

Yeast Sporulation and Tetrad Dissection (2/20/09)

Yeast Sporulation

Grow an overnight culture in YPD.

Allow saturated culture to stay on rollerdrum for 3 days—this is certainly true for *S. kudriavzevii*; not so sure for *S. cerevisiae*.

Spin 1 ml of super-saturated culture; aspirate media and resuspend in 1 ml 2% KAc.

Pour into tube containing 3 ml of additional 2% KAc. Sporulate on RT rollerdrum for 5-7 days.

Tetrad Dissection

Dissecting tetrads is a skill that takes time to develop.

Take 5 μ l of sporulated culture and add to 50 μ l of Zymolyase (150 μ g/ml). Vortex.

Digest for 10 min on 30° C rollerdrum. Add 150 μ l of cold H₂O to stop the reaction and keep on ice. *Do not vortex. From here on out, all pipetting should be done slowly to reduce mechanical stress on the cells (that way digested spores remain together).*

Spot 5 – 10 μ l of digested cells onto a YPD plate and allow the cells to spread out by gravity to form a streak across the center of the plate. *I avoid using a stick to spread out the cells to avoid breaking up cells, and, more importantly, to avoid tearing up the agar, which will make micromanipulation more difficult.*

Tip: Use dry YPD plates, it is difficult to get cells to stick to the dissecting needle if the plate is too wet.

Zymolyase Stock Solution (100 x)

5 mg/ml in 10 mM TrisCl, pH 7.5, 5% Glucose

Zymolyase Working Solution (3 x)

150 μ g/ml in 1 M Sorbitol (30 μ l of Stock Solution into 970 μ l 1 M Sorbitol)