

NIH AIDS Research & Reference Reagent Program
20301 Century Boulevard
Bldg. 6, Suite 200
Germantown, MD 20874
USA

Phone: 240 686-4740
Fax: 301-515-4015
www.aidsreagent.org

PROTOCOL FOR GROWING STOCK CLONES

Cosmid Clones Z2 and Z8

These clones are derived from a SuperCos 1 custom cosmid library (Stratagene), and are preserved in 100 μ l of a bacterial stock in 15% glycerol. The low percentage of glycerol allows for the freezing of stocks at -20°C when -70°C space is not available.

To grow, scrape some of the frozen culture onto a loop and streak onto an LB plate with 50 μ l/ml ampicillin, then treat as a regular plasmid clone.

Cosmid Clone Z6

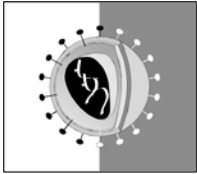
The starting material is in the form of glycerol stock. The cells should be streaked out on ampicillin plates as described above. To obtain DNA, pick colonies and grow overnight as mini-prep 3 ml cultures. DNA from several colonies should be analyzed for retention of insert. The donors' experience is that large scale cultures (250 ML) result in deleted cosmids; therefore, large amounts of DNA is difficult to obtain. Mini-preps can be combined if needed. We suspect that serial passage will also eventually result in deletion. If needed, DNA can be repackaged into cosmid arms using Stratagene's Gigapack Gold Kits to generate stock.

Phage Clones (L36, L47, L48, L54, L56, L74, L80)

These clones are derived from a Lambda FIXII custom genomic library (Stratagene), and preserved in SM buffer with 7% DMSO for freezing at -70°C. Their titers are approximately 10^3 – 10^4 pfu/ml.

To grow, plate 10 μ l and 100 μ l onto LB plates with infectable host cells in 0.7% top agarose and incubate at 37°C overnight. Pick plaque and grow as any other lambda phage. XL1-Blue MRA (P2) from Stratagene is the recommended host for amplification of the clones.

ALL RECIPIENTS OF THIS MATERIAL MUST COMPLY WITH ALL APPLICABLE BIOLOGICAL, CHEMICAL, AND/OR RADIOCHEMICAL SAFETY STANDARDS INCLUDING SPECIAL PRACTICES, EQUIPMENT, FACILITIES, AND REGULATIONS. NOT FOR USE IN HUMANS.



KSHV Genome

