Competitive Mating Assay (7/22/08)

For comparing the mating advantage in *MAT***a**

*MAT***a** and *MAT*α strains should have complementary auxotrophies and the diploids formed by the two *MAT***a** strains must be distinguishable. For instance:

Experimental strains:
E1 *MAT***a**, GPA1-W303, *his3, trp1, LEU2, ade2, can1-100*
E2 *MAT***a**, GPA1-RM::*NatMX*, *his3, trp1, LEU2, ade2, CAN1*

Tester strains:
T1 *MAT*α, GPA1-W303*, HIS3, TRP1, leu2, ADE2, can1-100*
T2 *MAT*α, GPA1-RM::*NatMX, HIS3, TRP1, leu2, ade2, can1-100*

Diploids can be selected on minimal media = adenine.
E1/T1 diploids are Can^R Nat^S; E2/T1 diploids are Can^S Nat^R.
E1/T2 diploids are Can^R Nat^R; E2/T2 diploids are Can^S Nat^R.

Grow overnight cultures of both *MAT***a** strains and the tester *MAT*α in YPD + Ade (extra adenine is necessary since experimental haploids are *ade2*). It couldn't hurt to filter the media to avoid getting crap on the filters.

Coulter count. Mix $5x10^6$ E1 cells, $5x10^6$ E2 cells in 10 ml YPD + Ade.

Dilute cells 1:500 in H_2O. Sonicate to break up cell clumps. Plate 100 λ onto –leu (~200 cells), -leu+canavanine, and ClonNat. Label plates "pre-mating."

Add $2x10^6$ Tester cells. Filter onto a 25 mm 0.45 micron nylon filter. Place filter onto a YPD + Ade plate (four filters per plate).

Incubate 30° C for 5 hours.

Remove filter from plate and wash off cells with 1 ml H_2O (Concentration of diploids should be ~10^6).

Dilute cells 1:$2x10^3$ in H_2O. Sonicate to break up cell clumps. Plate 100 λ onto and minimal+ade, and minimal+ade+can. Label plates "post-mating."

Incubate plates 30° C for two days.

Replica plate "pre-mating" –leu plates and "post-mating" minimal+ade plates to Canavanine and ClonNat.

Incubate plates 30° C overnight.

Count colonies on Canavainine and ClonNat plates. Determine the ratio of Can^R Nat^S to Can^S Nat^R pre- and post-mating.