ARI Crystal Gryphon Protocols

How to open, use, and modify a ARI Crystal Gryphon protocol

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Summary

This tutorial is for running a pre-existing _ARI Crystal Gryphon_ protocol. Later on this tutorial, I will show how to make a new or modify an existing protocol for setting up 96-reservoir (2 or 3 well) crystallization plate like _ARI Intelli-Plate_ and 96-reagent crystallization screen like _Emerald Screen_ using Art Robbins Instruments _Crystal Gryphon Robot_ located in KLB 412. The first part of the write-up will also show how to login into the computer and start the program. For more information, please contact XRF Director, _Dr. T. Somasundaram_.

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Running and modifying Crystal Gryphon protocol

Materials needed before running a typical protocol

- ARI INTELLI-PLATE g6-2 SHALLOW WELL PLATE (ARI CAT # 102-0001-20)
- HAMPTON RESEARCH HT CRYSTAL SCREEN (HR CAT # HR2-130)
- PROTEIN 10 mg/mL
- NANO PURE WATER FOR WASHING 1 GALLON (MINIMUM 1 LITER/RUN)
- CRYSTAL CLEAR 3” TAPE FOR SEALING THE INTELLIPLATE
- GREINER CAP MAT SEAL FOR DEEP WELL BLOCK (HR CAT # HR3-103)
- FOUR 0.2mL PCR-TYPE MICROFUGE TUBES (THERMO SCIENTIFIC CAT # AB-0337)
- FRESHLY PREPARED 10% ZYMUT SOLUTION IN 0.2mL PCR-TYPE TUBE WITH NO LID
- FRESHLY PREPARED 0.02M EDTA SOLUTION IN 0.2 mL PCR-TYPE TUBE WITH NO LID

At the Desk

ARI Crystal Gryphon is located in Kasha Laboratory Room Number 412. The first step is to remove the dust cover and set it aside. Next, on top of the table where Gryphon is sitting, locate the 4-outlet power strip labelled “Gryphon Power” and turn the switch to “ON” position on the power-strip. This power-strip energizes all the components that are needed for Gryphon to function except for the computer. The user may hear the sound of a motor running which is normal. A green light on the left-side of the Gryphon head assembly will light-up indicating all the components are energized.
Starting the Program

Now power-up the computer called kelvin.biophysics.fsu.edu with IP number 128.186.103.104 that controls ARI Gryphon. Inside the IMB network this computer is visible as Dell-pc. At the computer login using “Username” and “Password” [Ask Soma for the appropriate account and password]

- Username:####
- Password: xxxxxxxx

- Click on Gryphon 1.4.5.3 icon on the Desktop to start the program
- This will start the Gryphon program shown in next page
- In Gryphon 1.4.5.3 GUI, click the “Connect” icon
- The Icon will change into “Starting …” icon
- The Nano Nozzle will move and the Stage will move
- Eventually, the Icon will change to “Disconnect” icon indicating the program and computer are now connected to the Gryphon instrument and controlling it
- Now click the “DeckOut” Icon
- The Deck with labels “1” and “2” move toward the user revealing the wash station behind (“3”)
Clean & waste water and other hardware

Assuming that you are the first user in a while we need to confirm couple of things before proceeding:

- The tubes labelled Nano Fill and Head Fill or inserted into 2 gallon plastic carboy labelled “Clean water for Head and Nano Wash”
- There is at least 1 gallon of clean water in that carboy
- The tubes labelled Nano Wash and Head Wash or inserted into 2 gallon plastic carboy labelled “Waste water from Head and Nano Wash”
- There is at least 1 gallon empty space in that carboy
- You will use ~1½ liter of clean water and produce ~1½ liter of waste water per run

Clean water carboy

Waste water carboy
After the confirmation of the points mentioned above, the user needs to perform the following steps:

- Press and hold the black circular button on the RHS of the “Head wash pump”
- You will see the pump drawing water from “Clean” carboy and pushing water to “Waste” carboy
- While still holding the button, monitor the “Needle Wash Station” “3” to ensure it is fully covered with water and excess water is overflowing toward the “Waste” carboy (~ 45 seconds)
- Now let go of the black button
- Then, press and hold the black circular button on the RHS of the “Nano wash pump”
- You will see the pump drawing water from “Clean” carboy and pushing water to “Waste” carboy
- While still holding the button, monitor the “Nano Nozzle Wash Station” “12” to ensure it is fully covered with water and excess water is overflowing toward the “Waste” carboy (~15 seconds)
- Now let go of the black button

At the Gryphon

Now you are going to set-up the crystallization plate and screen buffer. The following steps are the most critical part of the experiment. So make sure the next steps are followed correctly. If you don’t follow them you are likely to damage the instrument and cause costly repair.

1. Correctly identify the crystallization plate (Note that there are variations in height, volume, and number of wells). If in doubt check with XRF Director.
2. If placed correctly, the metal clips should secure the crystallization plate in front and on the right. Eight screws; two to a side should secure the crystallization plate and be clearly visible
3. Correctly identify the screen buffer block (Note that there are variations in height, volume, and number of wells). If in doubt check with XRF Director.
4. If placed correctly, the metal clips should secure the buffer block in front and on the right. Eight screws; two to a side should secure the buffer block and be clearly visible.
5. Place the crystallization plate (in Position “1”) and screen buffer (in Position “2”) correctly. If in doubt check with XRF Director.
### Setting-up the protein in Position “10A1”

Now having secured the crystallization plate and screen buffer, it is time to add the protein. In principle, one can have four different proteins (200 µL each maximum) in Positions “10 A1, 10A2” and “11A1, 11A2”. In this write-up we are going to use one protein 60 µL in a 200 µL PCR tube. This protein (10 mg/mL concentration) will be placed in Position “10A1” in a 200 µL PCR tube with lid cut-off.
Now with everything set-up double check to make sure that the Intelliplate, Screen Buffer, Protein in PCR tube are all secured.

Now it is time to start the protocol. This will be covered in another Tutorial. YouTube video of the running of the protocol can be seen here: https://www.youtube.com/watch?v=ZwQ7P4MGHmU. Once all the sample protocols are completed the final wash protocol with zymit and EDTA should be run before stopping and switch off of the instrument.

**Stopping the program**

The final wash protocol with zymit and EDTA is called "Zymit 10 EDTA 11 Normal Wash.pro" and it should be run as the final step. Once it is complete, click the **DeckIn** Icon which will move the deck on top of the wash reservoir. Next, click the **Disconnect** Icon which will disconnect the computer and the robot. Next, turn the power off to the robot by flipping the off button in the “Gryphon Power” 4-outlet power strip. Finally, replace the dust cover on top of the instrument. Now it is time to start the protocol. This will be covered now.

**Opening and running an existing Protocol**

X-Ray Facility has made several ARI Crystal Gryphon Protocols (stored in “C:\Program Files\Gryphon\Protocols”) and is likely that you will be able to use one of them to set-up your protein. If you need more elaborate protocol please contact XRF Director Dr. T. Somasundaram.

Now let us look at how to open our first protocol and execute it. Later on in this tutorial you will see a table with each line of the protocol and its meaning. This will allow the new user to create their own protocol.

**Beginning of Explanation for 2 Well SW 1 Protein protocol**

From ARI Crystal Gryphon Graphical User Interface (GUI) shown in Figure 3, do the following:

File > Open Protocol

This action is shown in Figure 4 below.
This action will open a new window with multiple protocols as shown in the next Figure 5.

From the list of available protocols we are going to select "2 Well SW 1 Protein" protocol created by JOC on 07/28/2014 with "200nL" description (see Figure 6).
Select the protocol and click “Open Protocol”. This will open the protocol in the main GUI as shown in the Figure 7.

At this point, you want to make sure all the hardware is correct and the solutions are placed in their proper positions. To confirm all hardware and solutions, click on the “Protocol Start” line on the top of list of line items. This will highlight the current hardware as shown in the Figure 8.
From the above protocol list, note the following hardware and solutions and their positions:

- **Position 1**: Intelli-Plate 96-2 SW w/ A1 corner cut
- **Position 2**: Hampton Screen in Greiner Deep Well Block w/ A1 corner cut with 1 mL of screen buffer
- **Position 3**: Wash Flow-Through
- **Position 10 A1**: At least 100 µL of 10 mg/mL protein
- **Position 12**: Nano wash station

Once the hardware and solutions are placed, click the GO button to start running the protocol. But let us examine each step on this simple protocol to understand what we are doing. The understanding will allow new users to make their own protocols.

### Line by line explanation of the protocol

<table>
<thead>
<tr>
<th>Line in the Protocol</th>
<th>Detailed meaning of the line in the protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash 1 times 100 µL in Tray 3</td>
<td>All 96-needles will be washed by aspirating 100 µL of clean water in Position 3 while “Head Wash Pump” is running for a pre-selected time. See Figure 9.</td>
</tr>
<tr>
<td>Wash Nano Dispenser 1 time</td>
<td>The Nano nozzle will be washed in Position 12 while “Nano Wash Pump” is running for a pre-selected time. Figure 10.</td>
</tr>
<tr>
<td>Aspirate 60 µL from Tray 2</td>
<td>Aspirate (pick-up; arrow-up with clear background indicates screen pick-up) 60 µL each of 96-screen buffer solutions from Position 2 (Hampton Screen in Greiner Deep Well Block). See Figure 11.</td>
</tr>
<tr>
<td>Nano aspirate 30 µL from Tray 10</td>
<td>Aspirate (pick-up; arrow-up with dotted background indicates protein pick-up) 30 µL total of protein from Position 10 A1 (from 200 µL PCR tube with no lid). See Figure 12.</td>
</tr>
<tr>
<td>Step</td>
<td>Description</td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
</tr>
<tr>
<td>Dispense 58 µL in Tray 1 in location A,1</td>
<td>Dispense (delivery; arrow-down with clear background indicates screen delivery) 58 µL of screen buffer in each and every one of the 96-reservoirs of the Intelli-Plate. See Figure 13.</td>
</tr>
<tr>
<td>Dispense 0.2 µL in Tray 1 in location A,1</td>
<td>Dispense 0.2 µL of screen buffer in each and every one of the top 96-wells of the Intelli-Plate. See Figure 14.</td>
</tr>
<tr>
<td>Empty in Tray 1 in location A,1</td>
<td>Empty 1.8 µL (60-(58+0.2 µL)) of screen buffer back in each and every one of the 96-reservoirs. See Figure 15.</td>
</tr>
<tr>
<td>Nano dispense 0.2 µL drop in Tray 1</td>
<td>Nano nozzle will dispense (delivery; arrow-down with dotted background indicates protein delivery) 0.2 µL of protein in each and every one of the top 96-wells of the Intelli-Plate. See Figure 16.</td>
</tr>
<tr>
<td>Nano dispense purge in Tray 10.</td>
<td>Nano nozzle will purge the reminder (~10 µL) of the protein back to original PCT tube in Position 10 A1. Note we NEVER purge the buffer back to the Deep Well Block to avoid cross contamination. Only protein is returned. See Figure 17.</td>
</tr>
<tr>
<td>Wash 4 times 100 µL in Tray 3</td>
<td>All 96-needles will be washed by aspirating 4 x 100 µL of clean water in Position 3 while “Head Wash Pump” is running for a pre-selected time. See Figure 18.</td>
</tr>
<tr>
<td>Wash Nano Dispenser 1 time</td>
<td>The Nano nozzle will be washed in Position 12 while ‘Nano Wash Pump” is running for a pre-selected time. Figure 19.</td>
</tr>
</tbody>
</table>

![Figure 9 Needle Head Wash](image-url)
Figure 10 Nano Needle Wash

Figure 11 Aspirate (pick-up) screen buffer
Figure 12 Nano aspirate (pick-up) protein

Figure 13 Deliver screen buffer in 96-resevoirs
Figure 14 Deliver 200 nL screen buffer to top 96-wells

Figure 15 Empty the screen buffer in 96-reservoirs
Figure 16 Deliver 200 nL of protein to top 96-wells

Figure 17 Empty excess protein back to PCR tube
Now that we have seen how to open and execute an existing protocol, let us see how to make or modify a protocol.

Make or modify a protocol

Start the Crystal Gryphon 1.4.5.3 program as described in “At the Computer”. From ARI Crystal Gryphon GUI click the top line labelled “Protocol Start”. This will highlight the available hardware on the right hand-side panel as shown in Figure 20 below.
Then from the right hand-side hardware panel, drag the “Intelli-Plate 96-2 SW” icon to Position 1 (currently labeled 'empty') above the right hand side panel and drop it (now it will say ‘Intelli-Plate 96-2 SW’). Similarly, drag the “Emerald Screen” icon to Position “2” (currently labeled 'empty') and drop it (now it will say ‘Emerald Screen’), and finally drag the “Wash –Flow Thru” icon to Position “3” (currently labeled ‘empty’) and drop it (now it will say ‘Wash Flow Thru’).

With these steps we now defined hardware in Position 1 (Intelli-Plate 96-2 SW), Position 2 (Emerald Screen) and Position 3 (Needle Head wash station). This is shown in Figure 21.
Now from the left hand side panel drag the “Wash” icon and drop it in the space just below “Protocol Start”. This might give an error, “!Tray not valid Unknown liquid class” as shown in Figure 22. To rectify the error, while the line is high-lighted, manually click on icon labelled $3$ Wash Flow-Thru and in the “Volume (µL)” specify 100 µL and in the “Liquid Class” drop-down menu select “Water”. The error will disappear as shown in Figure 23. Also note that the Position 3 icon labelled $3$ Wash Flow-Thru “has a thicker border now.
Now, we will drag and drop “NanoWash” as the next step. This will obviously highlight “Position 12” since Nano Nozzle will be washed in that position. Now, drag and drop “Aspirate” as our next step. Once again we might see an error, “! No tray defined Unknown Liquid Class” as shown in Figure 24.

Like before, click on “Position 2”, in Volume (µL) enter “60 µL” and in Liquid Class drop-down menu, select “Screen”. This will rectify the error as shown in Figure 25.
Using the procedure outline above, the user can build rest of the protocol. The following steps are the minimum needed in a simple protocol:

1. Wash (96 Needle Head in Position 3)
2. Nano Wash (Nano Nozzle in Position 12)
3. Aspirate (Screen from Position 2)
4. Nano Aspirate (Protein from Positions 10 A1 or 10 A2 or 11 A1 or 11 A2)
5. Dispense (Screen in Position 1 in main reservoir)
6. Dispense (Screen in Position 1 in Well 1 or Well 2 or Well 3 or all or combination, as required)
7. Empty (Screen in Position 1 in main reservoir, if needed)
8. Nano Dispense (Protein in Position 1 in Well 1 or Well 2 or Well 3 or all or combination, as required)
9. Nano Dispense Purge (purge the excess protein back to Positions mentioned in Step 4 above)
10. Wash (96 Needle Head in Position 3)
11. Nano Wash (Nano Nozzle in Position 12)

**Example of an elaborate ARI Crystal Gryphon Protocol**

More elaborate protocols can be created by adding or modifying the simple protocols. An example for an elaborate protocol is shown in Figure 26 where protein is present in two wells, DNA in two wells, and protein and DNA in one well (with screen in all three wells) on an Intelli-Plate 96-3 (ARI 102-0001-13).
Figure 26 One of the elaborate protocols

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