

Mating

From S. Silverman and P. Gibney

MATING

1. Divide plate into sections for each haploid cross as shown. Using about half of an average colony, streak a little bit into the section for the appropriate parent. Streak the rest of the half colony into the mating section. Do the same with the other parent. Mix the two haploid parents in the mating section with the streaking device (stick, toothpick, loop, etc.)
2. Allow plate to incubate at 30°C for 3-4 hours.
3. Streak some of the mated section onto a new plate as a straight-line patch.
4. Using a dissection microscope fitted with a micromanipulator, remove zygotes from the line of cells to an isolated location on the plate. See below.
5. Remove 3-5 zygotes so they can form their own colony. Incubate plate at 30°C for 2-3 days until large single colonies have grown for surviving zygotes.

NOTE: Mating plate should be a dry YPD plate and the surface should be as level as possible – this will facilitate micromanipulation of diploids.

NOTE: Keep a record of each cross you make in a laboratory notebook. Include the date of the cross, names/genotypes of parent strains, and purpose of the cross.

