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Department of Forensic Science

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

FORENSIC BIOLOGY SECTION

QIAGEN® BIOROBOT® UNIVERSAL SYSTEM PROCEDURES MANUAL

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1 ISOLATION OF DNA

1.1 Technical Notes

1.1.1 The QIAamp® extraction procedure uses QIAamp® 96 plates to extract/purify DNA from both dried blood stains and buccal swabs.

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1.1.2 The QIAGEN® Buffer AL, included in the QIAamp® extraction kit, is a guanidine-based lysis buffer. The guanidine helps to set up the binding conditions needed for the DNA to adhere to the spin column membrane. The QIAGEN® AL lysis buffer also contains a detergent to rupture leukocyte and epithelial cell plasma and nuclear membranes, which exposes the nucleic acids.

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1.1.3 The QIAGEN® Protease is similar to, but less stringent than, Proteinase K. Both are used to break down proteins into their constituent amino acids. The DNA yield reaches a maximum after lysis for 10 min at 56°C. Longer incubation times have no effect on the overall yield of the purified DNA.

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1.1.4 The QIAGEN® Buffer AW1 is an ethanol-based stringent wash solution containing a low concentration of guanidine. This wash step removes any non-specific binding of inhibitors to the spin column membrane.

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1.1.5 The QIAGEN® Buffer AW2 is a Tris-based solution containing ethanol, which will wash away any salts that are present.

1.1.6 All Data Bank samples will be eluted in Type 1 water which captures the DNA attached to the membrane and serves as a storage medium.

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1.1.7 The QIAGEN® Top Elute Fluid enhances DNA recovery when applied to the membrane after the addition of the Type 1 water.

NOTE: If a Data Bank sample requires typing with the AmpFℓSTR® Yfiler™ System and cannot be extracted on the Qiagen Universal robot system (i.e. Q-tip type swab), then a cutting of the sample will be given to a casework examiner for analysis and typing.

1.1.8 A random sample will be run with each set of convicted offender and arrestee sample extractions (Y-STRs excluded) to serve as a verification that the samples are successfully being entered into Combined DNA Index System (CODIS) and the search algorithm is working properly. This sample serves as an internal laboratory control since the DNA profile is not known to the Data Bank analyst and must be verified by the Forensic Biology Program Manager or designee prior to the sizing data being considered acceptable. If a sample must be re-extracted a new random sample must be extracted along with the sample.

1.2 Equipment

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- QIAGEN® BioRobot® UNIVERSAL SYSTEM
- Carboys, 10 L and 4 L
- Microplate station blocks with MP Slots
- Reagent holders: 8-tube (1.2 mL), 24-tube (14 mL) and 5-trough
- Buffer bottles, 250 mL and 500 mL
- System liquid bottle, 1 L
- Black QIAplate holder and channeling adapter, MP Slot 16 (and 24 for two plates)
- Silver QIAplate holder, MP Slot 11 (and 26 for two plates)
- Blue elution microtube rack adapter, MP Slots 6 (and 7 for two plates)
- Silver vacuum base and top
- Scissors and tweezers
- BSD600-Duet Semi-Automated Dried Sample Puncher

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1.2 Materials

- 96-well S-block
- QIAamp® 96 plate
- Elution microtube rack with 96 elution microtubes
- Caps (strips of 8) for collection microtubes
- Adhesive tape
- Kimwipe®
- Troughs, 80 mL (for elution Type 1 water and AL)
- Round-bottom tubes, 14 mL (for QIAGEN® Protease)
- Disposable pipettes, 50 mL
- 1100 µL conductive disposable filter pipette tips

1.3 Reagents

- 1X Phosphate Buffered Saline (PBS), pH 7.2
- QIAGEN® Protease, lyophilized
- Protease Solvent
- QIAGEN® Buffer AL
- Ethanol (95%)
- QIAGEN® Buffer AW1 (reconstituted with 95% Ethanol)
- QIAGEN® Buffer AW2 (reconstituted with 95% Ethanol)
- QIAGEN® Top Elute Fluid
- Type 1 water

1.4 Preparation of Dried Blood Stain and Buccal Swab Samples

1.5.1 Using the BSD600-Duet Semi-Automated Dried Sample Puncher or scissors, punch or cut a 3.2 mm^2 and transfer to a labeled S-block using tweezers. If scissors are used, they should be cleaned between each use with a Kimwipe and 10% bleach then followed by isopropanol**.**

NOTE: If the sample is cut manually using scissors, a witness **MUST** be present to verify that the samples are placed into the correct wells of the S-block. Blood samples deposited on cotton swatches should ALWAYS be manually cut and placed into the designated well of the S-block.

The maximum number of samples that can be run at a time is 92 samples, which include 90 dried blood or buccal swab samples and 2 controls (one random sample and one reagent blank). The remaining 4 empty (unused) wells will be used for downstream applications.

1.5.2 Process a reagent blank and a random sample along with each set of samples.

1.5.3 Proceed to Section 1.6, Purification of Genomic DNA from Dried Blood Stains and Buccal Swabs Using the QIAGEN® BIOROBOT® UNIVERSAL SYSTEM.

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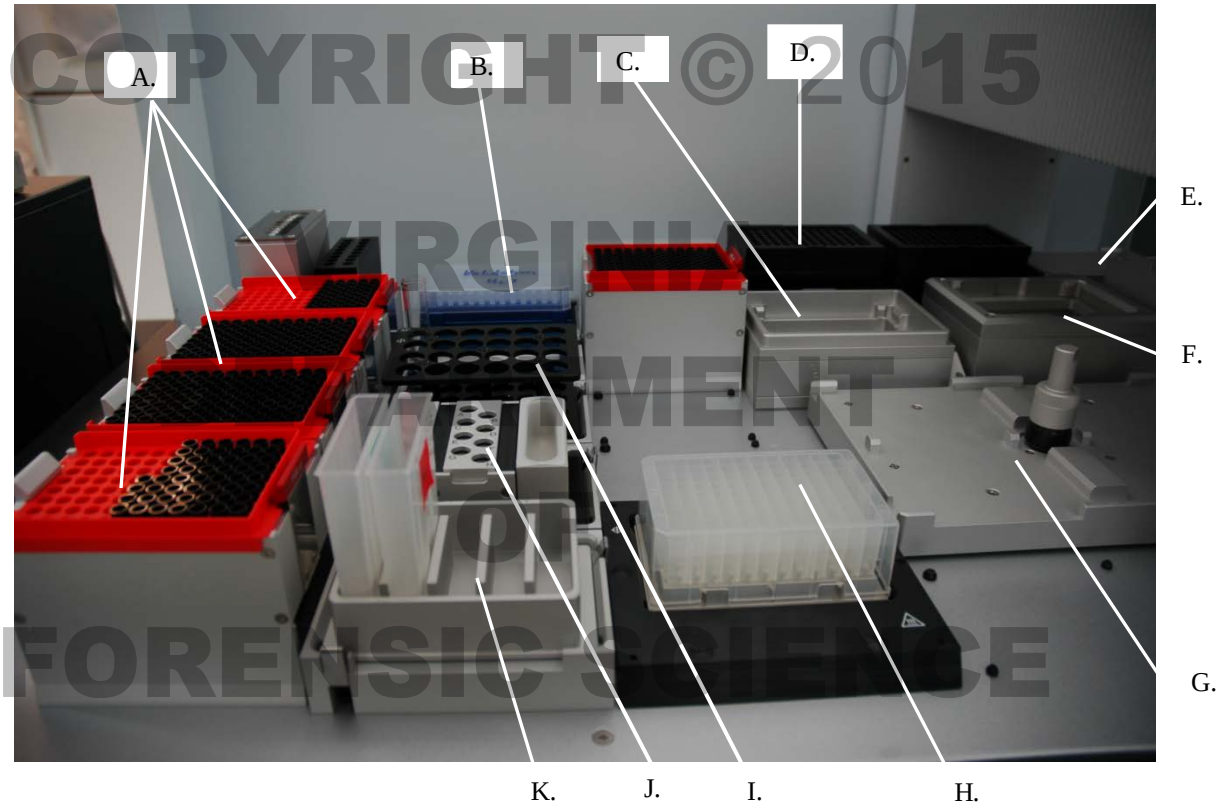
1.6 Purification of Genomic DNA from Dried Blood Stains and Buccal Swabs Using the QIAGEN® BIOROBOT® UNIVERSAL SYSTEM



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Front view of the deck

A. Tip-tray holders
B. Elution Microtubes CL
C. Vacuum base
D. Black multi-well-plate holder (channeling adapter)
E. Silver multi-well-plate holder (QIAamp 96 plate)
F. Vacuum top
G. High-speed shaker system
H. Cooling and heating system (S-Block)
I. Reagent holder for 24 tubes (Protease, position A1)
J. Reagent holder for 8 tubes (Top Elute Fluid, Sub-slot A)
K. Reagent holder for troughs (Elution Type 1 water and AL)

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A. Tip Disposal Unit
B. System Liquid Container
C. Waste Container
D. Condensate Trap
E. Vacuum Trap

1.6.1 Turn on the QIAGEN® BIOROBOT® UNIVERSAL SYSTEM using the switch on the front, right lower corner of the unit. A green light should appear to the left of the switch.

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1.6.2 Turn on the computer to the left of the instrument. The "username" field of the logon box should contain "Qiagen Instruments". Select 'OK' to continue, as a password is not needed to log onto the computer. Double-click on the QIAsoft program on the computer desktop. Log in with the appropriate username and password.

NOTE: Before the first run of the day, go under "Tools" and select the "Flush System" option to flush the system. It is not necessary to modify the settings.

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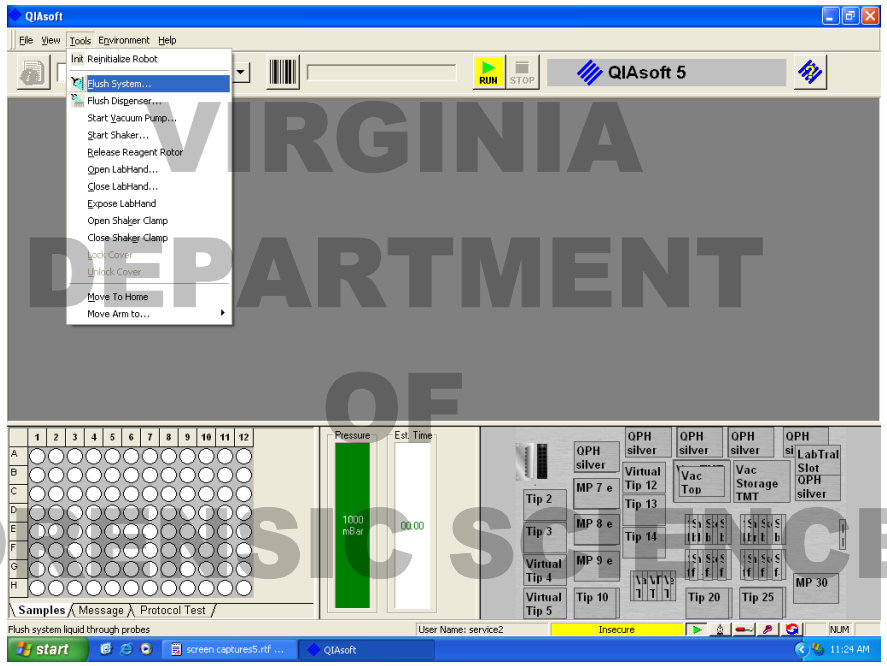
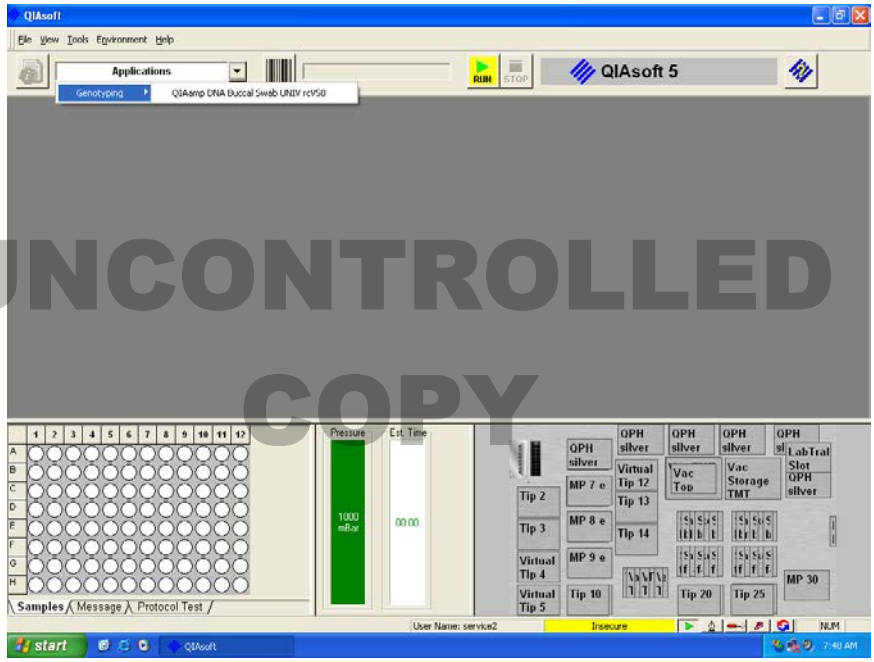


1.6.3 Click on the "Applications" dropdown menu located on upper left-hand corner, select "Genotyping" and then further select "QIAamp DNA Buccal Swab UNIV rcV50" program. The protocol is started by clicking on the "RUN" button in the middle of the toolbar.



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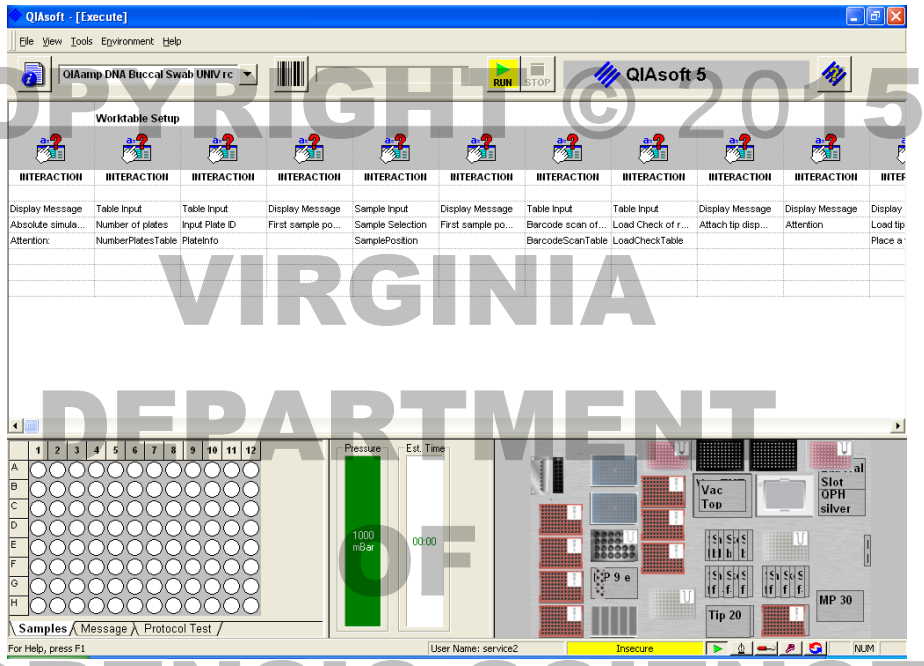
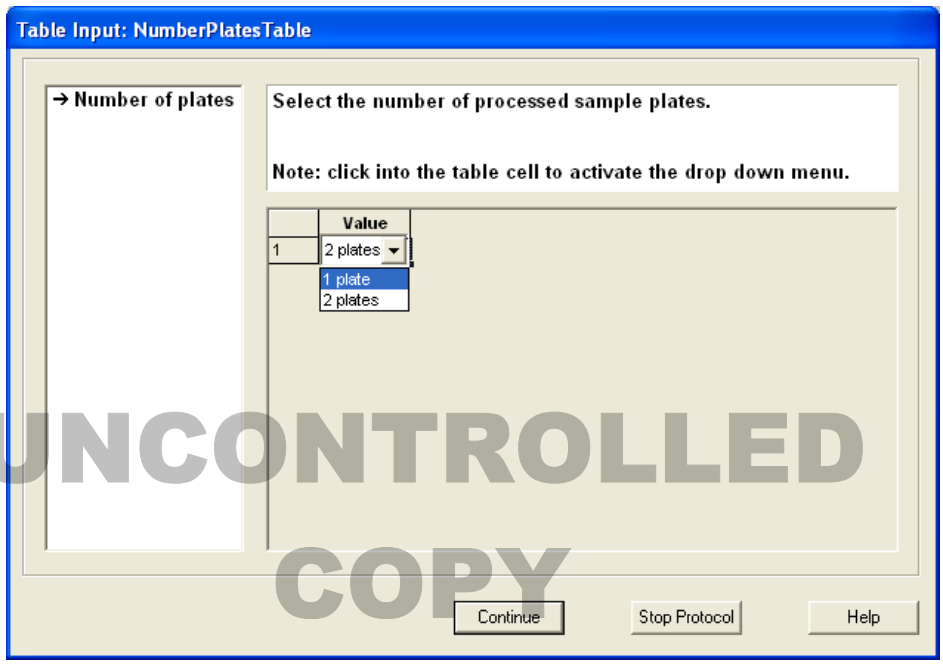
1.6.4 A series of dialog boxes will appear to begin the run. The Worktable Setup panel will display the current processes and their durations.



1.6.5 The dialog box "Table Input: NumberPlatesTable" will appear. Select the appropriate plate number from the drop-down menu and hit 'Continue' to proceed.



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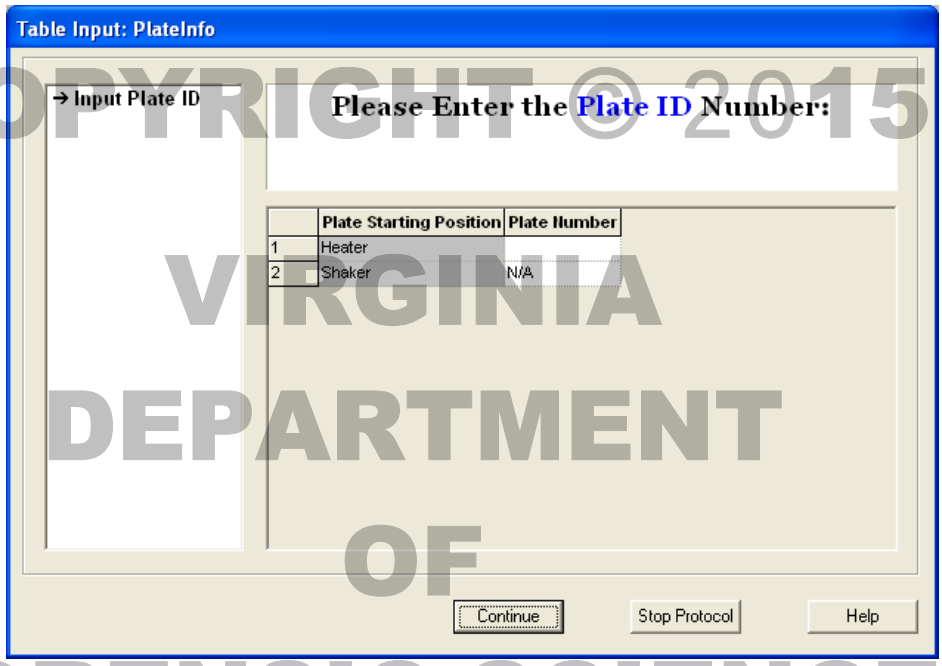
1.6.6 When the "Table Input: PlateInfo" dialog box appears, enter the Plate ID number(s) and then select 'Continue' to proceed.



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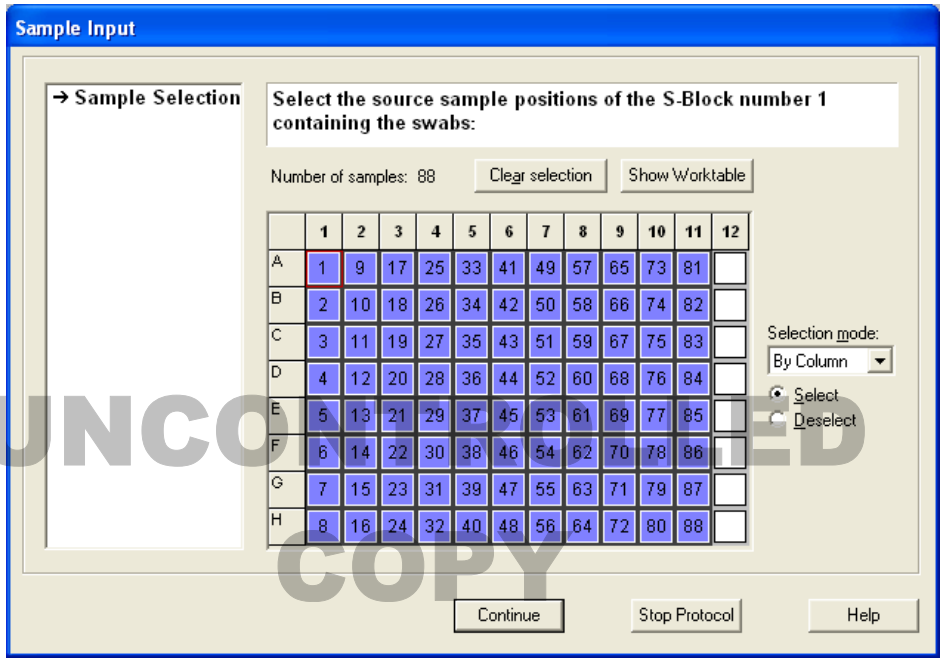
1.6.7 The "Sample Input" dialog box will appear. Highlight the plate position of samples to be processed. Be sure to include the appropriate controls in this number. Only complete rows of 8 may be selected. If you have a partial row, be sure to select the entire row. Select 'Continue' when finished.



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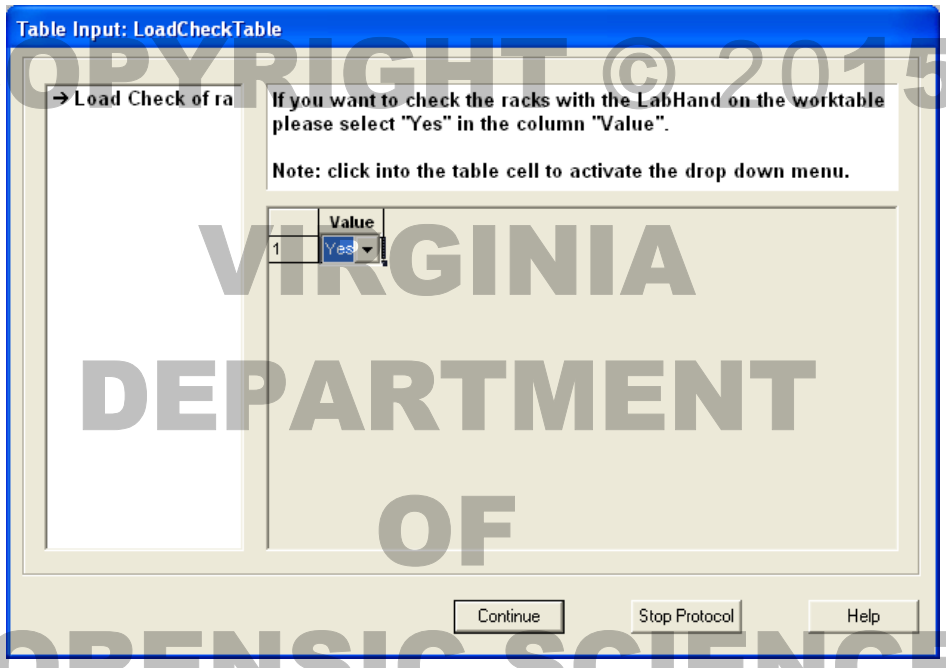
1.6.8 The “Table Input: LoadCheckTable” dialog box will appear. To have the LabHand on the worktable check the deck setup, select “Yes” from the dropdown menu; if not, select “No”. Click ‘Continue’ to proceed.



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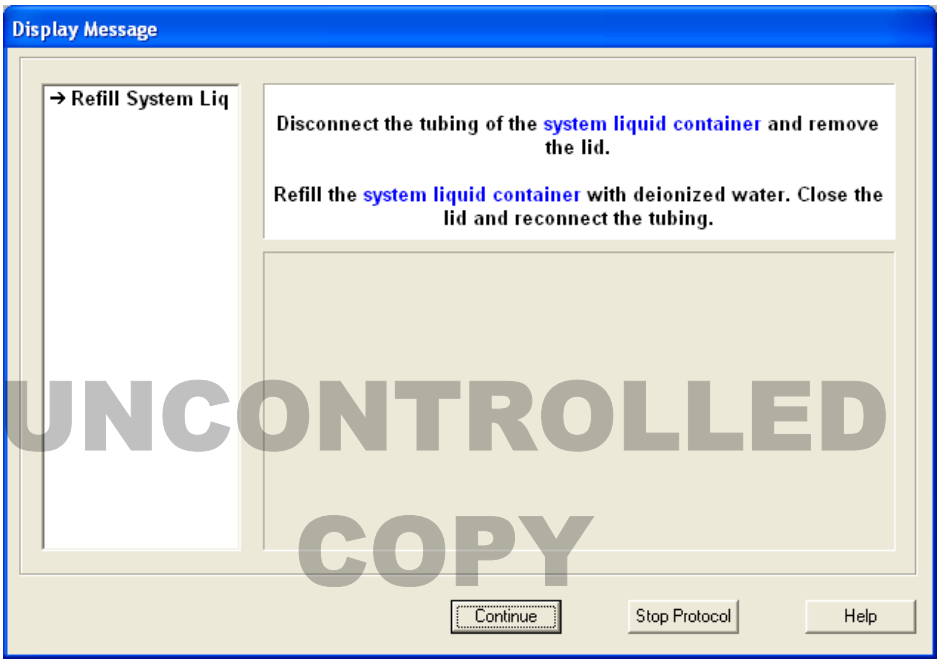
1.6.9 Fill the system liquid container (10 L carboy) with deionized water to the “MAX” line, and then select ‘Continue’ to proceed.



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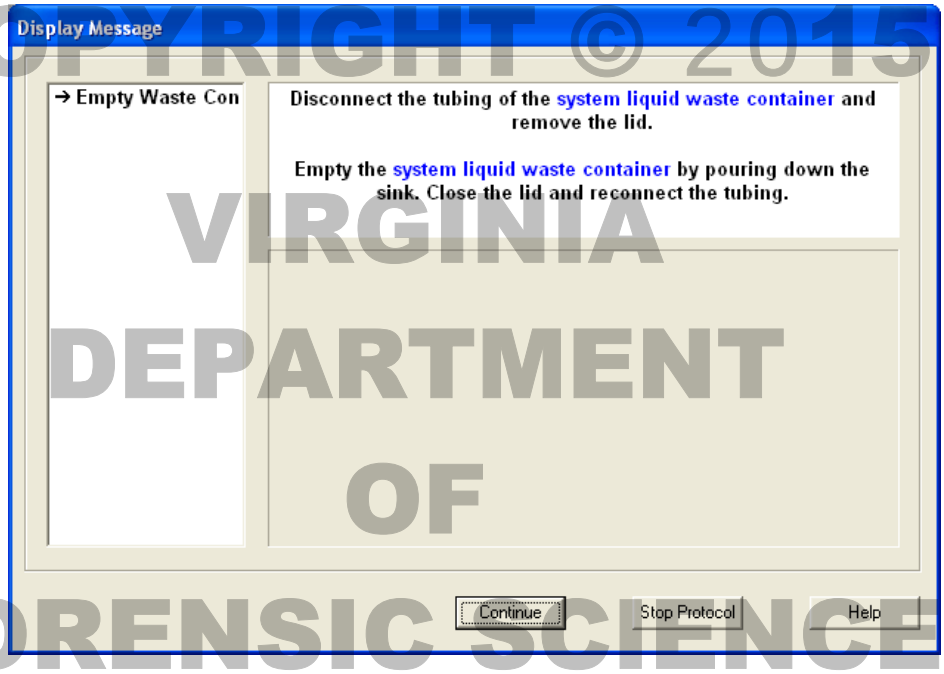
1.6.10 Empty the waste container, and then select 'Continue' to proceed.

NOTE: If the container labeled "Waste container" need to be emptied, the liquid (mainly water) can be poured down the drain and then flushed with water.



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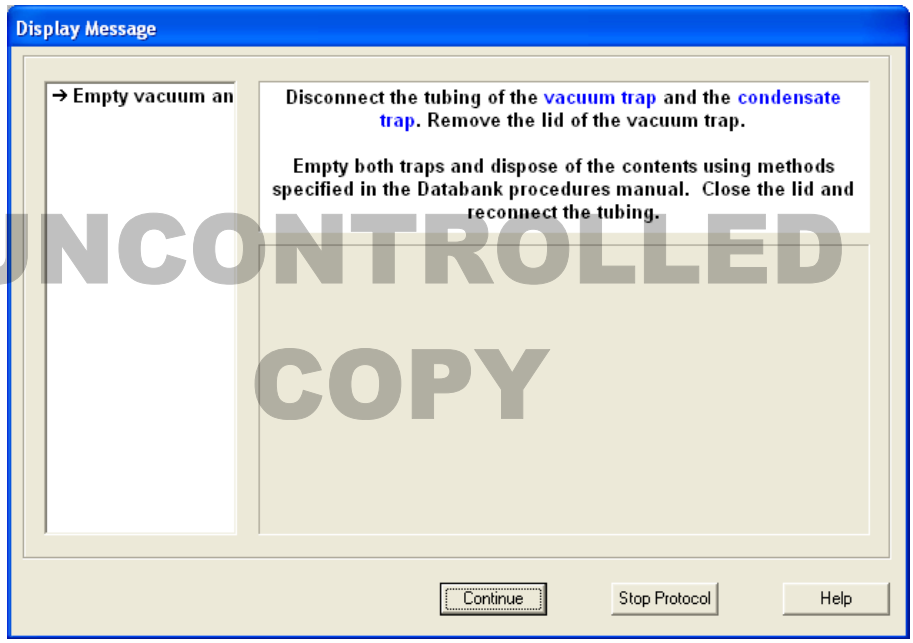
1.6.11 Empty the vacuum trap and condensate trap, and then select 'Continue' to proceed. Contents of vacuum and condensate traps must be treated with Pine Sol (approx 250 ml per 4 L waste) and allowed to sit for one hour prior to disposal down the drain. Use copious amounts of water during disposal.

WARNING!

The QIAGEN® Buffer AL contains Guanidine Hydrochloride. This chemical will produce chlorine gas when mixed with bleach. Exercise caution when cleaning up spills.



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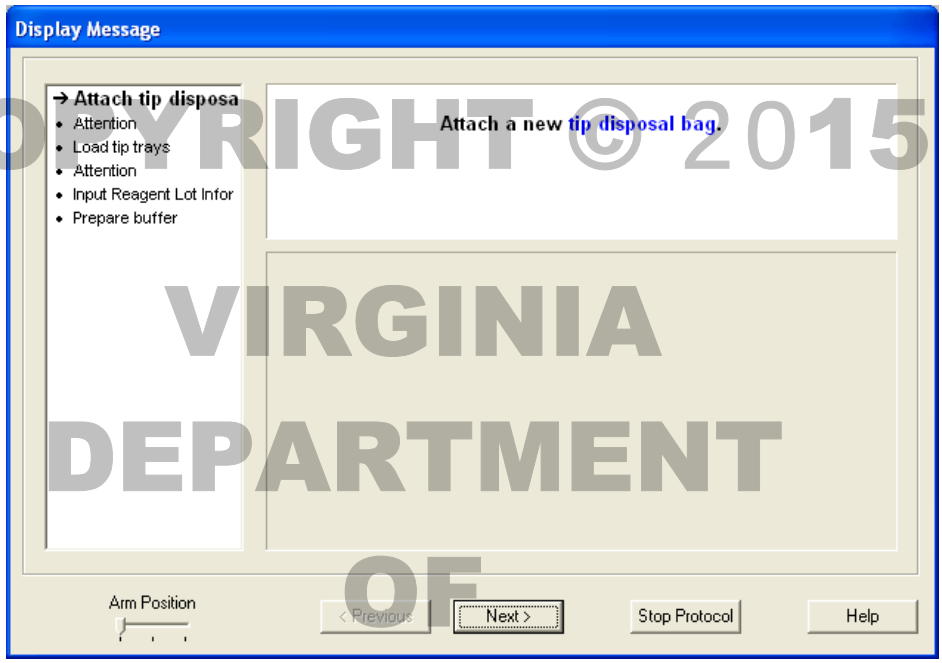
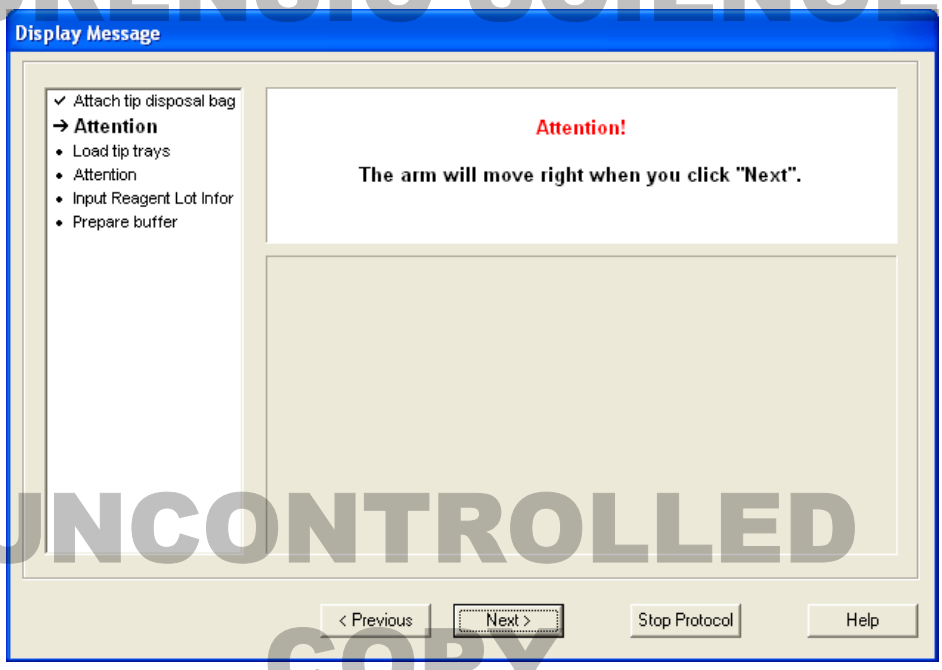
1.6.12 Attach a new tip disposal bag, and then select ‘Next >’ to continue.



1.6.13 The arm will move to the right when ‘Next >’ is selected.



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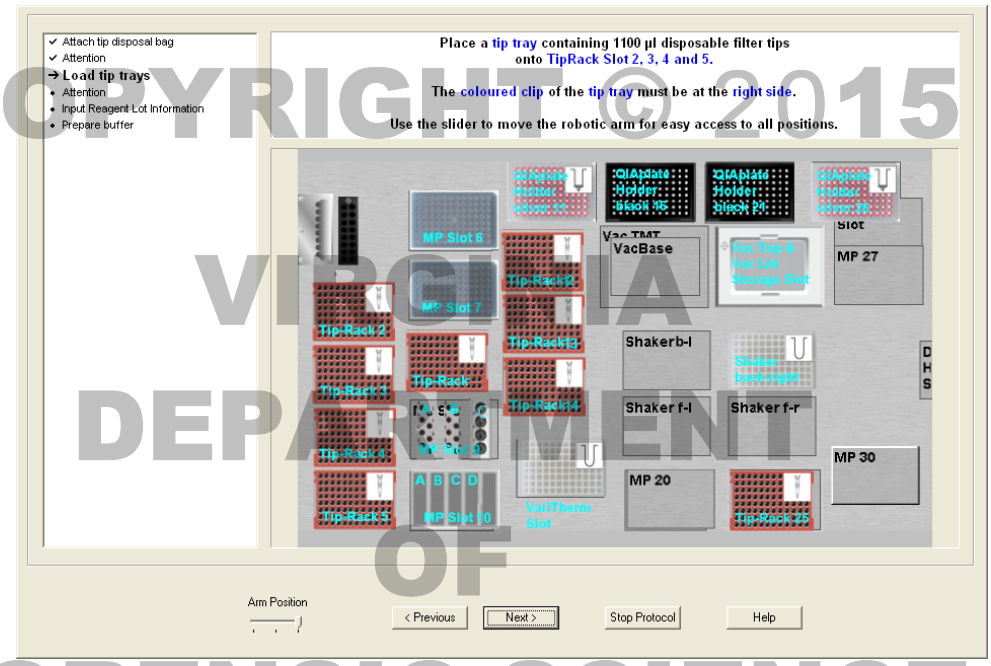
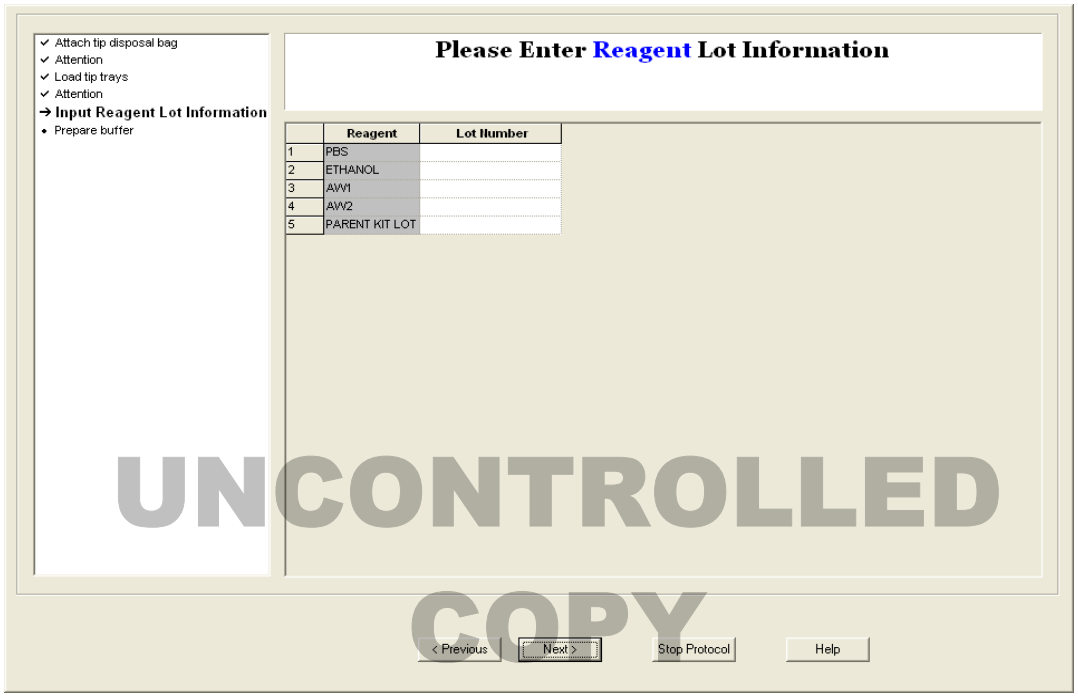
1.6.14 Place a tip tray containing 1100 µL disposable filter tips onto TipRack Slots 2, 3, 4 and 5. The colored clip of the tip tray must be on the right side. Select 'Next >' to continue.



1.6.15 Enter the appropriate reagent lot numbers in the next window, and then select 'Next >' to continue.



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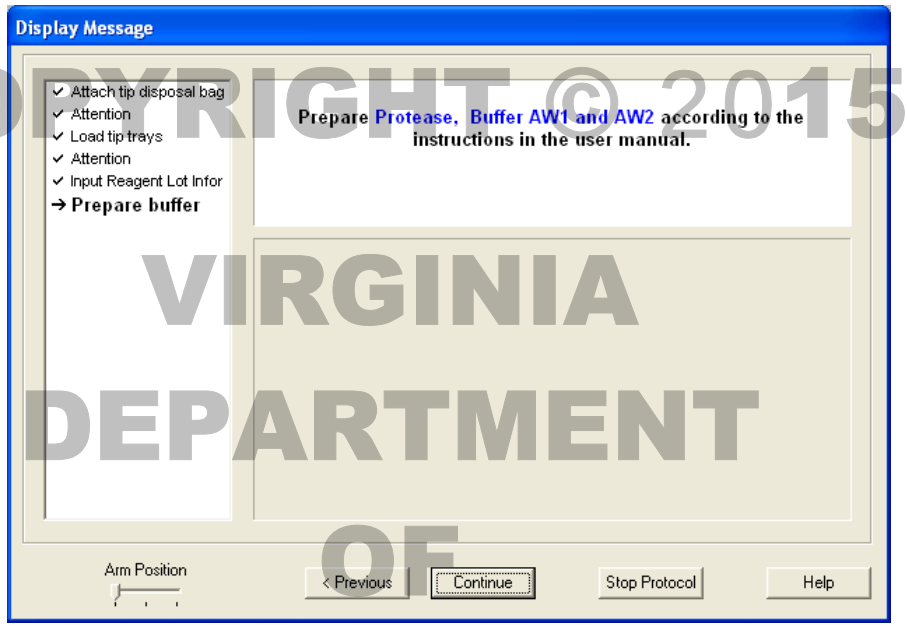
1.6.16 In the next message display prepare Protease and Buffers AW1 and AW2 according to instructions in Appendix A, then select 'Continue' to proceed.



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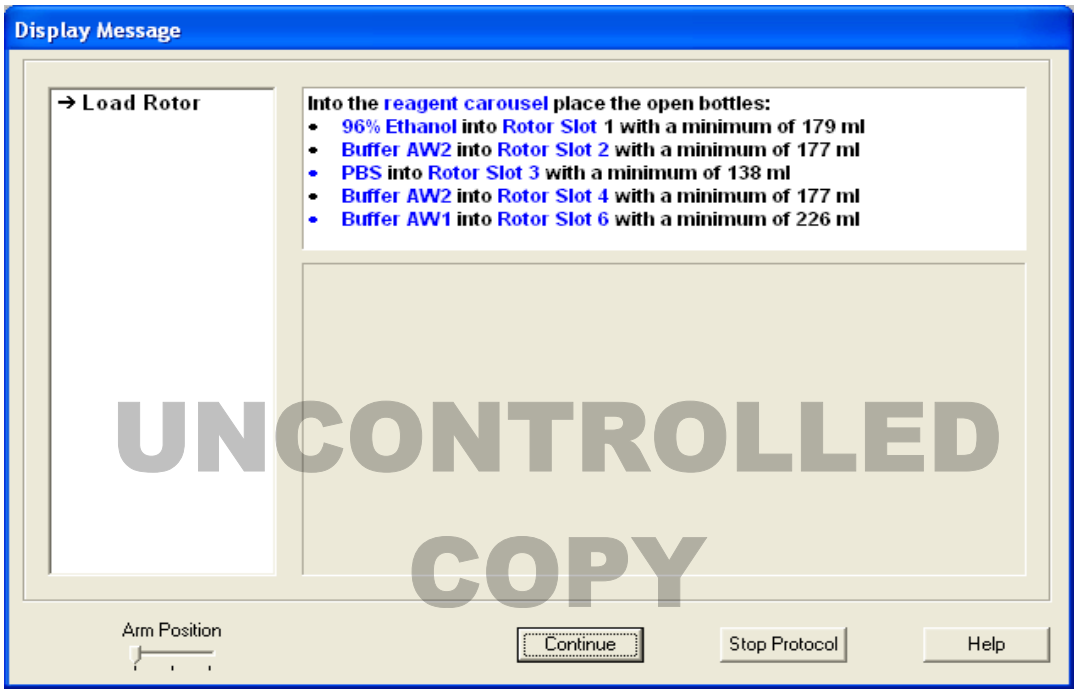
1.6.17 For the next message display, in the reagent carousel place the open bottles of reagents in their appropriate slots [95% Ethanol in Rotor Slot 1, Buffer AW2 in Rotor Slot 2 (also in Rotor Slot 4 for two plates), PBS in Rotor Slot 3 and Buffer AW1 in Rotor Slot 6]. The amount of reagent needed for the run is calculated by the software and displayed in this screen. Select 'Continue' to proceed.



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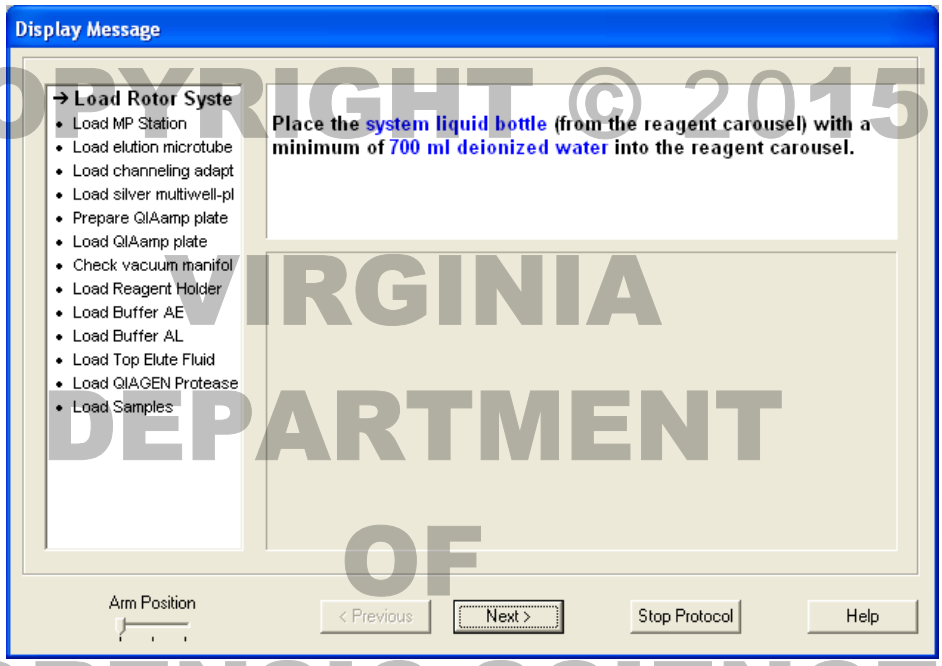
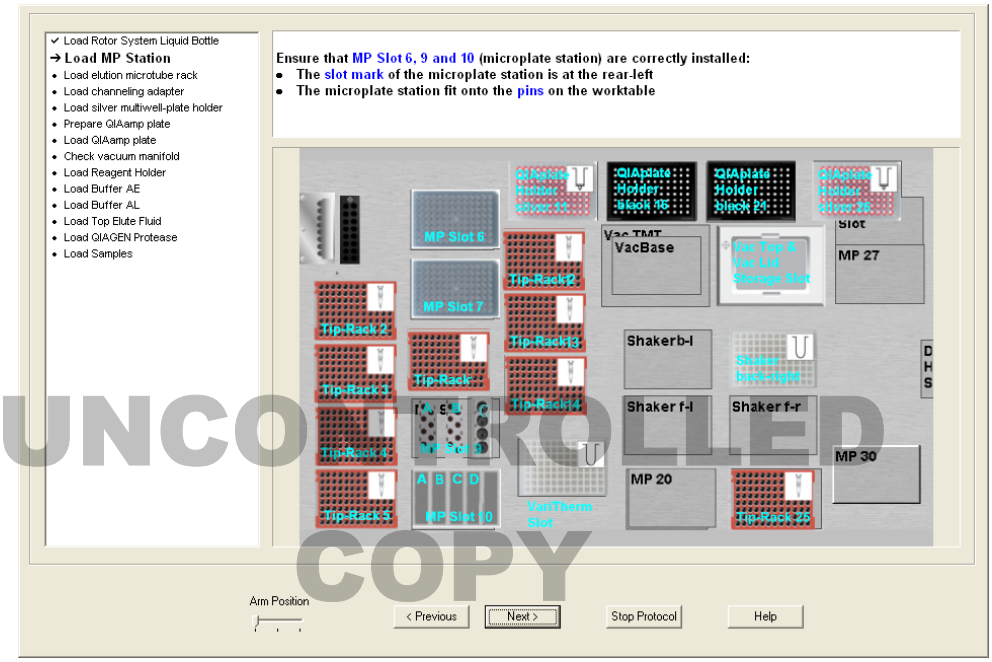
1.6.18 Place the smaller system liquid bottle (1 L flat bottle) with a minimum of 700 mL deionized water in the appropriate slot in the reagent carousel, and then select 'Next >' to continue.



1.6.19 Ensure that the microplate stations in MP Slots 6, 9 and 10 are correctly installed: the slot mark of the microplate station is at the rear-left, and the microplate station will sit flush when seated properly onto the pins on the worktable. Select 'Next >' to continue.



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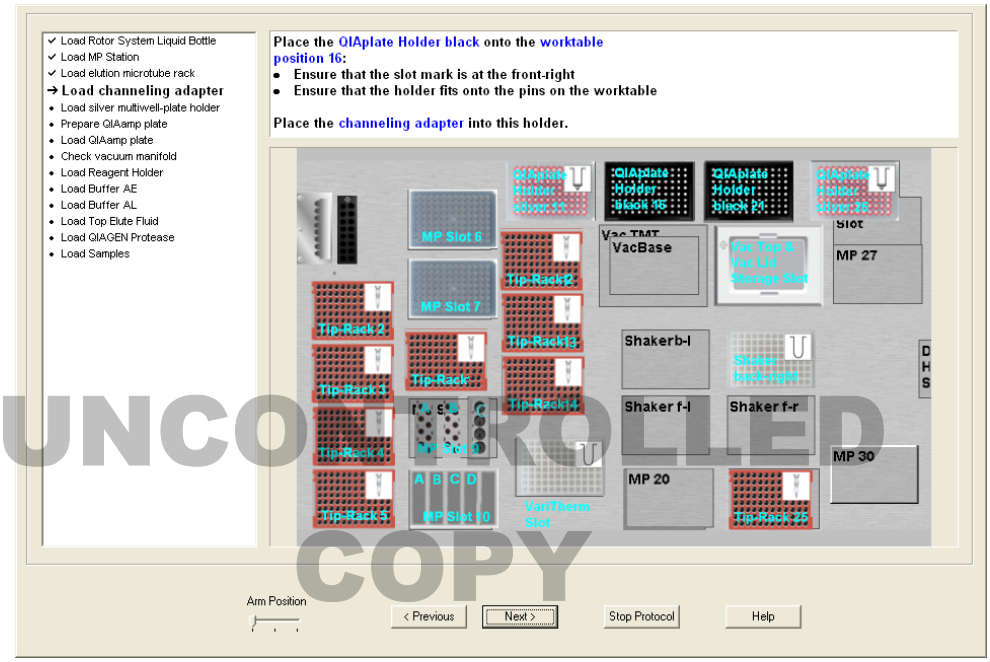
1.6.20 Place an empty elution microtube rack (with the lid and on the blue elution microtube adapter) onto MP Slot 6, with well A1 in the upper left corner. Select 'Next >' to continue.

1.6.21 Place the black QIAplate holder onto worktable position 16. Ensure that the slot mark is at the front-right and that the holder fits onto the pins on the worktable. Place the channeling adapter on top of this holder, and then select 'Next >' to continue.



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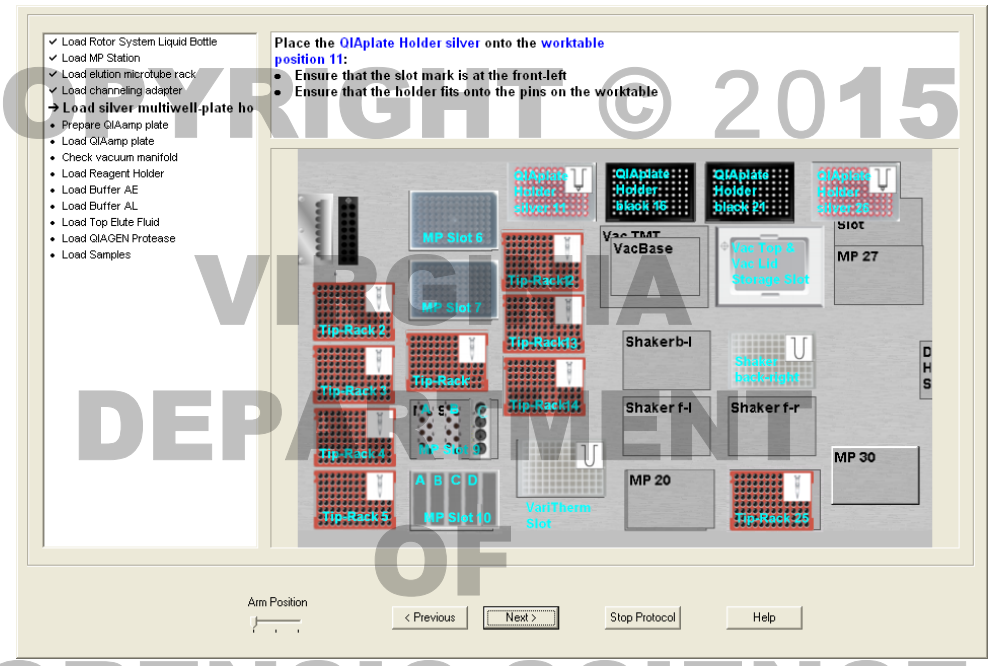
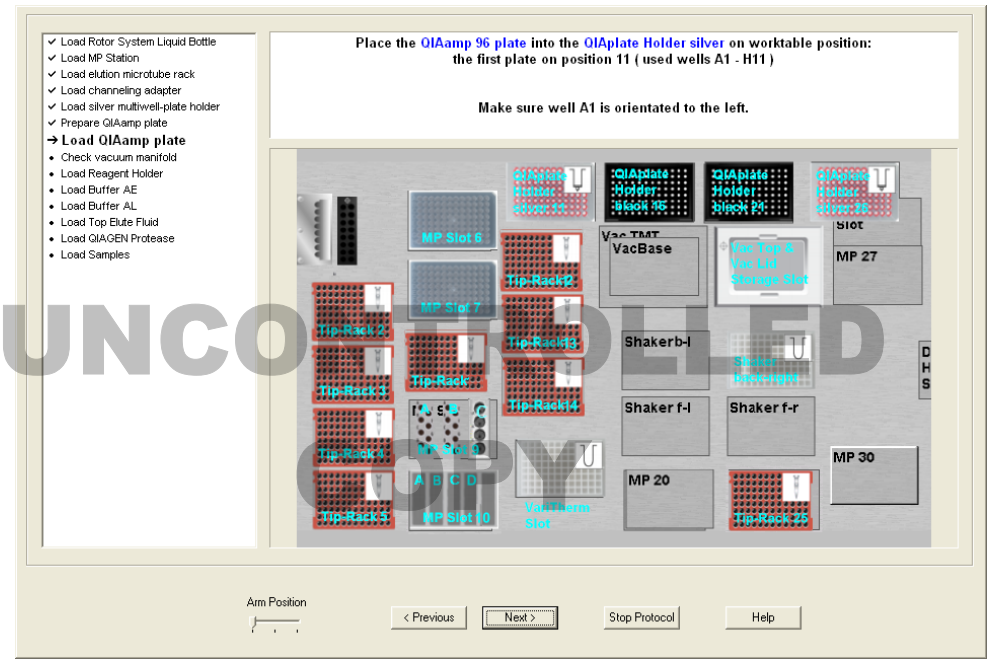
1.6.22 Place the silver QIAplate holder onto worktable position 11. Ensure that the slot mark is at the front-left and that the holder fits onto the pins on the worktable. Select 'Next >' to continue.



1.6.23 Seal any unused wells of the QIAamp 96 plate with adhesive tape. The majority of plates processed in the Databank will be full 96 sample runs. Select 'Next >' to continue.

1.6.24 Place the QIAamp 96 plate into the silver QIAplate Holder onto worktable position 11 (also onto worktable position 26 for two plates). Ensure that well A1 is oriented to the left. Select 'Next >' to continue.



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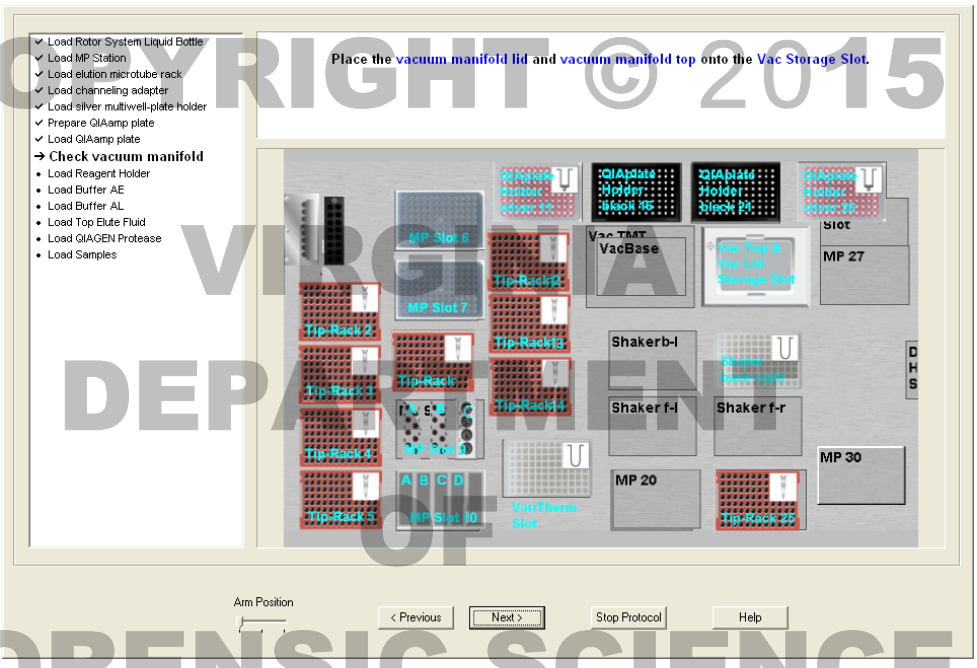
1.6.25 Place the vacuum manifold lid and vacuum manifold top onto the Vac Storage Slot (worktable position 22). The vacuum manifold lid should be **underneath** the vacuum manifold top. Select 'Next >' to continue.



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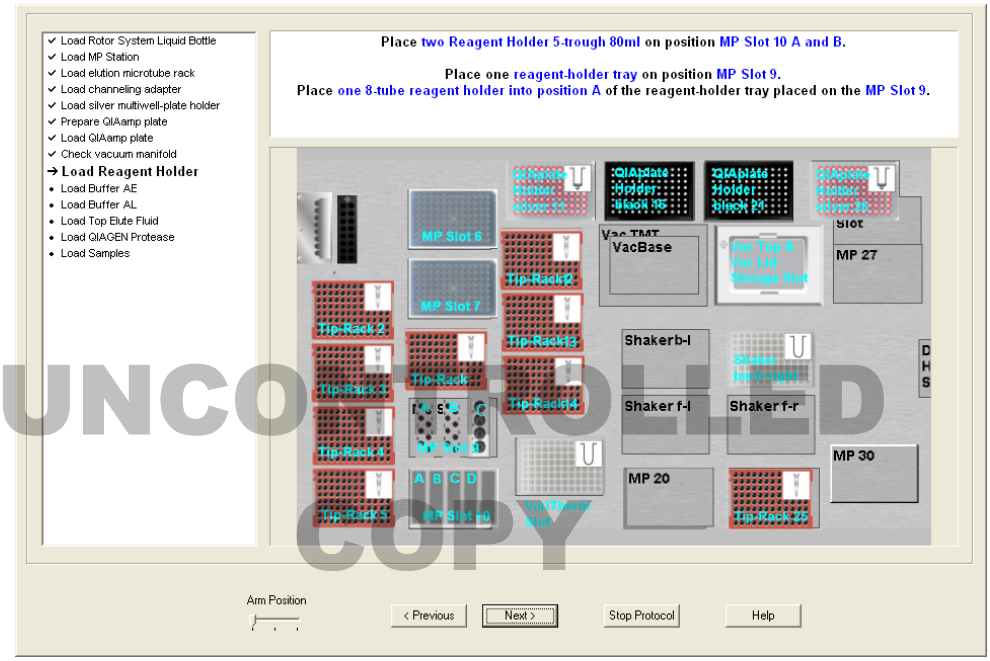
1.6.26 Place two 5-trough Reagent Holders at MP Slot 10 in positions A and B. Also, place one reagent holder tray at MP Slot 9 and place one 8-tube reagent holder in position A in this reagent holder. Select 'Next >' to continue.



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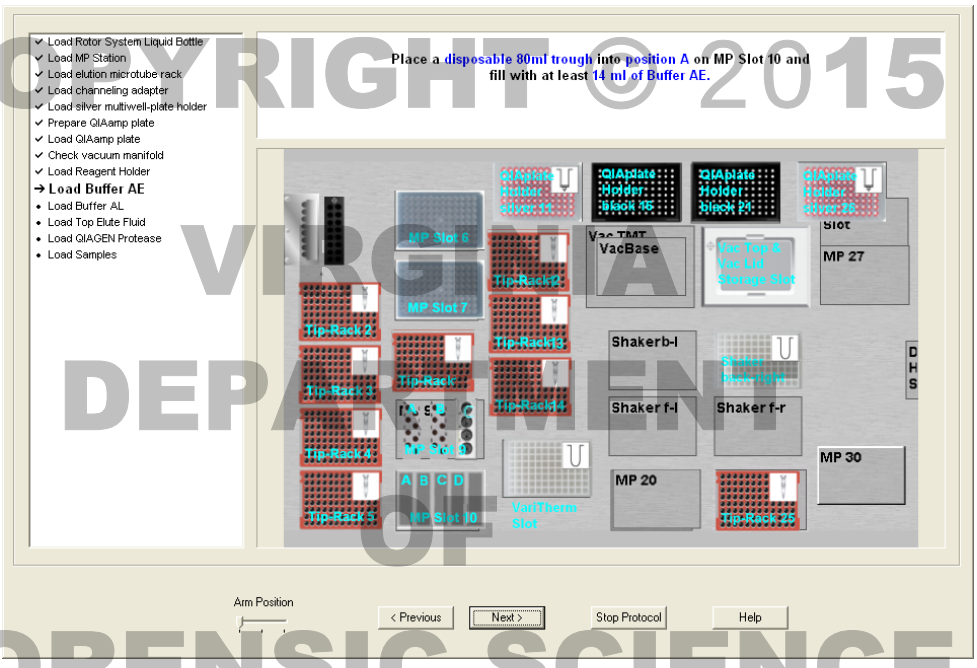
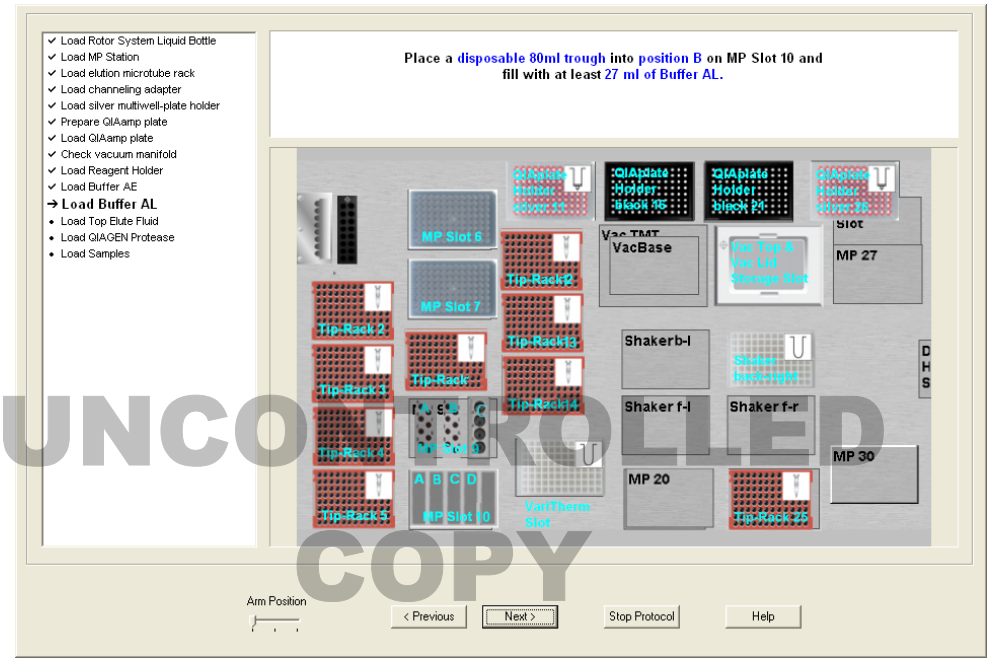
1.6.27 Place a disposable 80-mL trough into position A of MP Slot 10 and fill it with at least 14 mL of Type 1 water (23 mL for two plates). NOTE: AE buffer (included in the Qiagen kit) is not used for Data Bank samples. All samples are eluted in Type 1 water. Select ‘Next >’ to continue.



1.6.28 Place a disposable 80 mL trough in position B on MP Slot 10 and fill it with at least 27 mL of Buffer AL (49 mL for two plates). Select ‘Next >’ to continue.



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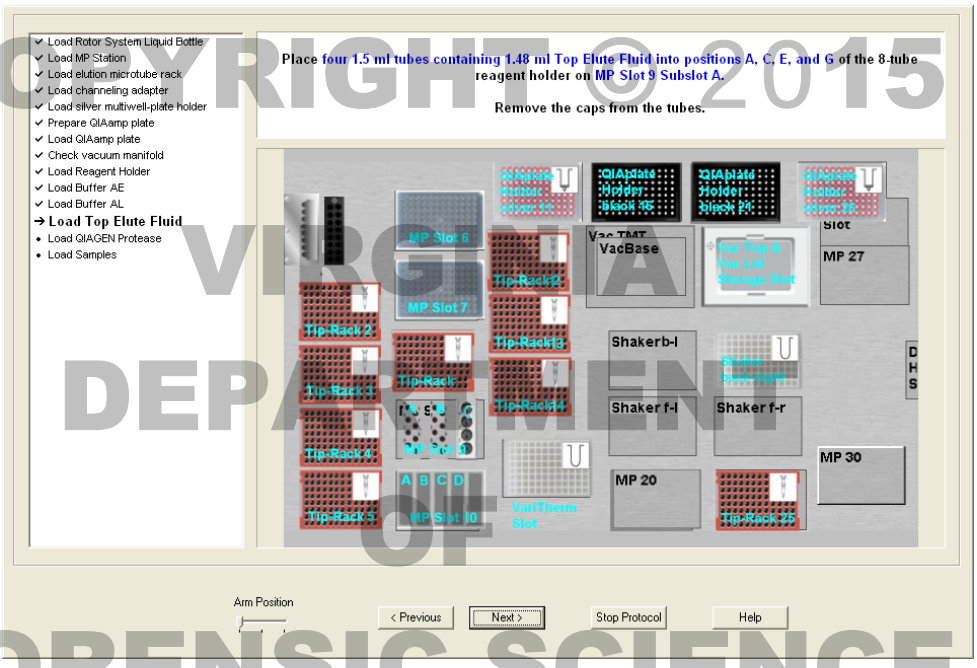
1.6.29 Place four 1.5-mL tubes containing 1.48 mL of Top Elute Fluid in positions A, C, E and G (an additional four tubes in positions B, D, F and H for two plates) of the 8-tube reagent holder on MP Slot 9, Subslot A. Remove the caps from the tubes. Select 'Next >' to continue.



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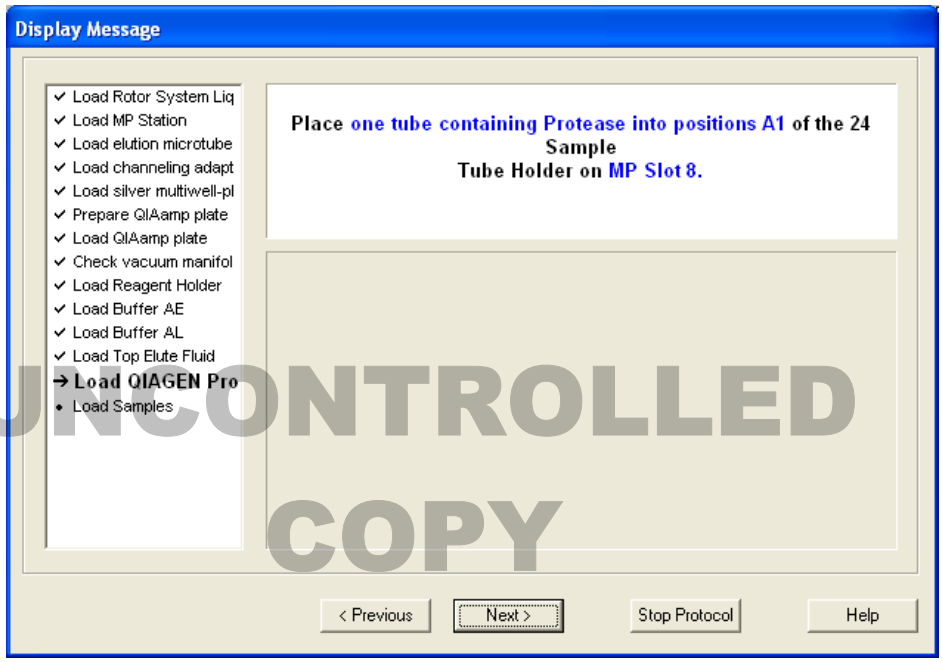
1.6.30 Place one round-bottom 14-mL tube containing reconstituted Protease in position A1 of the 24-Sample Tube Holder on MP Slot 8. For two plates, a second round-bottom 14-mL tube containing reconstituted Protease is placed in position B1 of the 24-Sample Tube Holder. Select 'Next >' to continue.



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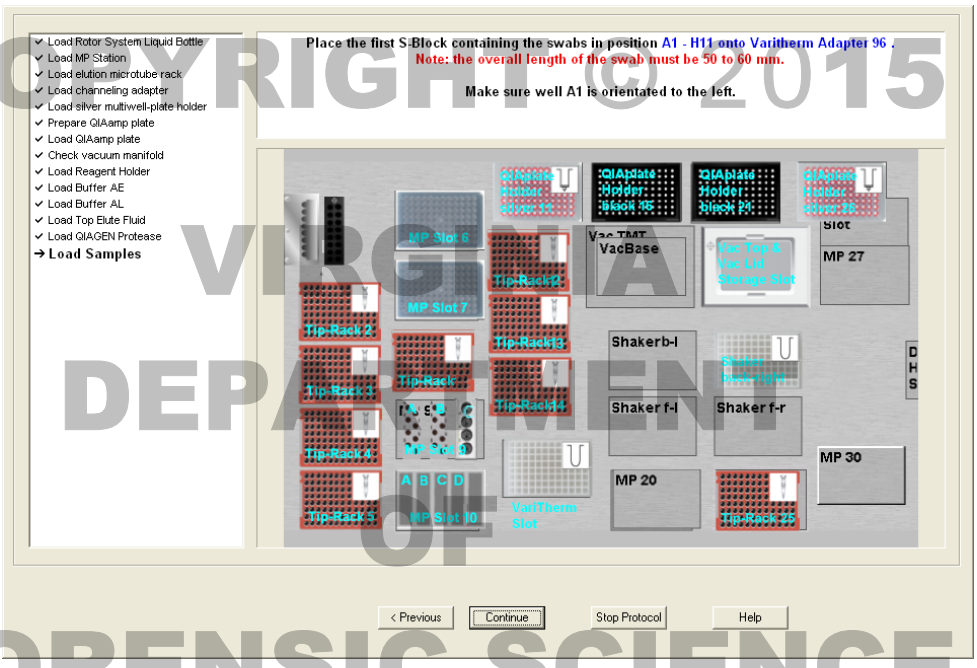
1.6.31 Place the first S-Block containing the swabs on the Varitherm Adapter 96. If a second S-Block is used, it is placed on the Shaker back-right position. Ensure that well A1 is oriented on the upper-left corner, and select 'Next >' to continue.



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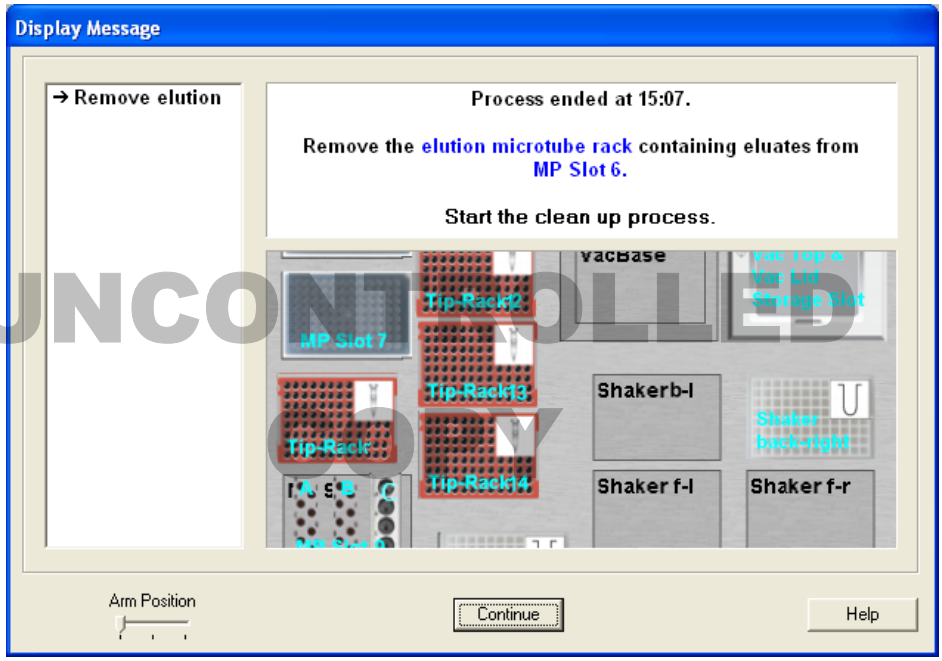
1.6.32 At this point, the robot will continue to operate and will not need user input until the processing of both plates has been completed.

1.6.33 When the run has been completed, the following message will be displayed to begin the cleanup process. The elution microtube rack can be removed from MP Slot 6 and the eluates are ready for amplification setup. Select 'Continue' to proceed.



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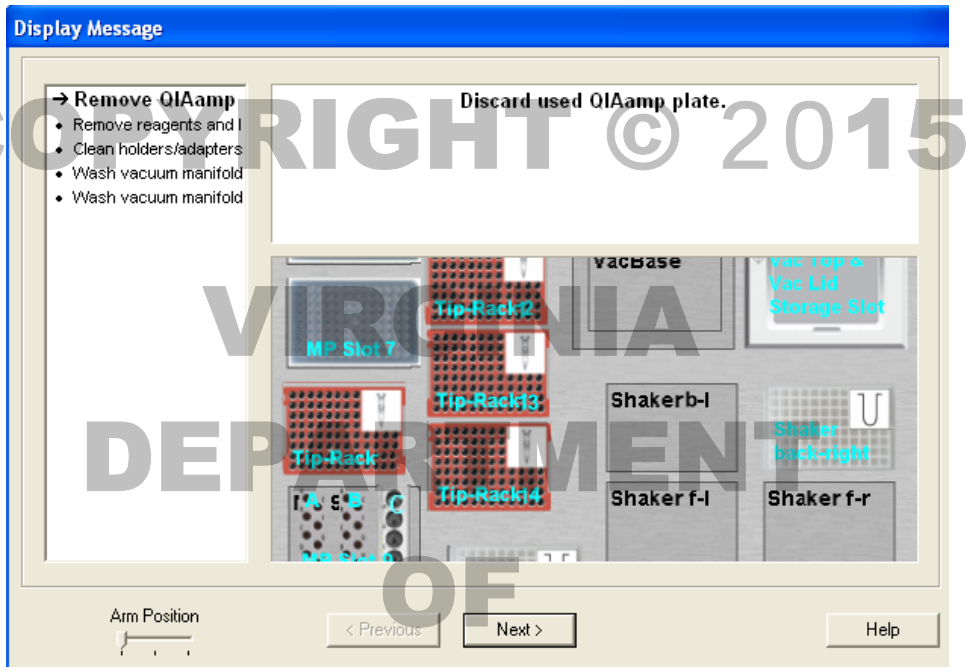
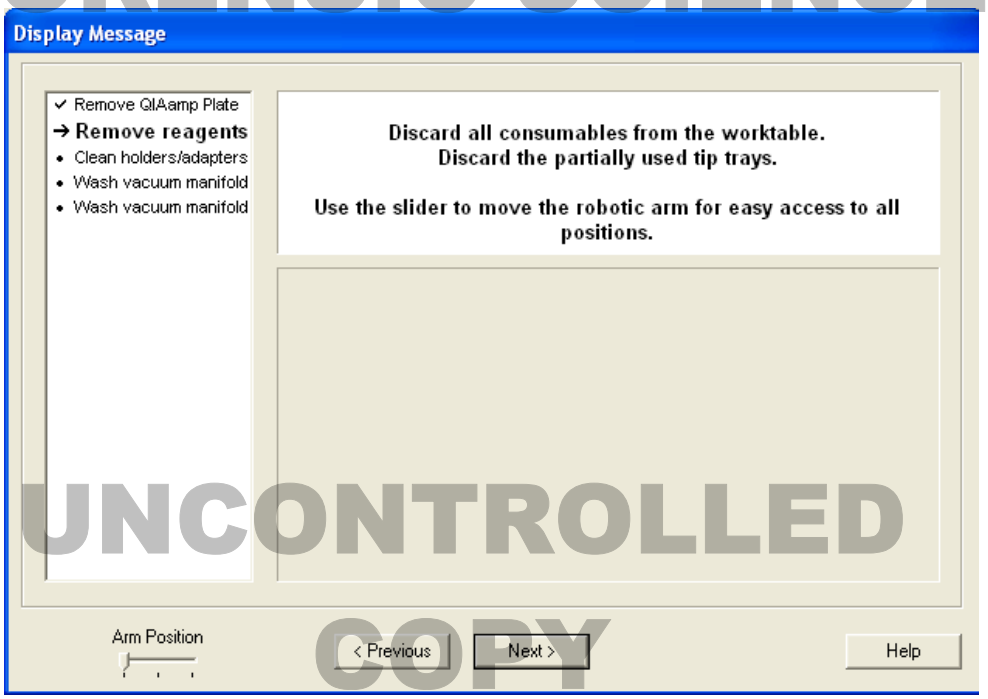
1.6.34 Discard the used QIAamp plate(s), then select 'Next >' to proceed.



1.6.35 Discard all consumables from the worktable, and then select 'Next >' to proceed.



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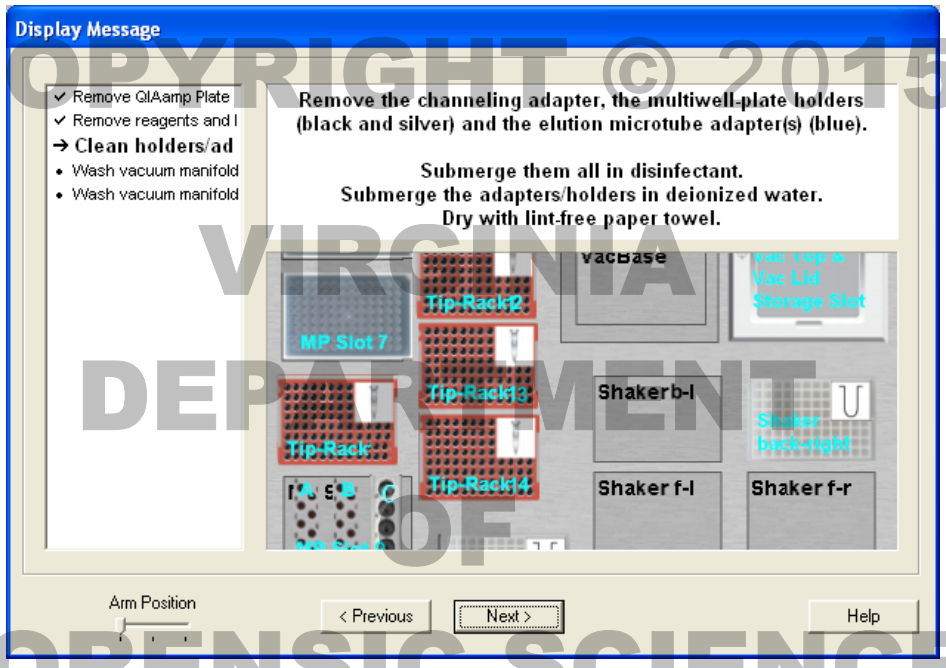
1.6.36 Remove the channeling adapter, multi-well plate holders (black and silver) and the elution microtube adapter(s) (blue). Submerge them all in disinfectant, then deionized water, and then dry them with lint-free paper towels. Select 'Next >' to proceed.



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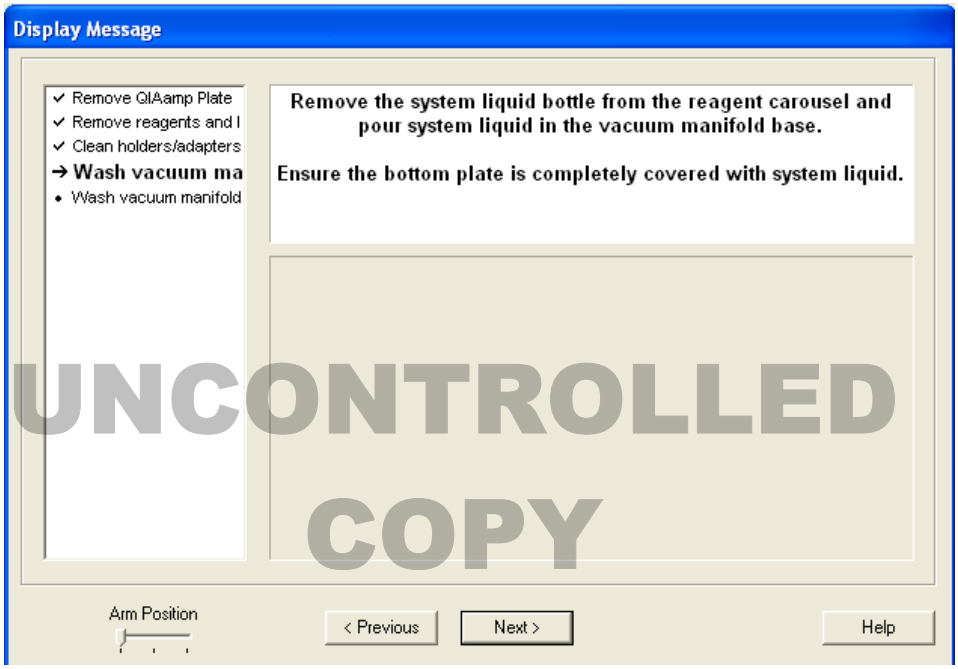
1.6.37 Remove the system liquid bottle from the reagent carousel and pour the system liquid over the heating plate in the vacuum manifold base. Ensure that the plate is completely covered with the system liquid. Select 'Next >' to proceed.



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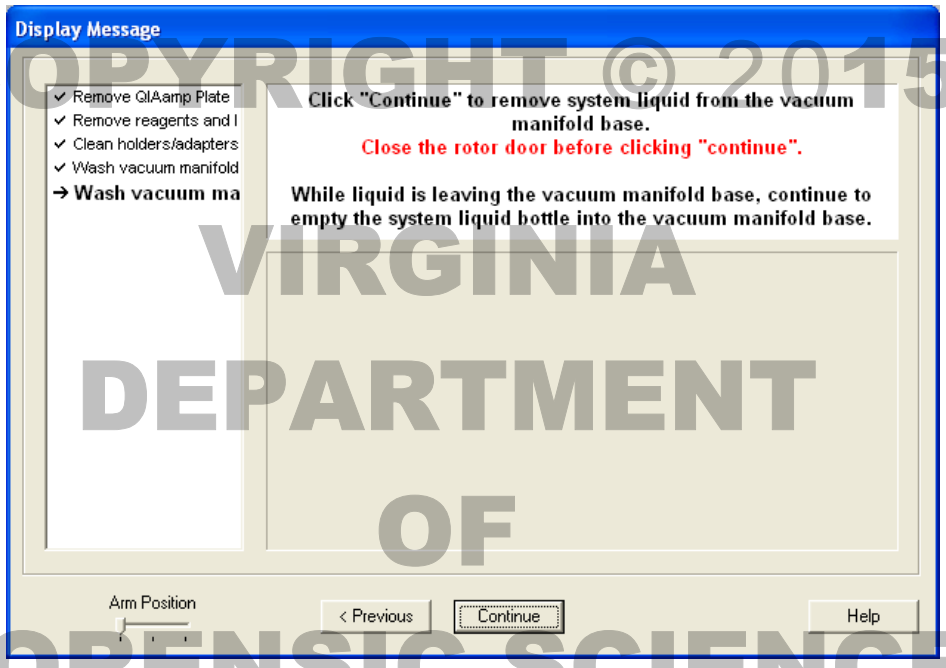
1.6.38 Ensure that the rotor door has been closed. Click 'Continue' to remove the system liquid from the vacuum manifold base. While the liquid is leaving the vacuum manifold base, continue to empty the system liquid bottle into the vacuum manifold base.



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1.6.39 Replace the caps on the bottles in the reagent carousel if no subsequent protocol will be performed, and then select 'Next >' to proceed.

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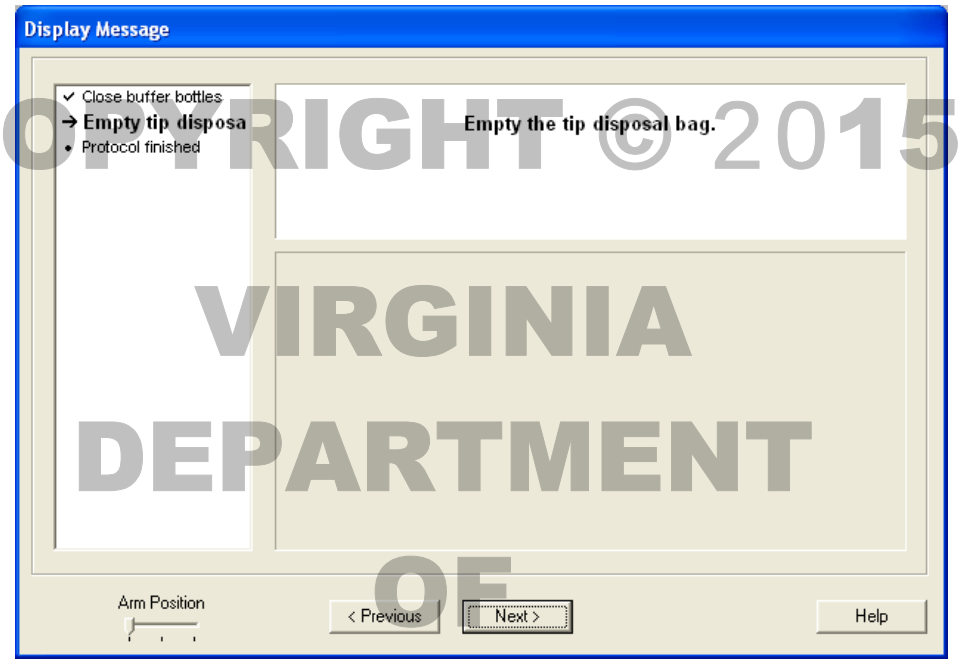
1.6.40 Empty the disposal bag and then select ‘Next >’ to proceed.



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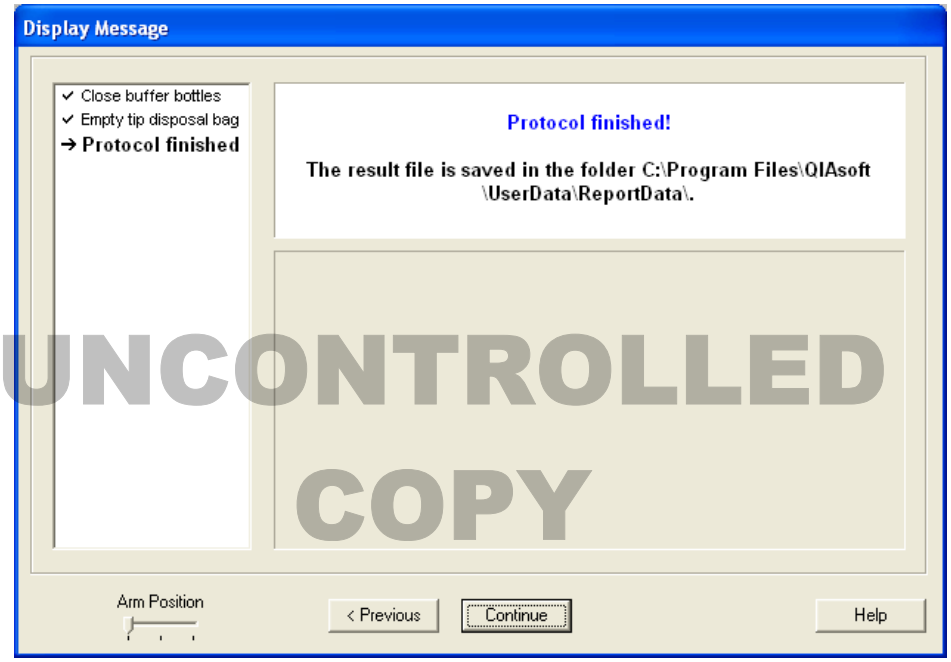
1.6.41 The protocol is now finished. The data file can be retrieved by selecting the “Shortcut to ReportData” icon on the desktop. Double-click on the .rtf file for the sample set by date (and subsequently by the time the run ended if there are multiple runs in a day). The date is located between the two underscores in a DD MM YY format. The process end time will be the last four digits of the filename.

1.6.42 Select ‘Continue’ to end the run.



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1.6.43 Properly shut down the computer by exiting all programs. Then click on the “Start” button in the bottom left corner and select “Shut Down”. In the next dialog box select “Shut Down the Computer” and hit “OK”.

1.6.44 The robot can be turned off by using the switch located on the front, right-hand side.

1.6.45 Proceed to the appropriate amplification portion of the Procedures Manual(s).

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APPENDIX A - REAGENTS

1. This appendix describes the preparation of reagents necessary for DNA analysis. For each reagent listed, the company and catalog number is included. As a reagent is prepared, it will be labeled to include the following information:

   Identity
   Concentration
   Lot number
   Date of preparation
   Initials of the preparer

   and if appropriate:

   Date of autoclaving
   Storage requirements
   Date of expiration

2. All chemicals and reagents will be stored according to manufacturer's specifications.

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ETHANOL, 95%

Expiration date: Manufacturer's expiration date.

NaCl, 0.9%

Expiration date: Manufacturer's expiration date.

PHOSPHATE-BUFFERED SALINE (PBS), 1X, pH 7.2

Expiration date: Manufacturer's expiration date.

QIAamp® DNA BLOOD BIOROBOT™ MDx KIT

Expiration date: Manufacturer's expiration date.

Purchased from QIAGEN, Inc., Valencia, CA, Catalog number 965162 for 12 plate kit.

QIAGEN® BUFFER AL

Expiration date: Manufacturer's expiration date.

Supplied with the QIAamp® DNA Blood BioRobot® MDx Kit.

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QIAGEN® BUFFER AW1 (concentrate)

Expiration date: Manufacturer's expiration date.

Supplied with the QIAamp® DNA Blood BioRobot® MDx Kit.

Before initial use, dilute concentrate with 95% ethanol according to the specifications on the bottle:

Once the QIAGEN® Buffer AW1 has been diluted with ethanol, store the buffer at room temperature.

QIAGEN® BUFFER AW2 (concentrate)

Expiration date: Manufacturer's expiration date.

Supplied with the QIAamp® Blood BioRobot® MDx Kit, and can also be purchased separately.

Before initial use, dilute concentrate with 95% ethanol according to the specifications on the bottle.

Once the QIAGEN® Buffer AW2 has been diluted with ethanol, store the buffer at room temperature

QIAGEN® PROTEASE

Expiration date: Manufacturer's expiration date.

Supplied with the QIAamp® Blood BioRobot® MDx Kit.

Reconstitute with supplied QIAGEN® Protease Solvent before initial use.

QIAGEN® PROTEASE SOLVENT

Expiration date: Manufacturer's expiration date.

Supplied with the QIAamp® Blood BioRobot® MDx Kit.

QIAGEN® TOP ELUTE SOLUTION

Expiration date: Manufacturer's expiration date.

Supplied with the QIAamp® Blood BioRobot® MDx Kit

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APPENDIX B - CALIBRATION AND MAINTENANCE OF THE QIAGEN® BIOROBOT® UNIVERSAL SYSTEM

The following tasks should be carried out on a monthly basis and documented on the appropriate form. The selection of procedures is listed in the "New Maintenance" tab of the "Maintenance" environment. User confirmation is required to ensure that they have been carried out.

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WARNING!

Do not use bleach to clean or disinfect the instrument. Bleach in contact with salts from the buffers can produce toxic fumes.

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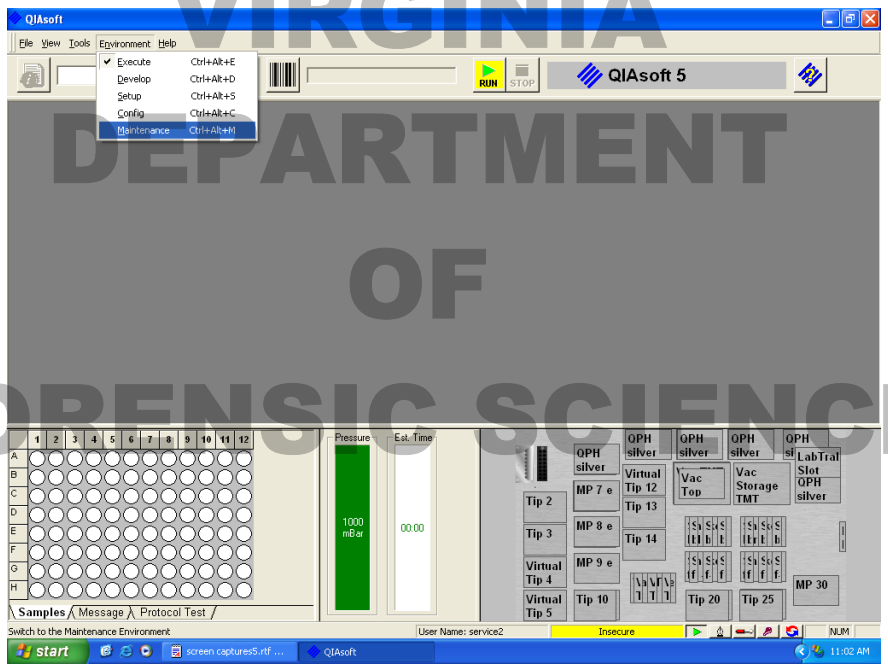

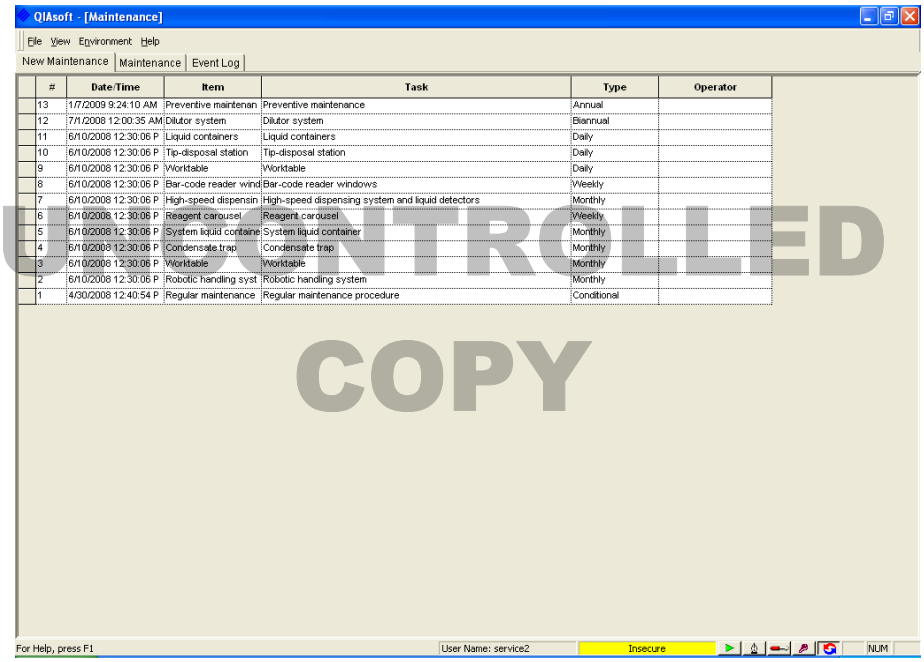

#	Date/Time	Item	Task	Type	Operator
13	1/7/2009 9:24:10 AM	Preventive maintenan	Preventive maintenance	Annual	
12	7/1/2008 12:00:35 AM	Dilutor system	Dilutor system	Biannual	
11	6/10/2008 12:30:06 P	Liquid containers	Liquid containers	Daily	
10	6/10/2008 12:30:06 P	Tip-disposal station	Tip-disposal station	Daily	
9	6/10/2008 12:30:06 P	Worktable	Worktable	Daily	
8	6/10/2008 12:30:06 P	Bar-code reader wind	Bar-code reader windows	Weekly	
7	6/10/2008 12:30:06 P	High-speed dispensin	High-speed dispensing system and liquid detectors	Monthly	
6	6/10/2008 12:30:06 P	Reagent carousel	Reagent carousel	Weekly	
5	6/10/2008 12:30:06 P	System liquid containe	System liquid container	Monthly	
4	6/10/2008 12:30:06 P	Condensate trap	Condensate trap	Monthly	
3	6/10/2008 12:30:06 P	Worktable	Worktable	Monthly	
2	6/10/2008 12:30:06 P	Robotic handling syst	Robotic handling system	Monthly	
1	4/30/2008 12:40:54 P	Regular maintenance	Regular maintenance procedure	Conditional	

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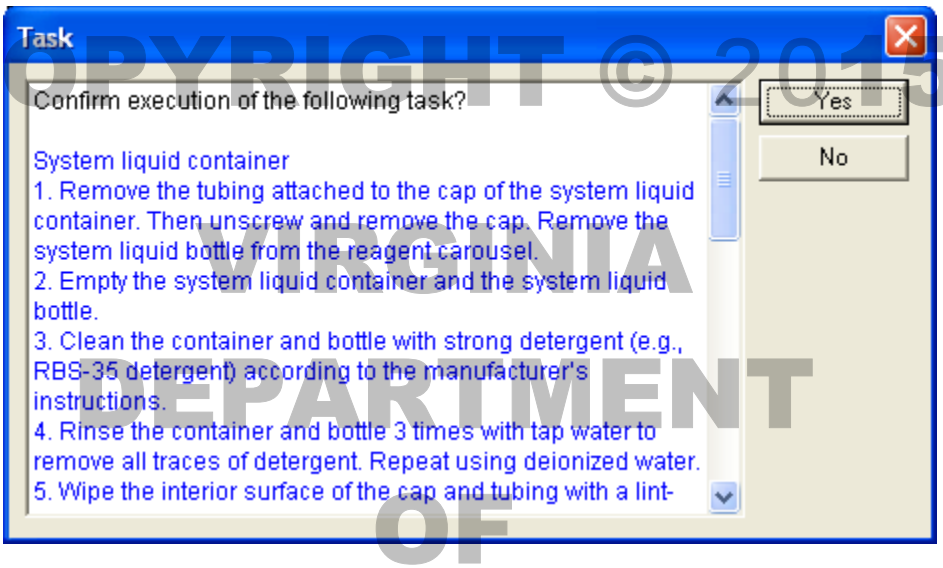
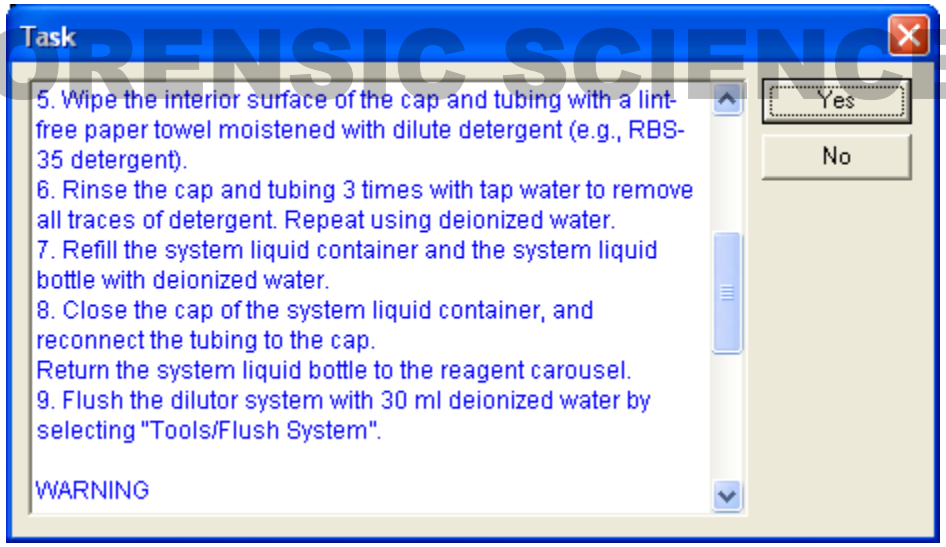
1 System liquid container

Double-click on the “System liquid container” task line, and the following will appear:





After completing all of the steps, select “Yes” to confirm the execution of this task.

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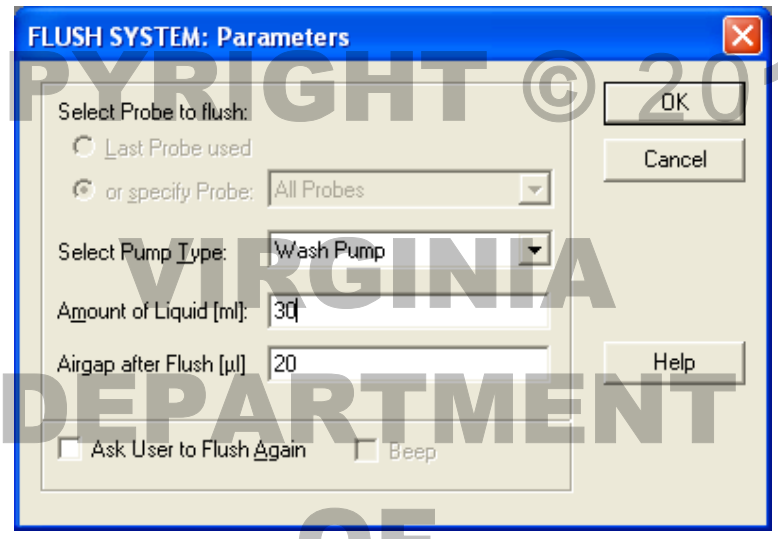
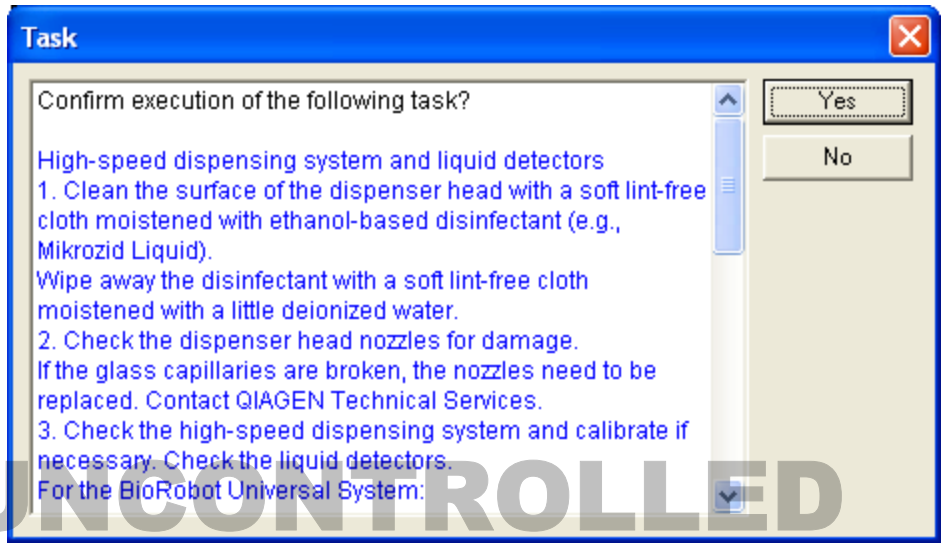
For step 9, the dilutor system can be flushed by going under “Tools”, selecting the “Flush System” option, entering “30” in the “Amount of Liquid (ml)” field and clicking “OK”.



2 High-speed dispensing system and liquid detectors

Double-click on the “High-speed dispensing system and liquid detectors” task line, and the following will appear:



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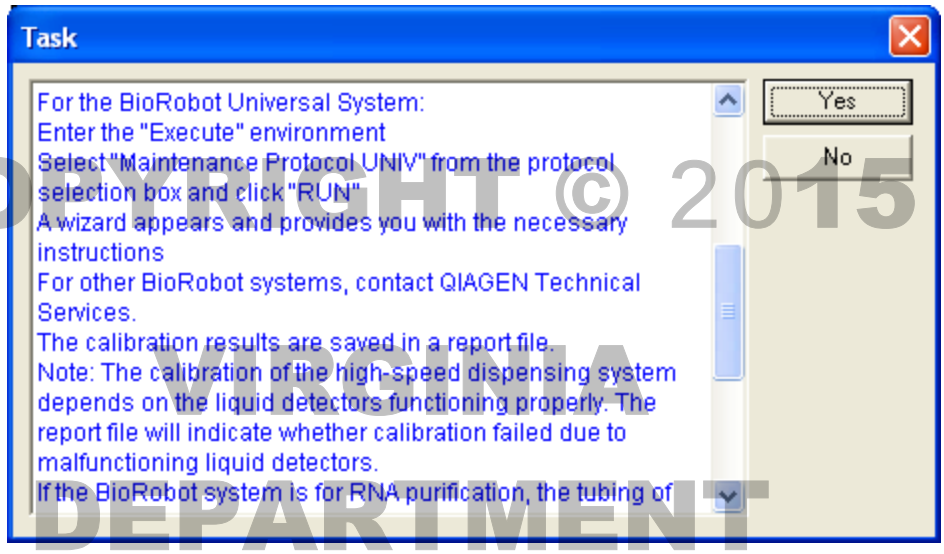

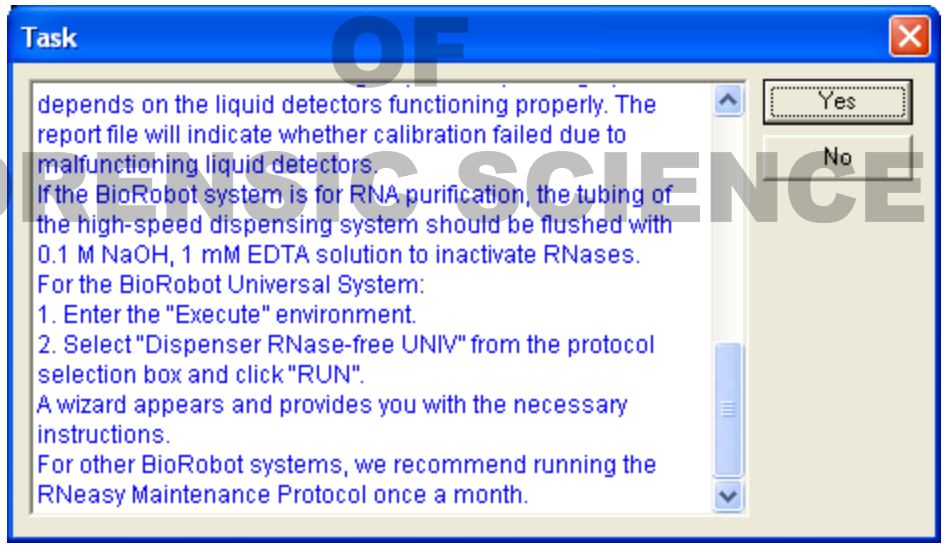

After completing all of the steps, select “Yes” to confirm the execution of this task.

** Record the maintenance tasks performed on the appropriate log sheets.

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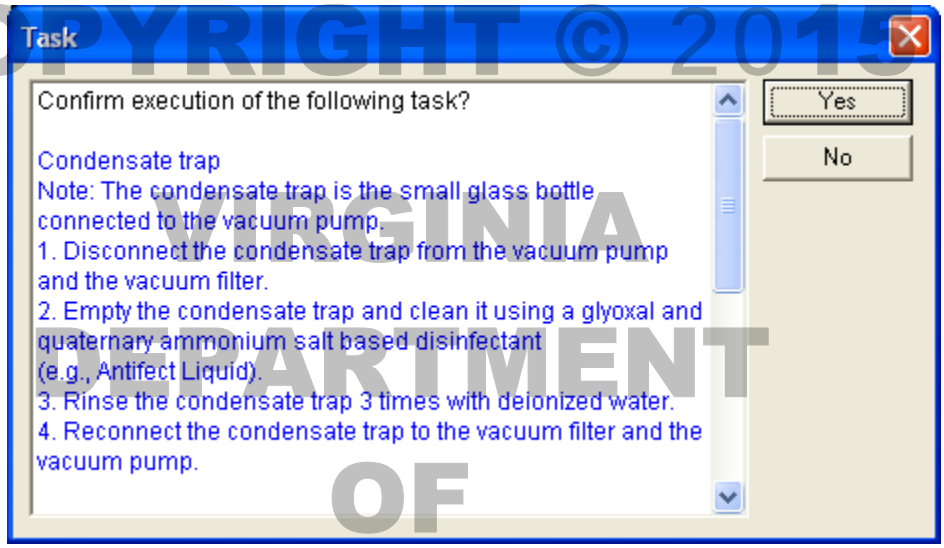
3 Condensate trap

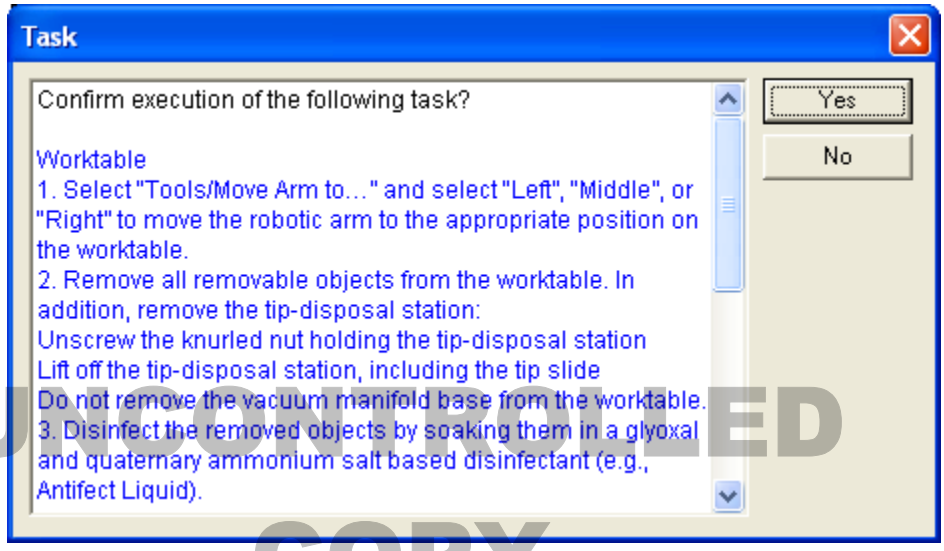
The condensate trap is a small glass bottle connected to the vacuum pump. Double-click on the "Condensate trap" task line, and the following will appear:



After completing all of the steps, select "Yes" to confirm the execution of this task.

4 Worktable

Double-click on the "Worktable" task line, and the following will appear:



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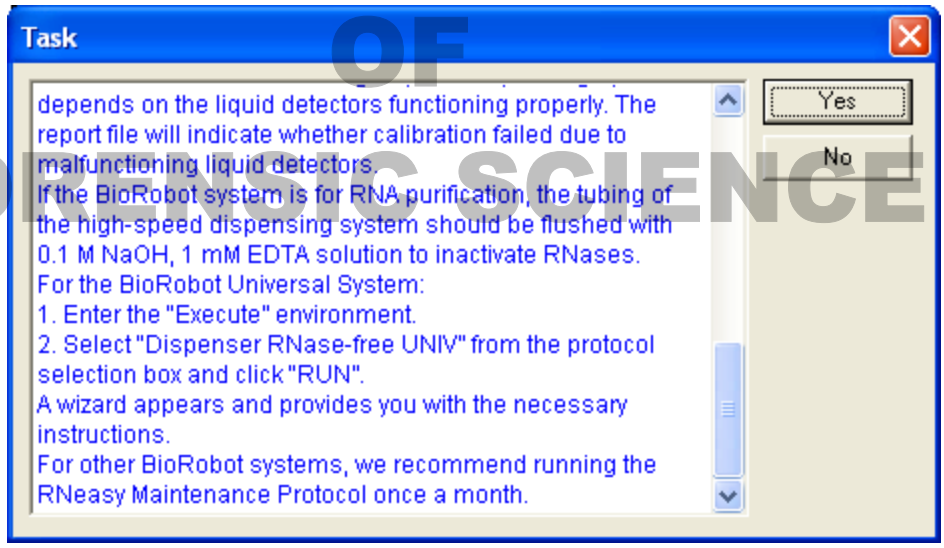

After completing all of the steps, select “Yes” to confirm the execution of this task.

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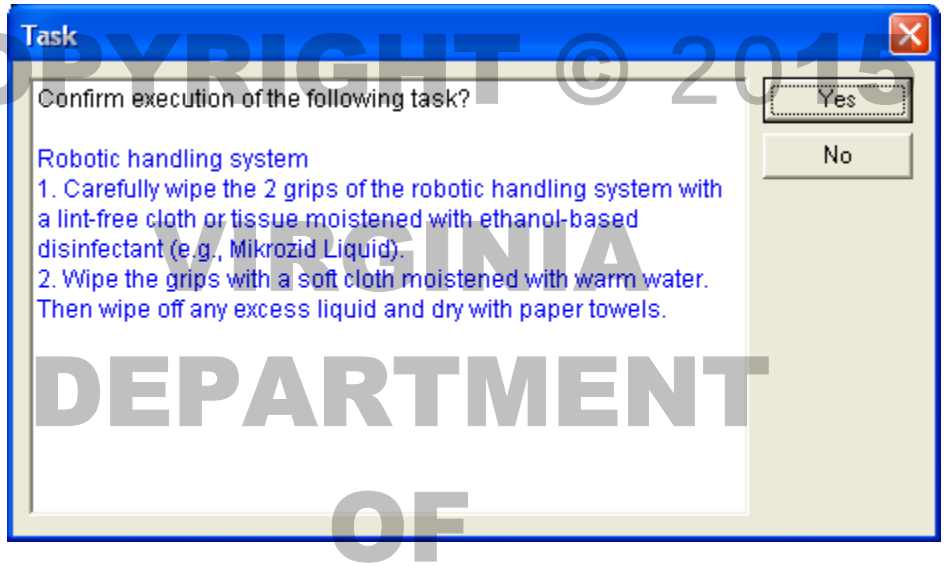
5 Robotic handling system

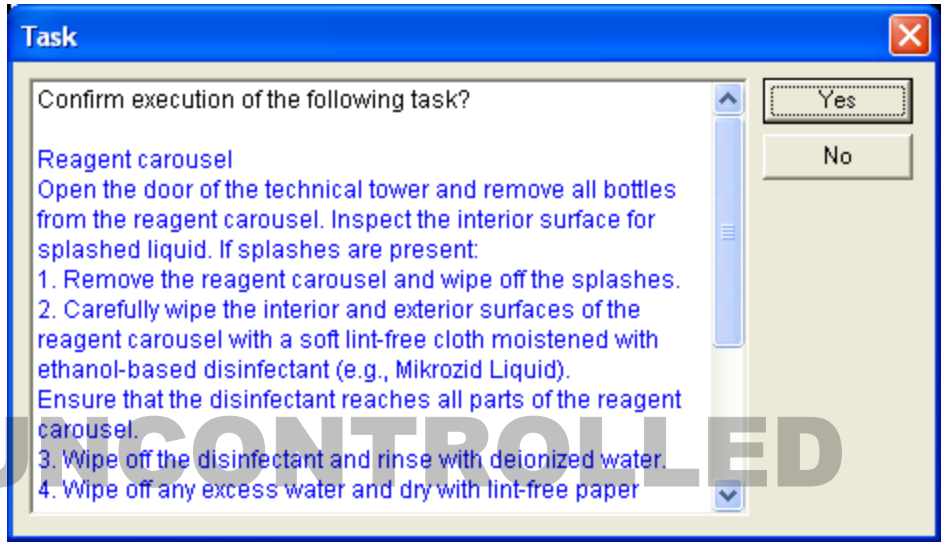
Double-click on the “Robot handling system” task line, and the following will appear:



After completing all of the steps, select “Yes” to confirm the execution of this task.

6 Reagent carousel

Double-click on the “Reagent carousel” task line, and the following will appear:



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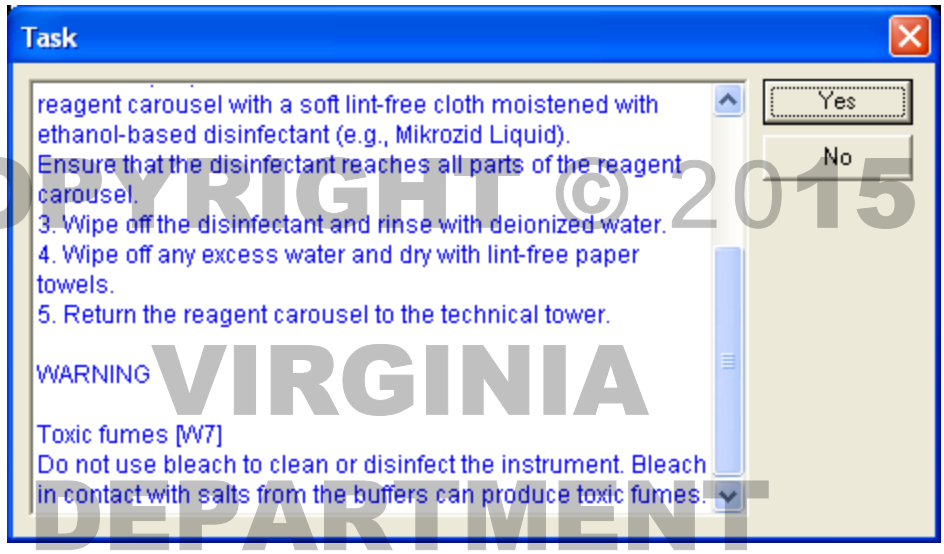

After completing all of the steps, select “Yes” to confirm the execution of this task.

7 Clean up old files

Delete message logs/data files that are older than 30 days.

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