



BioRobot 9604 Operating Procedure

Note: An import table (directions given in number 20) must be made prior to a robot run.

1. Prior to operating the BioRobot 9604, perform all daily and weekly maintenance as listed in the black notebook titled "BioRobot 9604 Maintenance Log." Check off every task completed and initial at the bottom of the form under the current date.
2. Prepare all reagents required for the protocol.

Note: Check Buffers ATL and AL for precipitates. If precipitates are present in either buffer, dissolve them by heating the buffer at 70° C.

- a. Add 1.3 ml of diluted Proteinase K (600 µl of Pro K plus 700 µL of distilled water) into each of four 2 ml tubes.
- b. Fill the empty 250 ml bottle for Buffer ATL with Buffer ATL.
- c. Aliquot 7.5 ml Buffer AL into each of four 15 ml tubes for Buffer AL (white screw-cap tubes).
- d. Check that Buffers AW1 and AW2 have been reconstituted with ethanol according to instructions.

Note: Save any unused Buffer ATL, AL, AW1, AW2, and diluted Proteinase K left after a run for use in following runs. DO NOT MIX LOT NUMBERS.

Note: Always switch on the high-speed pipetting system before switching on the BioRobot 9604.

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3. Ensure that the high-speed pipetting system (peristaltic pump) is switched on.
4. Ensure that the BioRobot 9604 is switched on. The power switch is located on the lower right of the rear panel.

Note: Prior to switching on the BioRobot 9604, gently push all four probes up as high as possible. Allow 1-3 seconds before releasing the probes to ensure that the BioRobot 9604 has completed probe orientation.

5. Switch on the computer and monitor.
Select operating mode "Windows NT Workstation Version 4.00"
6. Microsoft Windows NT Workstation Appears. Press Control + Alt + Delete to log on.
7. A window with Logon Information appears. The user name is "Administrator" and there is no password. Press enter.
8. From the Windows screen, double click on the Qiagen icon
 - a. Click on the Header and select "Custom Applications"
 - b. Select "Blood Card."
9. Click on "Run"
 - a. "Run Protocol: Slot Configuration" window appears
 - b. Select predefined slot configuration, "QIAamp 96 DNA Swab" and click "OK."
 - c. The "Run Protocol: No of Samples" dialog box appears.
10. Enter the number of samples to be processed (between 4 and 96), and the positions of the first and last samples.

Note: Only multiplies of 4 can be entered.

11. Wait for the BioRobot 9604 to initialize itself and perform the protocol test.
An "Enter Variable Value" dialog box appears when the BioRobot 9604 is ready.
12. Enter a name for the Report File that QIAsoft 3.0 will later generate. Click "Continue."
13. Another "Enter Variable Value" dialog box appears. Enter the Name of the Operator.

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Click "Continue."

14. A series of protocol messages will appear detailing the various preparation steps required before the BioRobot 9604 protocol can proceed. Follow each instruction given in the protocol message before proceeding.

Protocol Message: Place the Square well block containing the 1/8" blood card hole punches on the thermostat slot.

15. Click "Continue." An "Enter Variable Value" dialog box appears. Manually enter the identification code of the Square well block..
16. Click "Continue." A box appears allowing confirmation that the identification code entered is correct.
17. Click "Continue." A protocol message appears.

Protocol Message: Place a new rack of Collection Microtubes into MP-Slot 3.

18. Click "Continue." An enter variable value dialog box appears. Manually enter the identification code of the collection microtube rack.
19. Click "Continue." A box appears allowing confirmation that the identification code entered is correct.
20. A protocol message appears.

Protocol Message: Enter the name of the table to import.
The name of the import table must be in QiaSoft 3.0
User Data as a CSV file.
Prior to starting the BioRobot 9604 run, create the
import table.

Use the 3 ½" floppy disk titled "Worklist Transport Disk" to create an import table.

To create an Import Table:

A. Using a CODIS computer:

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1. Select "Specimen Manager"
2. Select "Reports"
3. Select "Donors by Specimen Number: Race"
4. Enter the beginning Donor I.D. number and the ending Donor I.D. number
5. Select "Export"
6. Rename and Save file in BioRobot 9604 Import folder as an excel file (.xls)
7. Close window when export is done
8. Open Excel, Select "File," "Open"
9. Select the C drive under "directories"
 - a. Open BioRobot 9604 Import folder
 - b. Select the excel file needed under "file name"
10. Double Click to open the needed file
 - **Select the specimen numbers and race
 - **Select Edit, "Copy"
 - ** Open template .xls and insert data into the template beginning with "B1"
11. Go to "File" and Select "Save As"
 - a. Select the "C drive" and change the file format from Microsoft Excel to CSV and save
 - b. Select the "A drive" with the "Worklist Transport Disk" in the A drive and change the file format from Microsoft Excel to CSV and save
12. Click Ok . Import table is then saved to C drive (as a back-up) and to the "Worklist Transport Disk"

B. Insert the "Worklist Transport Disk" into the BioRobot 9604 computer

1. Right Click on "Start"
2. Select "Explore"
3. Select 3 ½" floppy
4. Select current .csv file
5. Select "Copy"
6. Go to QIASOFT 3.0
7. Select "User Data"
8. Paste .csv file into User Data



21. Click “Continue.” A protocol message appears.

Protocol Message: Fill the system liquid container with distilled water.
Empty the waste container.
Empty the vacuum trap.
Empty the tip-waste bag.
Switch on the 96-well thermostat system.

22. Click “Continue.” A protocol message appears.

Protocol Message: Place four 2 ml tubes (each containing 1.3 ml diluted Proteinase K) into positions A1-A4 of the Protease block.
(1.3 ml diluted Pro K consists of 600 µl of Pro K and 700 µl of Distilled water). Open the 2 ml tubes.

23. Click “Continue.” A protocol message appears.

Protocol Message: Place the buffer bottles in the external bottle holder.
Make sure that Buffer ATL is attached to the black, AW1 to the green, Buffer AW2 to the red, and ethanol to the yellow connector.

24. Click “Continue.” A protocol message appears.

Protocol Message: Place four 15 ml tubes for Buffer AL, each containing 7.5 ml Buffer AL, into reagent slots B1-B4. Open the tubes.
Remove any large air bubbles present on the liquid surfaces.

25. Click “Continue.” A protocol message appears.

Protocol Message: Place a QIAamp 96 plate into the top plate of the vacuum manifold (which consists of a base plate equipped with a channeling block).
Seal any unused wells with an adhesive tape sheet and leave them sealed throughout the procedure.

26. Click “Continue.” A protocol message appears.



Protocol Message: Place sufficient tips into the tip racks.
A run of 96 samples requires 4 racks of 96 tips.
Check that tips are oriented and seated correctly in the holders.

27. Click “Continue.” A protocol message appears.

Protocol Message: Make sure that the cassettes for the peristaltic pump are fitted correctly.

28. Click “Continue.” A series of flushes will occur.

29. Click “Continue.” The QIAamp 96 DNA Protocol will start. A beeper will sound when it is time for the first user interaction. The BioRobot 9604 will perform an initial liquid-level check. If this indicates that there is insufficient Buffer AL or diluted Proteinase K for a run of 96 samples, additional protocol messages will appear warning of this and advising what steps should be taken to provide sufficient amounts of these.

User Interaction #1 (About 1 hour 45 minutes from start of protocol.)

30. Click on the speaker icon if the beeper does not stop. A protocol message will appear.

Protocol Message: Place the QIAamp 96 plate on top of an S-block, and put this assembly into the centrifuge. Centrifuge at 6000 rpm for 10 minutes.
Click “Continue” after starting centrifugation.

31. While centrifuging, click “Continue.” A protocol message appears.

Protocol Message: Remove the tubes containing Buffer AL from the tube holders in reagent slots B1-B4. Place four 15 ml tubes each containing 5 ml Buffer AE into the tube holders of reagent slots B1-B4. Open the tubes.

32. When the centrifugation process has finished, click “Continue.” A protocol message



appears.

Protocol Message: Place the 96 well centrifuge adapter onto the rack of collection microtubes. Place a QIAamp 96 plate onto the adapter. Place this assembly into MP-slot3.

33. Click “Continue.” The protocol continues. A beeper will sound when it is time for the next user interaction.

User Interaction #2

34. If necessary, click the button with the speaker icon to stop the beeper.

35. Click “Continue.” A protocol message appears.

Protocol Message: Seal the QIAamp 96 well plate with an AirPore Tape Sheet. Transfer the QIAamp 96 plate on top of the rack of collection microtubes into the centrifuge. Centrifuge at 6000 rpm for 3 minutes.

36. When the centrifugation is complete, click “Continue.” A protocol message appears.

Protocol Message: Remove the QIAamp 96 plate and the rack of collection microtubes from the centrifuge rotor. Discard the QIAamp 96 plate in the biohazard waste container. Seal the collection microtubes with the caps provided. Store the rack of collection microtubes containing the purified DNA at -20°C until required for use.

37. Click “Continue.” The Report File window appears, in which comments can be inserted. Click “Continue” to proceed if information is correct.

Wash Procedure

38. Click “Continue.” In the dialog box, enter “yes” to start the wash procedure.

Note: If you wish to perform another run immediately after the current run has

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finished, this wash step can be omitted. To omit the wash procedure, enter "no;" the procedure will continue from the protocol message in step 38.

39. Click "Continue." A protocol message appears.

Protocol Message: Attach the buffer bottle connectors to the adapters on the wash bottle filled with distilled water.
Click "Continue" to start the wash procedure.

40. Click "Continue." A protocol message appears.

Protocol Message: Please close all buffer bottles.
Release the peristaltic pump cassettes.
Switch off the 96-well thermostat system.

41. A beeper sounds when the procedure has finished.

42. Click "Continue." A protocol message appears.

Protocol Message: Process Done.

43. Click "Finish Protocol."

44. When Protocol is Finished, insert BioRobot 9604 "Text file" disk.

- a. Right Click on "Start"
- b. Select "Explore"
- c. Select "QiaSoft 3.0"
- d. Select "User Data"
- e. Select the text file for the current report
- f. Select "Edit," "Copy"
- g. Paste the text file onto the 3 ½" floppy disk

45. When Protocol is finished, complete the remaining tasks on the daily/weekly sections of the maintenance log. (See attached log)

Note: Wipe down all surfaces of the BioRobot 9604 with 96-100% Ethanol.

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46. If the BioRobot 9604 is not to be used again for **3-5 days**, switch it off. To ensure that the probes do not crash onto the surface of the robot, hold all four probes while switching the robot off. Gently release all four probes, allowing each to rest near the working surface of the robot.

Revision History		
Effective Date	Revision Number	Reason
August 30, 2001	00	Original Document
November 7, 2001	01	Procedure Update