Effect of lambda packaging extract *mcr* restriction activity on DNA cloning

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The negative effect of bacterial *mcrA* and *mcrB* restriction activity on the cloning of methylated DNA has recently been demonstrated (1,2,3,4). In order to determine the effect of these restriction systems on lambda and cosmid packaging, a *mcrA⁻,B⁻(5), hsd⁻(6), mrr⁻*(7) packaging extract strain was constructed by P1 transduction. The extract prepared from this strain, Gigapack II, was tested against the restriction positive (*mcrA+,B⁻*) extract, Gigapack I, by comparing efficiencies in constructing a cosmid library. The results indicate that these restriction enzymes are active in lambda packaging extracts and can affect cloning efficiencies. The elimination of *mcrA,B* restriction activity from Gigapack II allowed a 2-3 fold increase in the number of cosmid clones obtained. Similar effects have been observed with lambda libraries (8). This effect has also been shown to increase as the extent of DNA methylation increases (8,9). The results demonstrate the importance of utilizing restriction deficient lambda packaging extracts for improved cloning efficiency and possibly genomic representation. Optimal results are obtained when both *mcrA⁻,B⁻* packaging extracts and plating strains are used.

	Bacterial Strain			
	mcrA+B+ (DH1[10])	***mcrA+B⁻*** (HB101[11])	***mcrA⁻B+*** (JM109[12])	***mcrA⁻B⁻*** (PLK-A)
Methylated DNA (F5A DNA)				
Gigapack I	0	7	1	268
(*mcrA+,B⁻*)	3	5	3	240
Gigapack II	4	11	5	648
(*mcrA⁻,B⁻*)	2	16	5	504
Relative Plating Efficiency				
Nonmethylated DNA	1.3	1.0	2.0	4.3
Extract Efficiency				
Gigapack I	$2.4 \times 10^9 \pm 0.9 \times 10^9$ pfu/µg			
Gigapack II	$2.1 \times 10^9 \pm 0.8 \times 10^9$ pfu/µg			

Table Legend: The methylated F5A DNA, obtained from a somatic cell hybrid, contains a portion of human chromosome #1 on a hamster background. This DNA was restriction digested, size-selected and ligated into the pWE cosmid vector for evaluating the effects of methylation on cloning efficiency. Numbers listed for plating efficiency of cosmid library represent total number of colonies obtained per plate using the equivalent amount of starting DNA. Bacterial strains and extracts were all *recA⁻, hsd⁻*. The strain PLK-A is a *lac⁻* derivative of K802 (2). The *E. coli* plating efficiencies were determined by averaging the number of colonies obtained per plate when using a nonmethylated 45kb packaged cosmid. Colony numbers were normalized to the colony number obtained with HB101 to give relative plating efficiency. Extract efficiency was determined by comparing the plating efficiency on VCS257 cells from 8 independent packagings of nonmethylated lambda (*cI857,ind1,Sam7*) DNA.

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