

384-well plate prep

1 – 2 days beforehand:

1. Prepare YPD (+ glucose) + G418 medium. About 250 ml is required for a single set 14, 384-well plates. Also prepare YPD (+ glucose) + G418 + riboflavin medium for plates #14- 20 ml.
 - 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 14, 384-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 Matrix 16-channel multipipettor (swabbed with 95% EtOH prior) to transfer YPD/G418 to 384-well plates. See Matrix Pipettor Protocol (*typed*) to do this. Requires 16 ml per plate; reservoirs hold 48 ml vs. 100 ml.

TOTAL TIME REQUIRED: ~ 1 hour (Matrix Battery lasts 1.5 hr.)

To start, the volumes with be:	42 ul YPD/G418
	18 ul 50% glycerol 7

	60 ul total* (wells are 100 ul deep)

****N.B.: Total volume cannot exceed 60 ul per well since pinning @ a dip level = 0 (required!) will cause overflow into the adjacent wells!!***

2. Incubate o/n @ 30°C. All 14 plates can be stored in one plastic storage tray 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!!

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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.

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 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*)

Days 4 to 5:

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!)

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