Journal of Microbiology and Biotechnology
Vol. 4, No. 3, 233-234, 1994

Note

An Improved and Efficient Method for Cosmid Cloning

LEE, BAEK-RAK* AND BERNARD WEISBLUM[1]

Department of Microbiology, Inje University, Kimhae, 621-749, Korea
[1]Pharmacology Department, University of Wisconsin-Madison, 53706, USA

A general improved procedure for preparation of a cosmid library based on the use of a pBLcosT vector was described. The vector was modified to contain 2 tandem *Xcm*I sites and was digested with *Xcm*I to yield 2 terminal 3'T overhangs, capable of ligation with the insert that contains 2 terminal complementary 3'A overhangs. The resultant ligation mixture was packaged and a cosmid library in *Escherichia coli* was established.

Cosmids are hybrid plasmid-bacteriophage vectors which have been designed for cloning large DNA fragments. Cosmids accept DNA inserts of 30~45 Kb and utilize the lambda in vitro packaging system for effecient introduction of DNA into bacterial cells. Because of the high efficiency of transduction into bacteria and the large inserts which can be accommodated, cosmids are ideal vectors for construction of eukaryotic genomic libraries.

Despite these apparent advantages, various technical problems prevented cosmid cloning from being widely employed in the construction of genomic libraries. The main problems encountered were vector concatamerization resulting in cosmids lacking inserted DNA, recombinational rearrangements of the DNA caused by ligation of multiple inserts into a single cosmid and finally partial degradation of genomic DNA causing unsuccessful construction of a cosmid library (1).

We present here an improved and efficient method for preparation of a cosmid library based on the use of a pBLcosT vector.

Preparation of DNA to be Cloned

DNA to be cloned is mechanically sheared down to 40~50 Kb in length, or obtained in initially degraded form no shorter than this length. The resultant preparation is polished with mung bean nuclease and Klenow fragment of DNA polymerase to give flush ends. A single 3' deoxyadenosine is added to both ends of the flush-ended fragments by use of the Taq DNA polymerase and dATP (2, 3, 4).

Preparation of the Plasmid pBLcosT for Use in Cosmid Cloning

To construct pBLcosT, two 36 mer DNA oligonucleotides (5'-TCG-AGC-CAA-CAT-TTT-GTT-GGC-CAT-GTT-ATC-ACT-GGC-3', 5'-TCG-AGC-CAG-TGA-TAA-CAT-GGC-CAA-CAA-AAT-GTT-GGC-3') which contain 2 tandem *Xcm*I sites (CCANNNNNNNNNTGG) and flanking *Xho*I sites, were synthesized and subcloned to *Xho*I cut pMOcos X (obtained from M. Orbach). The resultant plasmid pBLcosT was verified by double digestion of *Xcm*I (New England Biolabs) and *Xba*I (Fig. 1). The pBL-

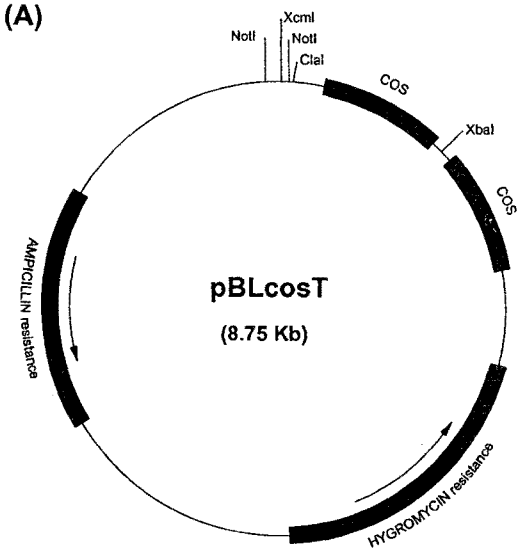

Fig. 1. (A) Genetic map of pBLcosT comid. (B) Representation of *Xcm*I double adaptor region. The underlined region will be removed upon digestion with *Xcm*I leaving 3' unpaired deoxythymidine residue at both ends.

*Corresponding author
Key words: cosmid T vetor, *Xcm*I double adaptor, Taq DNA polymerase

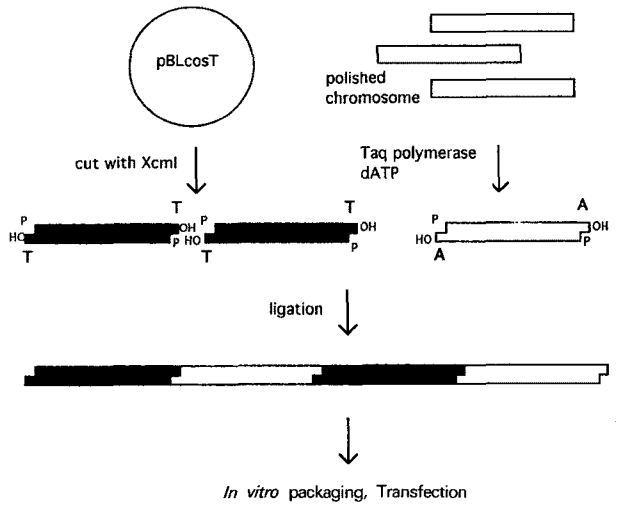

Fig. 2. Schematic represenation of cosmid cloning with pBLcosT.

cosT contained 2 tandem *Xcm*I sites and was prepared for cloning by digestion with *Xcm*I yielding 2 terminal 3'T overhangs (4, 5, 6) , capable of ligation with the 3'T deoxyadenosine residues used to tail the projected insert DNA. The prepared vector containing 2 terminal 3'T overhangs was ligated with the insert that contains 2 terminal complementary 3'A overhangs (Fig. 2). The resultant ligation mixture was packaged using the phage lambda system and used to infect cells of *E. coli* (1). Transformants were selected with ampicillin, and the cosmid DNA was prepared by the miniprep method. The cosmid DNA was examined for the presence of insert by digestion with a suitable restriction endonuclease (*Cla*I in the present case) followed by analysis of the resultant products by agarose gel electrophoresis.

In a specific implementation of this method, DNA from *Neurospora crassa*, degraded to a mean length of 40~50 Kb was obtained. Length was verified by fractionation of the preparation with pulse-field electrophoresis. After polishing and tailing with dA, 1.2 μg A-tailed chromsomal DNA was ligated with 2.5 μg with the plasmid vector pBLcosT. The resultant ligated DNA concatemer preparation was packaged (Packagene, Promega, Madison) and used to infect *E. coli*. The number of cosmid clones varied, depending on the ratio between DNA to be packaged and the amount of packaging reaction used. For example, the procedure yielded 9134 ampicillin-resistant transformants, or 500 transformants per μg input to the packaging reaction. Under these conditions, 12 out of 12 resistant cells contained cosmid clones that gave different DNA digestion patterns upon digestion with *Cla*I restriction endonuclease.

Advantages of the Method Described over Other Methods in Current Use

In usual practice, compatible cohesive ends in both the vector and insert are used.This results in self-ligation of either the vector or insert and a correspondingly reduced efficiency of the cloning. The method described in this paper will allow ligation in a way that favors concatemerization of alternating vector and insert fragments. This arrangement of vector and insert is optimal for the packaging reaction.

DNA to be used for cloning can often be obtained with lengths exceeding the 40~50 Kb size needed for packaging. Such DNA samples are usually degraded by partial digestion with restriction nucleases (most commonly *Sau*3A) to obtain the desired length fragments after size fractionation by agarose gel electrophoresis. If the DNA sample for cloning is already degraded (during preparation) down to 40~50 Kb in size, it is unsuitable for preparation of a library by the conventional method because partial digestion (with, e.g., *Sau*3A) will significantly reduce the DNA size below the desired 40~50 Kb size range; however, such a degraded DNA sample is optimal and ready for cloning by the method described above.

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(Received April 14, 1994)