

## High Efficiency Packaging of Methylated DNA for Genomic Library Construction using MaxPlax™ Lambda Packaging Extracts

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Successful construction of genomic lambda libraries from higher eukaryotes (i.e. highly methylated DNA) requires use of lambda packaging extracts devoid of restriction activities (e.g. *Mcr* and *Mrr*) that specifically degrade methylated DNA. All commercial suppliers of lambda packaging extracts claim high packaging efficiency when using a control DNA that is in no way representative of the DNA used for genomic library production. Our intent was to demonstrate the packaging efficiency that a user will likely find when preparing genomic libraries from highly methylated DNA using the MaxPlax™ Lambda Packaging Extracts. EPICENTRE's MaxPlax Lambda Packaging Extracts are derived from *E. coli* BHB2688 and a restriction-minus strain, NM759 to facilitate high efficiency packaging of methylated DNA.

**DNA Methylation.** T7 DNA was methylated *in vitro* by Sss I Methylase which specifically methylates the cytosine in the dinucleotide sequence 5'-CG-3'. This methylation pattern closely mimics that found in the genomic DNA of higher eukaryotes. Overnight digestion of methylated and untreated T7 DNA with *Hpa* II - a restriction endonuclease that digests unmethylated DNA at 5'-CCGG-3' but will *not* cut methylated DNA - was

used to assay for completeness of the methylation reaction (Figure).

**Lambda Packaging.** Untreated and methylated T7 DNAs were independently cloned into pWEB™ Cosmid vector (EPICENTRE) using Fast-Link™ DNA Ligase (EPICENTRE). Four microliters of each ligation reaction were individually packaged using MaxPlax Lambda Packaging Extract according to the product protocol. Dilutions of packaged DNA were used to infect *E. coli* EPI305 cells and infected bacteria grown on LB

plates at 37°C overnight. Plaques were counted and the packaging efficiencies calculated.

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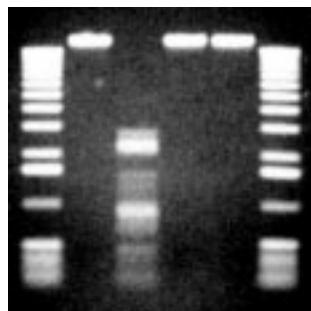
### High efficiency lambda packaging of methylated DNA.

The Figure demonstrates that methylation of T7 DNA at its 5'-CG-3' dinucleotide sequences by Sss I Methylase went to completion as determined by its resistance to overnight *Hpa* II incubation. Cosmid cloning of both the methylated and untreated T7 DNA and subsequent packaging using MaxPlax Lambda Packaging Extracts each generated libraries of >3 x 10<sup>7</sup> plaques per µg of DNA. Thus, MaxPlax Lambda Packaging Extracts produce cosmid libraries from methylated DNA with the same high efficiency as from unmethylated DNA.

**Table 1. Packaging of methylated and unmethylated T7 DNA using MaxPlax Lambda Packaging Extracts.**

Cosmid Insert	Plaques per plate	Pfu/µg DNA
T7 DNA	140	3.5 x 10 <sup>7</sup>
Methylated T7 DNA	155	3.9 x 10 <sup>7</sup>

M 1 2 3 4 M



**Figure. Complete methylation at 5'-CG-3' dinucleotides in T7 DNA was confirmed by its resistance to overnight incubation with *Hpa* II.** Lane 1, T7 DNA; Lane 2, T7 DNA after *Hpa* II incubation; Lane 3, Sss I Methylase treated T7 DNA; Lane 4, methylated T7 DNA after *Hpa* II incubation. M, DNA ladder.

### MaxPlax™ Lambda Packaging Extracts

*The highest efficiency and best value*

5 Extracts	MP5105-F73
10 Extracts	MP5110-F73
20 Extracts	MP5120-F73

*Each contains Extracts (individually dispensed), Control Lambda DNA, Control *E. coli* host cells.*

*Extracts are available in bulk quantity. Please inquire.*

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[www.epicentre.com/catalog/maxplax.htm](http://www.epicentre.com/catalog/maxplax.htm)