

***E. coli* C: a convenient host strain for rescue of highly methylated DNA**

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Host restriction systems, present in *E. coli* K12 strains, can prevent cloning of 5-methylcytosine modified eukaryotic DNA. Recently two host restriction systems, McrA and McrB, have been described (1). In order to circumvent host restriction a number of *E. coli* K12 strains, mutated in McrA, McrB or in both (2) can be employed. Although the use of these strains has been reported to increase the transformation and packaging efficiency of methylated DNA, it has become evident to us that not all methylated sequences can be rescued with such strains.

We have constructed transgenic mice, harbouring multiple copies of a lambda shuttle vector in a head-to-tail arrangement in their genome, for studying mutagenesis *in vivo* (3). Rescue of the vector from total genomic mouse DNA by *in vitro* packaging and plating of the phages on the *E. coli* K12 Y1090 (McrA<sup>-</sup>, McrB<sup>+</sup>) host, did not result in plaques. Southern analysis of liver, brain and testis DNA, digested with HpaII, MspI, HindIII or HindIII/XhoI revealed that the integrated lambda vectors were completely methylated at all HpaII sites and partially at XhoI sites (3). The hypermethylated state of the lambda vector could therefore be a main reason for the low rescue efficiency observed. We subsequently tested 2 different packaging extracts, derived from an *E. coli* K12 or *E. coli* C strain, and 3 different host strains, partially or completely lacking host restriction systems (4). Reasonable plating efficiencies were only observed when *in vitro* packaging was performed with an *E. coli* C-derived extract and the phages were subsequently plated on *E. coli* C as a host strain (Table 1). This indicates that host restriction systems present in *E. coli* K12 are responsible for the low rescue efficiency. On the basis of these results we recommend, in those cases when host restriction precludes cloning of certain methylated genomic DNA sequences, the use of *E. coli* C as a host strain.

Table 1: Rescue efficiency of lambda-gt10LacZ shuttle vector from 1 µg total genomic DNA of transgenic mouse strain 20.2.

In vitro packaging extract	Host strain		
	<i>E. coli</i> K12 Y1090 (McrA <sup>-</sup> , McrB <sup>+</sup> )	<i>E. coli</i> K12 KA802 (McrA <sup>-</sup> , McrB <sup>-</sup> )	<i>E. coli</i> C
<i>E. coli</i> K12-derived	0 pfu	0 pfu	2 pfu
<i>E. coli</i> C-derived	0 pfu	3 pfu	9100 pfu

**References**

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