

## Competitive Mating Assay (7/22/08)

For comparing the mating advantage in *MATa*

*MATa* and *MAT $\alpha$*  strains should have complementary auxotrophies and the diploids formed by the two *MATa* strains must be distinguishable. For instance:

Experimental strains:

E1 *MATa*, GPA1-W303, *his3*, *trp1*, *LEU2*, *ade2*, *can1-100*

E2 *MATa*, GPA1-RM::*NatMX*, *his3*, *trp1*, *LEU2*, *ade2*, *CAN1*

Tester strains:

T1 *MAT $\alpha$* , GPA1-W303, *HIS3*, *TRP1*, *leu2*, *ADE2*, *can1-100*

T2 *MAT $\alpha$* , GPA1-RM::*NatMX*, *HIS3*, *TRP1*, *leu2*, *ade2*, *can1-100*

Diploids can be selected on minimal media = adenine.

E1/T1 diploids are  $\text{Can}^R \text{Nat}^S$ ; E2/T1 diploids are  $\text{Can}^S \text{Nat}^R$ .

E1/T2 diploids are  $\text{Can}^R \text{Nat}^R$ ; E2/T2 diploids are  $\text{Can}^S \text{Nat}^S$ .

Grow overnight cultures of both *MATa* strains and the tester *MAT $\alpha$*  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  $5 \times 10^6$  E1 cells,  $5 \times 10^6$  E2 cells in 10 ml YPD + Ade.

Dilute cells 1:500 in H<sub>2</sub>O. Sonicate to break up cell clumps. Plate 100  $\lambda$  onto -*leu* (~200 cells), -*leu*+canavanine, and ClonNat. Label plates "pre-mating."

Add  $2 \times 10^6$  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<sub>2</sub>O (Concentration of diploids should be  $\sim 10^6$ ).

Dilute cells 1:2 $\times 10^3$  in H<sub>2</sub>O. Sonicate to break up cell clumps. Plate 100  $\lambda$  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 Canavanine and ClonNat plates. Determine the ratio of  $\text{Can}^R \text{Nat}^S$  to  $\text{Can}^S \text{Nat}^R$  pre- and post-mating.