

Set-up of a plate using the Mosquito

- Contact Mahmudul Hasan by email Mahmudul.Hasan@biochemistry.lu.se or by phone: 073-7262511 and book time on a Tuesday, Wednesday or a Friday. Time is available in 1-1.5 hour blocks.
- Book the screens you want to use as well.
- Show up at the booked time. If experiments are not finished on time the next user has the right to start and you will have to book another time.
- Pre-dispensed screens are available in the 12 °C incubator in the main laboratory. Take the screen(s) you want to set up.

- 1) Log on to the laptop next to the Mosquito

User: TTPFactory
Password: Factory01763

It will take some time to log on – be patient!

- 2) Double click on the <mosquito> icon.
 - a. Answer <Yes> on initialise question

Test run with coloured water

- To test for correct position of the plate, volume to load in the protein strip and to practice.
- Do not lean on the mosquito table during pipetting or sealing of the experiment!

- 1) Choose <Open> icon in the top menu bar.

- a. Under Program Files>mosquito>Protocols choose “**Rigaku 3-well test**”.

- 2) “**Setup**”-tab:

standard: pos. 1 “[None]” pos. 2 “XTL8 (low volume)” pos. 3 “SwissCi-Rigakulowprofile(UV+)3well”

Note: The numbers on the deck of the Mosquito explain which position has which number.

- 3) “**Protocol**”-tab:

standard:

Transfer Type	Source					Destination					Liquid		
	Position	Start	End	Row	Subwell	Position	Start	End	Row	Subwell	Volume nL	Dispense Type	Tip Changing
Aliquot	2	1				3	1	12		2	100	Contact first	Between transfers

Change “Volume nL” to the volume that will be used during experiment. Click on the number.

- 4) Put “Rigaku test plate” from bag on shelf in pos. 3 on the deck with the A1-well in the top left corner.
Note: Use gloves from here and on due to risk of contamination.

- 5) Put 2 or 5 µl well “TEST”-strip in pos. 2 with the wells upwards. 100 nl drops require a 2 µl strip, whereas larger drops require 5 µl strips.

- a. Pipette 1.8 µl water per well for 100 nl drops and 3.6 µl for 200 nl. In general pipett 50 % more protein solution per well than actually needed per drop.

Note: Take care to avoid air bubbles and do not to forget to fill all wells.

- 6) Immediately after pipetting start the program by pressing <Run>.

- a. Check that all the drops ended up in the correct wells.
- b. Check the wells in column 12 after run. If there is too little water in the “TEST”-strip wells these wells will be empty.

- 7) Put “Rigaku test plate” and “TEST”-strip back in bag.

Setting up Experiment

- Do not lean on the mosquito table during pipetting or sealing of the experiment!
- 8) Choose <Open> icon in the top menu bar.
 - a. Under Program Files>mosquito>Protocols, choose “**Rigaku 3 well same protein**” or “**Rigaku 3 well different protein**” depending on whether you want to set up the same or different proteins on the same plate. Multidispense of the reservoir is used for the same protein whereas the tips are exchanged when pipetteing the reservoir for different protein constructs.
 - 9) “**Setup**”-tab:

standard: pos. 1 “[None]” pos. 2 “XTL8 (low volume)” pos. 3 “Rigaku”

Note: The numbers on the deck of the Mosquito explain which position has which number.
Note: Never change protein strip type in pos. 2!
 - 10) “**Protocol**”-tab:
 - a. Change “**Volume nL**”, no. of columns pipetted, type of pipetting (in the fields on the screen).
Note: “Subwell 1” is always the reservoir and “Subwell 2”, “3” and “4” are the protein wells.

2	1
3	4

“Rigaku 3 well”

- Note: The machine can pipette down to 20 nl but to get good experiments it is recommended to use at least protein drops of 50 nl + 50 nl reservoir.
- b. Go to **Edit>Add transfer** to add command lines.
 - c. Start **Option>Simulation mode** and then press <Run>. Check that your protocol runs correctly.
 - d. Turn off **Option>Simulation mode**.
 - e. Go to **File>Save as** to save your edited protocol. Save with your own name, e.g. “**Rigaku 3 well_name**”. Don’t change the standard protocols “**Rigaku 3 well same protein**” etc.
- 11) Peel off the plastic cover on top of the reservoir plate and put it in pos. 3 on the deck with the A1-well in the top left corner.
Note: Use gloves from here and on due to risk of contamination.
 - 12) Choose a 2 or 5 μ l **well strip** and put in pos. 2 with the wells upwards. 100 nl drops require a 2 μ l strip, whereas larger drops require 5 μ l strips.
 - a. Pipette 1.8 μ l protein solution per well for 100 nl drops and 3.6 μ l for 200 nl. In general, pipette 0.6 μ l more protein solution per well than actually needed per drop.
Note: Take care to avoid air bubbles and do not forget to fill all wells.
 - 13) Immediately after pipetting start the program by pressing <Run>.
Note: Check the wells in column 12 after run. If there is too little protein in the protein wells these wells will be empty. If any, note which wells are empty.
 - 14) Immediately after run seal the wells with pressure adhesive **sealing tape** (ClearVue Sheets).
 - a. Put the tape on top of the plate.
 - b. Seal the tape with the brown plastic sealing tool.
Note: Take care to seal around all edges and corners. Check that you see the difference between sealed and not sealed when you look around the edges and corners. You should be able to see the seal when looking carefully around the borders of the plate.

- 15) Make a data base entry for your plate in the CrystalTrak computer (4, 12, 20 °C experiments that will be scanned using the Minstrel robot. Attach the **barcode to the plate (printed from the CrystalTrak program through the bar code labelling machine connected to the computer)**.
 - a. Put the barcode on the side of the plate close to the letters of the plate (A, B etc.). The text of the barcode should be closest to the A and the code furthest away from the A.
- 16) Put the plate on the shelf marked with your name in the coldroom or on the shelf “Plate to the hotel ” in the 20 °C robot room.
- 17) Throw away the protein strip and cut off the used pipettes with the pair of scissors on the shelf.
NOTE: Use gloves. Risk of dangerous chemicals in most screens!
- 18) **Close the mosquito program. Leave the computer on but close the lid of the computer.**
- 19) Fill in the report form (on the shelf). Please fill in the log book if you encounter any problems with the equipment.

Good Luck!

Mahmudul Hasan

Important:

Please refer to the crystallization facility if conditions are found that help determining a protein structure.

Suggested text:

Initial crystallization conditions were found at the Max-lab Crystallization Facility with the X screen set up in 96-well Rigaku plates using the Mosquito robot (TTP Labtech, UK).

A mention in the acknowledgements section would also be appreciated. Please send an electronic copy of the published article as well as the reference, to Mahmudul.Hasan@biochemistry.lu.se to help us with applications for future funding as well as staff for the crystallization facility.