Popping Vegetative Cells in Your Sporulated Culture

Pellet your sporulated culture (2000 rpm, 10 minutes)

Resuspend at 2 x 10^9 cells/ml (concentrate sporulated culture about tenfold) in "softening buffer"

Incubate 10 minutes at 30°C.

Pellet cells (1000 rpm, 10 minutes)

Respend cells at 1 x 10^9 cells/ml in spheroblasting buffer

Immediately remove 1 microliter: add to 100 microliter of 0.5% triton X-100 and place on ice as your "before" sample.

Incubate 30 minutes at 30°C with gentle shaking

Place cells on ice

Remove 1 microliter: add to 100 microliter of 0.5% triton X-100 as your "after" sample.

Compare vegetative/spore ratios of "before" and "after" cells using phase contrast microscopy of cells (using a hemocytometer helps).

If additional lysis of vegetative cells is desired incubate spheroblasting reaction an additional 10 minutes at 30°C.

Pellet cells (1000 rpm, 10 minutes).

Resuspend in 5 mls of 0.5% triton X-100 by vortexing

Chill cells on ice

Sonicate at a low setting (depends on your instrument) We use a Fisher Sonic Dismembranator Model 100 (sounds like Dr Dooffenshmirtz made it) setting 2, with 20% pulse time. Pulse about one minute.

Look at cells to monitor disruption of asci and bursting of remaining vegetaive cells.

We find that additional sonication is tolerated well by the spores but we limit pulse episodes to one minute or less with intermitent cooling. We do not exceed setting 4.

Pellet cells (1000 rpm, 10 minutes).

Resuspend in 1 ml 0.5% triton X-100. Count spores to determine concentrations. Spores retain over 90% viability when stored for two weeks at 4°C.

Good luck!

REAGENTS

Softening Buffer

10mM DTT
100mM Tris 9.4

recipe:

1.0 ml Tris 9.5 (1 M)
0.1 ml DTT (1 M)
8.9 mL H_2O

Spheroblasting Buffer

2M Sorbitol
10mM KPO_4 7.2
Zymolyase T-100 @ 3mg/mL

cheesy recipe:

add 0.1 ml KPO_4 (7.2) to 100 mL 2M sorbitol
add appropriate amount of zymolyase T-100