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Making copies of RNAi 96-well plates:

You will need sterile 96 well round-bottom dishes and LB supplemented with Ampicillin (1uM final).

1. Using sterile technique, pour LB supplemented with Amp into a trough.*
2. Use multichannel-pipette to aliquot 80 uL into each well of a 96 well dish.
3. **If using the metal prong method:** sterilize 96well prong by dipping in first in bleach, then water (slightly deeper than the bleach), then ethanol (slightly deeper than the water). Flame off the ethanol with a Bunsen burner, being very careful of the fireball that will form.
   If using reusable plastic replicator method: Open a sterile pack of 96 well replicators.
4. Dip in a thawed RNAi plate, then dip in deepwell plate with LB. It is an unfortunate necessity to use a thawed plate, because of the possibility that the ice chunk will come away with the prongs and cross contaminate the original plate.
5. Seal the plate with a foil lid making sure that each well is sealed from the wells around it (to prevent mixing via splashing).
6. Grow overnight in the 37°C shaker.
   a. Tape the deep 96-well plate to the bottom of the shaker to make sure it does not tip.
7. Next day, add 80ul of 60%glycerol (sterile) to each well using a multichannel pipette so that final concentration is 30% glycerol. Pipette up and down a few times to make sure the culture and glycerol are fully mixed.
8. Cover with foil lid making sure that each well is sealed from the wells around it.
9. Label and Freeze at -80C

*Alternatively, you can grow the RNAi in 30% glycerol from the start, but it will take longer in the shaker and some already slow-growing clones will take even longer. We typically do this when copying genome-wide libraries and other large libraries.

Ampicillin (100mg/mL) (80mL) • 8g Ampicillin sodium salt (stored at 4°C) • Add sterile ddH_2O to 80mL Filter sterilize with a 0.22μ syringe filter. Store at -20°C	**LB** H_2O 950 mL Tryptone 10 g NaCl 10 g Yeast extract 5 g Combine the reagents and shake until the solutes have dissolved. Adjust the pH to 7.0 with 5 N NaOH (~0.2 mL). Adjust the final volume of the solution to 1 L with H_2O. Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle.