GRIDDING

After startup procedure and opening program, prepare the Q-BOT for gridding as follows:

1. Check the humidifiers for signs of mold/mildew. Clean the humidifiers if necessary. Then fill the humidifiers and turn on at maximum output. The Q-BOT needs to be AT LEAST 50% humidity before gridding. Around 70-80% is best.
2. The bed should have only the rectangular metal pieces on the bed, making the bed completely flat.
3. Place clean and dry wash station and brush in the Q-BOT.
4. Place the gridding head in the Q-BOT, pins facing up.
5. Prepare the filter blocks as follows:
    a) Wipe the filter blocks down with 80% ethanol.
    b) Pour some sterile water onto the filter block.
    c) Lay the Whatman paper down onto the water so that it is centered on the block and there are no bubbles underneath.
    d) Add a small amount of sterile water on top of the Whatman paper if necessary, and them lay the membrane on the paper so that it is not hanging over the paper on any side.
    e) Using a sterile disposable pipet, gently roll the excess water off and remove the bubbles. Medium pressure should be applied. There should be no pools of water on the membrane, but the membrane should not be totally dry.
    f) Wipe the back of each filter block so that there is not a lot of water underneath it, and then place it in the Q-BOT. Check to make sure that

the membranes are centered on the block and that there are no puddles on top.

g) All 15 blocks must be in the Q-BOT in order to run the program. The first membrane can either be placed at the top right or bottom left when facing the robot from the computer side. Usually we use the bottom left as filter #1.

h) Secure the blocks in place by switching the Table Clamps button on the front of the Q-BOT several times on and off, then leave them on.

6. Open the gridding program, and sterilize the Q-BOT by selecting UV light for 20 minutes.
7. Prepare source plates by thawing in hood if necessary.
8. Place source plates in the hotel in the desired printing order, and place hotels in Q-BOT. Make sure they are all the way in place and the plates are pushed all the way back in the hotel.
9. Fill the wash bath with 80% ethanol, and secure the gridding head to the arm with the provided screw.
10. Select preferences in the program:
    - A 2x2 in duplicate will print 4,608 clones (12 384-well plates)
    - A 3x3 in duplicate will print 9,216 clones (24 384-well plates)
    - A 4x4 in duplicate will print 18,432 clones (48 384-well plates)
    - We use the CUGI setup 4x4, and the CGF for 2x2. The 3x3 is just called 3*3.
    - Leave the defaults that appear when the CUGI 6 field setup is selected.
    - Select the number of membranes that will be gridded. Select the location of filter #1.
11. Check the order of the plates in the hotel and make sure the filter blocks are locked into place.
12. Run the program. Check the humidity and ethanol level every 2 hours.
13. When the run is complete, remove the filter blocks one at a time to place the membrane on the medium.

14. Using forceps, carefully pickup the membrane by the diagonal corners. Carefully center the membrane over the fresh Q-tray medium. Lay down the center first, then slowly lower the membrane. It is critical that there are no

bubbles under the membrane, as no colonies will grow where there are bubbles. DO NOT shift the entire membrane on the medium, as this can lead to contamination. If bubbles appear, carefully lift the edge of the membrane nearest the bubbles, and lay down again. If this technique does not remove the bubbles, a small amount of liquid medium can be applied underneath the membrane.

15. One all filters have been placed on the media, place in 37 degree C incubator, upside down, with small spacer in between each tray.

16. Follow Q-BOT shutdown procedure.

17. Incubation times may vary depending on the library, but in general a minimum of 12 hours is required. Remove the Q-trays from the incubator when growth looks fairly uniform across the membrane, but the colonies are not yet touching. The membranes are ready for processing.