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Department of Forensic Science

VIRGINIA

DEPARTMENT

FORENSIC BIOLOGY SECTION

PROCEDURES MANUAL

FORENSIC SCIENCE

SECTION V

QIAGEN[®] BIOROBOT[™] 9604

PROCEDURE 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.
- 1.1.2 The QIAGEN® AL lysis buffer, included in the QIAamp® extraction kit, is a guanidine-based 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.
- 1.1.3 The QIAGEN® Biorobot™ 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.
- 1.1.4 The QIAGEN® AW1 wash buffer 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.
- 1.1.5 The QIAGEN® AW2 wash buffer is a Tris-based solution containing ethanol, which will wash away any salts that are present.
- 1.1.6 The QIAGEN® AE elution buffer is a Tris-EDTA solution, which elutes the DNA attached to the membrane and serves as a stable storage medium.
- 1.1.7 A random sample will be run with each extraction to serve as an extraction, amplification, and a typing gel migration control. The random sample is a previously analyzed convicted offender sample for which the DNA profile is known to the Forensic Biology Program Manger and Laboratory Directors. This sample serves as an internal laboratory control since the DNA profile is not known to the examiner/analyst and must be verified by the Forensic Biology Program Manger or the Laboratory Director 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.



A.

B.

C.

A. Tip Disposal Unit

B. External Bottle Holder

C. Peristaltic Pump

1.2 Equipment

- 1.2.1 QIAGEN® BioRobot™ 9604 and computer and printer
- 1.2.2 QIAGEN® BioRobot™ 9604 and computer and printer
- 1.2.3 QIAGEN® BioRobot™ 9604 and computer and printer
- 1.2.4 Carboys, 10 Liter, 4 Liter
- 1.2.5 Microplate station block with MP-slot
- 1.2.6 Reagent holders, 2.0 mL and 15 mL
- 1.2.7 Buffer bottle holder and buffer bottles, 250 mL and 500 mL
- 1.2.8 Wash bottle, 500 mL
- 1.2.9 Black orientation adapter
- 1.2.10 Elution microtube rack adapter for MP Slot 3
- 1.2.11 Hand held puncher
- 1.2.12 BSD 600- Duet Semi-Automated Dried Sample Puncher

1.3 Materials

- 1.3.1 96-well S-block (See Figure 1)
- 1.3.2 QIAamp® 96 plate (See Figure 1)
- 1.3.3 Elution microtube rack with 96 elution microtubes (See Figure 1)
- 1.3.4 Caps (strips of 8) for collection microtubes (See Figure 1)
- 1.3.5 Adhesive tape (See Figure 1)
- 1.3.6 Air-Pore Tape™ (See Figure 1)
- 1.3.7 Kimwipe®
- 1.3.8 Tubes (2mL) for QIAGEN® Biorobot™ Protease
- 1.3.9 Conical tubes, 15mL
- 1.3.10 Disposable pipettes, 10mL
- 1.3.11 1100 µL conductive disposable filter pipette tips

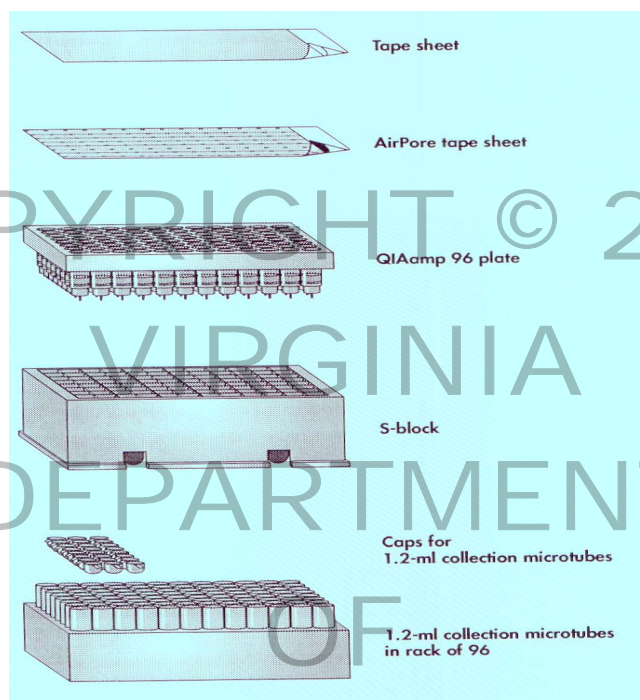


Figure 1: Accessories for extraction procedure

1.4 Reagents

- 1.4.1 1X Phosphate Buffered Saline (PBS), pH 7.2
- 1.4.2 QIAGEN® Biorobot™ Protease
- 1.4.3 Protease solvent
- 1.4.4 QIAGEN® AL lysis Buffer
- 1.4.5 Ethanol (95%)
- 1.4.6 Ethanol (70%)
- 1.4.7 QIAGEN® AW1 Buffer (reconstituted with 95% Ethanol)
- 1.4.8 QIAGEN® AW2 Buffer (reconstituted with 95% Ethanol)
- 1.4.9 QIAGEN® AE Buffer and four 15mL conical tubes
- 1.4.10 Type 1 water

1.5 Preparation of Dried Blood Stain and Buccal Swab Samples

- 1.5.1 Using the BSD 600- Duet Semi-Automated Dried Sample Puncher or a hand held puncher, punch a 6.0 mm² sample and transfer to a labeled S-block using tweezers. **If a hand held hole puncher and tweezers are used, they should be cleaned between each sample using a Kimwipe® and 70% ethanol.**

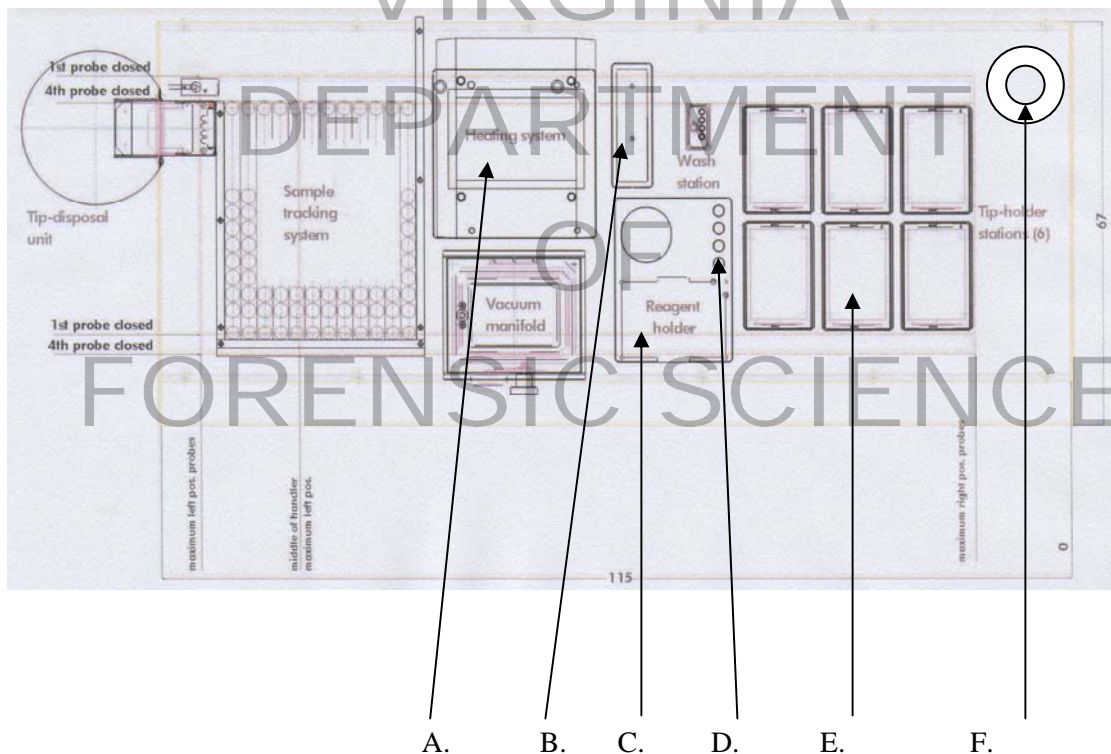
NOTE: If the sample is punched manually using a hand held hold puncher, a witness **MUST** be present to verify that the samples are placed into the correct wells of the S-block.

The maximum number of samples that can be run at a time is 92 samples, which includes 88 dried blood or buccal swab samples and 4 controls (two random samples and two reagent blanks). The remaining 4 empty (unused) wells will be used for PCR amplification controls.

1.5.2 Process two reagent blanks and two dried random samples 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™ 9604.

1.6 Purification of Genomic DNA from Dried Blood Stains and Buccal Swabs Using the QIAGEN® BIOROBOT™ 9604



- A. Thermostat Block
- B. Thermoblock with slots A1 to A8
- C. MP-slot 3 of the microplate station block
- D. Reagent holders B1 to B4 of the microplate station block
- E. Pipette tip racks 1 to 6
- F. Wash Bottle

1.6.1 Turn on the QIAGEN® BioRobot™ 9604 using the switch on the back, right lower corner of the unit. A green light should appear in the right front upper corner of the back panel of the robot, behind the wash bottle.

1.6.2 Turn on the peristaltic pump by pressing the black button on the front upper left corner of the gray box. The peristaltic pump is located between the robot platform and the computer. Snap the four white cassettes of the peristaltic pump into place.

1.6.3 Turn on the 96-well thermostat block using the switch on the back of the block in the upper right corner.

NOTE: Before the first run of the day wipe the Z-Bars with a dry Kimwipe while probes are down

- 1.6.4 Fill the carboy labeled “system liquid reservoir”, located on the floor below the robot, with distilled water to the “MAX” line. Make sure the liquid waste container is empty and that the liquid in the vacuum trap is below the “MAX” line.

NOTE: If the container labeled “waste container” needs to be emptied, the liquid (mainly water) can be poured down the drain and then flushed with water. If the container labeled “vacuum trap waste” needs to be emptied, pour the waste into a brown bucket (containing a stir bar) in a hood and add approximately 1 L of bleach to neutralize. Place the bucket on a stir plate and leave the waste in the hood overnight to allow the chlorine gas to dissipate out of the hood exhaust. The following day, pour the waste down the drain with plenty of water.

WARNING!

The QIAGEN® AL Lysis Buffer contains Guanidine Hydrochloride. This chemical will produce chlorine gas when mixed with bleach.

- 1.6.5 Fill the labeled buffer bottles, located in the external bottle holder between the robot and the peristaltic pump, with Phosphate Buffered Saline (PBS), QIAGEN® buffers AW1 and AW2, and 95% Ethanol. Remove the caps on the buffer bottles and connect the various colored adapters and tubing to the appropriate buffer bottle. The adapters and tubing are connected to the wash bottle located on the robot platform. The adapter color corresponds to the tape color on each bottle (i.e. PBS to the blue adapter, AW1 to the green adapter, AW2 to the red adapter and 95% Ethanol to the yellow adapter).
- 1.6.6 Ensure that pipette tip racks 1 to 4, containing 1100 µL pipette tips, are full.
- 1.6.7 Place the silver microplate station block, containing MP-slot 3 and reagent holders B1 to B4, in the space provided between the pipette tip racks and the vacuum manifold. The MP-slot 3 will face the front as the block can only fit one way due to the size and positions of the black knobs on the platform and corresponding holes in the microplate station block. Verify that the microplate station block is flush against the robot platform.
- 1.6.8 Using a 10 mL pipette, add 6.0 mL of QIAGEN® AL lysis buffer to four 15 mL conical tubes and remove all bubbles from the liquid surface. Place the uncapped tubes into reagent holders B1 to B4 of the microplate station located on the robot platform. **The AL lysis buffer is light sensitive and should not be left in the light longer than necessary.**
- 1.6.9 Place four 2 mL microcentrifuge tubes containing 1.25 mL of QIAGEN® Biorobot™ Protease. Place the uncapped tubes into slots A5 to A8 of the thermoblock, located to the right of the thermostat block.

NOTE: If the QIAGEN® Biorobot™ Protease is frozen, allow it to thaw completely before placing the tubes into the thermoblock.

- 1.6.10 Assemble the vacuum manifold in the silver slot located in front of the thermostat system. Place the flat surface of the white base plate into the slot, aligning the drain hole. Place the silver channeling block, with the notch oriented in the upper right corner, into the base plate. Situate the clear top plate on top of the base plate. The grey knob, used to open the lid, must face the front. Verify that the top plate is flush against the base plate.
- 1.6.11 Place a QIAamp® 96 plate into the vacuum manifold. **All unused wells MUST be sealed with adhesive tape to ensure a proper vacuum.** Lift the clear plastic lid of the vacuum manifold, by turning the grey knob clockwise, and situate the channels of the QIAamp® 96 plate into the silver channeling block, then gently lower the lid. In order for the lid to close properly, the QIAamp® 96 plate must be placed with the rack position A1 in the upper left corner.

- 1.6.12 Turn on the computer and press Ctrl + Alt + Delete to bring up the log-on information box. The username field, of the logon box, should contain “Administrator”. Select ‘OK’ to continue, as a password is not needed to log into the program.

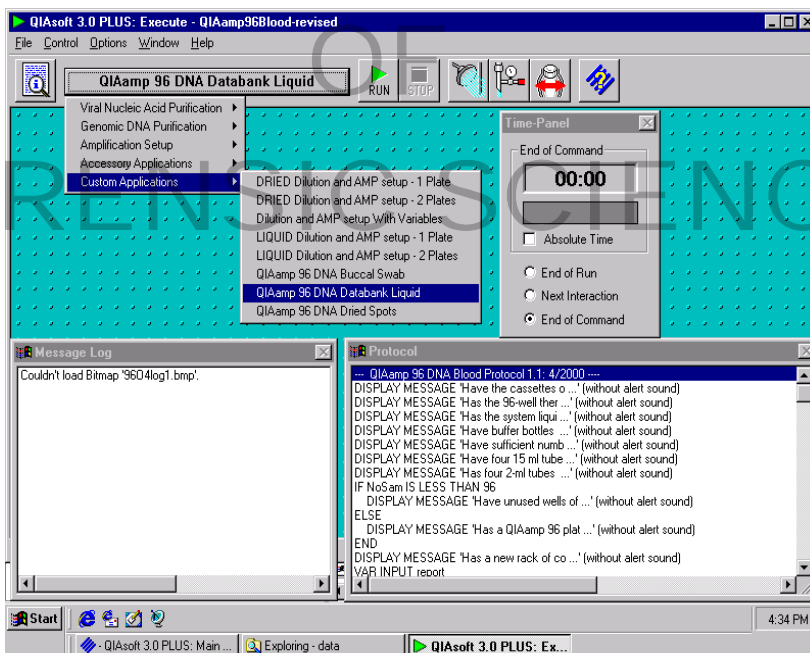
NOTE: Before the first run of the day use the shower icon to flush the system.

Settings: Peristaltic pump 15 mL
Dilutors 3 strokes

- 1.6.13 Click on the header button located on the toolbar to the left of the “Run” button. Select “Custom Applications” from the pull down menu (see diagram below), then proceed to step 1.6.13.1.

- 1.6.13.1 **Dried Blood and Buccal Swab Samples:** Select “QIAamp 96 DNA Dried Spots” from the menu.

The pull-down menus will disappear and the selected protocol title will appear on the header button. The protocol dialog box will contain the instructions for the selected procedure: --- QIAamp 96 DNA Dried Spots Protocol 1.1: 4/2000---



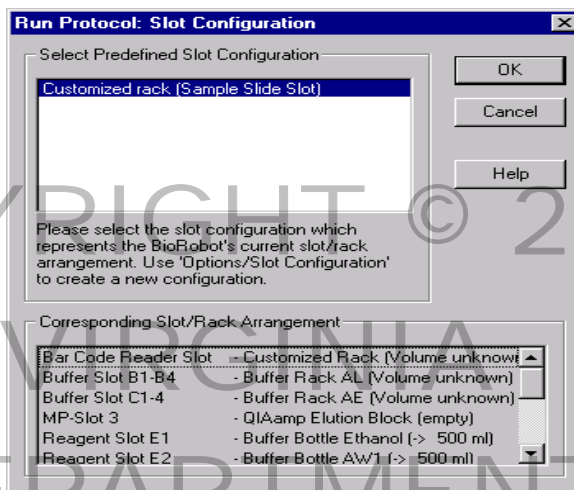
- 1.6.14 To start the protocol select the “Run” button from the toolbar or select “Start” from the “Control” menu.

NOTE: Once the protocol has been started the current procedure being performed by the robot will be highlighted in the protocol box. A dialog box will appear if user intervention is required. The robot will only continue after the action has been completed and the user has selected “OK” or “Continue”.

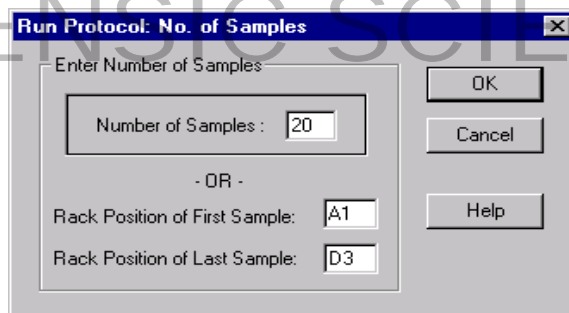
The Message Log box will display the commands and actions being performed as well as provide a history of the current protocol run.

The Time Panel box displays one of the following selections: time remaining until the end of the current protocol, the time until the next user interaction, or the time remaining until the current action is complete.

- 1.6.15 The dialog box “Run Protocol: Slot Configuration” with “Customized rack (Sample Slide Slot)” highlighted will appear. Select ‘OK’.

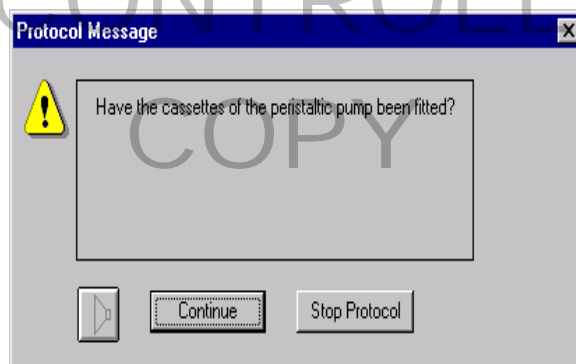


- 1.6.16 When the following dialog box appears enter the total number of samples that will be processed. **Only multiples of 4 can be selected. The maximum number that can be processed is 92, which includes 88 dried blood or buccal swab samples and 4 controls (two random samples and two reagent blanks).** The remaining 4 empty (unused) wells will be used for PCR amplification controls. Select 'OK' when finished.

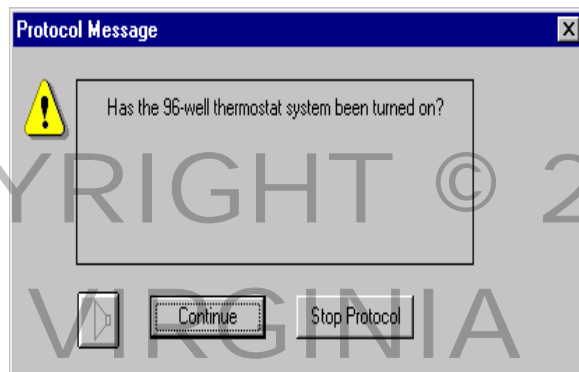


NOTE: If processing a partial rack of samples and the first sample is NOT in position A1 then it is necessary to enter the number of samples AND the rack position of the first sample (i.e. A3). The rack position of the last sample will automatically be calculated based on the number of samples and position of the first sample (i.e. H4 if there are 16 samples).

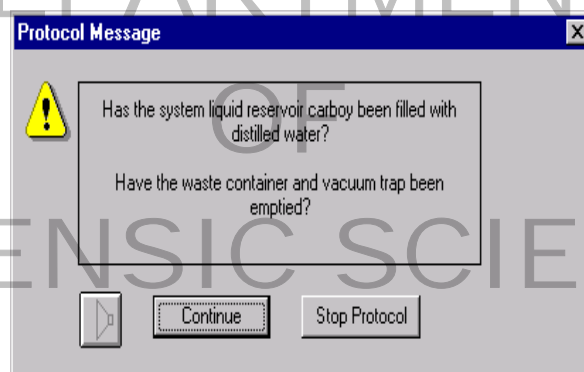
- 1.6.17 The following protocol message will appear, verify that the task has been completed, then select 'Continue'.



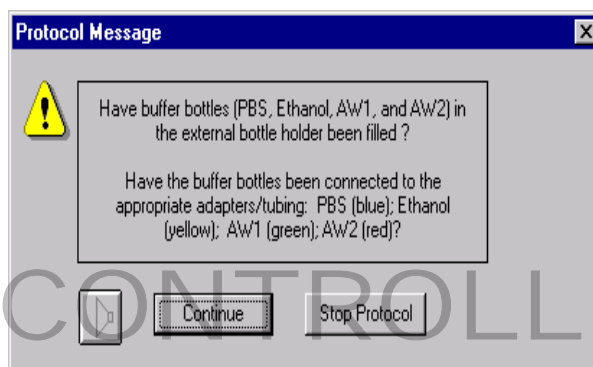
1.6.18 Select 'Continue' if the following task has been completed.



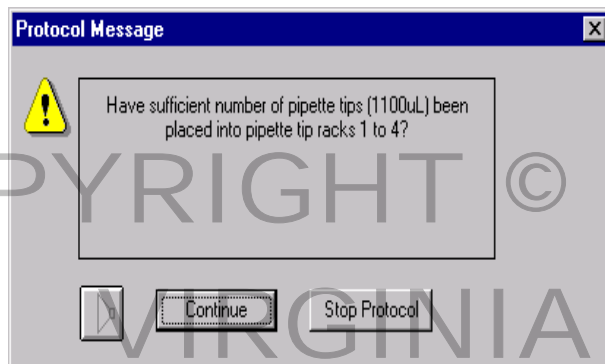
1.6.19 Ensure the tasks in the following protocol message have been completed, then select 'Continue':



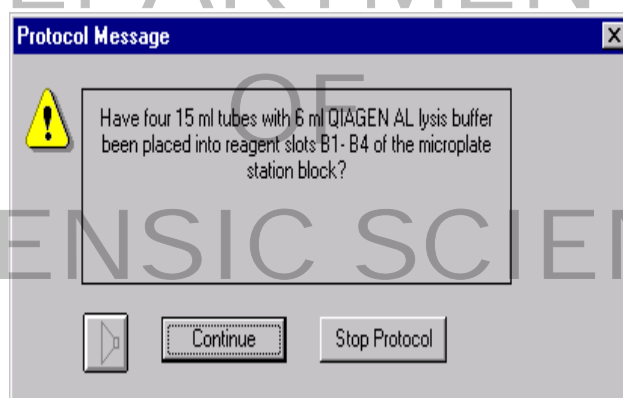
1.6.20 Verify that all buffer bottles have been filled and connected to their respective tubing. Enter the lot numbers and source for each reagent in the next 7 dialog boxes, then select 'Continue'.



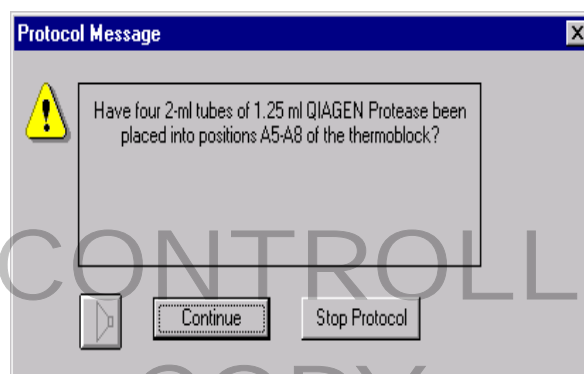
1.6.21 The next protocol message appears, verify that the tip racks have been filled, then select 'Continue'.



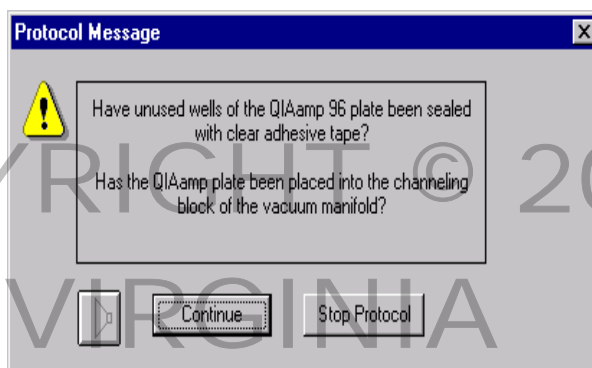
1.6.22 In the next dialog box verify that the QIAGEN® AL lysis buffer tubes have been uncapped and setup, then select 'Continue'.



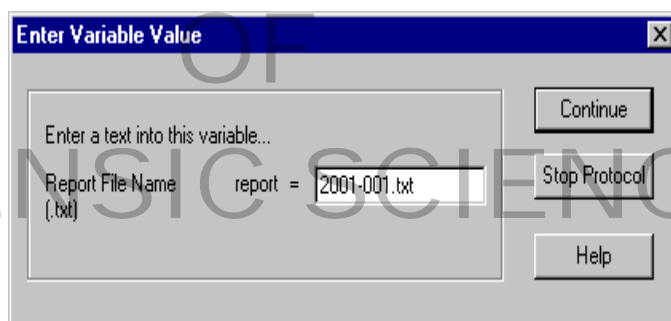
1.6.23 Next, verify that the QIAGEN® Biorobot™ Protease tubes have been uncapped and set up, then select 'Continue'.



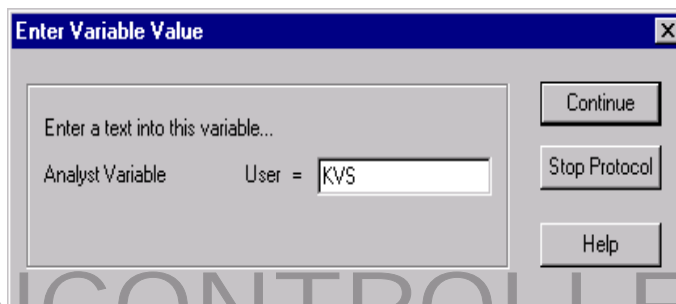
1.6.24 Verify that the tasks in the following protocol message have been completed, then select 'Continue'



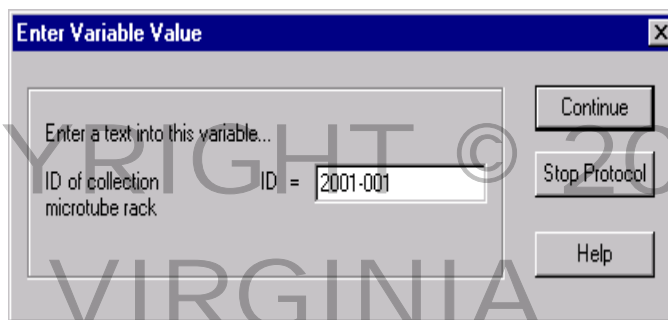
1.6.25 In the following dialog box enter a filename (it will be identical to the collection microtube rack number in step 1.6.28) including the file extension ".txt" (i.e., 2001-001.txt, 2001 corresponds to the year and 001 to the plate number), then select 'Continue'.



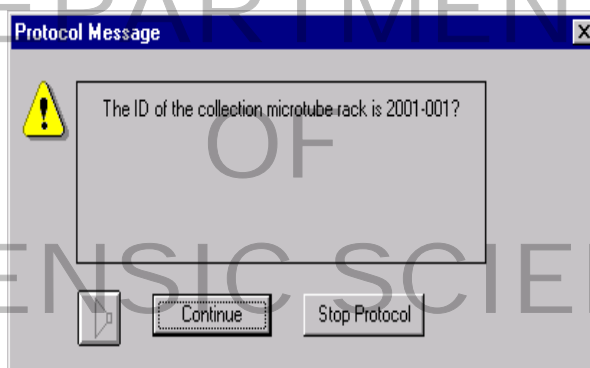
1.6.26 In the next dialog box enter the user's initials, then select 'Continue'.



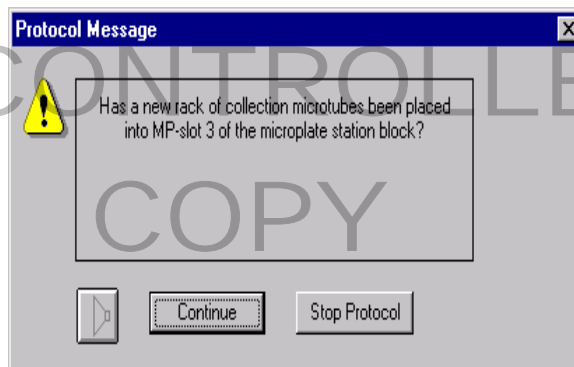
- 1.6.27 Enter the ID of the elution microtube rack in the next dialog box displayed. The ID should be identical to the file name in step 1.6.26 (i.e. 2001-001), then select 'Continue'.



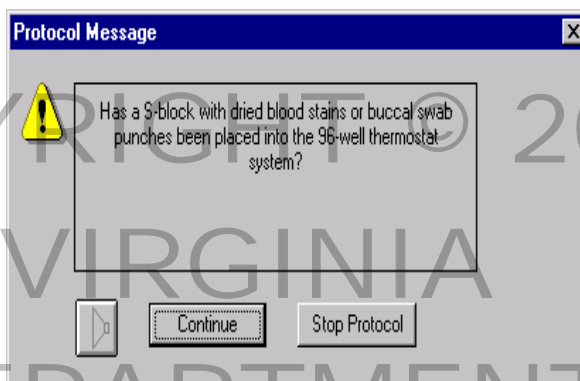
- 1.6.28 In the following dialog box, verify that the correct ID was entered. If incorrect, proceed to step 1.6.28.1. If the ID is correct then select 'Continue' and proceed to step 1.6.29.



- 1.6.28.1 If the wrong microtube rack was entered select 'Stop Protocol'. Then verify that 'VAR INPUT ID' is highlighted in the protocol dialog box. Select 'RUN' from the toolbar. A Protocol message box appears asking the user "Start protocol at command 'VAR INPUT' (Press no to start from beginning)". Select 'Yes'. Steps 1.6.16 and 1.6.17 will be repeated and followed by the dialog box from step 1.6.27. Enter the correct ID and select 'Continue'.
- 1.6.29 Label a new elution microtube rack (using the nomenclature in step 1.6.27). Place the elution microtube rack on top of the elution microtube rack adaptor located in MP-slot 3 of the microplate station block. Do not remove the plastic cover until step 1.6.36. **Ensure that rack position A1 of the collection microtube rack is in the upper left corner**, then select 'Continue' from the dialog box.



- 1.6.30 Place the S-block containing the dried blood stains or buccal punch into the thermostat block. **Ensure that rack position A1 of the S-block is in the upper left corner**, as the S-block can be placed into the thermostat in the reversed direction. Select 'Continue' and proceed to step 1.6.31.



INSTRUMENT OPERATION The robot will begin to flush the system and then proceed to add PBS to each well containing paper punches. The paper punches will incubate for 10 minutes at 56°C.

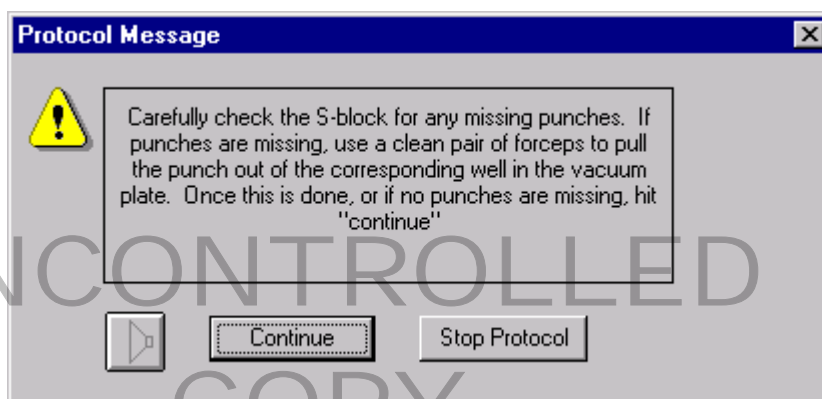
After the PBS incubation has elapsed, the robot will proceed to add QIAGEN® Biorobot™ Protease to each sample well.

The robot will proceed to add QIAGEN® AL lysis buffer to the dried blood and buccal swab samples in the S-block. Each sample is mixed and then incubated for 10 minutes while still on the thermostat block.

Subsequently, the robot will flush the probes and 95% ethanol is added to each sample.

The probes are flushed again. The samples are mixed before the sample mixture is transferred from the S-block wells to the corresponding wells in the QIAamp® 96 plate situated in the vacuum manifold.

- 1.6.31 After the sample transfer is complete, the following protocol message appears. Select "Continue" once the task has been completed and then proceed to step 1.6.32.



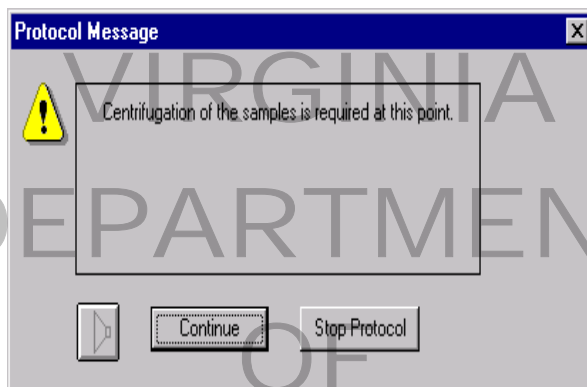
INSTRUMENT OPERATION QIAGEN® wash buffer AW1 is then added to each sample mixture in the QIAamp® 96 plate and the probes are flushed. The vacuum is turned on, and pumps continuously for 5 minutes.

An additional aliquot of QIAGEN® AW1 buffer is added to each well. The vacuum is turned on, and pumps continuously for 4 minutes.

The probes are flushed, and after the vacuum has finished, the robot proceeds to add QIAGEN® AW2 wash buffer to each sample after which the probes are flushed. The vacuum is turned on, and pumps continuously for 3 minutes.

An additional QIAGEN® AW2 buffer wash is added to the dried stain sample wells. The vacuum is turned on and pumps continuously for 2 minutes.

- 1.6.32 After the vacuum pump is turned off the following protocol message appears. Select 'Continue' once the task has been completed and proceed to step 1.6.33.



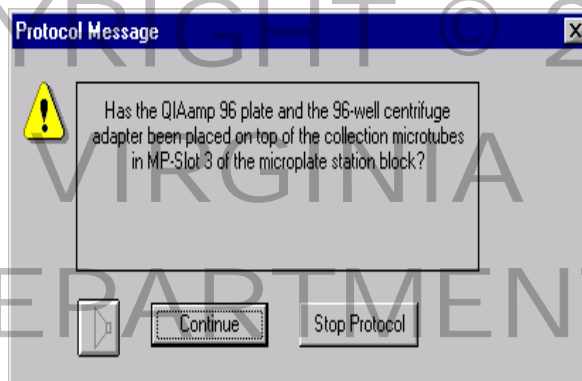
- 1.6.33 Remove the S-block from the thermostat block and the QIAamp® 96 plate from the vacuum manifold. Place the channels of the QIAamp® 96 plate into the wells of the S-block. Ensure that rack positions A1 of the QIAamp® 96 plate and S-block are aligned, then proceed to step 1.6.50.

- 1.6.34 Turn on the centrifuge, designated for 96 well plates, by pressing the switch in the back of the centrifuge in the upper left corner. Open the centrifuge by pressing the yellow lit button and lifting the lid. Remove the silver brackets from the two black centrifuge buckets. Place the assembly containing the samples into one rack and set it into a bucket. Assemble a similar set up using “balance parts” and place it in the opposing rack and bucket. Make sure that the QIAamp® 96 plate of each assembly is properly seated in the S-block. Close the lid to the centrifuge and verify that the speed is set to 6000 rpm and the timer has been set for 4 minutes. Press the green lit button to start the centrifuge. To stop the centrifuge at any time before the spin is over press the red lit button.

NOTE: To change a setting on the centrifuge (e.g., time) turn the blue knob on the front of the centrifuge until “time” is highlighted. Push the blue button in so that “set” is also highlighted. Turn the blue knob, right or left, to either increase or decrease the time. When the desired time has been selected push the blue button to set the time.

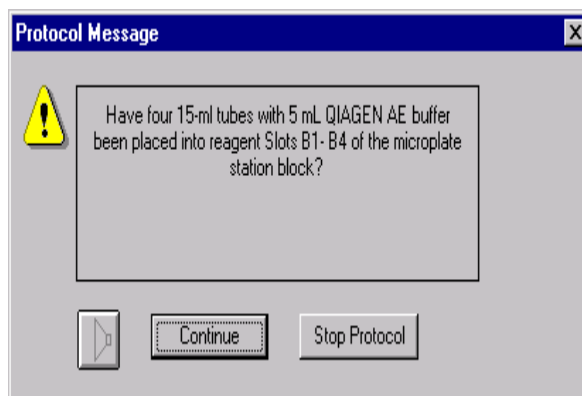
- 1.6.35 Once the centrifugation is complete remove the QIAamp® 96 plate and S-block assembly from the centrifuge.

- 1.6.36 Remove the plastic cover on the elution microtube rack, situated in MP-slot 3, and place the black orientation adapter onto the rack. Remove the QIAamp[®] 96 plate from the S-block and place it on top of the adapter. **The elution microtube rack, the adapter, and the QIAamp[®] 96 plate can only fit together correctly and snugly when rack position A1 of all 3 parts are aligned.** Place the 3-part assembly in MP-slot 3 with rack position A1 in the upper left corner, then select “Continue” from the dialog box once the task has been completed.



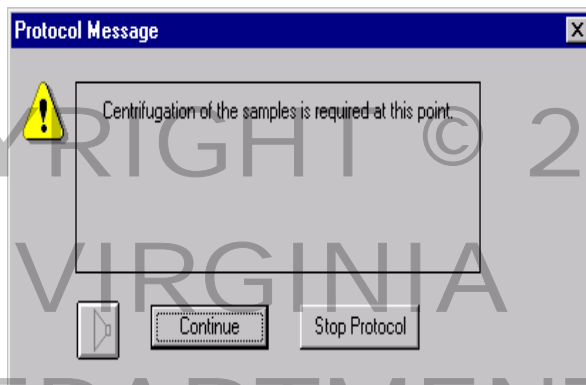
- 1.6.37 Remove the QIAGEN[®] AL lysis buffer tubes from reagent holders B1 to B4 of the microplate station block and discard. Place four, uncapped, 15 mL conical tubes filled with 5.0 mL QIAGEN[®] AE Elution Buffer (the AE buffer tubes are supplied with the QIAGEN[®] kit) or Type 1 water into reagent holders B1 to B4, then select ‘Continue’ from the dialog box to verify that the task has been completed.

NOTE: If a partial plate of samples is processed do not discard the QIAGEN[®] AL lysis buffer. Store the buffer and use with the next partial plate.



INSTRUMENT OPERATION the robot will then add QIAGEN[®] AE Elution Buffer or Type 1 water to each sample and set the timer for a one minute incubation at room temperature.

- 1.6.38 After the 1 minute incubation, the following protocol message will appear. Select 'Continue' and proceed to step 1.6.39.



- 1.6.39 Remove the 3-part assembly from MP-slot 3. Cover the wells containing sample with Air-Pore™ tape (off-white color). Place the assembly and a similar assembly for balance into the centrifuge. Centrifuge for 4 minutes at 6000 rpm.

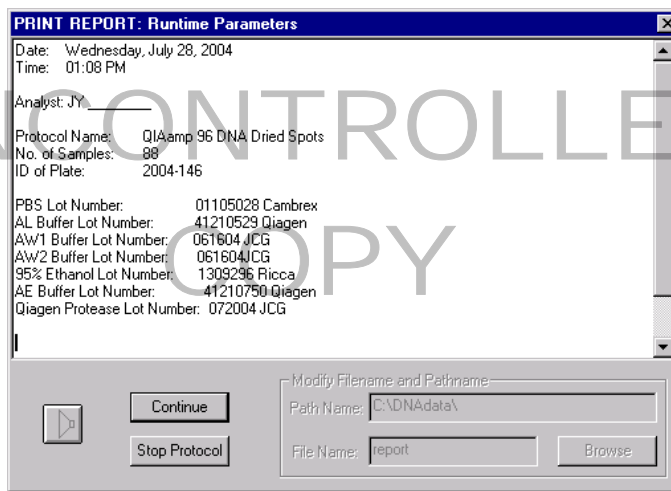
NOTE: To change a setting on the centrifuge (e.g., time) turn the blue knob on the front of the centrifuge until “time” is highlighted. Push the blue button in so that “set” is also highlighted. Turn the blue knob, right or left, to either increase or decrease the time. When the desired time has been selected push the blue button to set the time.

- 1.6.40 Once the centrifugation is complete remove the assembly from the centrifuge. The elution microtubes now contain the eluted DNA.

- 1.6.41 **CAREFULLY** remove the QIAamp® 96 plate from the assembly from the elution microtubes. After removing the QIAamp® 96 plate, remove the adapter and cap the elution microtubes.

1.6.41.1 **Storage:** Place the rack in the refrigerator for short-term storage (i.e., 1–3 days) or in the freezer for longer periods of storage (a few weeks).

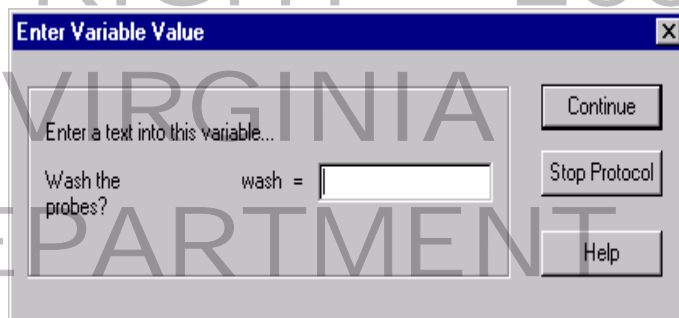
- 1.6.42 A “Print Report” dialog box will appear listing the user and witness initials, date, time, protocol name, number of samples, identification of the plate and reagent lot numbers. This report will be retrieved at a later time (See Section 2: DNA Isolation Report). Select “Continue” to proceed.



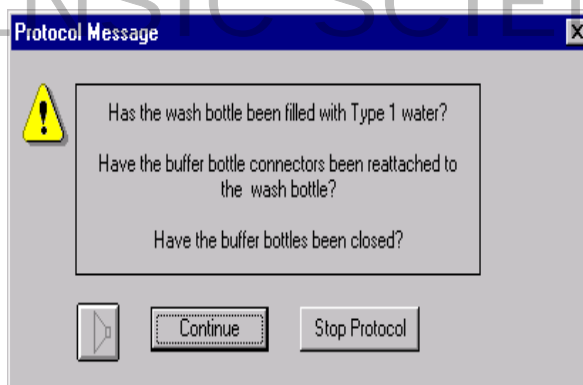
1.6.43 Depending on the next step of processing of the samples proceed to either step 1.6.43.1 or 1.6.43.2, as appropriate and enter “Yes” or “No” into the dialog box below as specified:

1.6.43.1 If another rack of samples will be extracted enter “No” into the dialog box below, then select ‘Continue’ and proceed to step 1.6.45.

1.6.43.2 If no other sample racks will be processed, enter “Yes” in the box, then select ‘Continue’ and proceed to step 1.6.44.



1.6.44 Fill the wash bottle located on the robot platform, with Type 1 water – this must be done before starting the wash procedure. Remove the tubing/adapters from the buffer bottles (PBS, Ethanol, AW1 and AW2) and re-attach them to the connectors on the wash bottle on the platform. Cap the buffer bottles, then select ‘Continue’ from the dialog box and proceed to step 1.6.45.

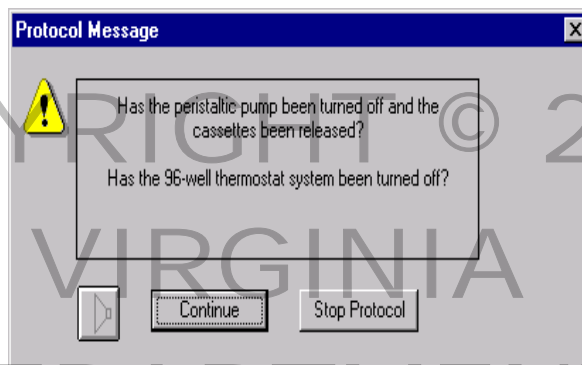


INSTRUMENT OPERATION The robot will proceed to flush the probes and tubing several times.

1.6.45 Remove and discard the tubes of QIAGEN® Biorobot™ Protease from slots A5 to A8 of the thermoblock and the tubes of QIAGEN® AE Elution Buffer or Type 1 water from reagent holders B1 to B4 of the microplate station block.

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- 1.6.46 Switch off the thermostat system, turn off the peristaltic pump and release the cassettes by squeezing the white clips located on the left of each of the four cassettes. Select 'Continue' when the tasks have been completed.



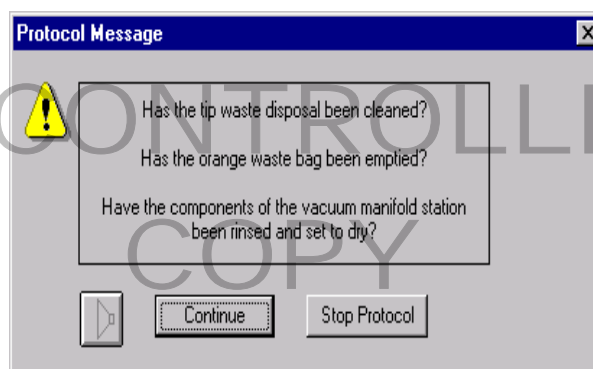
- 1.6.47 Dismantle and clean the vacuum manifold. To remove the vacuum manifold, lift and tilt the left side of the white base plate (drain whole side), cover the drain hole of the base plate with a Kimwipe[®], to prevent remaining liquid waste from flowing through. Move the base plate, containing the channeling block and clear top plate, to the sink and rinse the three components with distilled water. Wipe off excess water and allow the components to air dry. Clean off the area around the drain hole of the silver plate on the robot platform.

- 1.6.48 Clean the tip disposal unit and, if needed, replace the orange waste bag, both are located at the left end of the robot. To clean the tip disposal unit, set aside the bar code scanner and remove the silver cover by pulling it upward. Rinse the silver slide component of the disposal area with water and ethanol (CAUTION: DO NOT USE BLEACH). Allow the water and ethanol to drain into the waste bag and use a Kimwipe[®] to wipe off any blood and to dry the slide component. While the silver cover is off, the waste bag can be replaced. Remove the full bag and place it in a biohazard box, then tape the edges of a new bag to the silver hoop before reassembling the components of the tip disposal area. Replace the bar code scanner on the side of the tip disposal unit. When tasks are completed select 'Continue'.

- 1.6.49 Follow the appropriate step (1.6.49.1 - 1.6.49.2) depending on the variable selected in 1.5.45.

1.6.49.1 If the variable "no" was entered in step 1.6.43 and another set of samples will be extracted, then proceed to Section 2, DNA Isolation Report.

1.6.49.2 If the variable "yes" was selected in step 1.6.43, then proceed to step 1.6.50.



- 1.6.50 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".

- 1.6.51 To turn off the Robot, use one hand to support/hold the four probes and switch off the robot. **DO NOT** let go of the probes once the robot is off. **GENTLY**, guide the probes over to an area above the red tape on the robot platform, labeled “robot probes” (use the overhead cross bar to move the probes left and right), use the top of the Z-bar (above the overhead cross bar) to move the probes forward and back, and then **SLOWLY** lower the probes. The probes will rest at a position close to the platform. Once the probes are in their resting position the user can release the probes. **If the robot is turned off incorrectly the probes can be DAMAGED!**
- 1.6.52 Proceed to Section 2, DNA Isolation Report. Then proceed to the Fluorescent Detection PCR-Based STR DNA Protocol PowerPlex® 1.1, 2.1, and 16 BIO Systems Manual, Section III, Chapter 5, PCR Amplification.

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2 DNA ISOLATION REPORT

- 2.1 On the desktop select the “DNADData” icon. Once in Windows NT explorer select the labeled DNA data folder to find the file for the sample set (i.e. 2001-001). Click on the file with the small notepad icon next to the filename (txt file). Verify that the printer is on, then select File and then Print from the toolbar.
- 2.2 Once the reports have been printed, obtain the appropriate initials from the analyst and witness (see sample report below). Ensure that all DNA Isolation Report pages contain the file name and analyst’s initials.

Sample Report:

Date:	Monday, June 04, 2004
Time:	05:50 PM
Analyst:	KVS _____
Protocol Name:	QIAamp 96 DNA Dried Spots
No. of Samples:	88
ID of Plate:	2004-141
PBS Lot Number:	
AL Buffer Lot Number:	
AW1 Buffer Lot Number:	
AW2 Buffer Lot Number:	
95% Ethanol Lot Number:	
AE Buffer Lot Number:	
Qiagen Protease Lot Number:	
Lysate transferred manually to QIAamp plate for the following samples:	
Lysate transfer witness: _____	

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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.

ETHANOL, 95%

Expiration date: Dispose of when necessary

Purchased as Reagent Alcohol (190 Proof) from VWR, Bridgeport, NJ, Catalog number 6590-1, 4L bottle.

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

Expiration date: manufacturer's expiration date

Purchased from Cambrex, Walkersville, MD., Catalog # 17-516Q 1L bottle.

QIAamp® 96 BLOOD BIOROBOT™ KIT

Expiration date: Refer to individual kit component expiration dates.

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

QIAGEN® AE ELUTION BUFFER

Expiration date: Twelve months from date of receipt into the laboratory.

Supplied with the QIAamp® 96 Blood BioRobot™ Kit.

QIAGEN® AL LYSIS BUFFER

Expiration date: Twelve months from date of receipt into the laboratory.

Supplied with the QIAamp® 96 Blood BioRobot™ Kit.

QIAGEN® AW1 WASH BUFFER (concentrate)

Expiration date: Twelve months from date of dilution with 95% ethanol.

Supplied with the QIAamp® 96 Blood BioRobot™ Kit.

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

AW1 concentrate	Ethanol	Final volume
175 mL	230 mL	405 mL

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

QIAGEN® AW2 WASH BUFFER (concentrate)

Expiration date: Twelve months from date of dilution with 95% ethanol.

Supplied with the QIAamp® 96 Blood BioRobot™ Kit.

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

AW2 concentrate	Ethanol	Final volume
274 mL	640 mL	914 mL

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

QIAGEN® BIOROBOT™ PROTEASE

Expiration date: 6 months after sample is reconstituted if stored at –20°C.
2 months after sample is reconstituted if stored at 4°C.

Supplied with the QIAamp® 96 Blood BioRobot™ Kit.

Reconstitute with supplied QIAGEN® protease solvent before initial use according to the specifications below:

QIAGEN® Protease	Protease solvent	Final concentration
140 mg	10.2 mL	13.725 mg/mL

QIAGEN® PROTEASE SOLVENT

Expiration date: Twelve months from date of receipt into the laboratory

Supplied with the QIAamp® 96 Blood BioRobot™ Kit.

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Appendix B: Drying of DNA for Permanent Storage

- 1 Technical
 - 1.1 For permanent storage and preservation of extracted DNA, samples will be concentrated using a rapid evaporation system.
- 2 Equipment
 - 2.1 RC10.22 evaporator
 - 2.2 Rotor with 96 well plate holders (compatible with the RC10.22 evaporator)

3 Materials

- 3.1 Collection microtube rack (for balance)

4 Procedure

NOTE: If it is necessary to save an extract the following procedure should be used to dry down the sample down.

- 4.1 Turn on the vacuum pump by pressing the switch on the left side of the vacuum pump.

NOTE: The words “open the lid” will appear on the screen on the front of the evaporator.

- 4.2 Open the evaporator lid by sliding the lid back until it stops and then lifting the lid up.

NOTE: Once the lid has been opened, the screen will show a group of numbers.

- 4.3 Remove the lid from the collection microtube rack and uncap the collection microtubes, then place the collection microtube rack and a similar collection microtube rack, for balance, into the silver 96 well plate holders in the concentrator/evaporator.
- 4.4 Close the lid, by gently lowering the lid and then sliding the lid forward. The lid will drop into place.
- 4.5 Set the dry down time to 70 minutes by pressing the “F” on the key pad by the screen on the front of the evaporator. Then press either “-“ or “+” to scroll to “recall prog”. Once “recall prog” appears on the screen press “enter” and “recall prog: 08” will appear (the number after “recall prog” is the program number that was previously entered). Enter the number “08” and press enter. The next screen will show the program number and then the dry down time (70 minutes). Press “start” to begin the drying of the extracted DNA.

NOTE: If the incorrect program number was entered, start from the beginning by pressing “F”.

To stop the concentrator/evaporator at any time before the time is completed press the “stop” on the key pad.

- 4.6 Once the 70 minutes has elapsed remove the sample rack from the evaporator and verify that the samples are dry (no liquid remains). Recap the collection microtubes and replace the lid on the collection microtube rack. Store the dried samples under the appropriate conditions.

Appendix C: Resolubilizing Dried DNA Extract

1 Equipment

- 1.1 Pipettes, 100 μ L

2 Materials

- 2.1 Pipette tips, filter, 100 μ L

3 Reagents

- 3.1 Sterile Type 1 Water

4 Procedure

- 4.1 To resolubilize the dried DNA samples, add a volume of Sterile Type 1 Water, consistent with the samples volume prior to drying of the extracted DNA.

NOTE: In the dilution step, 2.0 to 2.5 μ L is removed for each dilution from DNA extracted from dried blood stains or buccal samples.

- 4.2 To dry down the resolubilized samples, return to Appendix B

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Appendix D: Calibration and Maintenance of the QIAGEN® BIOROBOT™ 9604

- 1 Flush System (As needed) –when build up is noted in the lines:

Flush the system (not the dilutors) with 20-50 mL of 70% Ethanol and then distilled water (20-50mL) to remove any potential build up in the tubing. In the execute environment, click on the shower head icon. Make sure that the peristaltic pump and the desired volume have been selected.

- 2 The peristaltic pump tubing (as needed):

In the execute environment, click on the header button located to the left of the “Run” button. Select Accessory applications and then select High speed pipetting (weekly) Select run and follow the directions of the protocol (may need to repeat)

To correct for any inaccuracies close the execute environment and open Setup (password: user). Go to Utilities and select pipetting system calibration Enter the value that was measured in the high speed test and select okay, this will adjust for any minor inaccuracies (50-100uL)

If the values are off by much more than 100uL replace the tubing

** Record date of check and if tubing was replaced in the log book

- 3 Dilutors/Syringes (monthly):

The dilutors will be qualitatively checked monthly and replaced as needed. The tightness of the plunger fit as well as wear on the plunger head will be evaluated.

** Record date of test and initials of the person performing the evaluation in the logbook. Also note the number of the probe if it is necessary for a dilutor to be changed.

After the dilutors have been checked or replaced, flush the system using the shower icon and the following parameters:

- Peristaltic pump 50 mL
- Dilutors 15 strokes

- 4 Clean up old files (monthly):

Delete message logs from previous month. Click on the DNA Data icon on the desktop. Determine which files can be deleted by verifying against the date processed log (worksheet QB-2 ROBOTUSELOG).

NOTE: If the Thermostat system or Vacuum System fails call Qiagen’s technical service at 1-800-426-8157 (800-DNA-PREP).