PICKING

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

1. Install plexiglass Q-tray holders into bed of Q-BOT. Make sure there are no large scratches or dirt on the plexiglass.
2. Place 96 picking head in center of Q-BOT, pins up.
3. Place clean and dry wash bath and brush in Q-BOT.
4. Turn on air filter.
5. Sterilize the Q-BOT for 20 minutes with UV light.
6. When sterilization is complete, turn off air filter.
7. Fill wash bath 1 with 80% ethanol. IMPORTANT: for picking, do not let the ethanol go over the tops of the brushes- it should hit about 1mm from the top.
8. Install head onto robot arm.
9. Place Q-trays onto holders as follows. First install the two back Q-trays and then the two front ones. Remove lid of Q-tray as installing, so that the tray fits all the way down onto the plexiglass. Slide the positioner piece of plexiglass to secure the Q-tray in place. The Q-tray should not have any room to move. Press down fairly hard on the edges of the Q-tray to ensure that the Q-tray is all the way down on the holder (**this is very important**).
10. Once all 4 Q-trays are installed, close doors and return to computer.
11. Usually all settings on the program are correct at the default.
12. Select “calibrate lens” button. Select a region on Q-tray 1 near the center. When calibration is complete, the computer will show you a score. The

score should be no less than 0.94. If it is, try the calibration again in another region of tray 1. Accept score when it is 0.94 or above.

13. Select “align camera” and select a region near the center of Q-tray 1. Robot will fire a pin in this region and then visualize the spot with the camera. If the pin has hit a colony, align again in a different region. Zoom in to 5x,

    and center image on spot. If the red cross is approximately in the center of the spot, accept alignment. It is not necessary to get the cross precisely in the center, because the alignment will vary depending on the location of the spot on the Q-tray. If the pin is significantly off center, or not touching spot, use the buttons indicated to center the cross on the spot. Then, check another area of the Q-tray for alignment to make sure the cross is on the spot.
14. Select “leave table light on”. Click on “firing test” and make sure that the head is clear of obstacles. Each pin should be fired and retracted. If there is a problem, check the head and make the necessary adjustments, then try again.
15. Turn off lights in room, and close the door.
16. Select “test image”. Select region **28** of Q-tray 1. The camera will show an image of that region. Click on “tools” at the bottom of that image. The axis ratio should be set at 0.7 and the roundness should be set at 0.7. The size should be between 7 and 20 (this can be adjusted if the colonies are very large). The “check proximity” should be selected, and set to 5. The “check overlap” should be selected and set to about 30. These parameters remain the same for all 4 Q-trays.
17. Select the “threshold” tab. The low threshold value can usually be set to 0, but may be adjusted as needed. The high threshold value is usually set to between 120-140, depending on the size of the colonies. Adjust threshold values and then click on “reprocess”. The goal is to get as many high quality colonies as possible, while avoiding colonies that are too near each other or otherwise poor quality. Do not try to get all the colonies in the

image selected. The green circle should not go too far outside or inside the boundaries of the colonies. Adjust threshold values as needed until the image is satisfactory. These settings are for this Q-tray only, and will be different for each q-tray. Close the “tools” window and return to the test

image. You can roll the arrow over the colonies to see why individual colonies are not selected for picking. Close image window.

18. Select region **16** of Q-tray 1 and follow the same procedure with the tab. Do not adjust the “criteria” tab settings at this point. Adjust threshold if needed, but avoid large changes, or you will have to start testing the first region again.
19. Continue testing Q-tray one in regions **8** and **20**.
20. Move to Q-tray 2. Again, do not adjust the values in the “criteria” tab. The threshold values may be significantly different that those in Q-tray 1. Adjust them so that as many high quality colonies are selected as possible. Continue with the same regions as on Q-tray 1, and then do the same for Q-tray 3 and 4. Then close out of “test image” screen.
21. Run is ready to start. Select “full” if there are 4 Q-trays, or “partial” if there are less than 4 Q-trays, or if there are regions on the Q-trays that you do not want to pick. If you select partial, highlight areas of each Q-tray that you want to pick, then continue as with “full” run.
22. Begin imaging Q-trays. The camera will image each region of each q-tray. When finished, it will ask “display image data?” Select yes only if you are concerned about the imaging on certain regions, otherwise, select no. The computer will then calculate the number of colonies it will pick. Select OK. Then press “continue”. Continue through the prompts. Fill hotel and place in Q-BOT. Make sure the plates in the hotel are pushed all the way to the back.
23. It is OK to turn the room lights on now. Check ethanol bath to make sure the level is not too high or low.

24. Begin picking. After first wash cycle, peek under head to see that there is no ethanol remaining. If it appears wet, change head, and redo imaging.
25. As picking continues, check ethanol level every hour or so and add more as needed.

26. When run is complete, computer will ask “carry over pin and well settings?”. Select yes if there are more Q-trays from this libraries to be picked today. Otherwise click no. The yes selection will allow you to just change the Q-trays and re-image without removing plates from the hotel. The head will begin inoculating into the la 

om the last run. Add plates to the hotel as neede
27. When picking is complete, remove all Q-trays and plates. Incubate plates at 37 degrees for 20 hours, and place a 384-well plate filled with water both on top and bottom of the stack of fresh inoculates in the incubator to minimize evaporation of media.
28. Follow SHUTDOWN procedure.