**OMP INCLUSION BODY PREP**

Revised January 2012 by S.K.

**Day One**

1. Inoculate 3 mL 2XYT (+3 µL Amp) with fresh colony from plate and grow at 37ºC for 6-8 hrs.
2. Inoculate 25 mL 2XYT (+25 µL Amp) with 50 µL of starter culture and grow overnight at 37ºC.
3. Autoclave 500 mL Terrific Broth in baffled flask (25.4 g TB powder + 500 mL water).

**Day Two**

1. Inoculate the 500 mL TB (+500 µL Amp) with 10 mL of overnight and grow at 37ºC.
2. Monitor growth by A600.
3. Induce with 500 µL 1M IPTG at OD ~ 1 (takes ~2 hrs).
4. After 4-6 hrs, harvest cells by centrifugation in JA-10 rotor at 5,000 rpm for 15 min at 4ºC. Pellets may be stored at -20ºC.

**Day Three**

1. Thaw pellets in bottles if previously frozen. Discard any liquid with a P1000. Resuspend cell pellet in 25 mL Lysis buffer (50 mM Tris pH 8.0, 40 mM EDTA pH 8.0). Keep on ice and resuspend by repipetting. Transfer to 50 mL conical.
2. French press 8 times to crack cells open (see French press directions for assembly instructions).
   1. If the French press does not draw up the entire solution it may be getting clogged. Try vortexing to get rid of any leftover clumps of cells.
   2. If there is leaking at the top of the cylinder around the piston, make sure the O-ring at the end of the piston is in good shape. It should fit snugly against the piston.
   3. The O-ring should be replaced periodically due to normal wear and tear.
3. Add 83.3 µL Brij-35 then invert several times to mix.
4. Collect inclusion bodies by centrifugation in 50 mL conicals in JA-10 rotor with adaptors at 5,500 rpm for 30 min at 4ºC. Pour off the supernatant. Allow residual liquid to settle by gravity for a few minutes and use a transfer pipet to discard any leftover supernatant. If there is any yellow/brown mucus-slime (DNA) on your pellet try to suck up as much as possible and discard.
5. Wash pellets with 25 mL Wash buffer (10 mM Tris pH 8.0, 1 mM EDTA pH 8.0). Resuspend in the same conical by repipetting.
6. Collect inclusion bodies again by centrifugation in JA-10 rotor at 5,500 rpm for 30 min at 4ºC. Repeat the supernatant discard process.
7. Repeat steps 12-13 to wash pellets again.
8. Resuspend pellets a third time in 25 mL of Wash buffer, and split suspension evenly into four or eight 15 mL conicals.
9. Collect inclusion bodies by centrifugation in JA-14 rotor with adaptors at 6,000 rpm for 30 min at 4ºC.
10. Pour off supernatant and pipet off excess liquid. Inclusion body pellets may now be stored at -20ºC for 1-2 years.