

Halo Mating Type Assay
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Protocol derived from Katja Schwartz's protocol.

In addition to your strains of interest, streak out:

DBY7730 (aka RC634a) MATa ade2 his6 met1 ura1 can1 cyh1 rme sst1-3

DBY7442 (aka XT1-20A) MATalpha leu- ura- ade- sst2

These strains were made in the Thorner lab. See Julius et al (1983) Cell, 32, 839-52 for documentation of DBY7730. DBY7442's exact genotype is unknown. The sst mutations make them super-sensitive to pheromone. When exposed to pheromone of the opposite mating type, the cells arrest.

Let streaks grow 2 days 30C.

Inoculate overnight YPD cultures of the mating type testers.

Dilute the overnights 1:10 with sterile media or water.

Spread ~200 μ l lawn on YPD plates, one for each mating type.

Incubate 30C 30 min.

Pin your strains of interest to the lawns, or use a toothpick to make small, well-separated patches on the lawn. Make sure to flame the frogger between each plate, or use a fresh toothpick for each plate.

Incubate 30C overnight.

Score whether or not the 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 a tester plate, the strain itself is alpha. The nice thing about having both the a and the alpha tester plates is that you can double-check your scoring by making sure there is a halo on only one tester plate.