

DMA REPLICATION PROTOCOL

A. PREPARATION OF DMA STRAINS.

1. Pin DMA strains from 14, 384-well plates (YPD/15% glycerol) to 14 corresponding **YPD agar**-Omnitrays. (Will be stored at 6°C for approximately 1 to 2 months, for continual re-use).

PROGRAM: *West1A/B*. C384S (**Dip Level = 0!**) → C384G (Dip Level = 3)

B. PREPARATION OF QUERY STRAIN.

1. Grow 10 ml of query strain in YPD o/n. Pour culture into a sterile, empty Omnitray.
2. Pin culture to 14 **YPD agar**-Omnitrays. → 30°C 2 days.

Position P1-A5

18 positions total!

PROGRAM: *West2*. C384G (empty; D.L. = 0) → C384G (YPD agar; D.L.=3).

C. CROSSING OF DMA STRAINS WITH QUERY STRAIN, etc.

1. Pin from ea of 14 DMA strain **YPD agar** Omnitrays (stored @ 6°C) to fresh YPD agar Omnitrays. Immediately thereafter, pin from ea of 14 Query strain **YPD agar** Omnitrays to the same set of 14 fresh YPD agar Omnitrays above. This allows equal numbers of both mating types to maximize mating interactions during colony growth. → 30°C, 1 day. (2 days does not improve diploidization efficiency!)

PROGRAM: *West4A/B from here down*. C384G (YPD agar; D.L.=3) → C384G.

2. Pin from ea of 14 **zygote** Omnitrays above to ea. of 14 **diploid-select** medium (**YPD + G418 + clonNAT** agar OR **SC - Ura + clonNAT** agar). → 30°C, 2 days.
3. Pin from ea of 14 **diploid-select** Omnitrays to 14 **sporulation medium** Omnitrays. → 30°C, 9 days.
4. Pin from ea of 14 **sporulation medium** Omnitrays to 14 **haploid-select medium** (SC-His-Arg+Can) Omnitrays. → 30°C, 2 days.
5. Pin from ea of 14 **haploid-select medium** Omnitrays to 14 **haploid-select medium** (SC-His-Arg+Can) Omnitrays again. → 30°C, 1 day.
6. Pin from ea of 14 **haploid-select medium** Omnitrays to 14 **SC medium + G418** (SC-His-Arg+Can; *w/o ammonium sulfate*)- double mutant select step 1. → 30°C, 1 day. OR **SC – Ura-His-Arg +Can!**
7. Pin from ea of 14 **SD medium + G418** Omnitrays to 14 **SC medium + G418 + clonNAT** (SC-His-Arg+Can; *w/o ammonium sulfate*)- Omnitrays. → 30°C, 1 day. **Switch to 0.5 mm pin heads here!!**
OR **SC – Ura-His-Arg +Can +clonNAT!**

126 Omnitrays/replication; 60 trays/case @ \$80.00/cs

Extra set of 14 Omnitrays for mishaps = 140 total needed total per replication (2 to 3 times as many needed if performed in duplicate or triplicate). 140/60 = 2.34; 2.34 X \$80.00 = \$190.00 total per repl.