

# MAKING PACKAGING EXTRACTS

## Freeze-Thaw Lysate

1. Streak a colony of BHB 2688 on LB and incubate at 32°C overnight.
2. Inoculate 3 x 50 ml LB + 10 mM MgSO<sub>4</sub>, incubate overnight at 32°C.
3. Test cultures by spreading 50 µl onto an LB plate and growing at 42°C overnight. No more than three colonies should grow.
4. Use 10 ml from best overnight culture to inoculate 500 ml LB plus Mg in a 2L flask. Shake at 32°C until OD<sub>650</sub> is 0.6.
5. Shift flask to a 90°C water bath until the culture temperature is 43°C. Immediately shift the flask to a 43°C incubator and shake vigorously for 15'.
6. Cool culture to 38°C on ice and then shake at 38°C for 2.5 hours.
7. Chill flask on iced water and then pellet cells at 0°C, 8K, 15'.
8. Pour off supernate and remove last few drops with a pipette.
9. Resuspend cells in 3 ml **ice cold** 10% sucrose, 50 mM Tris pH 7.5. Transfer to an SW50 ultracentrifuge tube.
10. Add 100 µl 2mgml<sup>-1</sup> lysozyme (make fresh), mix rapidly and immediately freeze in liquid N<sub>2</sub> for 15'. Thaw tube on ice water for 15'.
11. Add 500 µl **M1 buffer**, mix on ice, centrifuge 35K 25' at 0°C. Transfer supernate to a new tube.

12. Keep tube on ice and dispense 25  $\mu$ l per eppendorf. Drop in liquid  $N_2$ .  
Store at  $-70^\circ C$ .

### Sonic extract.

1. Grow BHB 2690 and test as above.
2. Grow and induce cells as above. Centrifuge and resuspend cells in 3 ml **ice cold** 20 mM Tris pH 8, 3 mM  $MgCl_2$ , 10 mM  $\beta$ -mercaptoethanol, 1 mM EDTA. Transfer to 50 ml Falcon tube.
3. Sonicate cells with a microtip at maximum microtip setting. Apply 5 sec blasts with 15 sec cooling pauses in between. Avoid foaming. Cell solution should lose viscosity and become iridescent after about 10 blasts. STOP HERE.
4. Centrifuge at 6K 15' to pellet cells (should be small). Add 600  $\mu$ l **M1 buffer** to supernate and distribute 20  $\mu$ l per tube. Drop in liquid  $N_2$  and store at  $-70^\circ C$ .

### Packaging

1. Thaw FTL and SE on ice.
2. Add 5  $\mu$ l SE to 25  $\mu$ l FTL and 3  $\mu$ l ligation mix (less than 1  $\mu$ g) and mix carefully with pipette tip. Incubate at room temperature for 2 h.
3. Add 200  $\mu$ l SM and a drop of chloroform. Vortex 1 sec and centrifuge 30 sec. Remove supernate and titer. Efficiency should be  $5 \times 10^8$ - $1 \times 10^9$  pfu/ $\mu$ g with CI857.

**M1 buffer** (make fresh). Add in order:

$H_2O$	200 $\mu$ l
$\beta$ -mercaptoethanol	2 $\mu$ l
0.5 M Tris pH 7.4	12 $\mu$ l
0.05 M spermidine, 0.1 M putrescine (pH7 with 1 M Tris base)	600 $\mu$ l
1 M $MgCl_2$	18 $\mu$ l
0.1 M ATP (pH7 with NaOH)	150 $\mu$ l

