

High efficiency, restriction-deficient *in vitro* packaging extracts for bacteriophage lambda DNA using a new *E.coli* lysogen

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Vectors carrying DNA modified in a mammalian host often do not propagate efficiently in *E.coli* due to bacterial restriction systems (1) capable of degrading DNA bearing a foreign methylation pattern. Restriction activity has been shown to interfere with cloning experiments involving mammalian genes (2, 3, 4) and with the rescue of shuttle vectors from transgenic mice (5, 6). In the case of shuttle vectors based on the bacteriophage λ (7), efficient propagation of vector DNA modified in the mammalian pattern requires that restriction activity be removed not only from the plating host strain (5, 6) but also from the lysogens from which the lambda *in vitro* packaging extracts are produced (6). Host strains deficient in all known restriction systems are available, such as *E.coli* C-derived strains and *E.coli* K12 strains with mutations in the *mcrA*, *mcrBC*, *mrr*, and *hsdR* loci. Restriction-deficient lysogens for making packaging extract, however, present a problem. A lysogenized derivative of *E.coli* C produced by Rosenberg can be used to prepare a restriction-free extract (8). However, this extract typically provides 5 to 15-fold less efficient packaging of λ DNA compared with the best available commercial extracts, making it sub-optimal for some experiments. For example, we and others have found this extract to be unsatisfactory for the purpose of rescuing λ phage shuttle vectors from the genome of transgenic mice (6).

An efficient extract is sold commercially (Gigapack Gold II, Stratagene, CA). This packaging system is based on that devised by Hohn and colleagues (9); it consists of two extracts derived from complementary lysogen pairs carrying mutations in the D and E genes of the prophage. Combined extracts contain all the factors necessary for excision and packaging of λ DNA to make viable virions. The Stratagene lysogens are described as being derived from the original Hohn *E.coli* K12 lysogens but are advertised to have been made deficient in all known restriction systems (6). However, only the extracts prepared from these strains, and not the strains themselves, have been made available, and use of these extracts can be prohibitively expensive for large scale work.

We report here the usefulness of a new packaging strain, NM759 [*E.coli* K12 *recA56* Δ (*mcrA*) *e14*^o Δ (*mrr-hsd-mcr*) (*lim434* *clts* *b2* *red3* *Dam15* *Sam7*)/ λ], which is a new,

restriction-free, K12-derived strain for making D⁻ (sonicate) extract by the method of Hohn (9). This strain, a derivative of W3110 (10), is free of known restriction systems by virtue of a deletion in the *E.coli* chromosome involving the *mrr*, *hsdR*, and *mcrBC* loci and an *e14*^o deletion including the *mcrA* locus (11). Its extract, when combined with freeze-thaw, E⁻ extract from the complementary Hohn lysogen (BHB2688), packages pure λ DNA as efficiently as the Stratagene system (Table 1). More importantly, this extract is particularly useful when used to package lambda DNA bearing the mammalian methylation pattern. For example, in our mutagenesis studies, extracts prepared from the NM759/BHB2688 strain pair were used to rescue λ phage shuttle vector DNA from the DNA of a transgenic mouse carrying multiple copies of the lambda DNA in its genome (12) (Table 1). Extracts from NM759/BHB2688 consistently delivered the highest efficiency of lambda vector rescue from mouse DNA, matching or exceeding that of the Stratagene extract at a fraction of the cost. Interestingly, despite the use of BHB2688, which was not specifically made deficient in restriction activity, this strain pair yielded extract with the same apparent lack of restriction activity as the Stratagene extract. This implies that the NM759/BHB2688 extract is functionally restriction deficient. It may be that the freeze-thaw process, used in making the BHB2688 extract, sufficiently inactivates restriction activity; however, this activity clearly survives in the sonication process, as in the BHB2690 preparation compared with that from NM759.

Several other strain pairs were also tested, including a set of lysogens derived from *E.coli* C (NM750 and NM751) which consistently yielded inferior extracts even though *E.coli* C strains are devoid of known restriction activity. It was also surprising that extracts prepared from several λ E⁻ lysogens derived from the same restriction-deficient *E.coli* K12 strain used to produce NM759 yielded freeze-thaw extracts inferior to that from BHB2688 (data not shown). The reasons for the exceptional efficiency of the extracts from NM759 and BHB2688 compared to those from these other strains have not yet been ascertained. In any case, the NM759 strain will be made freely available for research purposes and the NM759/BHB2688 strain pair ought to prove useful for workers who wish to make their own restriction-deficient extracts for packaging λ vectors carrying

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mammalian modified DNA. Those performing cloning experiments involving mammalian genes or shuttle vector rescue from transgenic animals may prefer this to the expensive commercial option.

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Table 1. Effects of packaging and plating strains on λ phage rescue efficiency

Sonicate strain	Freeze-thaw strain	pfu/ μ g λ DNA	pfu/ μ g mouse DNA <i>E. coli</i> C host	<i>E. coli</i> K12 hsdR ⁻ host
BHB2690	BHB2688	5×10^8	370	110
NM750	NM751	2×10^6	10	<1
NM759	BHB2688	2×10^9	6000	750
Stratagene	Stratagene	2×10^9	5200	680
Gigapack Gold II	Gigapack Gold II			