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Rapid processing of nitrocellulose filter lifts of bacteriophage lambda libraries

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We describe here an extremely quick and simple method of processing nitrocellulose filter lifts of bacteriophage λ libraries from agar plates. Screening lambda libraries involves making duplicate filter lifts of the phage plaques from agar plates, denaturing the phage DNA and fixing it onto the nitrocellulose filters. Conventionally this entails treating filters with 0.5 M NaOH-1.5 M NaCl to denature the DNA, then neutralisation with 0.5 M Tris.HCl, pH7.5-1.5 M NaCl and finally washing in 6 x SSC buffer[1]. The filters are then dried and baked *in vacuo*. Screening genomic DNA and cDNA libraries of 5 x 10^5 to 1 x 10^6 individual recombinants involves the processing of 20-40 filters. This is extremely time consuming and tedious. It has been reported[2] that filter lifts can be autoclaved to denature and fix the phage DNA, thus eliminating the usual alkaline denaturation/ neutralisation washing procedure. We have modified this procedure to produce a rapid and easy method of processing filters using a standard kitchen pressure cooker:

Lambda phage are incubated with suitable plating cells, mixed with 0.7% agarose in LB broth (containing 20 mM $MgSO_4$) and plated out onto LB agar plates (containing 20 mM $MgSO_4$). We routinely plate 50,000 phage onto 142 mm diameter plates for primary library screenings (≥ 10 plates). When the plaques have developed, the plates are left at 4°C for 60 min. Duplicate filter lifts are taken and left to air dry. When all lifts have been taken, the filters are stacked between pieces of Whatman 3MM paper and placed into envelopes of 3MM paper (≤ 20 per envelope) which are then wrapped in aluminum foil. Water is brought to the boil in a standard kitchen pressure cooker, the packets of filters placed inside the pressure cooker above the level of the water and autoclaved at full pressure for 3 minutes. The pressure weight is removed with forceps, to allow the steam to escape rapidly, and the filters are removed immediately and spread out to dry. Alternatively, the whole packet is dried under a hot lamp. The filters, baked *in vacuo* at 80°C for 1-2 hours are then ready for hybridisation. To prevent shrinkage of the filters, which can make aligning and picking of plaques difficult, we generally pretreat the filters before taking lifts by autoclaving them for 3 minutes exactly as described above. We have screened cDNA phage libraries in the vector λgt10[3] and genomic DNA libraries in the phage vector λFix[4] (a derivative of EMBL 3/4[5]) with the above procedure and have encountered no problems. The quality of signals obtained by hybridisation using nitrocellulose filters processed by the standard alkaline denaturation protocol and the rapid autoclave method described above are indistinguishable.

References

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