Mating Type switching (+ testing)

NOTE 1: Most lab yeast strains are homothallic, deleted for the HO endonuclease. This allows their stable propagation as haploids of a specific mating type. In this protocol the HO gene is under the control of a GAL promoter. After induction of the endonuclease, a mating-type switch is possible.

Required: Yeast strain transformed with pGal-HO.URA3 (Plasmid MKF251, mb262)
Minimal media (no URA) / 0.1% glucose / 2% raffinose
Minimal media (no URA) / 0.1% glucose / 2% galactose

1. Grow pGal-HO containing ($URA3^+$) transformants overnight 30°C (RT° if your strain is temperature sensitive, *ts*) in 5ml minimal media (no URA) / 0.1% glucose / 2% raffinose.

2. Next morning allow the tubes to sit on the bench for about 20 minutes. (This is a handy method of observing possible bacterial contamination. If the prep is yeast only they will settle quickly such that the top few mm of the culture tube will clarify).

3. Resuspend the overnight culture and sub 1ml to 5 ml minimal media (no URA) / 0.1% glucose / 2% galactose. Grow for 5 hours @ 30°C to induce mating type switching for approximately one generation; after 2, 3, 4 and 5 hours of growth in the presence of galactose take an aliquot of cells and place on ice. At the end combine all aliquots (optimal induction time varies per yeast strain and the likelihood of obtaining cells of the opposite mating type increases significantly by taking timepoints during the course of induction).

4. Determine $OD_{\lambda_{600}}$ (1 unit ≈ $2x10^7$ cells/ml) of pool and dilute to get ≈ 150-200 colonies per plate on YPD. Allow colonies to form @ 30°C (≈ 2 days).

NOTE 2: [MCK notebook pA1.51] $OD_{\lambda_{600}}$ of pool, 0.517 (≈10^7 cells/ml). Make 10-fold serial dilutions in YPD (1/10 ≈ 10^6 etc). Plate at least two of dilutions as you want isolated colonies to pick for the mating test (I usually plate 200µl of the 10^4, 10^3 and 10^2)

5. Test mating type of obtained colonies to determine the mating type (**NB.** you will find the occasional diploid: in exp on pA1.51 for an **a** to α switch I tested 32 colonies = 26**a**, 5α, 1**a**/α) …
   Option A: Pheromone production assay (halo-assay).
   Option B: Directly mating to tester strains (<u>much</u> easier)

Option A: Pheromone Production (Halo-assay)

NOTE 3: When exposed to opposite mating pheromone, cells arrest in G_1 in a characteristic schmoo form (thus *MAT*a cells arrest in reponse to α-Factor, which would normally be secreted by *MAT*α cells). Cells would normally degrade the pheromone and escape, but the <u>tester strains</u> below are deleted for Sst2, which regulates desensitization to α-Factor.

Required: <u>query strains:</u> for secretion of mating pheromones: a-factor or α-factor
<u>control strains</u>: *MAT*a, *MAT*α and a/α (diploid strains secrete neither factor)

tester strains: YF94: *MAT*a / *sst2*, YF95: *MAT*α / *sst2*
YPD plates

A.1 Streak the query strains (see above) in patches (≤ 8/plate) on YPD plates and incubate overnight. Likewise for the control strains.

A.2 In the AM inoculate a loopful of tester strains (YF94 MATa and YF95 MATα): into 2ml YPD. Grow four hours at 30°C.

A.3 Prepare tester strains on YPD plates: Dilute four hour cultures 1:100 in sterile ddH_2O. Spread 500µl onto YPD plate and allow to dry.

A.4 Replica plate query and control patches onto plates containing the tester-strain lawns. **NB.** use two different velvets so as not to mix a- and α- testers. Incubate overnight at 30°C.

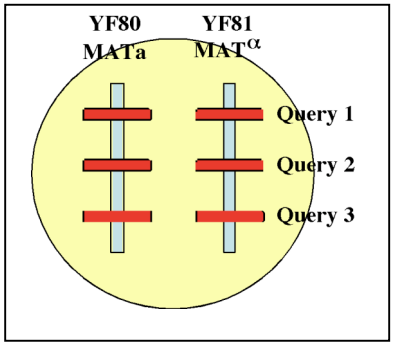
A.5 Check plates next day for halo-formation (a zone of clearance where cells aren't growing). A halo around a query strain on the YF94 (*MAT*a / *sst2*) plate indicates the query is *MAT*α. If not very pronounced, further incubation at 30°C usually helps.

Option B: Mating type testing

NOTE 4: Much easier. The tester strains in this case have a *lys9*- auxotrophy (unable to grow on LYS^- plates), shared by none of our common lab strains (if Lys is being used as an auxotrophy, it is usually *lys2*). The query strain must have another auxotrophy from the selection available on -6 dropout plates: his, trp, leu, ura, ade or lys.

Required: query strains: you want to know the mating type
control strains: known *MAT*a (KFY139), *MAT*α (KFY138) and a/α (KFY137); latter shouldn't mate
tester strains: KFY133: *MAT*a / *lys9*, KFY134: *MAT*α / *lys9*
YPD plates
- 6 dropout plates (his, trp, leu, ura, ade and lys)

A.1 Streak all strains out to fresh plates. Incubate overnight at 30°C.



A.2 On a YPD plate vertically streak testers YF80 and YF81 with sterile small wooden applicators. Then streak the query strains horizontally so that the queries and testers are well mixed at the intersection (**see figure**). Incubate plate overnight at 30°C. Next day replica the plates onto –6 dropout plates. Incubate plate overnight at 30°C. If successful mating occurred the resulting diploids will be WT for all auxotrophies represented on the plate.

NOTE 5: Some mutant strains will be defective for mating – check the literature to see if this is the case for the gene you're working with (**and keep it in mind**)