yMAT_3v1

Random Spore Analysis

(adapted from Susan L. Forsburg and Nicholas Rhind, Yeast 2006)

Requirements:

- Glusulase (Perkin-Elmer, Ref: NEE154001EA).
- Malt Extract plates (1 L: 30 g Malt Extract, 20 g agar, 300 mg each of uridine, histidine, adenine and leucine; adjust pH to 5.6 with NaOH) or SPAS plates (ID: M0136).
- YES plates (ID: M0043).
- Autoclaved bidistilled water.
- Autoclaved toothpicks.

Preparation:

2 days before crossing, patch cells of both strains on a YES plate (freshly growing cells are needed for good mating efficiency).

Crossing the strains:

1. Add a 5–10 µL drop of water to a mating plate (Malt Extract or SPAS). Take a toothpick of cells from Strain 1 and resuspend them in the drop of water. Take an equivalent amount of Strain 2, add it to the same drop, and mix well.
2. Incubate at 25–27 °C for 2–4 days. Monitor microscopically for formation of asci.

Random Spore Analysis:

1. Pick a toothpick full of cells from the mating plate into 100 µL sterile water. Add 10 µL 5% glusulase (Perkin-Elmer) and incubate overnight at room temperature.
2. Verify under the microscope that no complete asci remain.
3. Plate spores on YES plates. To obtain a suitable number of colonies per plate, plate several dilutions of the spore suspension (usually 1:100, 1:1,000 and 1:10,000 of the original suspension). The spore suspension can be washed free of the enzyme and stored at 4 °C in water for weeks if further plating is required.
4. Colonies will appear in 2–5 days. Replica-plate to assign markers.