

# HAND-PINNING DMA STRAINS

## *1 – 2 days beforehand:*

1. Prepare YPD (+ glucose) + G418 medium. About a liter is required for a single set of 53, 96-well plates. Also prepare YPD (+ glucose) + G418 + riboflavin medium for plates #70 and 71- 0.5 L.
    - A. To 900 ml of YPD (no “D”) add 100 ml sterile 20% glucose plus 0.2 g of G418 (200 ug/ml final). Swirl well.
    - B. Test the solution with several tester strains- those containing kanMX vs. those not- by inoculating ~ 2 ml and growing o/n @ 30°C with shaking. Don’t proceed further if this test doesn’t work perfectly!
  2. Label consecutively 53, 96-well plates on both top and sides with indelible markers. Cover o/n with bags the plates came in.
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## *Day 1 (assuming YPD/G418 solution test above worked):*

1. Use Gilbert’s 12-channel multipipettor (swabbed with 95% EtOH prior) to transfer YPD/G418 to 96-well plates. Requires 12 ml per plate; reservoir holds 48 ml.

TOTAL TIME REQUIRED: ~ 1 hour

To start, the volumes with be: 115 ul YPD/G418    Gilbert’s 12-channel (*push down hard on tips!*)  
50 ul 50% glycerol    Cross’s 8 channel Finpipette  
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165 ul total (wells are 360 ul deep)

2. Incubate o/n @ 30°C. All 53 plates can be stored in two plastic storage trays for convenience, and should be covered with Saran wrap to minimize evaporation.
  3. Check for contamination. Can incubate a second night, but this will increase evaporation from wells; volume is low to begin with.
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## *Day 2:*

1. *Thaw about half of the 53-plate DMA strain set from -70°C. Remove the aluminum seals while still frozen! Be careful of spattering while removing the seals- go slowly.*

TOTAL THAWING TIME REQUIRED: ~ 30’ to 45’’ per plate

2. Pin with a pre-sterilized pinning tool by using a repeated up-and-down motion into the plate held in place with a library copier. Pin slowest growing strains first (ResGen plates 70 and 71) since these likely will have to be incubated 3+ days. Pin-sterilization protocol between plates requires ~ 5’.

TOTAL TIME REQUIRED (@ 10 plates/hr.; 53/10) = ~ 5 to 6 hours (minus mishaps!)

*N.B. If you thaw 25 plates (half the set) for a 2.5 hr. pinning session, the plates will be thawed for that amount of time prior to re-freezing!!*

3. Incubate @ 30°C for 2 to 3 days. Evaporation will occur which must be accounted for. As above, all 53 plates can be stored in two plastic storage trays for convenience, and should be covered with Saran wrap to minimize evaporation.

## WEST LAB

4. Freeze the original DMA plates for >> 2 hours (overnight is OK, or even over the weekend, if wrapped tightly in plastic bag). Then seal with Aluminum tape, and place back in original ResGen boxes or respective plastic bags.

N.B.!!

1. Place a **band-aid** over index finger to to prevent cutting by sealing tape!! Then use this finger to smooth down foil on plate surface.

2. *Don't press down too hard. If you do, adhesive backing sticks to plate upon removing foil subsequently.*

TOTAL TIME REQUIRED: ~ 20' per 10 plates (*Don't thaw more than this at a time*)

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### **Days 4 to 5:**

#### **For 96-well plates:**

1. Add 50% glycerol (50 ul/ well) to all 53, 96-well plates, using a Finpipette with automatic mixing, or Gilbert's 12 channel manual pipettor (use LPS "Value Series" white tips, NOT YELLOW tips). Have backup resources available, such as tweezers, spatulas, extra reagent boats, etc. in case of trouble. If necessary, borrow Taffet's "ICN Titertek" 8 channel (50 to 300 ul) manual multichannel pipettor and use LPS Value Series pipet tips.

Requires 5 ml/plate (265 ml total).

Requires 1 rack of 96 sterile tips per 96-well plate (*i.e. need 53 racks of sterile tips!!*)

TOTAL TIME REQUIRED = ~ 5 to 10 hours (minus mishaps!)

2. Freeze the 53 plate set @ -70°C for >> 2 hours. Thaw in sets of ten, seal with Aluminum tape; store @ -70°C.

TOTAL TIME REQUIRED: ~ 20' per 10 plates (*Don't thaw more than this at a time*)  
1.5 to 2 hr. per full set

#### **For 384-well plates:**

1. Add 50% glycerol (18 ul/ well) to all 14, 384-well plates, using the Matrix 16-channel automatic pipettor with mixing.

TOTAL TIME REQUIRED = ~ 3 to 4 hours (~15'/plate; minus mishaps!)

N.B.!!

1. Withdraw Matrix tips from right to left so extra tips which are caught can be seen early.

2. Don't go more than three 384-well plates at a time prior to freezing @ -80C, since glycerol otherwise begins to settle out.

3. Matrix pipettor just makes it through six 384-well plates on a full charge prior to losing charge completely.