
Procedure for Qiagen BioRobot® Universal Using PowerPlex® Fusion

1.0 Purpose –To instruct users in the operation of the Qiagen BioRobot® Universal System (BioRobot) for the automated processing of database samples using PowerPlex® Fusion.

2.0 Scope – This procedure applies to DNA Database Section Forensic Scientists and trainees who perform DNA analysis using the Qiagen BioRobot® Universal.

3.0 Definitions

- **Database Sample** – A convicted offender or arrestee DNA sample.
- **System Liquid** – System liquid is specified as purified water, 18.2 MΩ-cm. Any water run through the robot shall be system liquid only. The software may prompt for the addition of deionized water in certain protocols. In these instances, purified water or an equivalent shall be used.
- See the Procedure for DNA Database Operations for additional definitions applicable to this procedure. Any reagent lists may be found in the reference documents.

4.0 Equipment

- Qiagen BioRobot® Universal System
- Disposable plastic ware
- 96 well amplification plates
- Qiagen QIAsoft Software
- System Liquid (Barnstead NANOpure Diamond Water Filtration Unit, Model D11901 or equivalent), deionized water (used for disinfection of robot components only)
- Various micropipettes and pipette tips
- Various laboratory equipment involved in DNA analysis (disposable tubes, gloves, trays, centrifuges, pipettes, vortexes, refrigerators, biohazard waste containers; reagents used for DNA amplifications)

5.0 Procedure

5.1 Basic Operation Instructions

5.1.1 DNA Database Forensic Scientists shall wear gloves, lab coat and face mask when handling robotic materials including robot components, disposables, worktable, pipette tips, and reagents. DNA Database Forensic Scientists shall wear gloves when using the robot computer system.

5.1.2 Turn on the computer and the robot.

5.1.3 If the computer is already on, but not logged in, the username is “Qiagen Instruments.” Click on the username. A password is not required.

5.1.4 Open QIAsoft software. Enter username and password.

5.1.5 Prior to operating the Qiagen BioRobot®, perform any required maintenance. Ensure that any air bubbles are cleared out of the system liquid lines. If the run button is yellow, any needed maintenance is found listed in the maintenance environment.

5.1.6 To run protocols, go to Environment > Execute. Protocols used for analysis of samples on the robot are listed in the Applications menu under the Promega Protocols section and include:

5.1.6.1 PPF Sample ID Scan and Process Sheets – This protocol is used to scan sample IDs, assign tray positions, and create pre-amplification worksheets. It is used for samples that will be amplified with PowerPlex® Fusion. Tray positions are automatically assigned by the Qiagen BioRobot® for ladders and required controls (reagent blanks, negative amplification controls, and 2800Ms (positive amplification controls)).

5.1.6.2 PPF Direct Amplification – 1 Plate – This protocol is used to set up single PowerPlex® Fusion amplification reaction trays before placement on the thermal cycler. It includes the addition of Punch Solution, mastermix, and 2800M to the reaction tray. This protocol can process 1 to 86 samples and corresponding controls.

5.1.6.3 PPF Direct Amplification – 1 to 4 Plates – This protocol is used to set up between 1 and 4 PowerPlex® Fusion amplification reaction trays before placement on the thermal cycler. It includes the addition of Punch Solution, mastermix, and 2800M to the reaction trays. This protocol will process 80 samples per plate along with corresponding controls.

5.1.7 Select the desired protocol from the Promega Protocols section of the drop down applications menu. To start each selected protocol, press the “run” button. For each protocol, follow the directions on the screen.

5.1.8 Any plate run on the robot shall be logged in the appropriate binder (including water runs). Note any maintenance done when logging the run. Record any robotic errors/error codes and follow-up actions in the Error Log.

NOTE: The Forensic Scientist Manager/Supervisor and Technical Leader shall be notified in the event of a technical issue and informed of any issues with robot function. The Technical Leader shall investigate root causes.

5.1.9 After the BioRobot has added PunchSolution and mastermix to the plate, verify that the expected wells have received each reagent. Manually add PunchSolution and mastermix to any wells that did not receive the expected amount.

5.2 PPF Sample ID Scan and Process Sheets Protocol

5.2.1 When prompted, enter the number of samples to be processed. This number includes the Database samples and NIST-TS controls (if applicable).

5.2.2 Enter the Database file name/batch number using naming conventions outlined in the Procedure for DNA Database Operations. This name generally includes a prefix + year + number (e.g., COB-2011-00001).

5.2.3 One by one, scan the bar codes for the samples with the hand barcode scanner. Sample ID numbers may be manually inputted only if the barcode scanner is inoperable, the sample

does not have a barcode (e.g., NIST-TS, employee sample), or if a small number of samples (e.g., proficiency test) are to be run on the Qiagen BioRobot®.

- 5.2.4 When the protocol is finished, open the pre-amplification worksheet (DNA Database PowerPlex Fusion Punch/Amplification Sheet). It can be found on the desktop. Open the shortcut: (C:\Program Files\QIAsoft\UserData\Pre Amplification Worksheets). Open the file corresponding to the run.
- 5.2.5 Review the sample numbers to correct any scanning issues. Use the pre-amplification worksheet as a guide for punching during the amplification protocol. Record Punch Information and Amplification Reagent Information for the amplification setup. A .pdf copy of the pre-amplification worksheet shall be maintained with the run file.

5.3 PPF Direct Amplification – 1 Plate Protocol

- 5.3.1 Follow the prompts on the screen to prepare the BioRobot for amplification setup of one plate.
- 5.3.2 When adding punches to the PCR plate, follow the instructions listed in the DNA Database Section Procedure for PCR Amplification with PowerPlex® Fusion.
- 5.3.3 When the protocol is complete, cap used wells in the 96-well reaction plate and spin the plate to ensure all punches and reagents are at the bottom of the wells and no bubbles are present. Place the 96-well reaction plate onto a thermal cycler and amplify the plate as described in the DNA Database Section Procedure for PCR Amplification with PowerPlex® Fusion.
- 5.3.4 When the protocol is finished, a result file (Qiagen Amplification Report) is created. This file can be found on the desktop. Open the shortcut: (C:\Program Files\QIAsoft\UserData\ReportData). Open the file corresponding to the run.
- 5.3.5 Adjust page layout as needed to minimize the number of pages. Ensure the report is complete and accurate. A .pdf copy of the result file shall be maintained with the run file. A detailed report can be found in (C:\Program Files\QIAsoft\UserData\LogFiles).

5.4 PPF Direct Amplification – 1 to 4 Plates Protocol

- 5.4.1 Follow the prompts on the screen to prepare the BioRobot for amplification setup of one to four plates.
- 5.4.2 When adding punches to each PCR plate, follow the instructions listed in the DNA Database Section Procedure for PCR Amplification with PowerPlex® Fusion.
- 5.4.3 When the protocol is complete, cap used wells in each 96-well reaction plate. Spin each plate for 1 minute at 2000 rpm to ensure all punches and reagents are at the bottom of the wells and no bubbles are present. Place each 96-well reaction plate onto a thermal cycler and amplify each plate as described in the DNA Database Section Procedure for PCR Amplification with PowerPlex® Fusion.

5.4.4 When the protocol is finished, a result file (Qiagen Amplification Report) is created. This file can be found on the desktop. Open the shortcut: (C:\Program Files\QIAsoft\UserData\ReportData). Open the file corresponding to the run.

5.4.5 Adjust page layout as needed to minimize the number of pages. Ensure the report is complete and accurate. A .pdf copy of the result file shall be maintained with the run file. A detailed report can be found in (C:\Program Files\QIAsoft\UserData\LogFiles).

5.5 Create the 3500xL setup sheet

5.5.1 Open the desktop shortcut: (C:\Program Files\QIAsoft\UserData\ExportData) and open the excel file (.csv file) corresponding to the file name/batch number.

5.5.2 Open a copy of the 3500xL setup sheet (found on section shared drive).

5.5.3 Starting at cell 2B in the .csv file, highlight and copy the sample names.

5.5.4 Paste the copied selection into 3C of the 3500xL Plate Map tab of the .xls file. Type the file name/batch number in cell B1. Type the initials of the 3500xL operator in cell M15.

5.5.5 Record lot number information for 3500xL setup. Save the file as a .xls file in the appropriate location (C:\Program Files\QIAsoft\UserData\3500 Files). A .pdf copy of the 3500xL setup sheet shall be maintained with the run file.

5.6 Create and import the .txt file for 3500xL software

5.6.1 In the 3500xL setup sheet, select the 3500xL Import Sheet tab at the bottom. The tab auto-populates from the information in the 3500xL Plate Map tab.

5.6.2 In the 3500xL Import Sheet tab, copy cells for all rows from row 1 through the row containing the last sample.

5.6.3 Open Notepad and paste the selection into a new file. Do not copy/paste empty horizontal wells as this prevents the file from importing. Hit enter after the last sample entry so the cursor is below the last sample for the run. Save the file using the corresponding file name/batch number as a .txt file in the appropriate location (C:\Program Files\QIAsoft\UserData\3500 Files).

5.6.4 Transfer the .txt file to the appropriate 3500xL computer.

5.6.5 On the appropriate 3500xL instrument, open the Data Collection Software, Click on **Library** in the menu bar. Select **Import** toward the top of the screen. Select the .txt file corresponding to the plate to be run and click **Open**. A pop-up will give notification of the import status. Click **OK** after successful import.

5.6.6 Follow the DNA Database Section Procedure for Use of the 3500xL Genetic Analyzer to set up a 3500xL plate and run.

5.7 Analysis of Database Samples

- 5.7.1** Follow the Database Section Procedure for DNA Database Analysis and Technical Review of Database Samples Amplified with PowerPlex® Fusion to analyze and upload database profiles.

6.0 Limitations

- 6.1** At a minimum, the Qiagen BioRobot® shall be run every ten working days. If a run is not scheduled, a water run shall be performed as described in the DNA Database Section Procedure for Instrument and Equipment Quality Control.

7.0 Safety

- 7.1** The use of the procedures in this document may cause exposure to the following, and precautions as noted in the Laboratory and Section Safety Manuals shall be used:

7.1.1 Blood borne pathogens

7.1.2 Chemical hazards

7.1.2.1 Formamide is both corrosive and a teratogen.

- 7.2** This instrument contains moving parts and their movement is not always indicated. Use caution during operation.

- 7.3** Use caution when operating the bar code reader as the beam can cause serious eye damage. Avoid looking directly at the beam.

8.0 References

3500 Data Collection Software

DNA Database Section Administrative Policy and Procedure

DNA Database Section Administrative Procedure for Safety and Hazardous Waste Disposal

DNA Database Section Procedure for Sample Processing Quality Control

DNA Database Section Procedure for DNA Database Analysis and Technical Review of Database Samples Amplified with PowerPlex® Fusion

DNA Database Section Procedure for DNA Database Operations

DNA Database Section Procedure for DNA Database Training for PowerPlex® Fusion

DNA Database Section Procedure for DNA Reagent Quality Control

DNA Database Section Procedure for Instrument and Equipment Quality Control

DNA Database Section Procedure for PCR Amplification with PowerPlex® Fusion

DNA Database Section Procedure for Use of the 3500xL Genetic Analyzer

Protocols for PPF Sample ID Scan and Process Sheets, PPF Direct Amplification – 1 Plate, and PPF Direct Amplification – 1 to 4 Plates

Qiagen BioRobot® 8000 User Manual

QIAsoft 5 Operating System User Manual

State Crime Laboratory Safety Manual

9.0 Records

- BioRobot® DB Runs Notebooks
- Performance Check QC Documentation Notebooks
- BioRobot® Log Notebooks
- BioRobot® Procedure Notebook

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
08/03/2015	1	Original Document
12/21/2015	2	5.1.9 include manually adding reagents; 5.4.3 specified spin time/rpm