

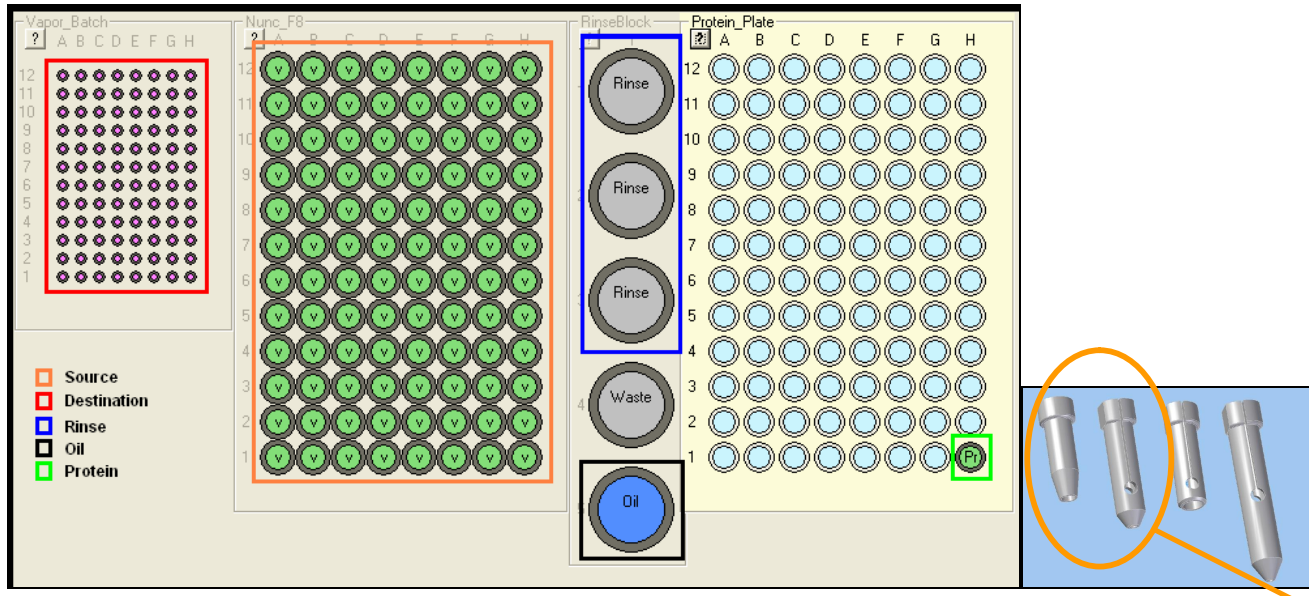
# Microbatch Screening

Douglas Instruments

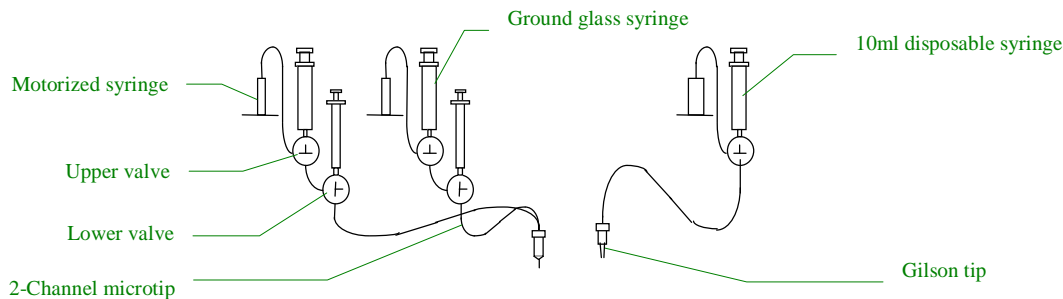
## Step-by-Step Instructions

### Hardware Preparation

1. Clean a Douglas Vapor Batch [VB] plate with compressed air, and place it on the top left corner of the Plate Loader.
2. Place a Nunc Frame to the right of the VB plate. Put up to 96 screening solutions in F8 strips as shown below
3. Place the rinse block to the right of the Nunc Frame, with bottles in wells 1 to 5. Fill the first 3 bottles to 1mm from the top with clean water for rinsing. Fill the 5<sup>th</sup> bottle 50% full with Al's Oil. Leave the 4<sup>th</sup> bottle empty for waste



4. Connect a 2-channel Microtip to the first two channels (green and red). Place the tip in the 2-channel "collet" (holder) on the left (Z) arm of the Plate Loader.
5. Fill the ground glass syringes of the upper valves with degassed pure water and replace them.



### Software Preparation

6. Switch on the computer and the MCC control unit. Launch the HTML Screens application by clicking on the "Screens" shortcut.



7. Navigate to Microbatch, General Microbatch Screen, then click on "Microbatch screening.xpp" or the image.
8. The program Wasprun will load. If desired, Change the size of the block on the Source plate and / or the location of the block on the Destination (VB) Plate. You can also change the drop volume, concentration and dispensing conditions.
9. When your experiment is ready, Select "Dispense".

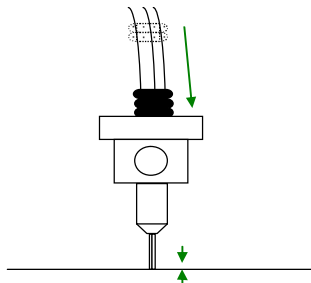
10. You will now move to a separate program walled **Wasp**. To the left of the start button is a button with an icon



showing the dispensing chassis. If you wish to debubble the system or install a new microtip, click on this button, which will in turn load the program **Front Panel**, which is used for controlling the robotics.

### Debubbling and Setting the Height of the Microtip

11. At the beginning of each day the system will require debubbling. Place a small bottle or vial under the Microtip in the Z-arm, select **Syringes, Debubble**, and follow instructions. Any air bubbles that were present at the top of the motorized syringes should have passed out into the connecting tubing.
12. Now follow instructions and remove the air bubbles from the connecting tubing as follows:
  - a. Remove the PTFE tubing from the needles of debubbled motorized syringes.
  - b. Expel water and air bubbles from the tubing using the ground glass syringe.
  - c. Reconnect the tubing carefully, ensuring no air bubbles re-enter.
13. Occasionally there may be bubbles between the upper and lower valves. Turn the top valves to the **flush position** (┆) and flush the bubbles out through the microtip with the ground glass syringes.
14. If you suspect that the 2-channel Microtip is not set to the correct height select **Plate Loader, Install Tip**. The arm(s) will move to its lowest position. Follow the instructions on the screen:
  - (1) Move one o-ring towards the tip and the other two away from the tip
  - (2) Adjust the height of the Microtip until it is just touching the table by pushing through the lower o-ring
  - (3) Mark the height by moving the top two o-rings down to the top of the collet.



- (4) Do not continue to the 'testing' phase of this routine.

### Running the Experiment

15. **Alt/Tab** to return to **Wasp**. Click on the large **Start** button.
16. The system will now check the positions of the motorized syringes. If necessary, you will be instructed to turn valves and the syringes will be moved in order to carry out the experiment.
17. You will now have the opportunity to adjust the tip position. Use the arrow keys and the 'Raise' / 'Lower' button to center the tip in the well for optimum drop location. Lower the tip until a slight movement can be detected between the collet and arm when in the lowered position. Save the configuration while in the 'Lowered' position.
18. A page of configuration information and instructions will now appear. Note that all air bubbles must be removed from all tubes of the first two channels including the microtip. Remember to remove any droplet on the end of the microtip to prevent this from being sucked in later on.
19. Air will now be loaded into the microtip to separate the protein from water already in the microtip. Click **OK** to begin the experiment.
20. After loading air, you will be asked to provide protein. Place the protein in the position chosen on the Protein Load Plate. The tip will automatically load the protein. Wipe the tip after the loading is complete if required.
21. Click **OK** to carry out the experiment automatically. When it is finished, "top up" the VB plate with 5 ml AI's Oils (50:50 silicone and paraffin mixture) or 100% paraffin to reduce evaporation. Place the plate in an incubator at the desired temperature.
22. Quit **Front Panel** before turning off your computer so that the motor positions are stored for the next use of the equipment.
23. Turn off the MCC by pressing the power button on the front of the machine.