Volume 15 Number 15 1987 Nucleic Acids Research

Preparation of high titer lambda phage lysates*

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Submitted June 29, 1987

The most time-consuming part of lambda phage cloning procedures is often the generation of a high-titer lysate. We have constructed a bacterial host strain that allows efficient growth of λ cloning vectors and describe a procedure for preparing high-titer lysates.

The host strain, TAP90 (pro, leuB6, thi-1, rpsL, lacY1, tonA1, supE44, supF58, hsdR$^-$, hsdM$^+$ recD1903::mini-tet) is restriction-deficient, has a supF allele, and carries a mini-tet insertion in recD (1). It has been shown that recD mutations allow for improved growth of Spi$^-$ derivatives of bacteriophage λ (1,2,3) and minimize loss of genomic sequences from recombinant DNA libraries (3). In addition, the host is resistant to phage T1 infection, an occasional problem during large-scale λ phage isolation.

To prepare a lysate, 10^5 to 10^6 plaque forming units (or a pure plaque soaked 1 hr in 0.1 ml TMG (10 mM $MgSO_4$, 10 mM Tris-HCl, pH 7.4, 0.01% gelatin)) are adsorbed to 1 ml of TAP90 plating cells on ice for 20 min. The plating cells are prepared by resuspending an overnight culture in 1/2 volume 10 mM $MgSO_4$. The phage and host are then added to 30 ml of warm (37°C) LB broth containing 5 mM $CaCl_2$ (LB + Ca^{++}). The culture is shaken vigorously at 39°C and monitored after 2 hr. If the bacteria reach a high density (OD_{650} >1.0) without lysing, the culture is diluted 1/2- to 1/4-fold with LB + Ca^{++} and incubation continued. After lysis has occurred, 0.1 ml chloroform is added and incubation continued for 10 min. The debris is then pelleted by centrifugation at 10,000 x g for 10 min. The lysate may be stored at 4°C with 0.1 ml chloroform or used to prepare phage DNA. We typically obtain titers of >10^{10}. Adsorption on ice and the use of Ca^{++} allows a more uniform infection and synchronous growth of phage. Lambda grows optimally at 39°C and this is a crucial part of the protocol.

Acknowledgements: Michael Dean is a fellow of the Leukemia Society of America, Inc.

*Research sponsored, at least in part, by the National Cancer Institute, DHHS, under contract NO1-CO-23910 with Program Resources, Inc. The contents of this publication do not necessarily reflect the views or policies of the DHHS, nor does mention of trade names, commercial products, or organizations imply endorsement by the U. S. Government.

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