Using the Phoenix Drop-Setter

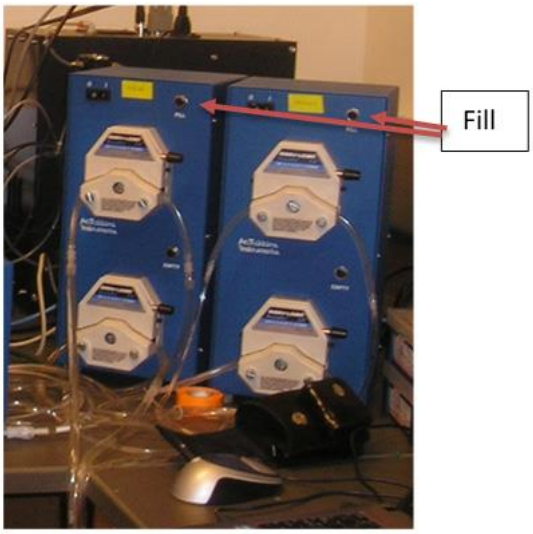
- **Before starting:** You **must** have one-on-one training with Annette Erbse and her approval to use the drop-setter independently. You can contact Annette at erbse@colorado.edu, (office C316, phone 2-0528). **No substitutions allowed**!

Note:

At all times, if the robot is going through a protocol, do not walk away. Stay with it and watch it so you can stop it if something goes wrong.

Before Starting

1. Check the carboys below the bench. Ensure the one labeled **distilled water** is at least ¼ full.



If it is not…

a. Fill it with distilled-water or equivalent
b. Empty the waste carboy, and
c. Before starting a protocol, make sure the tubes from the distilled water carboy are full by pressing the **Fill** buttons on both pumps.

2. Gently move the metal stage of the 98 syringe head up and down with your hands. Make sure no needles are stuck to the stage. If they are stuck, they can bend potentially damaging the needles.
3. Check the underside of the metal stage of the 98 syringe head for signs of crystallized stuff. Clean/soak the stage if necessary (see separate instructions in Binder next to the drop setter).

Your Sample:

1. There is a centrifuge by the instrument. If you have not done so, centrifuge or filter your sample. Then, put the appropriate volume in a PCR tube and centrifuge it to the bottom.
2. Put it (or them) in the A1 position (plus C1 and E1 if multiple samples) of the protein cooling block.
3. Put corresponding empty PCR tubes (for left over sample in the needle) in B1 (and D1 and F1 if needed) of the cooling block.
4. **Make sure the tube caps are removed**!!! We have had too many problems with needles getting stuck in tube caps.

The nano dispenser picks up an extra 5 λ (which it ultimately dispenses in your "leftover sample" tube—a good check on whether things worked correctly), plus 4 λ for **backlash** which it spits back in your sample tube in two 2 λ aliquots before dispensing the drops. The recommendation is to have a spare 10 λ of sample beyond what you need for the last sample dispensed, plus an extra 5 λ "leftover" for each separate dispensing.
For example:

- Single set, 0.1 λ drops: 25 λ sample (10 λ dispensed + 5 λ to leftover tube + 10 λ remain in sample tube)
- Single set, 0.2 λ drops: 35 λ sample (20 λ dispensed + 5 λ to leftover tube + 10 λ remain in sample tube)
- Two sets in which you aspirate sample once and dispense to both trays, 0.2 λ drops: 55 λ sample (20 λ for each tray + 5 λ to leftover tube + 10 λ remain in sample tube)
- Two sets in which you aspirate sample <u>separately</u> for each set, 0.2 λ drops: 60 λ sample (20 λ for each tray + 5 λ to leftover tube <u>twice</u> + 10 λ remain in sample tube) etc.

<u>Starting Up the Instrument</u>

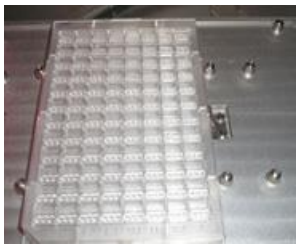
1. Turn on computer. Log into your user account.
2. Turn on instrument with the single switch on the small **Tripp-lite** surge protector behind monitor. Do not turn other switches on or off!
3. Double-click on **Phoenix** icon.
4. When the user interface comes up, click on **Connect** at the top right. This will start the motors and zero positions.
5. Watch until initialization is complete (status bar at bottom of screen will be blank), **Connect icon** will now show **Disconnect**.

<u>Deck Layout</u>

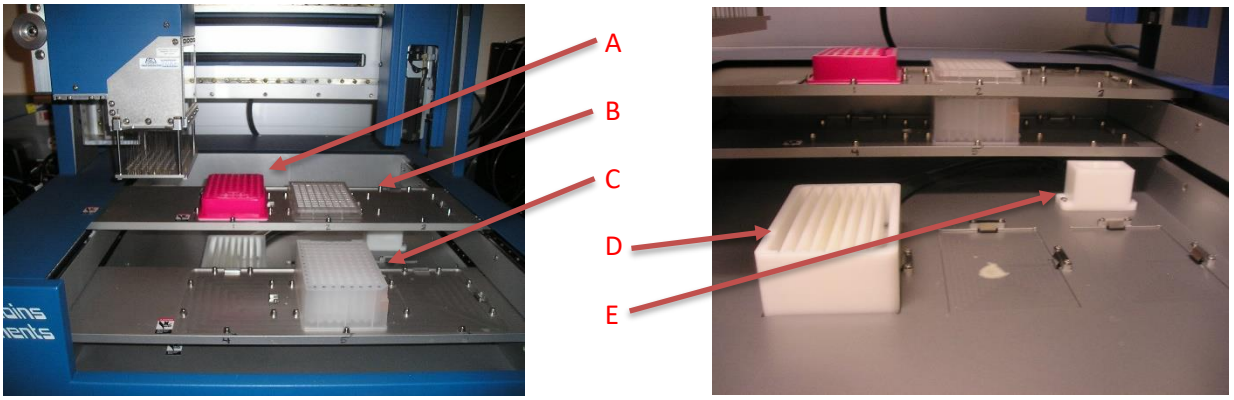
<u>Note: Decks will move!</u> Never have something on a deck that is not supposed to be there (only trays, deep-well blocks, protein chilling blocks and dedicated reservoirs allowed) or is not securely seated in one of the free positions. <u>This is not a storage space for clutter.</u> Collisions will damage the needles and/or trash the needle head and will cost us $$$. Remember you do not want to do this by hand.

1. Each location on the three metal decks of the robot are numbered from left to right:
    - 1,2,3 are on the top deck;
    - 4,5,6 are on the middle deck;
    - 7,8,9 are on the bottom deck.
2. Position 7 has the syringe head wash station. Position 8 will often hold an empty reservoir to empty the syringe head into. Position 6 and 9 are good positions to have cleaning solutions in (0.1 M NaOH, 40 % EtOH or 2% Micro 90). You will learn about cleaning during training!
3. The protein delivery needle can reach locations 1-6 but not 7-9 so there's no point putting either the tray or your protein there.
4. **Always place deep well blocks and trays in the desired position on the deck such that the A1 location is at the front left**.
5. There are spring-loaded locations in each position.

   Make sure that your trays, blocks and reservoirs are sitting level and secure in the locations since the robot uses basic geometry to go to where it expects tray wells (etc.) to be. Be very careful to make sure that the trays are sitting flush on the deck. Crooked trays can lead to damaged needles (very expensive!).



6. Always have the **protein chilling block in position 1** and the **deep well screen block in position 5.** Uniform use will make it easier for all users to avoid mistakes.



Running a Standard Protocol

Important:

Stay with the robot at all times during a run. If something looks to be going wrong click the stop icon to try and prevent collisions.

1. Go to **open protocol** and open the protocol that fits with the plate and samples you are using, e.g. "Intelli-plate 3 Well 1 Protein".
2. **Be sure you have the correct plate definition!**
3. If you change a standard protocol, e.g. to change the sample and screen volumes aspirated and dispensed, do not save and overwrite the standard protocol. If you want to save it, hit "save as" and save it with your name/identifier at the beginning of the file name in your protocol folder.
4. Always read the entire protocol, even if you are using the standard protocol, take the time to double check all the locations and to understand what it will do. Someone might have changed something by accident.
5. Make sure the positions in the protocol fit where you have your tubes, trays, blocks and reservoirs.
6. When you click the **GO** icon, a **Plate Loading** window comes up. Click **Show Top Decks** to load your screen blocks and crystallization plates at this time; the two top decks will move forward. Load your 96-well screen block in position 5, labeled **Emerald Screen** in standard protocols (don't change this).
7. Be sure your crystallization plates are loaded, with the "A1" position at the front left, in the correct positions.
8. **Be sure all plates on the tray are included in the plate loading menu; failure to do so can result in a collision!** The instrument only knows what you tell it.
9. **Things to watch out for while loading the decks (yes I have said it before but it really is important):**
    - Be certain all plates are pressed down flat on the decks.
    - Remove the lids from your protein tubes

    With screen blocks:
    - Make sure to remove the cover from the screen block!!!
    - If you removed an adhesive cover, be certain you have cleaned the adhesive from the top; otherwise, the plate on the 96-needle dispenser may stick to it.
    - Do not puncture an adhesive cover to allow access for needles; we had too many problems with needles getting stuck on the covers.
    - Instead remove cover and reseal if needed.

- Be sure you have the wash station and the waste reservoir in positions 7 and 8 of the bottom tray respectively, the protein chilling block in position 1 on the top tray and the deep well screen block in position 5 on the middle tray.

Running a Non-Standard Protocol

Don't, or ask for help!

Finishing Up

1. **Run the "after run wash" protocol. There is no exception.** If you are done or need to leave for longer than an hour you must run the after run wash protocol. **Failure to comply with this will result in loss of privilege to use the robot!!!**
    a) To run the wash you will need 3 PCR tubes, two filled with 150 uL NaOH placed in position A1 and C1 and one empty one in position A3 for the Nano Dispense purge.
    b) Remove the lids from the PCR tubes!!!
    c) You will need the water reservoir filled half with 40% EtOH in position 6.
    d) Make sure that the dest. water jug under the table is at least ¼ full and that the waste jug has enough empty space left. If not empty the waste and refill the dest. water. After refilling the dest. water press the fill buttons for both pumps to fill the lines.
    e) Stay with the robot during the wash. You have to be there in case something goes wrong to be able to stop it.
    f) Make sure that it blows bubbles into the wash reservoir of the nano needle during the nano needle wash.
2. **Wash the 98 syringe head:**
    a. Place the water reservoir in position 6 on the middle deck and fill it to about 2 cm hight with 40% Ethanol from the bottle provided.
    b. Run the "wash 98 syringe head" protocol.
    c. Remove the reservoir, empty it in store it upside down.
3. Move the stage carefully with your hands up and down, checking that no needles are stuck to the stage.
4. Check the underside of the stage for traces of crystallized stuff. Clean /soak the stage if necessary.
5. Sign the log book.

4. Hit the “disconnect” button, then close the “Phoenix” program and turn off the single switch on the small “Tripp-lite” surge protector.
5. Clean up and leave the area as clean as you found it.