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A novel method for isolation of large insert DNA from recombinant lambda DNA

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Genomic libraries are frequently made in Lambda vectors (1), such as Lambda 2001 (2), because of ease of construction, storage and handling. However, the resulting recombinants contain more than 60% vector sequence and they cannot easily be used for shotgun sequencing. Separation and purification of inserts by gel electrophoresis is difficult because of the relative sizes of insert and the two vector arms (10−20 kb, 20 kb, 10 kb), and removal of vector positive clones by hybridization is laborious and expensive. Here we describe a rapid and efficient method which allows the recovery of very pure insert regardless of size or sequence in less than two hours. The procedure exploits the cohesive ends of Lambda to remove the arms.

After melting of any annealed cohesive ends, the 12 bp overhangs are filled in using Klenow polymerase, bio-(11)dUTP, bio-(11)dCTP, dGTP and dATP as detailed in the legend to Figure 1. Very low concentrations (<1 μM) of all four dNTP's are used so that no precipitations are necessary. We have also carried out the procedure using one biotinylated dNTP. After incorporation, the DNA is bound to streptavidin coated magnetic beads. A large excess of beads is used as unincorporated bio-dNTP's are bound as well. The bound Lambda phage is then separated by a magnet and the beads are washed several times. They are resuspended in an appropriate restriction enzyme buffer and the DNA is cleaved using an enzyme that cuts in the phage polylinker but nowhere else in the vector arms. Cleavage within the insert is not important as this yields a number of fragments rather than one. The liberated insert is separated from the beads with the attached vector arms. The liberated DNA is used directly for shotgunning after shearing or digestion with a frequent cutter.

In the accompanying paper it is shown how this method may be applied for the isolation of end clones for walking.

Libraries were prepared from total genomic DNA by cloning 15−22 kb partial Sau3AI digests into the BamHI site of Lambda 2001.Individual clones were isolated and purified by cesium chloride gradient centrifugation or using the Qiagen Lambda purification kit (Qiagen). 1 μg. of recombinant phage DNA containing a 20 kb insert (Lambda A) was heated to 70°C for 5 minutes and then rapidly cooled on ice. The DNA was added to a 50 μl reacton mixture containing 0.3 μM each Bio-dCTP (Sigma) and Bio-dUTP (Enzo Diagnostics) and 0.5 μM each dGTP and dATP in standard Klenow reaction buffer. 5 units of Klenow polymerase were added and incubated at room temperature for 30 minutes. After heat killing at 70°C for 5 minutes, streptavidin beads (Dynabeads M-280,™ Dynal) (100 μl beads prewashed in 10 mM Tris−HCl pH7.4/ 2 M NaCl and resuspended in 20 μl TE (10 mM Tris−HCl pH7.4/0.1 mM EDTA)) were added and incubated at room temperature for 30 minutes with occasional gentle mixing. The beads were washed by pulling down on a magnet and washing in 100ul water or TE three times and then resuspended in XhoI digestion buffer. 20 units of XhoI were added and incubated at 37°C for 30 minutes. After heat killing the reaction at 70°C for 5 minutes the beads were pulled down once again and the aqueous phase removed.

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REFERENCES

1. Kaiser,K. and Murray,N. (1988) In Glover,D.M. (ed.), *DNA Cloning−A Practical Approach*. IRL Press, Oxford, Vol. 1, pp. 1−47.
2. Karn,J., Matthes,H.W.D., Gait,M.J. and Brenner,S. (1984) *Gene*, **32**, 217−224.

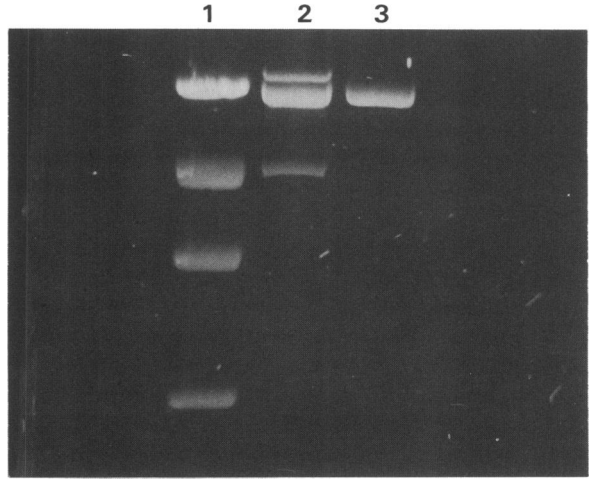

Figure 1. Top four bands of Lambda HindIII digest 2) XhoI digest of Lambda A. Note that the top band is undigested or concatenated and that the insert is unresolvable from the upper arm. 3) Biotin capture and XhoI digestion of Lambda A resulting in cleavage at the polylinkers and release of insert DNA.