and the nucleic acids were precipitated with 2 vol. of distilled, cold ethanol at –20°C for 60 min. The precipitate was collected by centrifuging the tubes for 10 min at room temperature. The pellets were air-dried and dissolved in 50 µl TE (Tris 10 mM, pH 8.0; EDTA 1 mM pH 8.0). Ten to 15 µl of the plasmid preparation was analysed on a 0.8% agarose gel. Figure 1 shows the effect of different concentrations of NaCl, glucose and sucrose. There were no differences in the pattern of plasmid bands prepared using either NaCl or sugar solutions except at 500 mM NaCl where two extra bands appeared at the expense of two regular bands. In the control (i.e.) in the absence of NaCl or sugar the top chromosomal DNA band appeared as a smear. When the preparations were treated with RNase and digested with the restriction enzyme HindIII, the plasmid was completely digested in all three preparations viz. NaCl, glucose and sucrose (data not shown).

For large scale isolation of plasmids, the plasmid was amplified as described by Maniatis *et al.*⁷ and extracted essentially as described above for small-scale isolation. The cell pellet from 500 ml amplified culture was resuspended in 10

ml TEN (the NaCl concentration was 150 mM) or TEG (the glucose concentration was 1%), lysed by adding 5 ml solution II and precipitated by adding 7.5 ml cold 3M potassium acetate. All centrifugations were done at 12,000 g for 10 min at 4°C. The final pellet was dissolved in 500 µl TE. The amount of DNA obtained from glucose and NaCl procedures was quantitated after RNase digestion and precipitation. The yields with glucose and NaCl were 3.45 mg/l culture and 7.41 mg/l culture, respectively. The 260/280 ratio was 1.95 in both the cases. DNA samples (obtained after RNase digestion) from both preparations were digested completely with HindIII.

The transformation experiment was done essentially as reported by Cohen *et al.*⁸. *E. coli* strain C600 was grown on LB containing 20 mM MgSO₄ to an A₆₀₀ of 0.5. Two hundred µl of CaCl₂-treated cells were transformed with 50 ng of plasmid DNA. Both preparations showed similar transformation efficiency (i.e.) 0.93 × 10⁶ and 1.27 × 10⁶ transformants/µg of plasmid DNA prepared using NaCl and glucose, respectively.

In order to find out whether the method is applicable to other plasmids, pBR322 was also prepared using different concentrations of NaCl and sugar solutions. There was no change in the pBR322 profile and the plasmid was completely digested irrespective of whether the plasmid was prepared using NaCl or sugar solutions (data not shown). We have also tested the method with a large plasmid (28 kb) from *Agrobacterium tumefaciens*. The modified procedure is equally efficient to isolate the large plasmid too and the plasmid DNA was readily digested

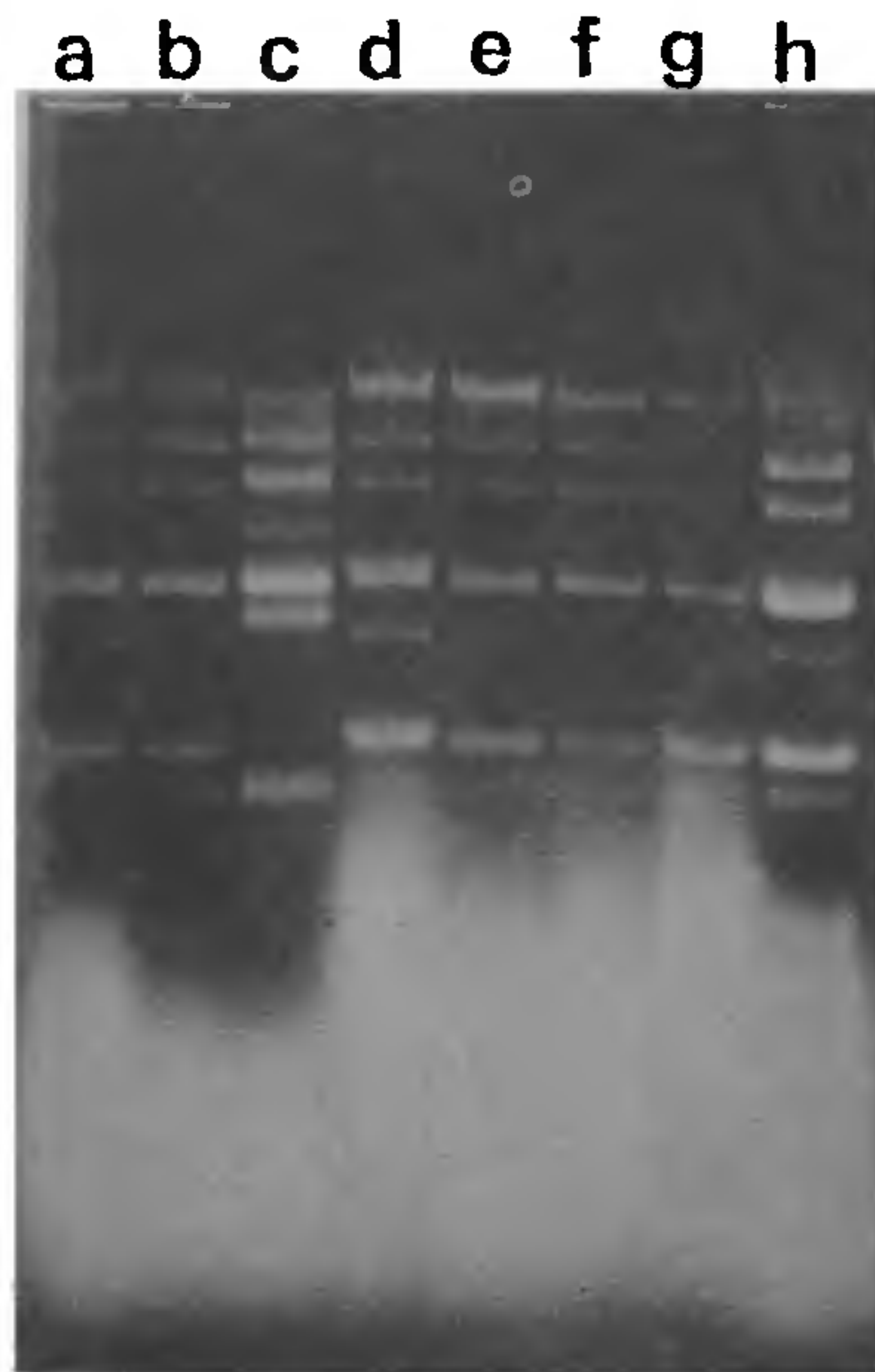


Figure 1. Effect of different concentrations of sodium chloride and sugar solutions on the isolation of pUC18. (a) 150 mM NaCl; (b) 300 mM NaCl; (c) 500 mM NaCl; (d) 1% glucose; (e) 3% glucose; (f) 5% glucose; (g) 8% sucrose; (h) Control.

with restriction enzymes (data not shown).

The method described here uses reagents that are stable at room temperature. In addition, this method uses lower concentration of NaOH and SDS for lysis

than that recommended by Birnboim and Doly¹. Milder conditions of lysis as the one employed here enable the release of small molecules including compact supercoiled plasmids while trapping larger

molecules such as denatured chromosomal DNA inside the cell. Moreover, the precipitation time with 3 M potassium acetate is also reduced to 15 min as longer incubations lead to slow leaching of the chromosomal DNA into the solution. The method is consistent, reproducible and gives better yields.

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ACKNOWLEDGEMENTS. We thank Mr V. Ramanathan for testing the procedure on large *Agrobacterium* plasmid, and DST, New Delhi, for financial support.

Received 18 October 1993; revised accepted 31 January 1994

SHAIK YAZDANI BASHA
P. PALANIVELU

*Department of Biotechnology
Madurai Kamaraj University
Madurai 625 021, India*

COMMENTARY

Paradoxes in the evolution of introns and genes

G. Naresh Kumar

The recent discovery of class II type of self-splicing introns in bacteria¹ has provided additional information to probe into the perplexing role of introns in the evolution of genes^{2–4}. The problem begins with the origin of introns. Introns can be clas-

sified into five different categories—class I, class II, class III, spliceosomal mRNA introns, and the unique small tRNA introns. Classes I and II introns both undergo self-splicing *in vitro* but require proteins for efficient splicing *in vivo*^{5,6}.

They differ from each other in their secondary structures and in their mechanisms of splicing. Class I introns are found in the genes of organelles, bacteria^{6–9} and in eukaryotic nuclear genes whereas class II introns, until their recent discovery in