Mating / mating type halo assay

Mating

1. ~2 days before, streak out all strains that you’re planning to mate (streaking into patches is useful here since you don’t need single colonies for mating).
2. Add 5uL of H2O to the “mating plate.”
3. Use a stick to pick up a clump of cells from one parental strain and gently mix into H2O.
4. Use a stick to pick up a clump of cells from the 2nd parental strain and gently mix into H2O and 1st parent.
5. Incubate mating plate for 3-4 hours at 30°C.
6. Use a stick to streak from your mixed parental strains to an area just below.
7. Use dissection scope to pull zygotes. The easiest zygotes to identify are those that have undergone mating and already produced a new daughter cell. They end up looking like “mickey mouse ears”.
8. 

9. Incubate 2-3 days at 30°C.
10. To confirm diploidization occurred, perform mating type halo assay and sporulation.

Mating type halo assay

When exposed to opposite mating pheromone, cells arrest in G1 in a characteristic schmoo form (thus MATa cells arrest in response to -Factor, which would normally be secreted by MAT cells).

1. ~3 days before, streak out DGY36 (MATa) and DGY37 (type = MAT).
2. ~1 day before, grow out DGY36 and DGY37 overnight in 2 mL YPD.
3. Use beads to plate 200uL of each onto separate “tester” plates.
4. Incubate tester plates for 30 minutes.
5. Either replica plate, patch, or pin (works with a toothpick or stick) your experimental strains onto both tester plates. Ensure that these patches are well-separated.
6. Incubate 2-3 days at 30°C. Halos become easier to see with longer incubation.
7. Score whether or not each patch has a halo of space around it. If it does, that means that the lawn strain responded to the pheromone emitted by the patch, and thus that they are of opposite mating type. So, if a halo formed around the patched strain on the MATa tester plate, the strain itself is MAT.
8. Double-check your scores by examining the opposite tester plate. If the experimental strain is haploid, it should only have a halo on one tester plate. If the strain is a diploid, halos will not form on either plate.