Mosquito Instructions

- 1. Check the two humidifiers to ensure there is water in them. One is located on the left-hand side of the instrument platform (Fig. 1) and the other is on the right side. If they are empty, use a squirt bottle to fill them with dl water.
- Log into your lab's account on the computer and double click the Mosquito Icon. Select "yes" when asked about initializing the instrument. The deck should move right and left, back to center.
- 3. Go to File → Open and double click to open the protocol that you need for your experiment. The protocol names indicate how many drops per well will be dispensed and the volumes. If you have questions about the protocol(s), please contact Prof. Fera. Do NOT edit any of the protocols!

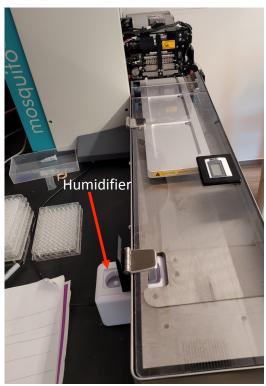


Figure 1. Humidifier

4. Advance the tips twice by clicking the tips icon at the top of the screen, circled in red in Figure 2. It's normal for the rest set of tips that exit the instrument head to be bent (this is why they are pushed out prior to the start of your experiment). This only needs to be done at the beginning of your session, so no need to do it between plates.

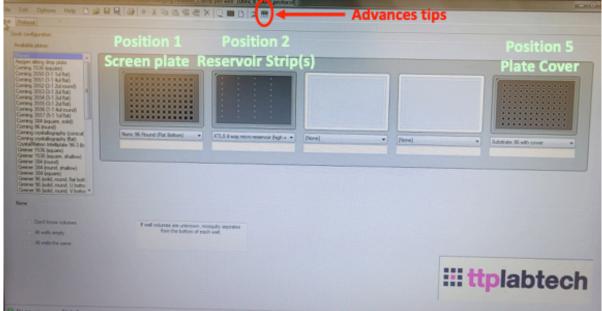


Figure 2. Setup tab options.

5. Tools for the robot are kept in the top-left drawer. You will need the two blocks, roller, and scissors. The rest of the reagents (reservoir strips, plate cover, screening plate, sample) come from your own lab.



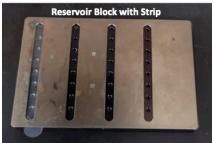




Figure 3. Supplies.

- 6. Place only a small amount of water on the block onto which your cover seal will go (Figure 3, left panel). Use a kimwipe if needed to remove some of the excess water. If there is too much water, the Mosquito tips might move the cover slip and your sample and reservoir drops may not be centered on one another.
- 7. Place reservoir strip(s) on the appropriate block (Figure 3, middle panel), aligning the wells of the strip with the holes of the block. Then place the silver plate over it, ensuring the pins fit in the holes. Note you will need to use the **5uL reservoir strip if you**
 - need to add more than 2uL of each sample per row. You will also need more than one strip if you are dispensing more than 1 drop per well.
- 8. Place your plates and blocks on the deck in the positions shown in Figure 4, and illustrated in Figure 2. If you need to move the platform, go to the "Protocol" tab on the screen and click "Park Left" or "Park Right" (Figure 5). Please make sure the plates and blocks do NOT sit on top of the metal on the bottom right corner of each slot!!! If they are, the tips will JAM! If this does happen, abort the run (in the protocol tab) and advance the tips twice, as described in step 4 above, before starting a new run.

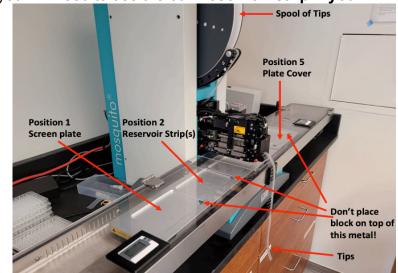


Figure 4. Mosquito Deck and Parts



Figure 5. Protocol Tab Options

- 9. Once everything is on the deck, you can pipette your sample into the reservoir strip. At the <u>very least</u>, you should pipette 1.8uL per well in the reservoir strip if dispensing 100nL in each well. If you are new to using the instrument or if you are doing multiple drops per well, you should pipette at least 2uL to avoid evaporation. Pipette at least 3.6uL per well if dispensing 200nL in each well, or at least 4uL if dispensing more than 1 drop per well. Close the lid of the deck chamber when you are finished dispensing your sample.
- 10. You can start the run by clicking the "Run" button on the bottom left of the "Protocol" tab. Make sure you see humidity vapors once the instrument starts running. If you don't see any, the chambers (Figure 1) might be in need of water, so you should fill them immediately (you can do so while the instrument is running). You should also watch to make sure the tips don't jam into your screening plate, your reservoir plate, or your cover. If they do, please indicate this in the logbook.
- 11. When the run is completed, place your reservoir screening plate (Figure 3, right panel) in the holder (Figure 3, left panel). Then, **FLIP** your block with the cover (don't worry, the cover won't fall off) and align it against the metal pins of the holder, and push down onto the reservoir screening plate. Slide the block (or pick it up) carefully, so as to not disturb the plate containing your drops. Use the roller to secure the plate cover on your plate, especially around the edges.
- 12. Repeat steps 6-11 if you are running additional screens. Note, if you are running the same sample but with different conditions, you can use the same reservoir strip and skip step 7.
- 13. After you complete your run(s), <u>cut the hanging tips</u> (Figure 4) with the scissors so they are not hanging on the floor, and throw them into the regular trash.
- 14. Make sure to remove everything from the deck and close the doors to the chamber. Throw out your reservoir strip. Place all the tools you used back into the top-left drawer.
- 15. <u>Sign the logbook</u>. Make a note of the tip count and of any problems encountered. If problems were encountered, or if the tips are running low, please contact. Prof. Fera to let her know in addition to writing it in the logbook.
- 16. Log out of your lab's account and please close the laptop. The instrument is to remain on.