Mating/Sporulation to Get Difficult Strains

1. Grow patches on YPD overnight.
2. Pick equal amounts of the two strains into YPD Eppendorf and rock gently or mix on YPD plate.
3. Mating takes 5 h to overnight, therefore can do it overnight but best to check for zygotes at 5-8 h to ensure there is mating going on (zygotes often very diluted after overnight, so more difficult to see).
4. Plate various dilutions on YPD to get separated colonies — grow till quite large.
5. Pick (20-40?) colonies and patch quite densely on sporulation plates (also spread used loop on YPD to keep strain for possible future use). N.B. Dips grow faster, so big colonies more likely dip but sometimes one MAT type gives bigger colonies so select a big-biased mixture.
6. Sporulation takes 1-5 d — check under microscope for tetrads.
7. Release spores from one whole positive patch (no need for high percentage of tetrads — separate protocol). N.B. If patch too small make bigger one of positives from YPD plate or mix several positive patches.
8. Plate spores in various dilutions to get separated colonies.
9. Pick random colonies (many, e.g. 100+ for rare combinations) into 100 µl dH_2O with yellow box unplugged tip and leave tip in Eppendorf.
10. Mix and drop 8 µl using same tip onto various selection plates including YPD for keeping strain.
    - e.g. Several YPD plates for ts test
    - e.g. YPgal for galHO + hmlΔ + hmrΔ (+ves are non-survivors)

    Or, instead, just patch colonies on YPD plates and replica plate onto selection plates after growth (but this loses a day and ts test and survival not so clear).