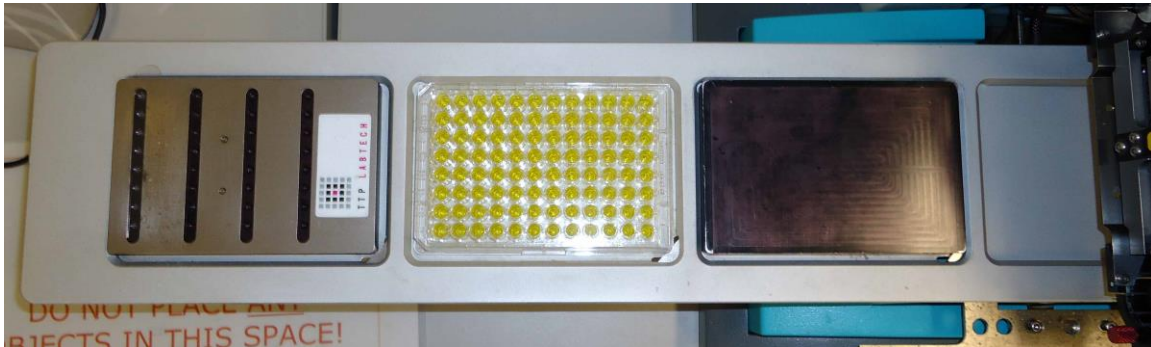


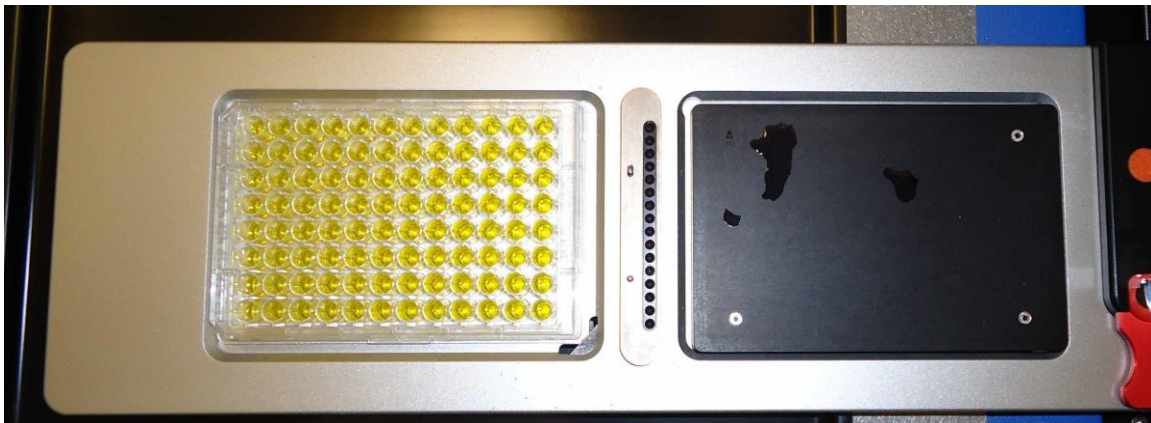
## Mosquito Use for Hanging Drop Experiments

There are two Mosquito instruments both can set up standard crystallisation experiments, one has an additional syringe that is used for LCP experiments. The instruments differ slightly in configuration but run using the same software.

The HTS Mosquito deck is shown below: the protein sample/s is placed in plastic strips in the holder on the left; the condition plate is in the middle; and the hanging drop seal is placed on the holder (substrate) on the right.



The LCP Mosquito deck is shown below: the condition plate is on the left; the protein sample/s is placed in plastic strips in the holder in the middle; and the hanging drop seal is placed on the holder (substrate) on the right.



## Protocols

UQROCX maintains just two standard protocols for hanging drop experiments. One for experiments using the standard seal and one for experiments using the UV seal.

However, the Mosquito can prepare many different types of experiments in a variety of formats if you wish to use a non-standard protocols please talk to UQROCX.

OPEN a protocol in aUQROCX Protocols/Hang Drop/

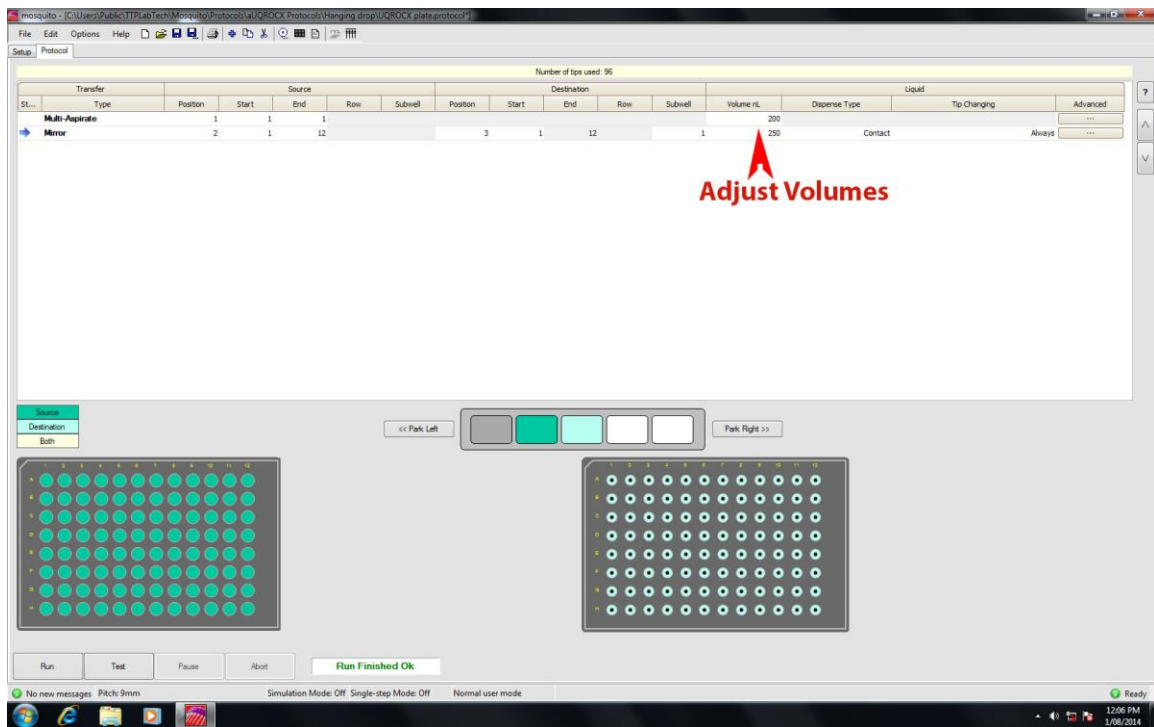
If using UV seals you must use the UV protocol as the UV seals are thinner UV\_UQROCXplate.protocol.

The HTS Mosquito and LCP Mosquito protocols will appear slightly different as is appropriate for their respective deck configurations.

NOTE the two tabs: the Protocol tab and the Set-Up tab

**On the Protocol tab** make sure all settings are correct. You should not need to change any setting except the volumes pipetted.

HTS Mosquito Protocol tab:



LCP Mosquito Protocol tab:

The screenshot displays the LCP Mosquito Protocol software interface. At the top, a menu bar includes File, Edit, Options, and Help. Below the menu is a toolbar with various icons. The main window is titled "Setup Protocol" and shows a table of protocol steps. A yellow banner at the top of the table indicates "Number of tips used: 96".

Transfer	Type	Source				Destination				Liquid				
		Posit...	Start	End	Row	Subwell	Posit...	Start	End	Row	Subwell	Volume nL	Dispense Type	Tip Changing
	Multi-Aspirate	2	1	1							200			
→	Mirror	1	1	12		3	1	12			200	Droplet	Always	...

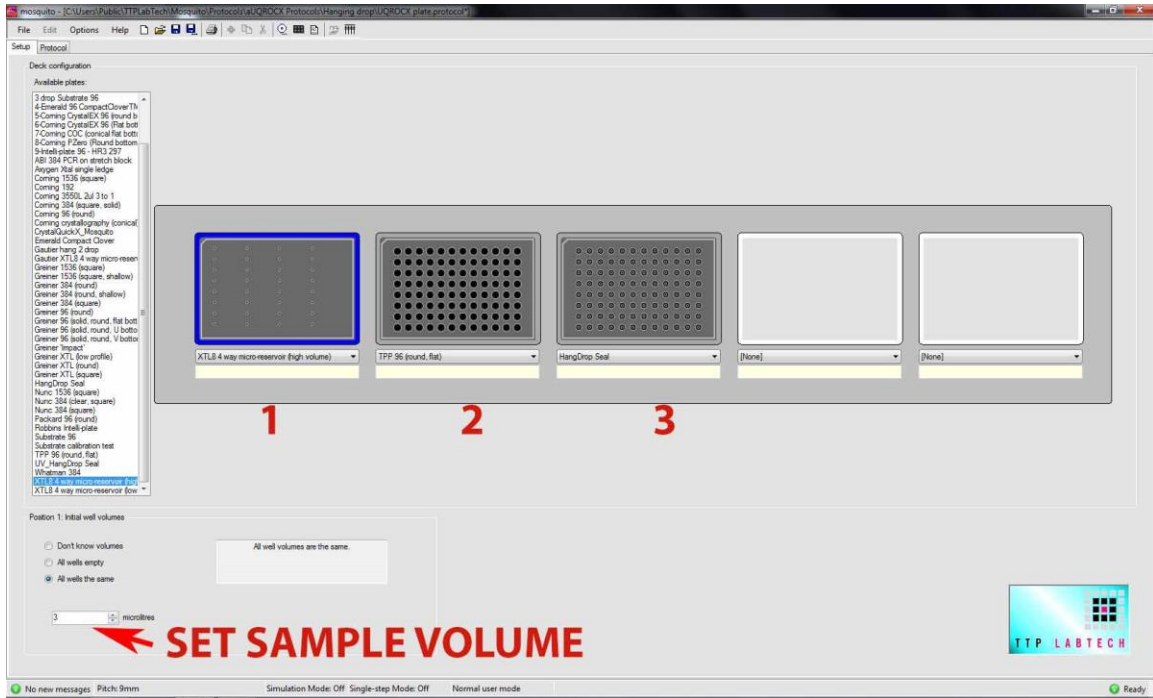
A large red arrow points to the "Volume nL" column of the "Mirror" step, with the text "ADJUST VOLUMES" overlaid in large red letters.

Below the table, there are controls for "Source", "Destination", and "Both" (highlighted in yellow). There are also buttons for "<< Park Left" and "Park Right >>".

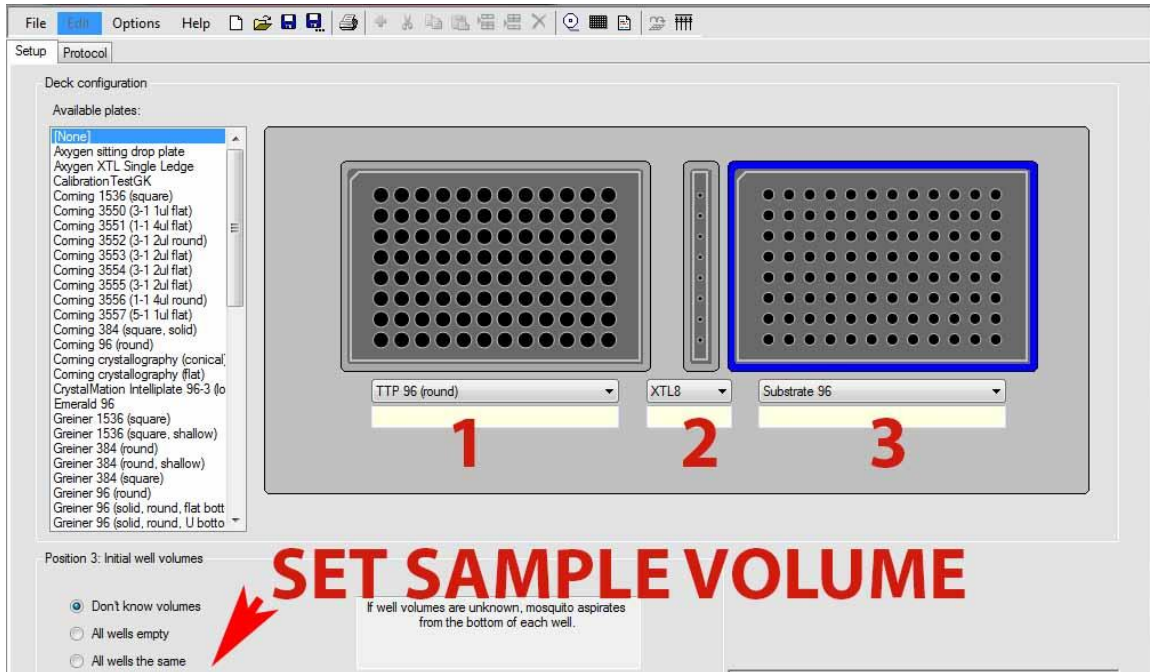
At the bottom, two 8x12 well plates are shown. The left plate has red 'X' marks in the first column of all rows (A-H). The right plate has red 'X' marks in the last column of all rows (A-H).

**On the Set-Up tab** make sure all positions are correctly assigned. Again you should not have to change anything here except for the volume of sample if you have 2 uL of sample use XLT8 (low volume) and if you have 5 uL of sample use XLT8 (high volume) .

HTS Mosquito Set-Up tab: The protein (sample) strip goes in position 1.



LCP Mosquito Set-up tab: The protein (sample) strip goes in position 2.



## Substrate

For both instruments the hanging drop seal is placed on a metal block called the substrate.

Apply the hanging drop seal to a small amount of water on the substrate block.

Make sure align the seal on the substrate block using the three spots (A12, H1 and H12).

To ensure you do not have excess water under the seal, tip the block and let excess water escape.

### **Running the protocol**

**Check that the protein strip, the condition plate and the seal substrate are all in their correct positions.**

**Press RUN.**

**NOTE: The Pause Button will stop the protocol which then can be restarted where it paused this is useful if you see you have made a mistake.**

Please do not save the changes you have made to the protocol.

### **Sealing the experiment**

Immediately after the Mosquito has finished take your condition plate and put it in the guide plate. Take the substrate with the seal on it and smoothly invert it using the pegs of the guide plate to position the seal on the condition plate. Slide the substrate off wipe the seal and roll to ensure good seal. Place plate in a RockImager.