ELSEVIER

Journal of Chromatography A, 827 (1998) 337–344

JOURNAL OF CHROMATOGRAPHY A

New approaches to the isolation of DNA by ion-exchange chromatography

Peter R. Levison[a,*], Stephen E. Badger[a], Prit Hathi[b], Martin J. Davies[b], Ian J. Bruce[b], Volker Grimm[c]

[a]*Whatman International Ltd., Springfield Mill, James Whatman Way, Maidstone, Kent ME14 2LE, UK*
[b]*School of Chemical and Life Sciences, University of Greenwich, Wellington Street, London SE18 6PF, UK*
[c]*Biometra GmbH, Rudolf-Wissell Strasse 30, D-37079 Göttingen, Germany*

Abstract

The performance of different anion-exchange media have been compared for the isolation of plasmid DNA and genomic DNA from bacterial cells and human whole blood. Whatman DEAE-Magarose, based on an agarose bead containing a paramagnetic component, has been compared with prepacked gravity-flow columns containing a derivatised silica matrix. In each case the DNA isolation at various scales of operation was similar both in terms of yield and quality. The magnetic susceptibility of DEAE-Magarose is very high, facilitating the use of this separation technique for rapid flexible batch chromatographic processes, a limitation of the prepacked column techniques.

Keywords: Magnetic sorbents; DNA

1. Introduction

The downstream processing of commercially important biopolymers has been gaining significance over the recent past. Traditionally, proteins were isolated from various sources including animal tissue, microbial culture and plants. More recently, with emerging techniques in biotechnology the isolation of recombinant proteins, peptides, carbohydrates and nucleic acids have gained importance. These separations have generally been based on established chromatographic techniques including ion exchange, affinity, hydrophobic interaction and size exclusion, predominantly at low pressure. The low-pressure media used for adsorption chromatography were initially based on polysaccharide supports including agarose, cellulose and dextran [1,2], with composite polymers gaining increasing importance [3]. A comparative study into the binding of various proteins and small ions to some 70 different commercially available ion exchangers was recently reported [4] and concluded that the manufacturers' proprietary chemical processing differentiated the performance of each medium regardless of base matrix or functionality.

Nucleic acids provide the genomic templates which code for the proteins associated with all cellular functions. DNA is a polymer of deoxyribonucleotides and RNA is a polymer of ribonucleotides [5]. DNA and RNA are anions at neutral pH and can therefore be isolated by anion-exchange chromatography [6–8]. Nucleic acids can also be

*Corresponding author.

0021-9673/98/$ – see front matter
PII: S0021-9673(98)00681-5

isolated by hybridization to specific oligonucleotide probes in an affinity mode [9,10] or by adsorption to silica surfaces under chaotropic conditions [11–13]. Typically nucleic acid isolation involves two stages. Firstly, the nucleic acid is released from its cellular compartment, for example the cell nucleus, and secondly, the nucleic acid is isolated by a chromatographic process. The first stage is typically an alkaline lysis for microbial cells [14,15] or a detergent-based system for mammalian cells, blood, tissue culture etc. [16,17]. The second stage will typically involve a nucleic acid enrichment process, often employing some selective contaminant precipitations [9,18], followed by the adsorptive step [6–13].

Purified DNA can be used for a variety of purposes including polymerase chain reaction (PCR) [19], sequencing [14], forensics and molecular diagnostics [10] and for the emerging opportunities in gene therapy [18].

In order to achieve the isolation of DNA from the cell extract anion-exchange techniques may be carried out using either a column or batch mode. Following adsorption, desorption of DNA is generally carried out stepwise and consequently a batch process may be considered more effective, particularly as the viscosity of the nucleic acid containing solutions may be restrictive in permitting adequate flow through a column contactor. To this end paramagnetic supports have gained increasing popularity in such processes [9,10]. In the present study we report the use of Whatman DEAE-Magarose, an anion-exchange agarose bead containing a paramagnetic component [9] for the isolation of plasmid DNA from a bacterial cell lysate and genomic DNA from bacterial cells and blood. In this study we have assessed the suitability of a magnetically driven separation process as compared to a traditional anion-exchange column-based process, for each isolation. The magnetically driven separation is a batch adsorption/desorption process where the solid–liquid separation between adsorbent and mobile phases is effected by adsorbent sedimentation using an applied magnetic field, to which the Magarose beads respond. Once the field is removed, the adsorbent being paramagnetic refluidises for the next stage in the batch chromatographic process.

2. Experimental

2.1. Materials

DEAE-Magarose was obtained from Whatman (Maidstone, UK). Magnetic Beads Plasmid Mini, Midi and Maxi Kits (containing DEAE-Magarose) were obtained from Biometra (Göttingen, Germany). Plasmid Mini, Midi and Maxi Kits, QIAamp Blood Kit, Blood and Cell Culture DNA Mini and Maxi Kits, Genomic-tip 20/G, 100/G and 500/G and Genomic DNA buffer set were obtained from Qiagen (Crawley, UK). Ribonuclease A and Proteinase K were obtained from Sigma (Poole, UK). All chemicals were of molecular biology grade.

2.2. Plasmid DNA isolation

Escherichia coli JM109 cells expressing the plasmid pBluescript were grown to late log phase in Luria-Bertani broth containing 100 μg/ml ampicillin. The bacterial cells were harvested either from 1.5 ml (mini), 25 ml (midi) or 100 ml (maxi) of cell culture by centrifugation at 10 000 *g* for 1 min (mini) or 7500 *g* for 15 min (midi and maxi). The pellets were resuspended in 0.05 *M* Tris–HCl buffer (pH 8.0) containing 0.01 *M* EDTA and 400 μg/ml ribonuclease A (100 μl, mini; 1.75 ml, midi; 4 ml, maxi). Cell lysis was performed by gently mixing the resuspended cell pellet with 0.2 *M* NaOH containing 1% (w/v) SDS (200 μl, mini; 3.5 ml, midi; 8 ml, maxi) and placing the mixture on ice for 5 min. Genomic DNA and other contaminants were precipitated by addition of 3 *M* potassium acetate (pH 5.5) previously cooled to 4°C (150 μl, mini; 2.5 ml, midi; 4 ml, maxi). The mixture was stood on ice for 10 min, and then centrifuged at 10 000 *g* for 5 min to sediment the precipitated protein, cell debris and denatured chromosomal DNA. The supernatant was placed in an appropriate centrifuge tube and a 4% (w/v) suspension of DEAE-Magarose in 0.01 *M* Tris–HCl buffer (pH 8.0) containing 0.1 *M* NaCl, 0.001 *M* EDTA and 1% (w/v) Triton X-100 (STET buffer; 150 μl, mini; 860 μl, midi; 3.5 ml maxi) added. The suspension was gently mixed for 5 min at room temperature. The beads were immobilized using a Magnetic Separator Stand (Biometra, Gött-

ingen, Germany) and the supernatant removed. The beads were washed by resuspension in 0.01 *M* Tris–HCl buffer (pH 8.0) containing 0.4 *M* NaCl and 0.001 *M* EDTA (400 μl, mini; 7 ml, midi; 28 ml, maxi). After immobilization of the beads, the supernatant was discarded and the plasmid DNA desorbed by addition of 0.01 *M* Tris–HCl buffer (pH 8.0) containing 1 *M* NaCl and 0.001 *M* EDTA (200 μl, mini; 3 ml, midi; 10 ml maxi). The beads were immobilized and the supernatant transferred to a fresh centrifuge tube. Absolute ethanol pre-cooled to −20°C (2.5 volumes) and 7.5 *M* ammonium acetate (0.1 volumes) were added to the supernatant containing the eluted plasmid DNA and the mixture stored at −20°C for 10 min. The precipitated DNA was collected by centrifugation at 15 000 *g* for 30 min. at 4°C and the supernatant discarded. The pellet was washed with 70% (v/v) cold ethanol (50 μl, mini; 100 μl, midi; 100 μl, maxi) and centrifuged at 15 000 *g* for 10 min at 4°C. The supernatant was discarded and the pellet allowed to air dry at room temperature for 10 min. The pellet was re-dissolved in 0.01 *M* Tris– HCl buffer (pH 8.0) containing 0.001 *M* EDTA (50 μl). The DNA solution was assayed by absorption measurement at 260 nm and 280 nm and by agarose gel electrophoresis.

In a parallel study, pBluescript was isolated from the cell pellets harvested from 1.5 ml, 25 ml and 100 ml cell culture using the Plasmid Mini, Midi and Maxi Kits, respectively (Qiagen).

2.3. Genomic DNA isolation from bacterial cells

E. coli JM109 cells expressing the plasmid pBluescript were grown to late log phase in Luria-Bertani broth containing 100 μg/ml ampicillin. The bacterial cells were harvested from either 400 μl (mini), 2 ml (midi) or 10 ml (maxi) cell culture by centrifugation at 10 000 *g* for 20 s. The pellets were resuspended in 0.05 *M* Tris–HCl buffer (pH 8.0) containing 0.05 *M* EDTA, 0.5% (w/v) Triton X-100 and 200 μg/ml ribonuclease A (75 μl, mini; 260 μl, midi; 750 μl, maxi). The cell suspensions were incubated with 20 mg/ml proteinase K solution in sterile water (5 μl, mini; 20 μl, midi; 50 μl, maxi) at 37°C for 30 min. The suspensions were clarified by vortexing with 3 *M* guanidinium hydrochloride (pH 5.5) (25 μl, mini; 90 μl, midi; 250 μl, maxi) and incubated at 50°C for 30 min. Sterile water (0.8 ml, mini; 2.8 ml, midi; 8 ml, maxi) was added to each lysate and vortexed for 10 s. The diluted lysates were placed in an appropriate centrifuge tube and a 4% (w/v) suspension of DEAE-Magarose in STET buffer (150 μl, mini; 500 μl, midi; 1.5 ml, maxi) added. The suspensions were gently mixed for 5 min at room temperature. The beads were immobilized using a Magnetic Separator Stand and the supernatant removed. The beads were washed by resuspension in 0.01 *M* Tris–HCl buffer (pH 8.0) containing 0.4 *M* NaCl and 0.001 *M* EDTA (400 μl, mini; 1.4 ml, midi; 4 ml, maxi). After immobilization of the beads the supernatant was discarded, and the genomic DNA desorbed by incubation with 0.05 *M* arginine free base containing 1.0 *M* NaCl (200 μl, mini; 700 μl, midi; 2 ml, maxi) at 65°C for 5 min with end-over-end mixing. After immobilization of the beads the supernatants were transferred to a fresh centrifuge tube. Additional genomic DNA was isolated by two further desorption steps as described above and the supernatants pooled. Absolute ethanol (2.5 volumes) pre-cooled to −20°C and 7.5 *M* ammonium acetate (0.1 volumes) were added to the supernatants containing the eluted genomic DNA and the mixtures stored at −20°C for 15 min. The precipitated DNA was collected by centrifugation at 15 000 *g* for 30 min at 4°C and the supernatant discarded. The pellet was washed with 70% (v/v) cold ethanol (50 μl, mini; 100 μl, midi; 100 μl, maxi) and centrifuged at 15 000 *g* for 10 min at 4°C. The supernatant was discarded and the pellet allowed to air-dry at room temperature for 10 min. The pellets were resuspended in 0.01 *M* Tris–HCl buffer (pH 8.0) containing 0.001 *M* EDTA (200 μl, mini; 500 μl, midi; 500 μl, maxi) and re-dissolved by heating at 50°C for 60 min with very gentle mixing. The DNA solutions were assayed by absorption measurement at 260 nm and 280 nm and by agarose gel electrophoresis.

In a parallel study genomic DNA was isolated from *E. coli* JM109 cell culture (400 μl, mini; 2 ml, midi; 10 ml, maxi) using the Genomic Tip 20/G, 100/G and 500/G, respectively and the Genomic DNA buffer set (Qiagen).

2.4. Genomic DNA isolation from whole blood

Human whole blood stored in anticoagulant was used as supplied by the National Blood Service (Tooting, UK). Blood (0.2 ml, mini; 1.0 ml, midi; 10 ml, maxi) was lysed by addition of ice-cold 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.32 *M* sucrose, 0.005 *M* $MgCl_2$ and 1% (w/v) Triton X-100 (lysis buffer) (0.2 ml, mini; 1.0 ml, midi; 10 ml, maxi) and ice-cold sterile water (0.6 ml, mini; 3 ml, midi; 30 ml, maxi). The suspension was mixed by inversion and the translucent lysate incubated at 0°C for 10 min. The lysates were centrifuged at 1300 *g* for 15 min. at 4°C using a centrifuge fitted with a swing-out rotor and the supernatants discarded. The pellets were resuspended in ice-cold lysis buffer (0.25 ml, mini; 0.25 ml, midi; 2.0 ml, maxi) and ice-cold sterile water (0.75 ml, mini; 0.75 ml, midi; 6.0 ml, maxi) and vortexed for 1 min. The suspensions were centrifuged at 1300 *g* for 15 min at 4°C using a centrifuge fitted with a swing-out rotor, and the supernatants discarded. The pellets were resuspended in 0.03 *M* Tris–HCl buffer (pH 8.0) containing 0.8 *M* guanidinium hydrochloride, 0.03 *M* EDTA and 0.5% (w/v) Triton X-100 (100 μl, mini; 100 μl, midi; 1.0 ml, maxi) and vortexed for 30 s. The suspension was incubated with 20 mg/ml proteinase K solution in sterile water (2.5 μl, mini; 2.5 μl, midi; 25 μl, maxi) at 50°C for up to 60 min. Sterile water (0.8 ml, mini; 0.8 ml, midi; 8.0 ml, maxi) was added and the suspensions vortexed for 10 s. The lysates were transferred to an appropriate centrifuge tube and a 4% (w/v) suspension of DEAE-Magarose in STET buffer (150 μl, mini; 150 μl, midi; 1.5 ml, maxi) added. The suspension was gently mixed for 5 min. at room temperature. The beads were immobilized using a Magnetic Separator Stand, and the supernatant discarded. The beads were washed by resuspension in 0.01 *M* Tris–HCl buffer (pH 8.0) containing 0.4 *M* NaCl and 0.001 *M* EDTA (0.4 ml, mini; 0.4 ml, midi; 4.0 ml, maxi) and mixed for 1 min. After immobilization of the beads, the supernatant was discarded, and the genomic DNA desorbed by incubation with 0.05 *M* arginine free base containing 1.0 *M* NaCl (200 μl, mini; 200 μl, midi; 2 ml, maxi) at 65°C for 5 min. with end-over-end mixing. After immobilization of the beads the supernatants were transferred to a fresh centrifuge tube. Additional genomic DNA was isolated by two further desorption steps as described above and the supernatants pooled. Absolute ethanol (2.5 volumes) pre-cooled to −20°C and 7.5 *M* ammonium acetate (0.1 volumes) were added to the supernatants containing the eluted genomic DNA and the mixtures stored at −20°C for 15 min. The precipitated DNA was collected by centrifugation at 15 000 *g* for 30 min at 4°C and the supernatant discarded. The pellet was washed with 70% (v/v) cold ethanol (50 μl, mini; 50 μl, midi; 100 μl, maxi) and centrifuged at 15 000 *g* for 10 min at 4°C. The supernatant was discarded and the pellet allowed to air-dry at room temperature for 10 min. The pellets were resuspended in 0.01 *M* Tris–HCl buffer (pH 8.0) containing 0.001 *M* EDTA (200 μl, mini; 400 μl, midi; 500 μl maxi) and re-dissolved by heating at 55°C for 60 to 120 min with very gentle mixing. The DNA solutions were assayed by absorption measurement at 260 nm and 280 nm and by agarose gel electrophoresis.

In a parallel study genomic DNA was isolated from whole blood (200 μl) using the QIAamp Kit (Qiagen) and using the Blood and Cell Culture DNA Mini and Maxi Kits (Qiagen) for 1 ml and 10 ml samples, respectively.

2.5. Agarose gel electrophoresis

Samples for electrophoresis were mixed with a 0.1 volume of loading dye [40% (w/v) sucrose, 0.25% (w/v) bromophenol blue in water] and electrophoresed in 1% (w/v) agarose gels (type I, low electroendosmosis; Sigma) at a constant voltage of 75 V in a Mini-gel tank (Bio-Rad, Paisley, UK). Gels were stained using 0.001% (w/v) ethidium bromide and visualised on a UV transilluminator.

3. Results and discussion

In any adsorption chromatographic process, it is apparent that the surface chemistry and the physicochemical characteristics of the base matrix affect the chromatographic performance of the medium, at least in terms of binding capacity and selectivity of the separation [4]. Moreover the physical properties of the matrix influence their mode of operation. In

Table 1
Binding of pBluescript plasmid DNA to ion exchangers

Volume of cell culture used (ml)	DEAE-Magarose			Column based adsorber		
	DNA yield (μg)	A_{260}/A_{280} ratio	Time of purification (min)	DNA yield (μg)	A_{260}/A_{280} ratio	Time of purification (min)
1.5	8.2	1.94	90	7.7	1.90	90
25	100	1.94	120	100	1.85	180
100	668	1.88	120	505	1.85	180

this study we have compared established column based ion-exchange techniques for nucleic acid isolation based on a silica matrix with those based on a magnetic field separation. The magnetic field simply provides the motive force to effect a solid–liquid separation between, in our case, the Magarose bead and the mobile phase. Separations of this type are gaining importance in chromatography [10,20,21]. We have previously reported the properties of DEAE-Magarose and oligo-dT Magarose [9] and their suitability for batchwise purification of nucleic acids.

The binding properties of DEAE-Magarose for pBluescript plasmid DNA isolated from 1.5 ml, 25 ml and 100 ml *E. coli* JM109 cell culture are summarized in Table 1 and compared with the column-based Plasmid Mini, Midi and Maxi Kits (Qiagen). The data indicates that the yield of plasmid DNA isolated from cell cultures following alkaline lysis is similar for both types of ion exchanger at each scale of application. Furthermore the DNA was of high quality for each product type as indicated by the A_{260}/A_{280} ratio. Agarose gel electrophoresis confirmed this to be the case (Fig. 1). As the scale of the isolation increased to midi and maxi, it was evident that the magnetically driven batch separation was significantly faster than the gravity flow-based column system (Table 1). This may offer some advantages to the end-user although it should be noted that the magnetic technique is slightly more labour intensive than the column devices.

The binding properties of DEAE-Magarose for *E. coli* strain JM109 genomic DNA isolated from 0.4 ml, 2 ml or 10 ml cell culture are summarized in

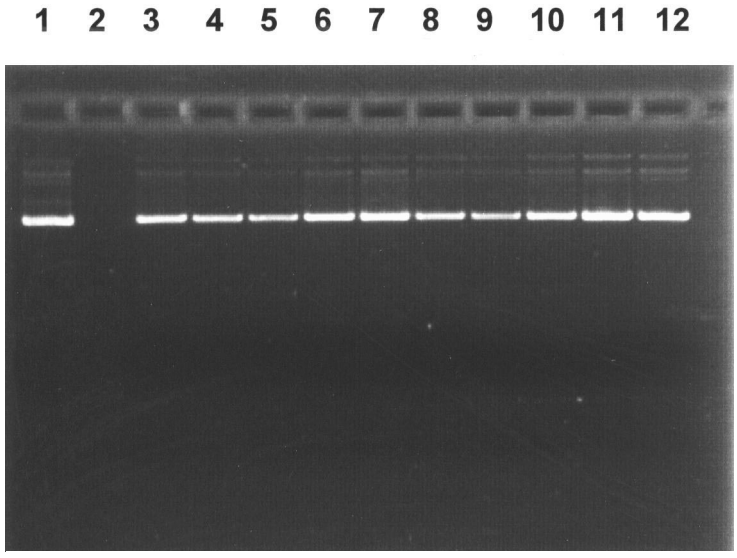

Fig. 1. Agarose gel electrophoresis of pBluescript plasmid DNA isolated from 1.5 ml of *E. coli* JM109 cell culture. Lane 1: 10 μl of product from a Plasmid Mini Kit (Qiagen). Lanes 3–12: 10 μl of product from ten replicates using DEAE-Magarose.

Table 2
Binding of *E. coli* JM109 genomic DNA to ion exchangers

Volume of cell culture used (ml)	DEAE-Magarose			Column based adsorber		
	DNA yield (μg)	A_{260}/A_{280} ratio	Time of purification (min)	DNA yield (μg)	A_{260}/A_{280} ratio	Time of purification (min)
0.4	9	1.51	180	6.5	1.78	390
2.0	40	1.44	200	23	1.68	360
10.0	107	1.51	180	45	1.72	210

Table 2 and compared with the column based Genomic Tip 20/G, 100/G and 500/G systems (Qiagen). The data indicates that the yield of genomic DNA isolated from the cell lysates is greater for the Magarose albeit at a slightly reduced purity compared with that isolated using the column adsorbers (Table 2). In each case agarose gel electrophoresis demonstrated similar DNA quality. As for the plasmid isolations the magnetically driven separations were faster than the column based ones, primarily due to the viscosity of the lysates causing severe flow restrictions in the gravity driven column process. This effect was most evident with the smaller diameter column devices.

The binding properties of DEAE-Magarose for human genomic DNA isolated from 0.2 ml, 1.0 ml or 10 ml whole blood are summarized in Table 3 and compared with the silica-based QIAamp Blood Kit for 0.2 ml blood and the column-based Genomic Tip 20/G and 500/G Blood and Cell Culture Mini and Maxi Kits (Qiagen). The data indicates that the yield and purity of the genomic DNA isolated from 1 ml and 10 ml whole blood using the ion-exchange protocols was comparable for each mode of contacting. For 0.2 ml of blood, the yield of genomic DNA was superior for the faster silica spin system compared with the ion-exchange process, although DNA purity was superior using the latter technique. One explanation for this loss of yield is that the silica-bound DNA elutes directly with water, while the salt-eluted DNA from DEAE-Magarose required ethanol precipitation. At such low concentrations of DNA this presents practical difficulties in visualising the pellet, and it is likely that during removal of the ethanol supernatant following centrifugation, some of the pelleted material may be accidentally aspirated away. In each case DNA quality as indicated by agarose gel electrophoresis was consistently high. As was observed for the other isolations the magnetically-driven separations were quicker than the gravity flow column systems (1 ml and 10 ml blood).

In the field of nucleic acid purification, particularly at a miniprep scale, silica in an underivatised form, has traditionally been the matrix of choice. Under chaotropic conditions, DNA adsorbs to silica [11,12], presumably by hydrogen bonding to the matrix. The DNA may then be eluted with water. We have introduced Silica Spin Kits for plasmid DNA minipreparations (Biometra) using a particulate silica matrix in suspension and more recently a Silica Spin Disc Kit (Biometra) where the silica matrix is in a laminar form. Following alkaline lysis of 1.5 ml of *E. coli* JM109 cell culture, we have reported the isolation of between 10 and 20 μg pBluescript plasmid DNA using each of these products [13]. However while silica supports offer speed and

Table 3
Binding of human genomic DNA from whole blood to ion exchangers and silica adsorbents

Volume of blood used (ml)	DEAE-Magarose			Column/spin based adsorber		
	DNA yield (μg)	A_{260}/A_{280} ratio	Time of purification (min)	DNA yield (μg)	A_{260}/A_{280} ratio	Time of purification (min)
0.2	3.1	1.80	240	6.7	1.73	30
1.0	30	1.61	240	25	1.58	300
10.0	282	1.77	240	315	1.72	330

flexibility and the DNA may not require precipitation prior to use, it is a non-selective technique which may not differentiate nucleic acids from other hydrophilic solutes in the loading buffer. Consequently purity of the DNA may be reduced, which while acceptable at small scale, may be undesirable for larger scale purification where sequencing and transfection studies require DNA to be of high purity and quality.

To address these issues of selectivity and consequently purity, ion-exchange techniques are gaining importance for isolation of larger quantities of DNA, be it plasmid for transfection studies and developments in gene therapy products, to genomic DNA for studies of genomics or forensic applications. By manipulation of the mobile phase conditions both during adsorption and desorption the separation efficiency achieves a degree of control which may be absent when using underivatised silica. We have previously reported significant differences between the chromatographic performance of some 70 different commercially available ion exchangers used for protein separations [4]. Our observations supported the thinking that these differences were manifestations of the proprietary chemical processes used in their manufacture and consequently different media are best suited for individual applications and media screening would be recommended as part of method scouting. For the same reasons, it is reasonable to assume that nucleic acids will bind differently to anion exchangers due to surface charge differences, both on the nucleic acid and the surface of the anion exchanger. We previously reported the development of the Magarose matrix [9], and in this study have shown that the DEAE-Magarose is effective in the isolation of plasmid DNA and genomic DNA from bacterial cells and whole blood. Our data demonstrate that the performance of DEAE-Magarose is similar to the column adsorbers containing an anion-exchange silica material (Qiagen) in terms of DNA yield and quality, while Magarose benefits from a shorter purification time. Notwithstanding the handling benefits of a magnetic separation, the chemical nature of Magarose is distinct from the Qiagen matrix and accordingly, Magarose offers the molecular biologist another matrix for nucleic acid isolation, which may, for specific applications, offer selectivity benefits over competitive media. This is a key issue if purity and quality of the nucleic acids is a pre-requisite. Furthermore, being a batch technique, isolations using DEAE-Magarose may be tailored to individual needs, giving efficient use of the media in the separations. On the other hand the prepacked gravity-flow columns are supplied in discreet sizes, reducing their flexibility in process development, which may affect their efficiency.

In terms of scaleability, the batch techniques described here should in principle scale-up linearly. Logistical issues associated with a large-scale magnetically driven separation process may pose a challenge for contactor designers, but such processes have recently been reported for specialised applications in the nuclear industry [22]. Other challenges for the large-scale isolation of plasmid vectors for example in gene-therapy will be DNA free of endotoxin. This is outside of the scope of this study but may nonetheless be an integral consideration either in media selection or in process design and optimisation. While anion exchange may offer low discrimination between these two ionic species, subsequent processing by size-exclusion chromatography or affinity chromatography may be a means of reducing such contamination from the product.

References

[1] D. Friefelder, Physical Biochemistry, Freeman, San Francisco, CA, 2nd ed., 1982, p. 249.
[2] E.F. Rossomando, Methods Enzymol. 182 (1990) 309.
[3] L.L. Lloyd, J.F. Kennedy, in: G. Subramanian (Ed.), Process-Scale Liquid Chromatography, VCH, Weinheim, 1995, p. 99.
[4] P.R. Levison, C. Mumford, M. Streater, A. Brandt-Nielsen, N.D. Pathirana, S.E. Badger, J. Chromatogr. A 760 (1997) 151.
[5] L. Stryer, Biochemistry, Freeman, San Francisco, CA, 2nd ed., 1981, p. 511.
[6] T. Kaczorowski, M. Sektas, B. Furmanek, Biotechniques 14 (1993) 900.
[7] C.L. Harris, C.J. Kolanko, Anal. Biochem. 176 (1989) 57.
[8] N. van Huynh, J.C. Motte, J.F. Pilette, M. Decleire, C. Colson, Anal. Biochem. 211 (1993) 61.
[9] P.R. Levison, S.E. Badger, J. Dennis, P. Hathi, M.J. Davies, I.J. Bruce, D. Schimkat, J. Chromatogr. A 816 (1998) 107.
[10] N. Chadwick, I. Bruce, M. Davies, B. van Gemen, R. Schukkink, K. Khan, R. Pounder, A. Wakefield, J. Virol. Methods 70 (1998) 59.

[11] B. Vogelstein, D. Gillespie, Proc. Natl. Acad. Sci. USA 76 (1979) 615.
[12] K. Melzak, C.S. Sherwood, R.F.B. Turner, C.A. Haynes, J. Colloid Interface Sci. 181 (1996) 635.
[13] P.R. Levison, J.W. Dennis, K.D. Jones, R.W. Philpott, S.L. Taylor, V. Grimm, Clin. Chem. (1998) in press.
[14] T.R. Utterback, L.A. McDonald, R.A. Fuldner, Genome Sci. Technol. 1 (1995) 1.
[15] N. Sheibani, W.A. Frazier, Anal. Biochem. 250 (1997) 117.
[16] U. Michel, A. Rau, P. Rieckmann, Anal. Biochem. 249 (1997) 246.
[17] J. Duan, X. Cai, L. Zhou, J. Wang, Anal. Biochem. 251 (1997) 291.
[18] P. Wils, V. Escriou, A. Warnery, F. Lacroix, D. Lagneaux, M. Ollivier, J. Crouzet, J.-F. Mayaux, D. Scherman, Gene Therapy 4 (1997) 323.
[19] I.J. Bruce, Sci. Progr. 77 (1993) 183.
[20] K. Rudi, F. Larsen, K.S. Jakobsen, Appl. Environ. Microb. 64 (1998) 34.
[21] C. Albretsen, K.-H. Kalland, B.-I. Haukanes, L.-S. Havarstein, K. Kleppe, Anal. Biochem. 189 (1990) 40.
[22] L. Nunez, M.D. Kaminski, Filtration Sep. May (1998) 349.