Automated DNA IQ™ Extraction from Bloodstains & Other Casework Samples

1 General Information

1.1 **WARNING: THE LYSIS BUFFER IN THE DNA IQ KITS IS CORROSIVE AND TOXIC. IT CAUSES SEVERE SKIN BURNS AND EYE DAMAGE AND IS HARMFUL IF INHALED OR SWALLOWED**. If on skin: take off all contaminated clothing and rinse with water and soap. If in eyes: rinse with copious amounts of water for several minutes. Keep lysis buffer bottle tightly closed.

1.1.1 The waste plates containing residual reagents can be discarded in the regular laboratory garbage.

1.1.2 The remaining residual Wash buffer and Elution buffer reagents in the troughs after a run can be disposed of in the laboratory sink flushed with copious amounts of water.

1.1.3 However, the remaining residual **Lysis Buffer and Lysis Buffer with Resin must be collected in a properly labeled waste container and be properly discarded via a chemical waste vendor**.

1.1.3.1 Contact QA to collect waste containers and expired bottles for proper disposal.

1.2 **CAUTION: DO NOT ADD BLEACH OR ACIDIC SOLUTIONS DIRECTLY TO ANYTHING CONTAINING LYSIS BUFFER INCLUDING SAMPLE WASTE**. Exposure to strong acid or bleach will result in the generation of toxic gases.

1.2.1 If liquid containing these buffers spill, clean with suitable laboratory detergent and water.

1.3 **CAUTION: DO NOT ADD BLEACH TO ANY PART OF THE HAMILTON® ROBOT INCLUDING THE DECK**. Bleach will cause the robot to rust. For any spills on the robot, use ethanol and water for cleanup.

1.4 This extraction is applicable for exemplar samples and all casework evidence samples EXCEPT suspected semen samples. Refer to the Evidence Examination manual to determine the appropriate sample size to submit for extraction.

1.5 Do not extract evidence samples and exemplars together.

2 Sample Preparation and Incubation

2.1 Obtain two empty 1.5ml microcentrifuge tubes for the extraction negatives and label one as Extraction Negative 1 and the other as Extraction Negative 2. Obtain labeled cuttings in 1.5mL microcentrifuge tubes. Compare your sample labels and tube tops to the input sample list in LIMS, and confirm that you have the correct samples.

2.2 Print input labels and place on spin basket units (consisting of spin basket and click fit tube) for each sample. Also print LIMS output labels and label 1.5ml screw cap tubes to be used as the elution tubes.

2.3 Prepare digest buffer master mix as per the calculated amounts in the Reagents tab in LIMS. Record the lot# of each reagent in LIMS.

Stock Solution	1 sample
Diluted ATL (Qiagen)	192 µL
ProteinaseK (Qiagen)	8µL

2.4 Vortex the master mix well.

2.5 Add 200ul of digest buffer master mix to each of the sample tubes including the extraction negative tubes. If necessary, take a clean pipette tip and push the substrate down into the digestion liquid.

2.6 Incubate at 56ºC for 30 minutes with shaking at 1400 rpm in the thermomixer.

2.7 Record the temperature of the thermomixer in LIMS. The temperature should be within ±3ºC of 56ºC.

2.8 Prepare 10% bleach, distilled water, and 70% alcohol in three 50ml conical tubes. Clean a pair of forceps by dipping the forceps in each of the three tubes briefly and then drying with a fresh lint free wipe.

2.9 While samples are incubating, proceed with the daily maintenance of the robot, if needed, in Section **5** Daily Maintenance below.

2.10 Remove the tubes from the thermomixer and briefly centrifuge the samples for a few seconds at <3000rpm.

2.11 Have a **witness** verify the order of the spin basket units and the sample digestion tubes by reading the tube-top label and the entire input sample ID number for each sample. This is recorded as your extraction witness.

2.12 For each sample, transfer the substrate or scrapings using the forceps to its associated spin basket unit. Pipette mix the sample lysate within the incubation tube a few times to disturb any pellet that may have formed, and then pipette the entire lysate volume (~200ul) over the top of the substrate in the spin basket. Close the tube top over the spin basket. Repeat this step for each sample, cleaning and drying the forceps between each sample.

2.13 Centrifuge the substrates in spin baskets at 13,200 rpm to 15,000 rpm for 2 minutes.

2.14 Using clean and dry forceps (or a fresh lint-free wipe), remove and discard the spin baskets (including the swab remains), taking care to avoid bubbles at the rim of the open tube. Clean and dry forceps between each sample. Close the tube.

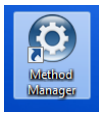
2.15 Proceed to setting up the Hamilton STAR Robot.

3 Setting up the Hamilton® STAR Robot:

3.1 Make sure the green power switch on the robot is turned on before logging into the computer.

3.2 Daily maintenance is to be performed prior to the first run of the day.

3.2.1 Proceed with the daily maintenance of the robot, if needed, in Section 5 Daily Maintenance below

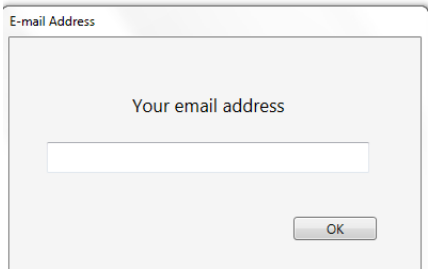
3.3 Once Daily Maintenance is done, click on the Method Manager icon. 

3.4 Pull out all tip carriers for loading except for the stationary carrier and leave them in the out position. The robot will bring all tip carriers back in as it scans the tip barcodes.

3.5 Click on the Extraction button.

3.6 Then Click the Run icon. 

3.7 The following pop up box will appear:

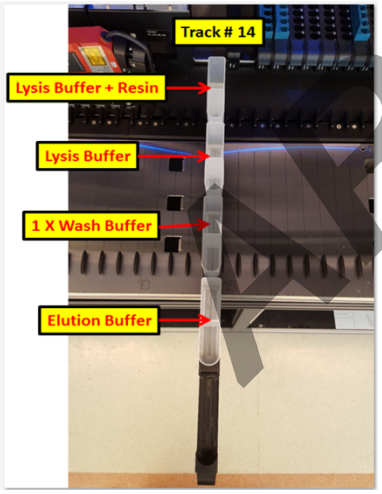


3.8 Enter your email address. Click OK. This will allow you to receive an email message from the robot when your run is done, or if the run is aborted. The robot can also send you a text message by entering your 10 digit phone number in an email format:

Verizon: number@vtext.com
AT&T: number@txt.att.net
T-Mobile: number@tmomail.net
Sprint: number@pm.sprint.com

3.9 In the next window, enter the total number of samples of your batch. Include extraction negatives. Maximum number of samples is 84. Click OK.

3.10 Record the lot numbers for the DNA IQ extraction reagents in LIMS.



3.11 Load the indicated volume of each reagent into new 60ml reagent containers on track #14 as indicated by the arrows above. The calculation of the amount of reagents is calculated by the robot software. The following tables may also be used for reference.

**The software rounds the amount of resin to the nearest thousandth of a mL

	Amount to add in mL		
Buffer	**8 samples**	**16 samples**	**24 samples**
Lysis Buffer & Resin**	5.4	7.4	9.4
	0.151	0.207	0.263
Lysis Buffer	4.4	5.2	6.0
1X Wash Buffer	6.2	7.8	9.4
Elution Buffer	5.4	5.9	6.3

Buffer	**32 samples**	**40 samples**	**48 samples**
Lysis Buffer & Resin**	11.4	13.4	15.4
	0.319	0.375	0.431
Lysis Buffer	6.8	7.6	8.4
1X Wash Buffer	11.0	12.6	14.2
Elution Buffer	6.8	7.2	7.7

Buffer	**56 samples**	**64 samples**	**72 samples**
Lysis Buffer & Resin**	17.4	19.4	21.4
	0.487	0.543	0.599
Lysis Buffer	9.2	10.0	10.8
1X Wash Buffer	15.8	17.4	19.0
Elution Buffer	8.1	8.6	9.0

Buffer	**80 samples**	**84 samples**
Lysis Buffer & Resin**	23.4	24.4
	0.655	0.683
Lysis Buffer	11.6	12.0
1X Wash Buffer	20.6	21.4
Elution Buffer	9.5	9.7

3.11.1 Prepare the Promega Lysis Buffer and DNA IQ™ Resin Solution fresh before each run as per the calculations in the DNA IQ program. Before pouring the resin, thoroughly mix the resin by inversion many times before adding. Do not store solution after run.

3.11.2 Add Promega Lysis buffer to the second reagent container as per the calculations in the DNA IQ program. Remember to re-cap the bottle tightly to prevent crystallization!

3.11.3 If Promega 2X wash buffer has not already been diluted, prepare as follows and add Promega 1X wash buffer to the third reagent container as per the calculations in the DNA IQ program.

3.11.3.1 Note: The wash buffer comes from the manufacturer at a concentration of 2X in each DNA IQ kit. It needs to be diluted to 1X. If you are opening a new DNA IQ kit proceed with the following:

3.11.3.1.1 Add 35ml of 95–100% ethanol and 35ml of 99% isopropyl alcohol directly to the 70 ml 2X Wash Buffer bottle. Replace the cap, and mix by inversion several times.

3.11.3.1.2 Label as 1X Wash Buffer, indicating the addition of ethanol and isopropyl alcohol. These lot numbers do not need to be recorded in LIMS. Label the date of the dilution and your initials.

3.11.3.1.3 Store at room temperature (22–25°C). Make sure bottle is closed tightly to prevent evaporation.

3.11.4 Add Promega Elution buffer to the fourth reagent container as per the calculations in the DNA IQ program.

3.11.5 Label the reagent containers with the reagent name.

3.12 Set up the Robot Platform:

3.12.1 IT IS VERY IMPORTANT THAT VWR PLATES AND PROMEGA PLATES ARE ONLY USED WHERE DIRECTED**. DO NOT INTERCHANGE THE DIFFERENT TYPES OF PLATES AS THIS WILL RESULT IN A Z CRASH ERROR!**

3.12.2 All plates should be oriented with well A1 in the top left corner.

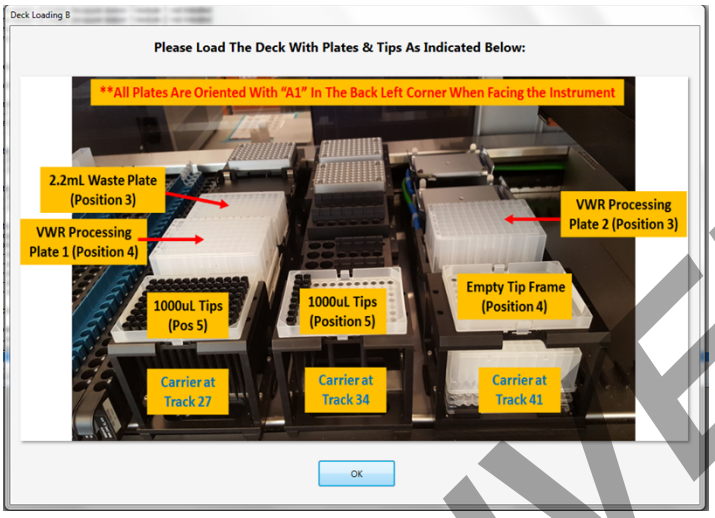
3.12.3 Load one new VWR deep well processing plate to position 4 on the carrier located on tracks 27-32. Make sure that the VWR plate is seated flush on the magnetic plate so that the plate wells are aligned with the magnetic wells.

3.12.4 Add another VWR plate to position 3 on carrier located on tracks 41-47.

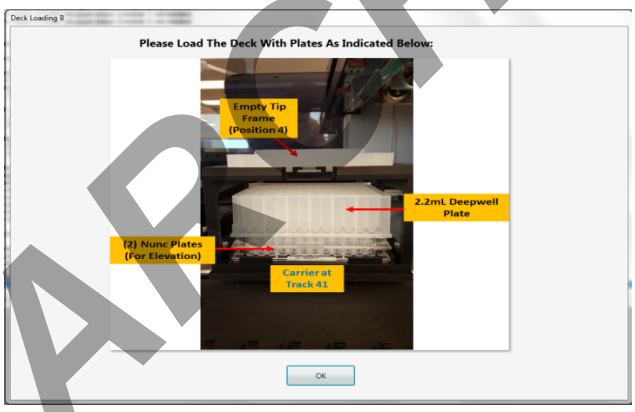
3.12.5 Add one Promega 2.2ml deep well plate for waste on position 3 of carrier located on tracks 27-32.

3.12.6 Place a clean empty tip frame in position 4 of carrier located on tracks 41-47.

3.12.7 Click OK.



3.12.8 Add another Promega 2.2ml deep well plate under position 4 on carrier on tracks 41-47. The plate should rest on top of two Nunc plates (used for elevation only) as seen in the photo below.



3.12.9 Check if the number and location of tips on the deck matches what is shown in the tip-counter window below. Refill tips only if the program indicates tips are needed.

3.12.9.1 The program has a built-in tip-counter that will remember which tips it used in previous runs, the number of tips used, and where those tips are located on the deck. The program calculates the number of tips needed for each tip size in order for the run to complete, based on the number of samples indicated in step 3.9.

3.12.9.2 If the tips on the deck match what is shown in the program, click OK.

3.12.9.3 If there are discrepancies between the number and location of tips on the deck compared to what is shown in the program, you will need to change the display in the program to match the deck.

3.12.9.3.1 Click and drag the lasso tool in the program to indicate where the tips are located for each tip size. When a circle on a tray is highlighted orange, it means a tip is present at that location.

3.12.9.4 When the program matches the deck, click OK.

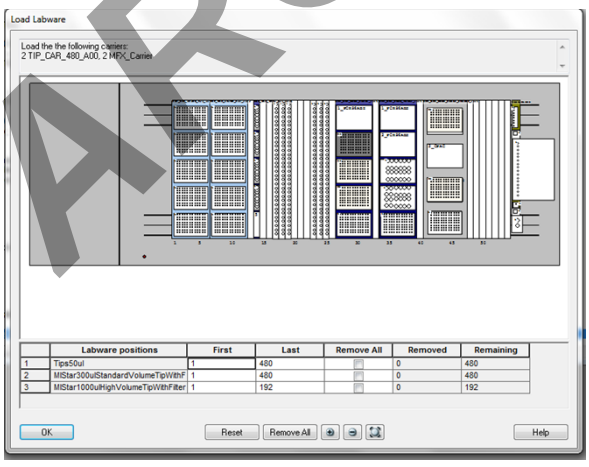
3.12.9.5 After the instrument pulls in the carriers, the program calculates the number of tips needed for the run. If there aren't enough tips, the program will give a warning message indicating which tip size needs to be filled.

3.12.9.6 Click OK and a tip-counter window will appear. At this point, the window will not let you highlight any tips. Click OK and the instrument will push out the carriers, enabling you to add tips to the deck.

3.12.9.7 Ensure that the correct size tips go in the correct carrier. Ensure that the bar code on the side of the tip tray is facing to the right.

3.12.9.8 Another tip-counter window will appear in the program and will allow you to highlight where tips were filled on the deck.

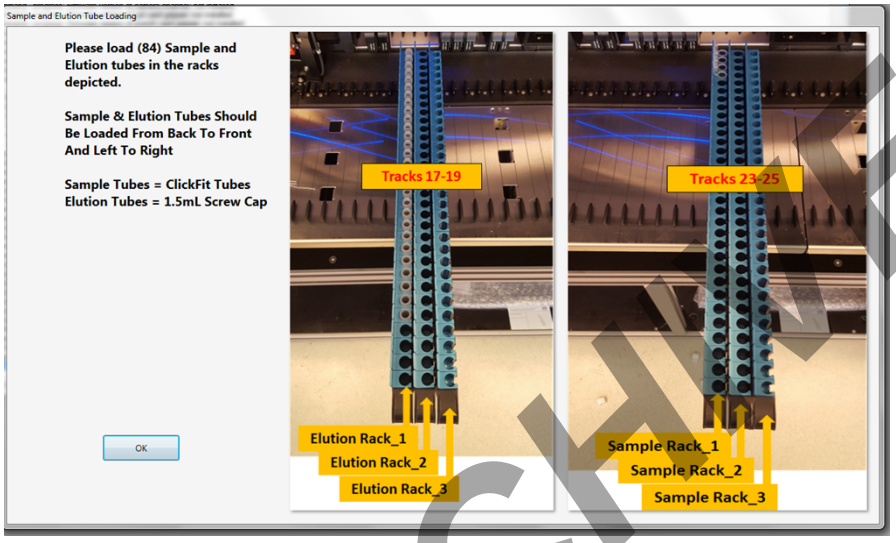
3.12.9.9 If full trays of tips have been added, click the Reset button. Then Click OK.
*****NOTE: Only hit reset if ALL 3 tip types have FULL TRAYS.***



3.13 A series of prompts will ask for confirmation of the carriers located on the instrument deck. Verify the correct location of the carriers and click YES to continue.

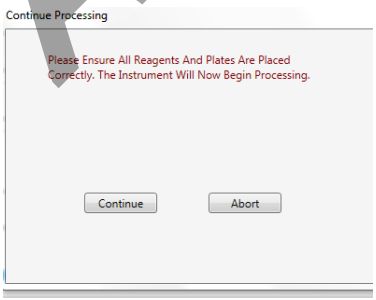
NOTE: If multiple tip sizes don't have enough tips, another warning message will appear stating which tip size needs additional tips. Follow 3.12.9.6through 3.13for each tip size that needs to be filled.

3.14 Have a **witness** verify the "robot setup" by confirming the sample order while loading the sample lysates in open click-fit tubes into the blue tube adaptors on the sample carriers located in tracks 23-25. The samples should be loaded from back to front.



3.14.1 The witness must also confirm the elution tube order while loading the uncapped labeled elution tubes into the carriers located in tracks 17-19 from back to front.

3.14.2 Finally, the witness must also make sure that the carriers are on the correct tracks, the deck layout is correct, VWR plates and Promega plates are in the right positions, elution tubes are uncapped, input tubes are open, and the robot door is closed. This will be your "robot setup" witness in LIMS.



3.15 Click Continue to begin run. Make sure to watch the run for the first few minutes to make sure it is running ok.

3.16 When the run is complete, an email (or text) will be sent to you. Once the Run is complete:

3.16.1 Cap the elution tubes and store in a cryobox in the appropriate fridge. Visually inspect the volume of the elution tubes to ensure that all appear to have the same volume (40µL). If any samples have less than this amount, alert QA.

3.16.2 Discard input tubes, plates, and the empty tip frame in position 4 along with its Promega deep well plate underneath it on the carrier on tracks 41-46. If a tray of tips is depleted, do not discard the tip tray. These should be saved for future runs in labeled sealable plastic bags, to be used as the tip holder at position 4 (track 41-46).

3.16.3 Reagent troughs may be discarded or saved for reuse. If saved, each reagent trough must be rinsed only with deionized water and fully dried prior to reuse. Troughs must be labeled with the name of the reagent and reused only with that reagent. Refer to steps **1.1.2** and **1.1.3** in regards to disposal of residual reagents.

3.16.4 In the LIMS system, navigate to the Data Entry page, indicate if the DNA IQ extraction passed or failed.

3.16.5 Ensure that the Hamilton usage log has been filled out in LIMS.

3.16.6 Close the Method Manager software and logout of the computer.

3.16.7 If it is the last run for the day, turn off the instrument.

4 Troubleshooting

4.1 DNA IQ

Poor yield	Too much sample was used. Excessive amount of sample can reduce the efficiency of DNA binding to the resin. Use less sample.
Inconsistent yield	Inconsistent amounts of resin. Vortex resin stock thoroughly before adding to reagent trough. Do not allow the resin to dry out.

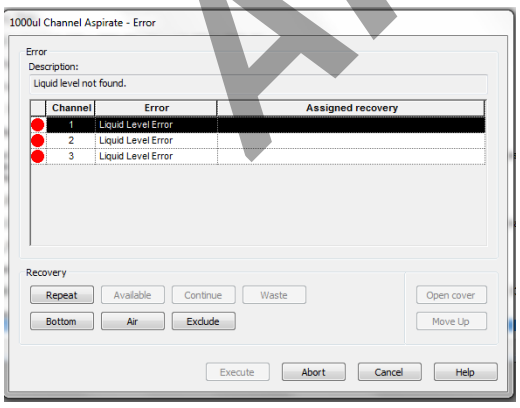
4.2 STAR Robot

4.2.1 While some errors may be corrected by the user, most should be handled by QA. Error email messages are sent to ocmerobotics@ocme.nyc.gov. If an error is encountered, contact QA to check for an email. If the run is aborted, an email is sent to the email address that was entered at the beginning of the run.

4.2.2 Below are some common error codes that may be resolved by the analyst. For further troubleshooting errors, contact QA:

Main Error Code	Error	Description
5	Barcode Error	Barcode could not be read or is missing
6	Insufficient Liquid Error	Not enough liquid available
7	Tip Present Error	A tip has already been picked up
8	No Tip Error	Tip is missing use the next tip in sequence or fill in tips
9	No Carrier Error	No carrier present for loading
16	Cover Open Error	Cover not closed or cannot be locked
18	Wash Liquid Error	Waste is full or wash liquid is empty
100	Wrong Carrier Error	Wrong carrier barcode, a wrong carrier is loaded.
102	Liquid Level Error	Liquid surface not detected.
103	Not Detected Error	Carrier not detected at end position on deck

4.2.3 If a liquid level error occurs, for example, (like in the following pop up window), click repeat and execute to determine which trough has an insufficient volume of liquid. The error message should appear again. You can then add additional reagent and click repeat and execute again to continue the run.

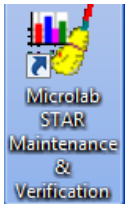


4.2.4 The following describes the actions of each recovery option in the pop up window when an error is encountered. Choose the appropriate action or contact QA for assistance:

Recovery Button Text	Description
Abort	Run is aborted
Cancel	Run is canceled and a defined user error handler is started
Repeat	The command is repeated
Exclude	The channel or position is excluded until a new tip pick up is called.
Waste	The tip is ejected to the default waste
Air	Rest of missing volume is filled up with air
Bottom	Repeats the aspiration on container bottom
Continue	Continue without any change
Barcode	Barcose is assigned manually
Next	Repeat the command on next sequence position
Available	Aspirate & dispense the available volume.

5 Daily Maintenance

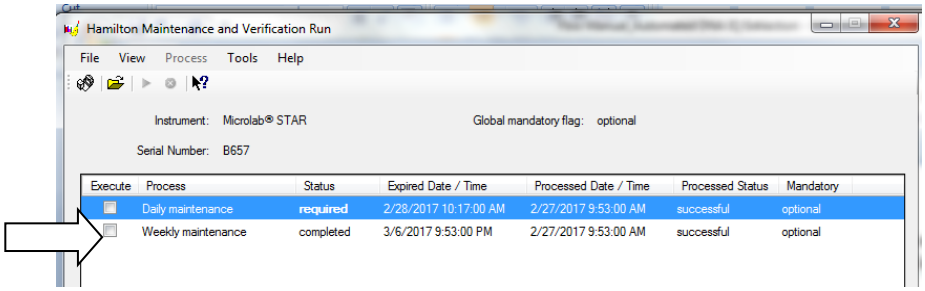
5.1 Maintenance should be performed before the first run on a calendar day. Check to see if the daily maintenance was performed by clicking on The Microlab STAR Maintenance & Verification icon on the desktop.



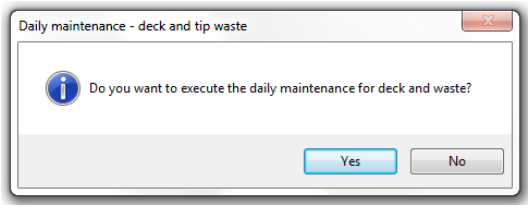
5.2 Check the Processed Date/Time to determine the date maintenance had last been completed.

5.2.1 Note that the Expired Date/Time is registered based on a 24hour time frame; therefore, the system may not say required even if the Maintenance has not been performed that day.

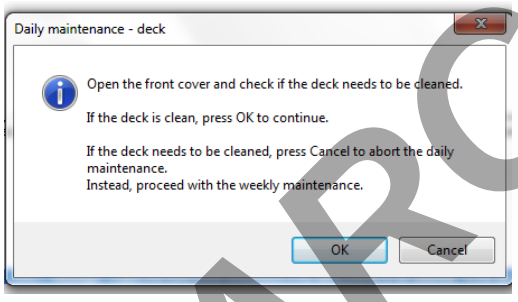
5.3 If the maintenance had not been completed for the day, follow the prompts as described below.



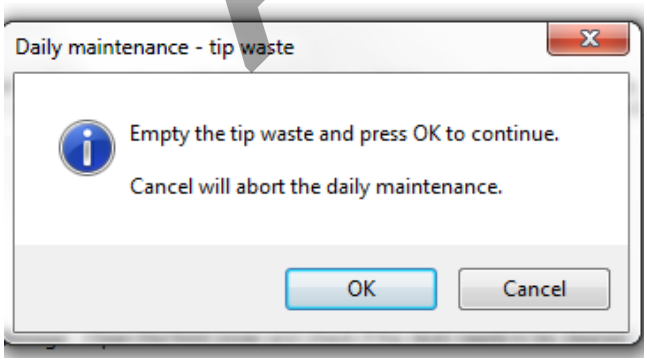
5.3.1 A window will appear after the robot initializes. Click "Yes."



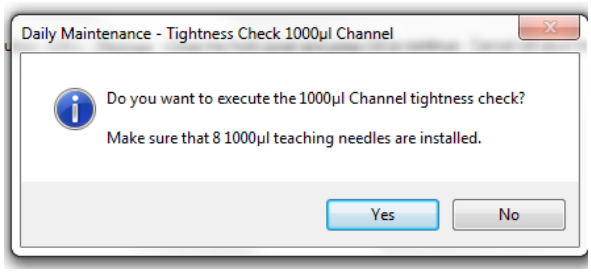
5.3.2 Inspect the deck to make sure it is clean. If the deck is clean, press OK. If not, press Cancel and alert QA to run the weekly maintenance.



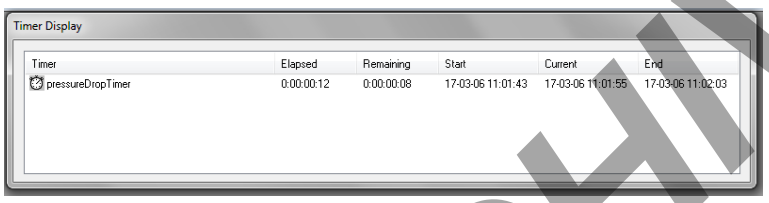
5.3.3 If the tip waste is full, empty it; otherwise, press OK.



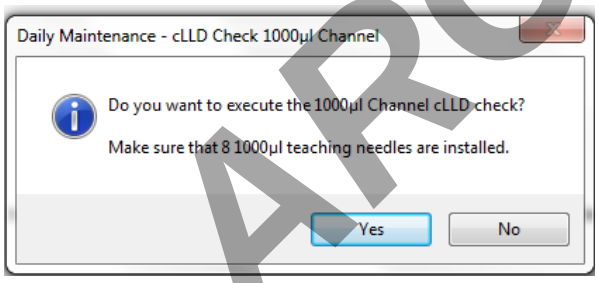
5.3.4 When prompted "Do you want to execute the 1000ul Channel tightness check?" click Yes.



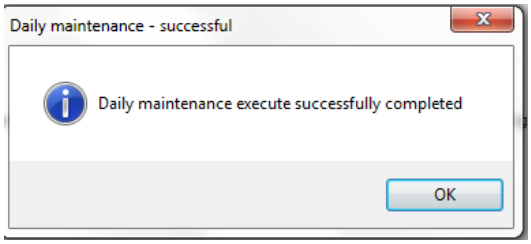
5.3.5 The window below will then appear. Wait while the channels perform a pressure check.



5.3.6 When prompted, "Do you want to execute the 1000ul Channel cLLD check?" click Yes.



5.3.7 Click OK once the maintenance has successfully completed.



5.4 Update the daily maintenance log in LIMS by doing the following:

5.4.1 Navigate to the LIMS tramstop "Other", then instrument→ *instrument name*→ select instrument→ click [maintenance log]→ Add→ select "daily maintenance performed" in the Process Performed from the dropdown menu and click Save Entry.