PACKAGING EXTRACTS FOR λ CLONING

A simple method of preparing λ packaging extracts is described. Good extracts will yield 5 x 10^8 pfu/µg DNA.

Materials:

Cells : Freeze thaw lysate - BHB 2688 Dam
Sonicate extract - BHB 2690 Eam

10% Sucrose/50 mM Tris pH 7.5

Lysozyme solution:
2 mg/ml in 0.25 M Tris pH 7.5

Buffer A :
20 mM Tris pH 8.0
3 mM $MgCl_2$
0.05% ß-Mer
1 mM EDTA pH 7

Buffer M1:
110 µl dH_2O
6 µl 0.5 M Tris pH 7.5
300 µl 0.05 M spermidine neutralized w/Tris Base 0.1 M putrescine
9 µl 1.0 M $MgCl_2$
75 µl 0.1 M ATP neutralized with NH_4OH
1 µl ß-Mer

Procedure:

A. Freeze Thaw Lysate (FTL) from BHB 2688 Dam.

1. Grow 3 x 250 ml cultures of BHB 2688 in L Broth at 31°C in 2 L flasks.
2. AT OD = 0.6, turn up incubator to 37°C, add an equal volume of 65°C L Broth to each flask.
3. Grow at 37°C with vigorous shaking for 60 minutes.
4. Cool cells on ice.
5. Collect cells in Sorvall GS3 rotor at 5 K for 10 minutes in 6 bottles.
6. Drain off all media, drain pellet well.
7. Resuspend each pellet in 0.5 ml cold 10% sucrose/50 mM Tris pH 7.5
8. Pool all supernatants
   Dispense into two - 10 ml oakridge tubes (3 ml each).
   To each tube add 75 µl fresh lysozyme soln. Mix gently but well.
9. Quick-freeze in liquid N_2, store at -80°C.
10. Thaw at room temp, then at 4°C until pellet completely thawed.

11. To each tube add 75 µl Buffer M1. Mix gently but well.
12. Spin at 35 K for 35 minutes at 4°C.
13. Remove supernatants, aliquot into 100µl amounts. Freeze in liquid N_2 store at -80°C.

B. Sonicate Extract (SE)

1. Grow 250 ml cultures in L Broth at 31°C. Induce as for FTC.
2. Grow for 1 hour at 38°C with vigorous shaking.
3. Collect cells in Sorvall GS3 rotor at 5 K for 10 minutes and pour off supernatant.
4. Suspend each pellet in 0.5 ml Buffer A. Pool, transfer to a single tube, dilute with 2.6 ml Buffer A.
5. Sonicate on ice using 3 second blasts until no longer viscous. Do not allow foaming.
6. Pellet debris at 6 K for 6 minutes.
7. Aliquot supernatant in eppendorf tubes (suggested volume is 50 µl). Quick freeze in liquid N_2.

C. Packaging Reaction

1. Thaw FTL and SE on ice.
2. Mix reaction in order:

Buffer A	7 µl
DNA	1-2 µl
Buffer M1	2 µl
SE	6 µl
FTL	10 µl

3. Incubate for 60 minutes at 25°C.
4. Dilute with 500 µl phage buffer, absorb, plate.

Comments:

1. These extracts are only as good as the effectiveness of the 31°-37°C temperature shift, so work fast.
2. Be extra careful in preparation of the SE to prevent foaming. Accidental denaturation of extracts can reduce packaging efficiency by 10^2 - 10^3.
3. For trial packaging reaction used 10-100 ng of DNA.
4. The amounts of FTL should be varied (for example from 5-10 µl) to determine the optimal proportions.
5. Remember to use a good prep of Charon 4a, 30 to check the efficiency of packaging.
6. Oligomerize at least 4 hours at 42°C.

Reference: *Scalenge et al. Chromosoma 82, 205-216.*