



BIOROBOT 3000 OPERATING PROCEDURE

The Qiagen Biorobot 3000 has been customized to set up amplification reactions using as DNA source DNA samples extracted from the Qiagen Biorobot 9604 and contained in the 96 microtube collection racks. Alternatively, eppendorf tubes can be used (24 tube rack) using a specific protocol "PCR setup (24 well cooling block)", which is still under preparation.

The slot lay out of the Biorobot 3000 is as follows:

MP1	MP4	T1	T3
MP2	MP5	T2	T4
MP3	MP6		T5

A	1	2	3	4	5	6
B						
C						(E block)
D						

OR

B5	B1	B2	B3	B4
B6				
B7		B9		B10 (B block)
B8				

MP = sample rack positions

T = tip rack positions

E = Eppendorf reagent block, used for **PCR set up** protocol

B = reagent tube block, used for **PCR setup_1plate at MP2** protocol



MATERIALS

PCR tubes: Robbins Scientific Corporation, Cycleplate-96ET, Thin wall PCR plate non sterile (Cat # 1055-00-0) or equivalent.

PCR Caps: Robbins Scientific Corporation, Strip-ease-12 PCR Caps, colorless non sterile (Cat # 1044-72-0) or equivalent.

Tips: Qiagen 960 Disposable Filter tips, Conductive 300 uL (Cat # 990232). It is important that **filter** tips be used to prevent any possible leak from the probes following the washing steps into the sample tubes.

PROTOCOLS

Two protocols are currently in use:

1. PCR setup and
2. PCR setup_1 plate at MP2.

PROTOCOL: PCR setup

This protocol is designed to provide more flexibility and automation to the user as compared to the “PCR setup_1 plate at MP2” protocol. With this protocol, up to two DNA source racks can be used (at MP1 and MP4, respectively) to set up from one to four destination plates for Powerplex1.1 (PP1.1) and Powerplex2.1 (PP2.1) systems as follows:

1. MP1 → MP2 + MP3
DNA source PP1.1 samples PP2.1 samples
2. MP4 → MP5 + MP6
DNA source PP1.1 samples PP2.1 samples

The first step of the protocol is the distribution of 12 µl of the master mix prepared as indicated in the “Robot amp setup” lab form. The second step is the transfer of 1 µl of DNA from each tube from the source DNA rack to the respective tube of the PCR rack. The probes are washed extensively during transfer between DNA samples. The source DNA rack positions E12 and F12 are reserved for negative PCR control (no DNA) whereas the G12 and H12 positions are reserved for the positive PCR control (K562 DNA solution). Each DNA source rack contains only water in these four positions. Each PCR plate is



completed before a second one begins to be processed in the order indicated by the diagram above. Also, the DNA source rack at MP1 is processed first and after its completion for both systems (PP1.1 and PP2.1), MP4 is processed.

Another feature of this protocol is that the control K562 is added automatically. For this purpose, diluted K562 DNA (1 to 8 dilution, i.e. 125 μ l K562 DNA added to 875 μ l TE for a total volume of 1000 μ l) is placed in an eppendorf tube at the D6 position of the E reagent block. If two source plates are used, A1 and A2 of the E block are reserved for PP1.1 master mix and B1 and B2 of the E block are reserved for PP2.1 master mix (2,400 μ l from each master mix are prepared in a 15 ml conical tube and then 1,200 μ l is aliquoted to each of two eppendorf tubes).

Note: The protocol "PCR set up" is designed for use of **identically selected** wells on both DNA source plates, if two source plates are desired. For example, the robot cannot process a run with all 96 wells selected on the first plate and some wells on the second.

Protocol Run:

A step to step operation of the PCR setup protocol is as follows:

1. Empty the Waste Liquid Bottle
2. Fill the System Liquid Reservoir with distilled water up to the maximum level.
The tubing that connects these tanks with the robot needs to be taut to prevent back flashes.
3. Dispose of the tip waste bag in the biohazard waste if full and replace with a new one. If half full make sure that the used tips are not clogging the entrance to the bag (push the used tips all the way to the bottom of the bag).
4. Make sure that T racks have tips (you will need all T racks filled if all 4 destination slots are used)
5. Place the DNA source rack(s) at MP1 and MP4 slots (secure the tubes on the racks with tape to prevent lifting of the tube during probe movement)
6. Place the PCR tube racks at MP2, MP3, MP5 and MP6 slots (similarly secure the plastic racks on the metal racks of the slots). MP2 and MP5 are for PP1.1 setups whereas MP3 and



MP6 are for PP2.1 reaction plates.

7. **NOTE: The E reagent block is used with this protocol!** Place the master mixtures (1,200 μ l in each of two 1.5 ml eppendorf tubes) at A1 and A2 positions (for PP1.1) and B1 and B2 positions (for PP2.1) of the E block. The diluted K562 DNA solution is assigned to position D6 of the E block.
8. Turn the robot on (the switch is on the back in the bottom right corner)
9. Turn computer on and follow the sign “**Press Ctrl+Alt+Delete to log on**”
10. Screen displays: **Logon Information**
 User name: Administrator
 Password: (leave blank)
 Click **OK**
11. **Shortcut to Qiasoft 4.0** opens automatically.
At this point you need to import your file. Your file is originally generated as an excel file and saved in a csv format on a floppy disk titled “Worklist transport disk”. This is the same format that is used for the Biorobot 9604. Therefore you need to copy this file in the User Data folder of the program as follows: Go to Start, select Programs, select Windows NT Explorer, select C: drive, select Program Files, select Qiasoft 4.0 and select User Data. Copy your file in this folder. Exit the window and you are back to the program.
12. If the correct protocol is displayed then click **RUN**. Otherwise, select the protocol by clicking the black arrow left from the green arrow for **RUN**

From the different choices displayed select **Custom Applications** and then **PCR setup**
13. From **Tools** select **Flush System**
Flash system: parameters opens up
By clicking the arrow next to **Select pump type** : select **Wash Pump**
Amount of liquid (mL): 15 Click **OK**
14. Again from **Tools** select **Flush System**
Flash system: parameters opens up
Again from **Select pump type** : select **Dilutor(s)**
Number of strokes: 15



- Also from **Select Probe to flush:** leave marked the **Specify probe: All Tip probes**
Click **OK**
15. Repeat steps 13 and 14 making sure that there are no bubbles in the pump and dilutor tubing
 16. Click **RUN**.
 17. Screen displays: **Run Protocol: Layout Configuration**
Collection tubes+plates in cooling blocks
Click **OK**
 18. Screen displays: **Run Protocol: Select samples** (Select the wells)
Selection mode: By column
Click **OK**
 19. On screen **Display message:**
This message is a reminder (see step 11) to save the data/report in the User Data directory. Ignore the rest of the message. Click **Next**
 20. On screen: **Display message:**
Fill system liquid reservoir with distilled water.
Empty waste container. Refill tip racks with 300 µl tips

This is a reminder to make sure that steps 1-4 have been followed.
Click **Next**
 21. On screen: **Variable Input:**
Operator: (Enter your initials) Click **Next**
 22. On screen: **Variable Input:**
report: (Enter the name of your report, i.e. "DB01-01.txt" for one source DNA rack, or "DB01-01 and DB01-02.txt" if two source DNA racks are used)
Click **Next**
 23. On screen: **Variable Input:**
SourcePlates= (Enter the number of DNA source plates)



Click **Continue**

24. On screen: **Variable Input:**
DestPlatesforS1= (enter the number of destination plates to create from the first DNA source plate) Click **Continue**
25. On screen: **Variable Input:**
DestPlatesforS2= (enter the number of destination plates to create from the second DNA source plate) Click **Continue**
26. On screen: **Variable Input:**
Template ID1= (Enter the first DNA source ID, i.e. "DB01-01")
Click **Continue**
27. On screen: **Variable Input:**
Template ID2= (Enter the second DNA source ID, i.e. "DB01-02")
Click **Continue**
28. On screen: **Variable Input:**
PosTabName= (Enter the name of the imported csv file (see step 11) of the first DNA source plate, i.e. "DB01-01.csv")
Click **Continue**
29. On screen: **Variable Input:**
PosTab2Name= (Enter the name of the imported csv file (see step 11) of the second DNA source plate, i.e. "DB01-02.csv") Click **Continue**
30. On screen: **Variable Input:**
PCRID11= (Enter the ID of the first PCR plate you wish to generate from the first DNA source, i.e. "DB01-1-01" for PP1.1)
Click **Continue**
31. On screen: **Variable Input:**
PCRID12= (Enter the ID of the second PCR plate you wish to generate from the first DNA source, i.e. "DB01-2-01" for PP2.1) Click **Continue**



32. On screen: **Variable Input:**
PCRID21= (Enter the ID of the first PCR plate you wish to generate from the second DNA source, i.e. "DB01-1-02" for PP1.1) Click **Continue**
33. On screen: **Variable Input:**
PCRID22= (Enter the ID of the second PCR plate you wish to generate from the second DNA source, i.e. "DB01-2-02" for PP2.1) Click **Continue**
34. On screen: **Display message:**
This is a reminder that steps 5-7 have been followed
Click **Continue**
35. Run begins.
- The run lasts approximately 40 min. The progress of every step is displayed and described on the screen.
- The wells change to yellow on the screen as master mix is added in them. When DNA is transferred they change to green. Temporarily, in blue color are shown the ones ready to be processed next.
36. At the end of the protocol click on the beeper icon to stop beeping. Note at the bottom of the screen where your report can be retrieved from: C:-Program files-Qiasoft 4.0-User data-Reports. The report has been saved as a text file i.e. "DB01-01.txt" (for one DNA source plate) or "DB01-01 and DB01-02.txt" (for two DNA source plates). Then click **Continue**
37. On screen: **Display message:**
Protocol finished! Click **Continue**
38. Exit the program, go to "Reports" under "User data" and copy your report on a floppy titled "Reports" to print it later.

Notes:



-It is important to hold the probes and the robot arm with one hand while turning the robot off with the other. Gently direct these parts to rest on the same level anywhere on the platform. If this measure is not taken the probes will rapidly crash on the platform and that may damage the robot functions permanently.

-If during the probe washes the wash tank overflows, a fine regulation may be necessary at the pump: adjust the flow rate by tightening the screw located at left bottom of the pump.

PROTOCOL: PCR setup_1 plate at MP2

This protocol uses one source slot (MP1) and one destination slot (MP2). The DNA source rack is positioned at MP1 slot and the 96 PCR tube rack at MP2 slot. In the beginning of the protocol, 12 μ l of the master mix prepared as indicated in the "Robot amp setup" lab form are distributed from an eppendorf tube at B5 slot of the B reagent block to each of the PCR tubes at MP2 slot. The following step is the transfer of 1 μ l of DNA from each tube from the rack at MP1 to the respective tube of the rack at MP2. The probes are washed extensively during transfer between DNA samples. The rack positions E12 and F12 are reserved for negative PCR control (no DNA) whereas the G12 and H12 positions are reserved for the positive PCR control (0.13 μ l of K562 DNA solution per tube) which are added manually when the protocol finishes its run. The DNA source rack contains only water in these four positions. Besides the manual K562 addition, another drawback for this protocol is that only one DNA rack can be processed at a time which produces only one master mix setup, either PowerPlex1.1 or PowerPlex2.1.

Protocol Run:

A step to step operation of the PCR setup_1 plate at MP2 protocol is as follows:

1. Empty the Waste Liquid Bottle
2. Fill the System Liquid Reservoir with distilled water up to the maximum level. The tubing that connects these tanks with the robot needs to be taut to prevent back flashes.



3. Dispose the tip waste bag in the Biohazard waste if full and replace with a new one. If half full make sure that the used tips are not clogging the entrance to the bag (push the used tips all the way to the bottom of the bag).
4. Make sure that T1 and T2 racks have tips
5. Place the DNA source rack at MP1 slot (secure the tubes on the rack with tape to prevent lifting of the tubes during probe movement)
6. Place the PCR tube rack at MP2 slot (similarly secure the plastic rack on the metal rack of the slot)
7. Place the master mix at B5. **NOTE: Reagent block B is used for this protocol!**
8. Turn the robot on (the switch is on the back in the bottom right corner)
9. Turn computer on and follow the sign "**Press Ctrl+Alt+Delete to log on**"
10. Screen displays: **Logon Information**
User name: Administrator
Password: (leave blank)
Click **OK**
11. **Shortcut to Qiasoft 4.0** opens automatically.
At this point you need to import your file. Your file is originally generated as an excel file and saved in a csv format on a floppy disk titled "Worklist transport disk". This is the same format that is used for the Biorobot 9604. Therefore you need to copy this file in the User Data folder of the program as follows: Go to Start, select Programs, select Windows NT Explorer, select C: drive, select Program Files, select Qiasoft 4.0 and select User Data. Copy your file in this folder. Exit the window and you are back to the program.
12. Select the protocol to run by clicking the black arrow left from the green arrow for **RUN**
(If the correct protocol is displayed then click **RUN**)
From the different choices displayed select **Custom Applications**
and then **PCR setup_1 plate at MP2**



13. From **Tools** select **Flush System**
Flash system: parameters opens up
By clicking the arrow next to **Select pump type** : select **Wash Pump**
Amount of liquid (mL): 15 Click **OK**
14. Again from **Tools** select **Flush System**
Flash system: parameters opens up
Again from **Select pump type** : select **Dilutor(s)**
Number of strokes: 15
Also from **Select Probe to flush**: leave marked the **Specify probe: All probes**
Click **OK**
15. Repeat steps 13 and 14 making sure that there are no bubbles in the pump and dilutor tubing
16. Click **RUN**
17. Screen displays: **Run Protocol: Layout Configuration**
Default configuration Click **OK**
18. Screen displays: **Run Protocol: Select samples** (Select the desired wells)
Selection mode: By column Click **OK**
19. On screen: **Display message:**
Fill system liquid reservoir with distilled water.
Empty waste container. Refill tip racks with 300 µl tips

This is a reminder to make sure that steps 1-4 have been followed.
Click **Next**
20. On screen: **Variable Input:**
Operator: (Enter your initials) Click **Next**
21. On screen: **Variable Input:**
report: (Enter the name of your report, i.e. "DB01-01 ")
Click **Next**
22. On screen: **Variable Input:**
sourceno= 1 (is the number of DNA source plates)



Click **Continue**

23. On screen: **Variable Input:**
templateID1= (enter the name of the DNA source plate, i.e. "DB01-01 ")
Click **Continue**
24. On screen: **Variable Input:**
plateno1=1 (is the number of destination plates from the DNA source plate)
Click **Continue**
25. On screen: **Variable Input:**
PCRID11=(enter the name of the destination plate, i.e. "DB01-1-01 " for Powerplex 1.1 or "DB01-2-01 " for Powerplex 2.1)
Click **Continue**

Steps 26-28 below are reminders of the preparation steps 5-7

26. On screen: **Display message:**
Place the first 96 well collection tube rack (ID= i.e. "DB01-01 ") containing sample DNA into the position MP Slot1
Click **Continue**
27. On screen: **Display message:**
Place the first PCR amplification plate (ID= i.e. "DB01-1-01 ") into the MP Slot 2 position
Click **Continue**
28. On screen: **Display message:**
Place the 1.5 ml tube containing 1267.20 µl of the master mix and water for PCR plate 1 into Reagent Slot B5
Click **Continue**

Note: the program calculates the theoretical volume needed for the master mix adding extra buffer to account for the waste volumes. Empirically, 1200 µl have been shown to be adequate.

29. Run begins.



-The run lasts approximately 10 min. The progress of every step is displayed and described on the screen.

-The wells change to yellow on the screen as master mix is added in them. When DNA is transferred they change to green. Temporarily, in blue color are shown the ones ready to be processed next.

30. At the end of the protocol click on the beeper icon to stop beeping. Note at the bottom of the screen where your report can be retrieved from: C:-Program files-Qiasoft 4.0-User data. The report has been saved as a text file i.e. "DB01-01.txt". Then click **Continue**
31. On screen: **Display message:**
Protocol finished! Click **Continue**
32. Exit the program, go to User data and copy your report on a floppy titled "Reports" to print it later.
33. Do not forget to add manually 0.13 uL of K562 to G12 and H12 positions of the PCR rack.

Notes:

-It is important to hold the probes and the robot arm with one hand while turning the robot off with the other. Gently direct these parts to rest on the same level anywhere on the platform. If this measure is not taken the probes will rapidly crash on the platform and that may damage the robot functions permanently.

-If during the probe washes excess the wash tank overflows, a fine regulation may be necessary at the pump: adjust the flow rate by tightening the screw located at left bottom of the pump.

Revision History		
Effective Date	Revision Number	Reason

NCSBI MOLECULAR GENETICS SECTION
Technical Procedures Manual
BioRobot 3000 Operating Procedure- Revision 01



August 30, 2001	00	Original Document
November 26, 2001	01	1) Update Protocol for clarity; no substantiative changes