Chopped inserts: a convenient alternative to agarose/DNA inserts or beads

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Embedding cells in agarose prior to lysis produces high molecular weight DNA suitable for molecular karyotyping (1,2) and restriction analysis using pulsed electrophoresis (3,4,5). There are currently two types of embedded DNA: 1) inserts, which are small blocks of DNA/agarose molded to fit gel slots, and 2) beads, which are made by dispersing molten cell/agarose into oil, producing uniform spheres. Inserts are often favored for karyotype analysis, since they are easy to load consistently into gel slots while also producing good restriction digests after overnight incubation. Beads are often used for restriction digestion and other biochemical manipulations because diffusion time into the bead is rapid, so that reactions are completed in less time. However, bead production can be messy and residual oil may also inhibit diffusion into the bead if not carefully removed. We report here a simple method, that combines the ease of insert making with the short enzyme diffusion times that beads offer.

Suprisingly, we found that inserts can be chopped into fragments that are the same size as beads, without any appreciable DNA breakage. Consider that a typical 8 mm insert is chopped only 20 times, and that a typical DNA relaxed coil here is approximately 5 μ m in diameter. Consequently only a small percentage of molecules are potentially cleaved. Figure 1 lane 1–5 shows the molecular karyotype of *Candida albicans* (6) and *Saccharomyces cerevisiae*. Since chopped inserts and beads are similarly sized, we expected that restriction enzyme diffusion times should be equivalent. Figure 1 lane 6–7 shows a complete 1 hour EcoRI digestion of *Candida albicans* chopped inserts, as evidenced by the total elimination of chromosomal bands and the appearance low molecular weight species. We also found that chops work well for RARE analysis (RecA-Assisted Restriction Endonuclease cleavage, 7, data not shown). As with all gel embedded DNA, care must be given to small fragments, since they can diffuse out of the matrix during manipulations.

To conclude, chopped inserts are an easy and effective way to do biochemistry with large DNA molecules, and should find utility in a wide range reaction condition.

40 μ l agarose inserts (Seakem Low Gelling Temperature Agarose, 1% concentration) prepared by the standard method (1), are chopped into bead-sized pieces using a cover slip (18 $\times$ 18 mm, Fisherbrand). The extent of chopping has little effect on the quality of DNA, as shown on Figure 1, lanes 2–4. Chopped inserts (chops) are packed into a 1.5 ml Eppendorf tube and washed by 1 ml 1 $\times$ EcoRI buffer (100 mM Tris-HCl pH 8.0, 50 mM NaCl, 20 mM MgCl₂, 1.5 μ M DTT and 200 μ g/ml nonacetylated BSA (8)) 5 times, 10 minutes each. Chops settle

during the course of washing so that buffer changes are simple. Washed chops are now ready for restriction digestion. In our hands, 40 μ l of chops of *Candida albicans* can be digested completely by 20 units EcoRI in 1 hour, as shown in Figure 1. Digestion is stopped by addition of EDTA to 0.1 M and loaded on to the gel by pipetting with wide-bore tips. Chops also work well for *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* digestions using SgrAI, NotI, SrfI and RsrII under similar conditions with appropriate buffers (data not shown).

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REFERENCES

- Schwartz,D.C. and Cantor,C.R. (1984) *Cell* **37**, 67–75.
- Cook,P.R. (1984) *EMBO J.* **3**, 1837–1842.
- Schwartz,D.C. (1985) Ph.D thesis. Columbia University.
- Schwartz,D.C. et al. (1982) *Cold Spring Harb. Symp.* **47**, 189–195.
- Carle,G.F. and Olson,M.V. (1987) *Methods Enzymol.* **155**, 468–482.
- Scherer,S. and Magee,P.T. *Microbiological Reviews*, Sept.1990, 226–241.
- Ferrin,L.J. and Camerini-Otero,R.D. (1991) *Science* **254**, 1494–1497.
- Wilson,W.W. and Hoffman,R.M. (1990) *Anal. Biochem.*, **191**, 370–375.

Figure 1. Pulsed electrophoresis of *Candida albicans* insert, chops and digestion. Lane 1: yeast *S. cerevisiae* chromosomal DNA insert as the marker; lanes 2–4: *C. albicans* chops, showing the effect of chop size on DNA integrity. Chop size decreases from 2 to 4. Lane 5: *C. albicans* insert; lane 6: 1 hour digestion of *C. albicans* chop by 40 units EcoRI (New England Biolab); lane 7: 1 hour digestion of *C. albicans* chop by 20 units EcoRI; lane 8: λ concatamer DNA ladder.

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