4932–4933 Nucleic Acids Research, 1995, Vol. 23, No. 23 © 1995 Oxford University Press

Selective recovery of DNA fragments from silica particles: effect of A-T content and elution conditions

Rupinder Kaur, Raj Kumar and Anand K. Bachhawat*

Institute of Microbial Technology, Sector 39-A, Chandigarh 160 014, India

Received August 3, 1995; Revised and Accepted October 23, 1995

Silica particles and silica-based resins are widely used for the purification of DNA fragments from agarose gels, from reaction mixtures and for the purification of plasmids (1–5). Several commercial and 'home-made' kits have been described using these matrices (6,7). Despite their wide use, the conditions for DNA elution and the recovery yields obtained vary widely amongst users. Elution temperatures range from 50 to 65°C and the recommended elution buffer is either TE buffer or water. A discussion highlighting these different claims was recently summarized (6), where it was also mentioned that the best conditions for elution appeared to be at 65°C with water. We have observed, however, that the elution conditions can be crucial to the recovery of a fragment and that elution, with water in particular, reproducibly results in distinctly different yields with different DNA fragments. This puzzling observation has led us to examine whether the DNA sequence of the fragment itself affects the recovery during elution from silica particles. Since one possibility was differences in the A-T content of the different fragments, we have used in our study fragments generated from plasmid pVc8 (8). Plasmid pVc8 contains a 9.3 kb fragment from an A-T rich region of yeast chromosome XI (9) cloned into the yeast vector YCp50 (Fig. 1). We carried out *Eco*RI and *Pst*I digests of plasmid pVc8 and the fragments generated were bound and then eluted from silica particles at 45 and 55°C with either Milli-Q water or TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). Our results, shown in Figure 2, reveal the strikingly different elution profiles of the different DNA fragments. The 2.1 kb fragment of the *Pst*I digest and the 2.8, 2.5 and 1.8 kb fragments of the *Eco*RI digest which are all solely derived from the A-T rich region of the insert recover extremely poorly when eluted in water at 55°C in the absence of any salts or buffers. The remaining fragments however were recovered in good yield. To determine at what stage this selective loss of DNA fragments was occurring, we first bound the DNA fragments to silica particles, eluted with water at 55°C and then subsequently repeated the elution with TE buffer. If the fragments were still bound to the silica particles after the first elution with water at 55°C, they would be eluted during the second extraction with TE buffer. However, the second extraction yielded only negligible amounts of DNA indicating that only negligible amounts were remaining bound to the silica. The poor yields were therefore not a consequence of poor elution, but were probably a result of these fragments being lost due to thermal denaturation. Furthermore, since these fragments spanned the entire insert region (Fig. 1) it appeared that base composition rather than actual sequence was possibly responsible for this selective recovery. We therefore estimated and compared

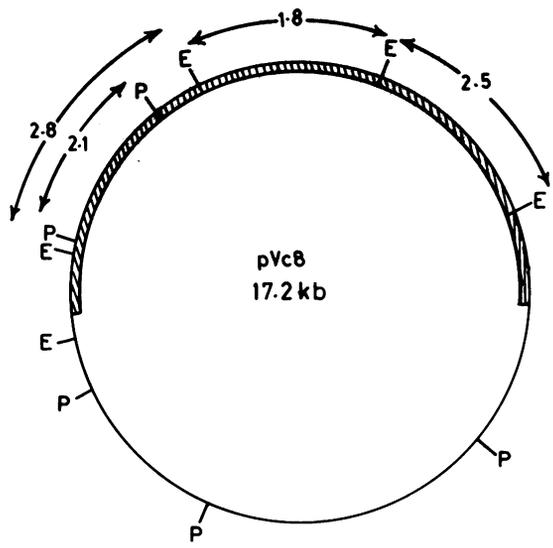

Figure 1. Map of plasmid pVc8 indicating the *Eco*RI and *Pst*I sites. Shaded region indicates the A-T rich insert from yeast chromosome XI. The map has not been drawn to scale. E, *Eco*RI; P, *Pst*I.

the A-T content of the various fragments (Table 1). The fragments that were poorly recovered had an A-T content between 62.5 and 63.5%, while the fragments that recovered well had lower A-T contents ranging from 49.5 to 60.3%. As the 3.8 kb *Pst*I fragment (60.3% A-T) had only a 2% lower A-T content than the poorly recovered fragments and yet recovered well, we decided to examine the A-T (G-C) profiles of this fragment and compare it with that of the 2.5 kb *Eco*RI fragment (62.5% A-T). We observed in the %G-C profiles of the 3.8 kb fragment, but not in the profiles of the 2.5 kb fragment, a patch (~500 bp) of high G-C content (>50%) (data not shown). It is possible that this acts as a nucleation point allowing reannealing of regions that have melted thereby resulting in increased recovery. In the absence of such high G-C patches the DNA may fail to recover to the original structure and may either remain as single stranded DNA or may form other secondary structures, either of which would account for the loss of the original fragment. The additional bands which appear on the gel (and which vary with each experiment) are probably a result of the formation of novel secondary structures. Cooling to room temperature very gradually did not help in increasing the recovery of the fragments.

* To whom correspondence should be addressed

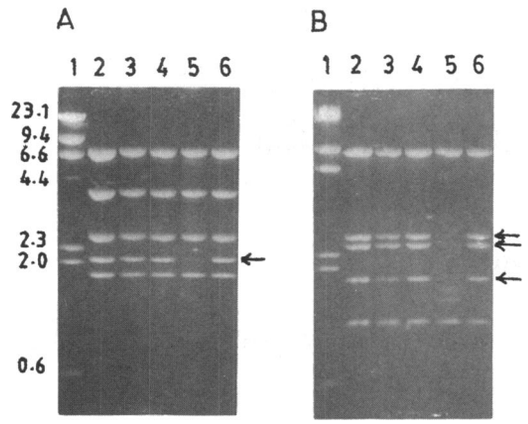

Figure 2. Agarose gel electrophoresis of restriction fragments of pVc8 recovered from silica after elution with either water or TE buffer at different temperatures. (**A**) *Pst*I digest. (**B**) *Eco*RI digest. The digestion mixes were bound to silica particle (Sigma Chemical Co., Cat # S5631) suspensions made in 6 M potassium iodide (in 50 mM Tris, 20 mM EDTA, pH 7.5) washed with 50% methanol, and then with acetone. The silica pellet was dried and then eluted in water at 45 and 55°C (lanes 3 and 5) or in TE buffer at 45 and 55°C (lanes 4 and 6). Lanes 1 contain standard λ *Hin*dIII markers and lanes 2 contain the *Eco*RI and *Pst*I digestion mixes that were applied to the silica particles. The sizes of the markers are indicated on the left hand panel. The arrows indicate the fragments that are eluted poorly.

Table 1. A-T content and recovery pattern of DNA fragments of pVc8

Fragment digest (kb)	Source of fragment	A-T content	Recovery at 55°C in water
*Eco*RI (9.0)	Vector/Insert	55.8%	Good
*Eco*RI (2.8)	Insert	63.5%	Poor
*Eco*RI (2.5)	Insert	62.5%	Poor
*Eco*RI (1.8)	Insert	62.5%	Poor
*Eco*RI (1.1)	Vector/Insert	57.2%	Good
*Pst*I (7.0)	Vecor/Insert	59.2%	Good
*Pst*I (3.8)	Vector	60.3%	Good
*Pst*I (2.5)	Vector/Insert	58.9%	Good
*Pst*I (2.1)	Insert	62.6%	Poor
*Pst*I (1.8)	Vector	49.5%	Good

Interestingly, when all these experiments were carried out with double distilled water prepared in our laboratory (instead of Milli-Q water), we found that the selective recovery of DNA fragments could be observed only at 65°C and not at lower temperatures (45 or 55°C). We believe that this is a reflection of the small amount of ions that have remained in our double distilled water preparation that were sufficient to prevent melting of the A-T rich fragments. In the absence of counterions DNA melts at lower temperature and over a broad range of temperatures, unlike the sharp transitions seen in the presence of sufficient counterions. This may also explain why the selective recovery phenomenon can be seen from 45 to 65°C although it is more pronounced at the higher temperatures.

To determine whether buffers or salts other than TE buffer could recover the poorly recovered fragment,we also carried out elution with other salts and buffer conditions. We found that 0.1 × TE buffer and 10 mM NaCl were sufficient to allow recovery of the A-T rich fragments even at 65°C. $MgCl_2$ (10 mM) also prevented selective loss of the A-T rich fragments but the recovery yields of all fragments were lower when elution was carried out with 10 mM $MgCl_2$.

Based on the experiments described in this report, we feel that we have identified many of the causes for variable recovery seen during elution from silica particles. We conclude that elution from silica particles should take into consideration the A-T content (and profiles) of the DNA fragment, especially if experiments demand that the DNA be recovered in water. Otherwise the preferred elution would be in TE buffer, or in water that contains sufficient amount of counter-ions (such as 10 mM NaCl or even 0.1 × TE buffer) that would prevent melting of the DNA fragments while still allowing efficient elution from silica particles. Covalently closed circular plasmid DNA yields that are purified by elution from silica particles, however, do not appear to be affected by elution at different temperatures in water. This includes plasmid pVc8 that has a large insert of A-T rich sequence, but we have not examined whether localized secondary structure changes could occur in such plasmids containing large segments of A-T rich regions.

ACKNOWLEDGEMENTS

We would like to thank Dr K. Ganesan for invaluable suggestions during the course of this work. R. Kaur is a Junior Research Fellow of the Council of Scientific and Industrial Reseach, India.

REFERENCES

1 Vogelstein, B. and Gillepsie,D. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 615–619.
2 Marko, M. A., Chipperfield, R. and Birnboim, H.C. (1982) *Anal. Biochem.*, **121**, 382–387.
3 Boom, R., Sol, C. J. A,, Salimans, M. M. M., Jansen, C. L., Werthem-van Dillen, P. M. E. and van der Noorda, J. (1990) *J. Clin. Microbiol.*, **28**, 495–503.
4 Boyle, J. S., and Lew, A. M. (1995) *Trends Genet.*, **11**, 8.
5 Carter, M.J. and Milton, I.D. (1993) *Nucleic Acids Res.*, **21**, 1044.
6 Hengen, P.N. (1994) *Trends Biochem. Sci.*, **19**, 182–183.
7 Hengen, P. N. (1994) *Trends Biochem. Sci.*, **19**, 388–389.
8 Bachhawat, A. K., Manolson, M. F.,Murdock, D.G., Garman, D. J. and Jones, E.W. (1993) *Yeast*, **9**, 175–184.
9 Dujon, B. *et al.* (1994) *Nature*, **369**, 371–378.