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An improved method for mapping recombinant λ phage clones

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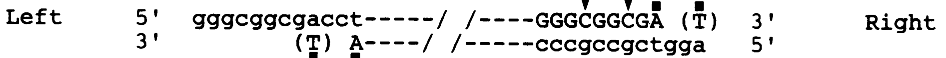
Here we describe a method derived from that of Rackwitz *et al.* [1] for mapping recombinant λ-phage inserts, in particular those of the EMBL3 and 4 series (see [1]). The left or right cos ends are uniquely labelled using a specific combination of deoxy-, dideoxy-, and α-^{32}P-deoxynucleotides, followed by partial restriction enzyme (RE) digestion, electrophoresis and autoradiography. The resulting ladder of bands shows the relative order and spacing of the RE sites from either end. This method requires niether oligonucleotides nor γ-^{32}P-labelled compounds, using reagents already on hand in laboratories performing dideoxy DNA sequencing.
1):Specific cos end labelling. Denature phage cos ends (1μg/10μl) by heating for 10min/65°C and chill on ice. Add half to tube "L" (containing 1μl 10mM ddGTP, 1μl 10mM ddCTP, 0.5μl (5μCi) α-^{32}P-dATP, 1μl 10xRE buffer, 1μl water and 0.5μl (2U) Klenow polymerase), and half to tube "R" (1μl 10mM ddATP, 1μl 10mM ddTTP, 1μl 0.5mM dGTP, 0.5μl (5μCi) α-^{32}P-dCTP, 1μl 10xRE buffer and 0.5μl (2U) Klenow polymerase). Incubate 10min/37°C, heat 10min/65°C and chill on ice. 2):Partial restriction. To each tube on ice: add 95μl 1xRE buffer, mix, and dispense 15μl to 5 tubes, leaving 30μl in tube 1. Add 1 unit RE to tube 1, mix, dispense 15μl to tube 2, mix, dispense 15μl to tube 3 and so on, leaving 30μl in tube 6. Incubate 30 min/37°C, return to ice, add 1μl 200mM EDTA per tube and pool. Ethanol precipitate and resuspend in 10μl TE pH 8.
3):Electrophoresis. Load 5μl sample with 1μl loading buffer into a 5 x 1mm slot in a 3mm thick, 20 x 20cm 0.4% agarose gel made in 40mM Tris, 30mM Acetic acid, 20mM Na Acetate, 2mM EDTA (pH∿7.8). Electrophorese in the same buffer for 42 hours at 30 V (our inter electrode distance is 30cm), changing buffer after 21 hours. Fix gel in 12% Acetic acid/10 min, dry and expose to X-ray film. Size standards are end-labelled BRL high molecular weight markers.

DISCUSSION: This method relies on the specificity of the end-labelling mixtures given above. In practice the ddNTP blocking may not be absolute and some labelling of the other cos end may occur. The use of two ddNTPs in high concentration minimises this problem. EMBL right arm cos labelling gives better resolution due to its small size. Figure: Right cos end labelling. ▪=dd, ▾=^{32}P, cos ends in lower case, filled ends in upper case.



Reference: [1] Rackwitz, H-R *et al.* (1984) Gene 30, 195-200.

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