SPORULATION AND DISSECTION OF YEAST TETRADS

(also random sporulation)

NOTE 1: Most lab yeast strains are homothallic, deleted for the HO endonuclease. This allows their stable propagation as haploids of a specific mating type. >90% of the time we work with diploids, it is only after mating two haploid strains and sporulation is intended. To track marker segregation two methods are possible; (i) tetrad dissection, or (ii) random sporulation. Both methods are described in this protocol.

A. Sporulation

A.1 Grow diploids overnight on GNA or YPD media at 30°C (Sporulation efficiency can sometimes be improved by pre-growing the cells on rich media, so it is generally recommended that you use YPD).

A.2 Patch cells to either SPOR or NGS plates. Try to cover much of the plate surface so that the cells rapidly deplete the few nutrients in the plate.

SPOR plates: 10g Potassium Acetate
1.25g Yeast Extract
1g Glucose
20g Agar
ddH_2O to 1 liter, mix well and autoclave

A.3 Leave the plate at room temperature for one day, then transfer to 30°C for 2-7 days. Sporulation can be monitored by scraping off a small sample of cells and looking at them under the microscope. Sporulating cells first become large and round, with a large, gray vacoule in the center. Tetrads are easily recognizable by their diamond or tetrahedral shape. Many "tetrads" will contain less than four spores. When a reasonable fraction of well-formed tetrads are visible (at least 5%), they are ready for dissection.
NOTE: >50% tetrads recommended if you're planning random sporulation

B. Dissection

B.1 Put 40µl of **Zymolyase-100T** (1 mg/ml in 1M sorbitol) in a sterile ependorff.
NOTE: Zymolyase is very expensive. There is a lab stock at 4°C. The stock is dated and kept to a small volume (10 ml or less) so the enzyme doesn't undergo long storage times.

B.2 With a sterile stick, scrape up some cells from the SPOR plate. There should be enough cells to form a small, but visible, ring on the end of the stick. Resuspend cells in the zymolyase solution. Gently swirl the stick around to break up any clumps.

B.3 Incubate for 15-20 minutes at 37°C. The incubation time is somewhat empirical (depends on the strength of the zymolyase, how "hard" the tetrads are).

B.4 At the end of the incubation, add 500µl sterile ddH_2O to the tube. **The tetrads will be fragile**, so the tube should be handled gently and the ddH_2O should be added slowly by running it down the side of the tube.

B.5 Using a sterile loop, the cell suspension can be gently mixed. Take a loopful of cells and spread them in a straight line along one edge of the dissection plate. Under the dissecting microscope, ensure the line of cells is within the reach of the needle and that tetrads are still visible.

B.6 Use the manipulator to pick up a tetrad and move it into the center of the plate. Use the numbers on the platform to guide placement. Tetrads should be placed at intervals of five along the x-axis.

B.7 After placing the tetrad on the surface of the plate, use the needle to break apart the spores. Place the needle on the tetrad and vibrate it by tapping the microscope base. **Appropriately digested tetrads should break without too much banging.** If tetrads fail to break apart, the may be underdigested and the zymolyase treatment can be repeated for a longer time. If tetrads break apart as soon as they are touched, they may be overdigested.

B.8 Once the four spores are broken apart, use the needle to pick up single spores and place them a intervals of 5 in the y-axis. The manipulator has "stops" in the this axis to make this process easier. Dissection takes a great deal of patience, but improves markedly with practice. Some common mistakes are to press the needle too hard and break it or embed it in the agarose. Also, when moving the platform, the needle should be lowered since the plate is probably not perfectly level and the needle may drag along the plate.

B.9 Incubate plates 2 days at 30°C. Photograph plates before replica plating to determine marker segregation.

C. Random sporulation

C.1 Put 25µl of **Zymolyase-100T** (1 mg/ml in 1M sorbitol) in a sterile ependorff.
NOTE: Zymolyase is very expensive. There is a lab stock at 4°C. The stock is dated and kept to a small volume (10 ml or less) so the enzyme doesn't undergo long storage times.

C.2 With a sterile stick, scrape up some cells from the SPOR plate. There should be enough cells to form a small, but visible, ring on the end of the stick. Resuspend cells in the zymolyase solution. Gently swirl the stick around to break up any clumps.

C.3 Incubate for 20-30 minutes at 37°C. The incubation time is somewhat empirical (depends on the strength of the zymolyase, how "hard" the tetrads are) – for this approach you want the efficient digestion.

C.4 At the end of the incubation, add 500µl sterile ddH_2O to the tube. **Vortex vigorously to break up the tetrads.**

C.5 Plate a series of 10-fold dilutions on plates (want ≈ 150-250 separated colonies per 8cm plate – this makes it easier to pick isolated colonies). **The media choice is dependent on which phenotype you're chasing.** Incubate plates 2 days at 30°C (or RT° if you're tracking a temperature sensitive allele).

C.6 Pick >40 well isolated colonies and streak patches on plate of the same type. Incubate overnight.

C.7 Replica to other plates to track all phenotypes / auxotrophies of interest. Also determine the mating type of clones of interest.