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c2X75, a derivative of the cosmid vector c2XB

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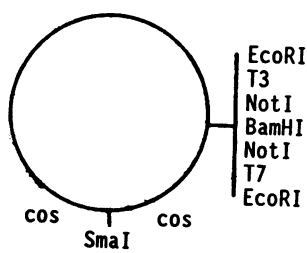
Long stretches (38-53kb) of genomic DNA may be cloned with high efficiency in cosmid vectors. Packaging of the recombinant plasmid in lambdaphage heads accounts for the efficient delivery (relative to that seen with conventional transformation methods) of such large DNA into the E.coli host. A substantial improvement in the efficiency of packaging the recombinant molecules was achieved by the inclusion of two cos sites on the vector (1,2). The problem associated with cloning MboI partial digests into a BamHI site is the frequent failure to regenerate the BamHI site, precluding the release of the insert as a large restriction fragment(s). This problem has been overcome by flanking the BamHI site with synthetic NotI sites (2,3), which in many instances will allow the insert to be released intact. A double cos vector, pcos6EMBL, containing the flanking NotI sites and based on the R6K replication origin has been constructed (2). However, there exists no analagous construct based on the ColEI replication origin. Such a construct, c2X75, derived from the cosmid vector c2XB, is described below.

The BamHI site originally used for cloning in c2XB was removed by restriction with BamHI, blunt ending with Klenow DNA polymerase and religation. The c2XB derivative lacking the BamHI site was linearised with EcoRI and ligated to the 75bp EcoRI fragment from pWE15 (ATCC# 37503). The resulting construct, c2X75, now contains a BamHI site flanked by both NotI and EcoRI sites. Maxam-Gilbert chemical sequencing was used to confirm the presence of a single insert and the orientation of the T3 and T7 RNA polymerase promoters, which are also contained on the pWE15 EcoRI fragment. The restriction map of c2X75 showing the pertinent features is:



Acknowledgements
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References 1. Bates, P.F. and Swift, R.A. (1983) Gene 26:137-146. 2. Ehrich, E., Craig, A., Poustka, A., Frischauf, A.-M. and Lerach, H. (1987) Gene 57:229-237. 3. Wahl, G.M., Lewis,K.A., Ruiz, J.C., Rothenberg, B., Zhao,J. and Evans, G. (1987) Proc. Natl. Acad. Sci. USA 84:2160-2164.