Biomek® 3000 GenomeLab Gene Expression Methods

Quick Start Guide

Beckman Coulter PN A48441
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Beckman Coulter, Inc
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Beckman Coulter, SAGIAN Operations
Indianapolis, Indiana
www.beckmancoulter.com

Warranty and Returned Goods Requirements

All standard Beckman Coulter, Inc. policies governing returned goods apply to this product. Subject to the exceptions and upon the conditions stated below, the Company warrants that the products sold under this sales agreement shall be free from defects in workmanship and materials for one year after delivery of the products to the original Purchaser by the Company, and if any such product should prove to be defective within such one year period, the Company agrees, at its option, either (1) to correct by repair or at the Company's election by replacement, any such defective product provided that investigation and factory inspection discloses that such defect developed under normal and proper use, or (2) to refund the purchase price. The exceptions and conditions mentioned above are as follows:

a. Components or accessories manufactured by the Company which by their nature are not intended to and will not function for one year are warranted only to reasonable service for a reasonable time. What constitutes a reasonable time and a reasonable service shall be determined solely by the Company. A complete list of such components and accessories is maintained at the factory.

b. The Company makes no warranty with respect to components or accessories not manufactured by it. In the event of defect in any such component or accessory, the Company will give reasonable assistance to Purchaser in obtaining from the manufacturer's own warranty.

c. Any product claimed to be defective must, if required by the Company, be returned to the factory, transportation charges prepaid, and will be returned to Purchaser with transportation charges collect unless the product is found to be defective, in which case the product must be properly decontaminated of any chemical, biological, or radioactive hazardous material.

d. The Company shall be released from all obligations under all warranties, either expressed or implied, if any product covered hereby is repaired or modified by persons other than its own authorized service personnel, unless such repair by others is made with the written consent of the Company.

e. If the product is a reagent or the like, it is warranted only to conform to the quantity and content and for the period (but not in excess of one year) stated on the label at the time of delivery.

It is expressly agreed that the above warranty shall be in lieu of all warranties of fitness and of the warranty of merchantability, and that the company shall have no liability for special or consequential damages of any kind or from any cause whatsoever arising out of the manufacture, use, sale, handling, repair, maintenance, or replacement of any of the products sold under the sales agreement.

Representatives and warranties made by any person, including dealers and representatives of the Company, which are consistent or in conflict with the terms of this warranty, shall not be binding upon the Company unless reduced in writing and approved by an expressly authorized officer of the Company.

Parts replaced during the warranty period are warranted to the end of the instrument warranty.

Note: Performance characteristics and specifications are only warranted when Beckman Coulter replacement parts are used.

Safety Information

WARNING: If the equipment is used in a manner not specified by Beckman Coulter, Inc., the protection provided by the equipment may be impaired.

Warning and Caution Definitions

All Warnings and Cautions in this document include an exclamation point, a lightning bolt, or a light burst symbol framed within a triangle.

The exclamation point symbol is an international symbol which serves as a reminder that all safety instructions should be read and understood before installation, use, maintenance, and servicing is attempted.

WARNING: A WARNING calls attention to a condition or possible situation that could cause injury to the operator.

CAUTION: A CAUTION calls attention to a condition or possible situation that could damage or destroy the product or the operator's work.

When this symbol is displayed in this manual, pay special attention to the specific safety information associated with the symbol.

Electrical Safety

To prevent electrically related injuries and property damage, properly inspect all electrical equipment prior to use and immediately report any electrical deficiencies. Contact a Beckman Coulter Service Engineer for any servicing of equipment requiring the removal of covers or panels.

High Voltage

This symbol indicates the potential of an electrical shock hazard existing from a high voltage source and that all safety instructions should be read and understood before proceeding with the installation, maintenance, and servicing of all modules.

Do not remove system covers. To avoid electrical shock, use supplied power cords only and connect to properly grounded (three-holed) wall outlets. Use only multiplug power strips provided by the manufacturer.

Disposal of Electronic Equipment

It is important to understand and follow all laws regarding the safe and proper disposal of electrical instrumentation.

The symbol of a crossed-out wheeled bin on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates that:

- the device was put on the European Market after August 13, 2005.
- the device is not to be disposed via the municipal waste collection system of any member state of the European Union.

For products under the requirement of WEEE directive, please contact your dealer or local Beckman Coulter office for the proper decontamination information and take back program, which will facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.

Laser Light

This symbol indicates that a potential hazard to personal safety exists from a laser source. When this symbol is displayed in this manual, pay special attention to the specific safety information associated with the symbol.

Laser Specifications

Laser Type: Class II Laser Diode

Maximum Output:11 mW

Wavelength: 670 nm

Chemical and Biological Safety

Normal operation of the instrument may involve the use of materials that are toxic, flammable, or otherwise biologically harmful. When using such materials, observe the following precautions:

- Handle infectious samples according to good laboratory procedures and methods to prevent the spread of disease.
- Observe all cautionary information printed on the original solutions containers prior to their use.
- Dispose of all waste solutions according to your facility's waste disposal procedures.
- Operate the instrument in accordance with the instructions outlined in this manual, and take all the necessary precautions when using pathological, toxic, or radioactive materials.
- Splashing of liquids may occur; therefore, take appropriate safety precautions, such as using safety glasses and wearing protective clothing, when working with potentially hazardous liquids.
- Use an appropriately contained environment when using hazardous materials.
- Observe the appropriate cautionary procedures as defined by your safety officer when using flammable solvents in or near a powered-up instrument.
- Observe the appropriate cautionary procedures as defined by your safety officer when using toxic, pathological, or radioactive materials.

Note: Observe all warnings and cautions listed for any external devices attached or used during operation of the instrument. Refer to applicable external device user's manuals for operating procedures of that device.

Moving Parts

To avoid injury due to moving parts, observe the following:

- Never attempt to exchange labware, reagents, or tools while the instrument is operating.
- Never attempt to physically restrict any of the moving components of the instrument.
- Keep the instrument work area clear to prevent obstruction of the movement.

Cleaning

Observe the cleaning procedures outlined in this user's manual for the instrument. Prior to cleaning equipment that has been exposed to hazardous material:

- Appropriate Chemical and Biological Safety personnel should be contacted.
- The Chemical and Biological Safety information contained in this user's manual should be reviewed.

Maintenance

Perform only the maintenance described in this manual. Maintenance other than that specified in this manual should be performed only by service engineers.

Important

It is your responsibility to decontaminate components of the instrument before requesting service by a Beckman Coulter Service Engineer or returning parts to Beckman Coulter for repair. Beckman Coulter will NOT accept any items which have not been decontaminated where it is appropriate to do so. If any parts are returned, they must be enclosed in a sealed plastic bag stating that the contents are safe to handle and are not contaminated.

安全信息

本文件中的所有 “警告”和 “当心”信息均附有惊叹号、闪电符号或三角形内的闪光符号。请特别注意与此类符号相关的具体安全信息。

警告：如果以 Beckman Coulter,Inc. 未说明的方式使用器械，器械提供的保护可能受到损害。

警告和当心定义

本文件中的所有 “警告”和 “当心”信息均附有惊叹号、闪电符号或三角形内的闪光符号。

惊叹号符号是国际通用符号，用于提醒用户在尝试安装、使用、维护和修理之前阅读和理解所有安全说明。

警告：警告提醒用户注意可能对操作员造成伤害的状况或可能的情形。

小心：当心提醒用户注意可能损坏或毁坏产品或操作员工作的状况或可能的情形。

当本手册中显示该符号时，请特别注意与该符号相关的具体安全信息。

电气安全

为了预防与电气相关的伤害和财产损坏，请在使用前以适当方式检查所有电气设备，并立即报告任何电气缺陷。任何要求移除盖板或面板的器械维修操作均须与 Beckman Coulter 维修服务人员联系。

高压

该符号表示可能存在高压电源电击危险，在进行所有模块安装、维护和修理之前应阅读和理解所有的安全说明。

请勿移除系统盖板。为了避免电击，仅限使用提供的电源线，并与适当接地（三孔）壁式插座连接。仅限使用制造商提供的多插头电源板。

电子设备弃置

理解和遵循所有有关电气设备安全和适当弃置的法规十分重要。

欧盟《废弃电气和电子设备（WEEE）指令》要求产品带有打叉轮箱符号。产品上显示该符号表示：

- 该设备于 2005 年 8 月 13 日之后投放欧洲市场。
- 该设备不得通过欧盟任何成员国的市政废品收集系统弃置。

如需获得有关 WEEE 指令要求产品的适当防污染和回收计划信息，请与您的经销商或地方办事处联系，他们可向您提供适当的搜集、治疗、康复、设备回收和安全弃置的信息。

化学与生物安全

设备的正常操作可能涉及使用有毒、易燃或在其他方面具有生物有害性的材料。使用此类材料时，请遵守以下注意事项：

- 依照妥善的实验室程序和方法处理传染性样品，以防传播疾病。
- 使用前遵守原溶液容器上印制的所有注意事项信息。
- 按照您所在设施的废品弃置程序弃置所有废溶液。
- 按照本手册中的说明操作设备，在使用病理性、有毒或放射性材料时，采取所有必要的防范措施。
- 液体可能溅洒；因此，在使用具有潜在危险的液体时采取适当的安全措施，例如佩戴安全镜和穿戴保护服。
- 在适当隔离的环境中使用有害材料。
- 在通电器械中或附近使用易燃液体时，应遵守安全管理人员规定的适当防范程序。
- 在使用有毒、病理性或放射性材料时，应遵守安全管理人员规定的适当防范程序。

附注：在设备操作过程中遵守所有附加或使用设备的警告和当心提示。查阅适用的器械和设备使用手册中有关该设备的操作程序说明。

移动部件

为了避免移动部件造成伤害，请遵守以下规定：

- 切勿在器械操作过程中尝试更换实验室器皿、反应物或工具。
- 切勿尝试以物理形式限制设备的任何移动部件。
- 保持工作场所无障碍物，防止阻碍设备移动。

清洁

遵守本用户手册中规定的清洁程序。清洁接触有害材料的器械之前：

- 应与适当的化学和生物安全工作人员联系。
- 应查阅本用户手册中包含的化学和生物安全信息。

维护

仅限执行本手册中说明的维护程序。本手册中未说明的维护程序应由 Beckman Coulter 维修服务代表执行。

重要事项

在请求 Beckman Coulter 维修服务代表维修或将部件送回 Beckman Coulter 修理之前，您有责任对部件消毒。 Beckman Coulter 不接受需要消毒但尚未消毒的任何物品。如果退回任何部件，必须将部件装入密封的塑料袋内，并说明袋内的物品无污染，可安全处理。

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1.1 Overview

The Biomek® 3000 Gene Expression Suite of Methods is designed to quantitate and normalize extracted RNA, prepare a reaction plate, and prepare sample and buffer plates for analysis with the GenomeLab™ GeXP Genetic Analysis System. The platform for these methods is the Biomek® 3000 Automation Workstation with a Static Peltier Device, a Shaking Peltier Device and an optional on deck DNA Engine (PTC-200) Thermocycler.

Three methods make up the Biomek® 3000 Gene Expression Suite of Methods:

- *Quantitation and Normalization Method* (Section 1.2)
- *Reaction Setup Method* (Section 1.3)
- *Plate Preparation Method* (Section 1.4)

1.2 Quantitation and Normalization Method

The Quantitation and Normalization method utilizes either absorbance at 260nm or sensitive fluorescent dyes to quantitate nucleic acids in a full or partial 96-well plate. An integrated calculations template provided within the method offers quick quantitation results for the sample plate, and conversion of this data for normalization. Other options available in the user interface allow the extraction to be customized for specific needs. A guide to reagent volume and location is given in the Quant and Norm Reservoir Volumes step and is based on user defined parameters from the user interface.

This section of the quick-start guide is divided into the following sections:

- *Materials Required* (Section 1.2.1)
- *Quantitation and Normalization Method Procedure* (Section 1.2.2)

1.2.1 Materials Required

Performing the Quantitation and Normalization method on the Biomek 3000 Workstation requires the following equipment, reagents, labware, and other supplies.

Note: Part numbers are listed in the following sections for convenience. Third party vendors may change part numbers. Confirm part numbers with the vendor before ordering.

1.2.1.1 Equipment

Running the Quantitation and Normalization method requires the following instruments:

- Biomek 3000 Laboratory Automation Workstation with integrated:
  - Static Peltier Device
  - Shaking Peltier Device
  - DNA Engine (PTC-200) Thermocycler (optional)
  - MP200 Tool
  - P200L Tool
  - Gripper Tool
- DTX-880 Multimode Detector
- Microsoft Office Excel

Note: The macro security in Microsoft Excel must be set to **Medium** or **Low** to work with this method. The macro security setting is found on the Security tab of the Options dialog in Microsoft Excel.

1.2.1.2 Reagents

The following reagents are used in the Quantitation and Normalization method. The volume of reagent required varies depending on the number of samples processed:

Table 1-1. Reagent Requirements

Reagent	Ordering Information
Quant-iT™ PicoGreen® dsDNA Assay Kit	Invitrogen, PN P-7589
Quant-iT™ RiboGreen® RNA Assay Kit	Invitrogen, PN R-11490
Nuclease-Free Deionized Water	

1.2.1.3 Tips and Labware

The following tips and labware are used in the Quantitation and Normalization method. The quantity of each required depends on the number of samples processed.

Table 1-2. Tip and Labware Requirements

Tips and Labware	Ordering Information
Biomek AP96 P250 Pipette Tips, presterile	Beckman Coulter, PN 717252
Biomek AP96 P20 Pipette Tips, presterile	Beckman Coulter, PN 717255
96-Well Sample Microtiter V-Bottom Thermocycler-Compatible Polypropylene Plate	Beckman Coulter, PN 609801
24-Position Tube Rack with 1.5 mL (white) Inserts	Beckman Coulter, PN 373661
Diameter Insert, 11mm, White, for 1.5 mL Microfuge Tubes	Beckman Coulter, PN 373696
Polypropylene Tubes with Snap-On Caps, 1.5 mL	Beckman Coulter, PN 356090
Modular Reservoir, Half Module	Beckman Coulter, PN 372709
Modular Reservoir Frame	Beckman Coulter, PN 372795
Costar 96-Well Clear Round Bottom Polystyrene Plate	Corning, PN 3795
Corning 96-Well Half Area UV Plate	Corning, PN 3679
Greiner 96-Well UV Plate	Greiner Bio-One, PN 655801
Greiner CellStar 96-Well Plate	Greiner Bio-One, PN 655079

1.2.1.4 Other Supplies

The following additional supplies are used in the Quantitation and Normalization method.

- Calibrated pipettors—P20, P200, and P1000.
- Nuclease-free barrier pipette tips.
- Pipette-aid.
- Sterile, plugged disposable serological pipettes (5 mL, 10 mL, and 25 mL).
- Sterile conical tubes, 15 mL and 50 mL (BD Falcon 352095/352073 or equivalent)
- RNase ZAP solution, 250 mL bottle (Ambion 9780).
- RNase ZAP Wipes (Ambion 9786)
- DNA Away (Molecular BioProbes, cat#7010)

1.2.2 Quantitation and Normalization Method Procedure

The Quantitation and Normalization method is a Biomek Software method that quantitates and normalizes nucleic acid samples. To set up the instrument and run the Quant and Norm method:

Note: Prior to the setting up and running the method, wipe down the instrument, deck, and tools with RNase zap or DNA Away to ensure work is done in a nuclease-free environment.

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1. Turn on the Biomek 3000 instrument and open **Biomek Software**.
2. Turn on power to the Shaking Peltier Device and the Static Peltier Device.
3. From the Instrument menu, select **Home All Axes**.
4. Open the Quantitation and Normalization method in Biomek Software.

5. Select the **Quant and Norm User Interface** step (Figure 1-1).

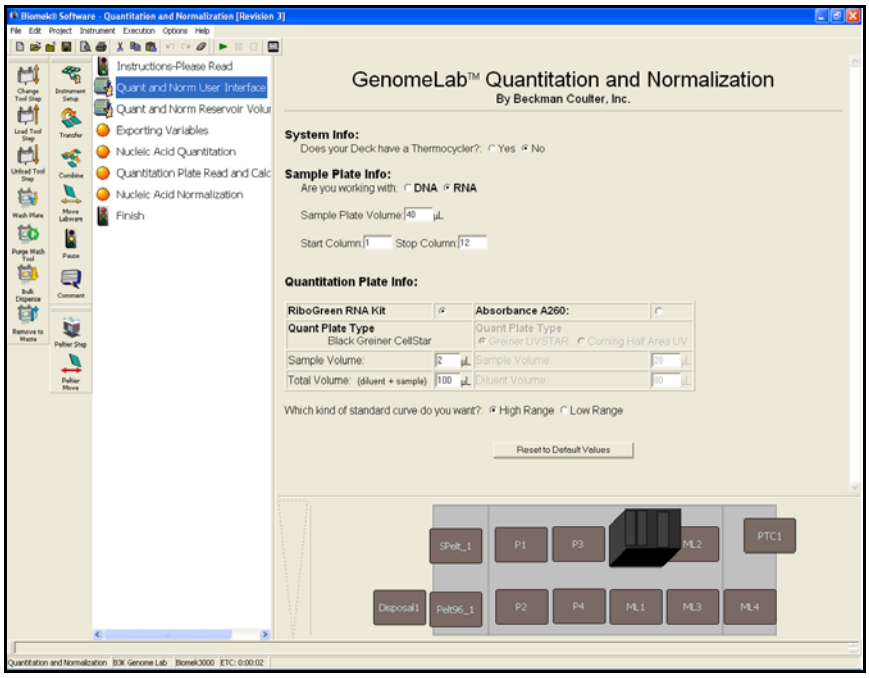


Figure 1-1. Quant and Norm User Interface step

6. In System Info, specify whether or not the deck includes an integrated DNA Engine (PTC-200) Thermocycler.
7. In Sample Plate Info, specify the nucleic acid type: **DNA** or **RNA**.
8. In Sample Plate Volume, enter the volume of sample in the sample plate in µL.
9. In Start Column, specify the first column that contains samples.
10. In Stop Column, specify the last column that contains samples. The Stop Column must be equal to or greater than the Start Column and less than or equal to the total number of columns on the plate (12).

Note: Samples must be contained in adjacent columns.

11. In Quantitation Plate Info, select to quantitate using fluorescence and configure the fluorescence options:

Note: The fluorescence option changes between PicoGreen DNA Kit or RiboGreen RNA Kit, depending on the the sample type selected in Sample Plate Info.

a. In Sample Volume, enter the volume of sample to transfer to the quantitation plate.

Note: Choose an appropriate Sample Volume for the quantitation plate such that the expected concentration falls within the range of the selected standard curve.

b. In Total Volume, enter the total volume of sample plus diluent to transfer to each well of the quantitation plate.

c. Specify the type of standard curve to use, based on information from the Quant iT RiboGreen or PicoGreen protocols: **High Range** or **Low Range**.

Note: Concentrations for the standard curve can be found in the Quant and Norm Reservoir Volumes step.

OR

In Quantitation Plate Info, select to quantitate using absorbance and configure the absorbance options:

d. In Sample Volume, enter the volume of sample to transfer to the quantitation plate.

e. In Diluent Volume, enter the volume of diluent to transfer to each well of the quantitation plate.

f. Specify the Quant Plate Type:

- Greiner UV STAR
- Corning Half Area UV

Note: At any time, choose the **Reset to Default Values** to restore all options in the Quant and Norm User Interface step to the default values:button resets the Sample Plate volume to 40 µL and the columns to 1-12. The Quantitation Plate Information is reset to 2 µL of sample, 100 µL total volume, and High Range standard curve for fluorescence, or 20 µL of sample, 80 µL of diluent, and Greiner UV STAR for absorbance.

12. Select the **Quant and Norm Reservoir Volumes** step (Figure 1-2). The **Quant and Norm Reservoir Volumes** step provides basic instructions for preparing the reagents and filling labware for the method.

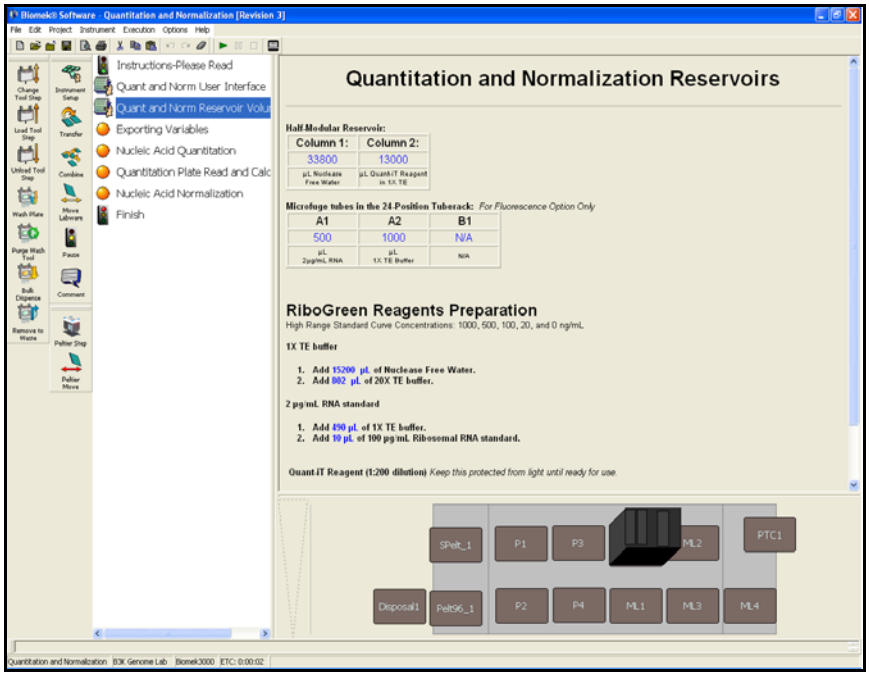


Figure 1-2. Quant and Norm Reservoir Volumes step

13. If using Fluorescence, prepare all kit reagents as described in the Quant and Norm Reservoir Volumes step.

    a. Prepare a 1X TE buffer solution by diluting the 20X TE buffer solution included in the kit with nuclease-free water.

    b. Prepare the kit standard according to the formulation for the RNA or DNA standard and place in a 1.5 mL microfuge tube.

    c. Prepare the Quant-iT reagent.

14. Add reagents to the half-modular reagent reservoir:

    a. Add the volume specified of nuclease-free water to the first column of the modular reservoir.

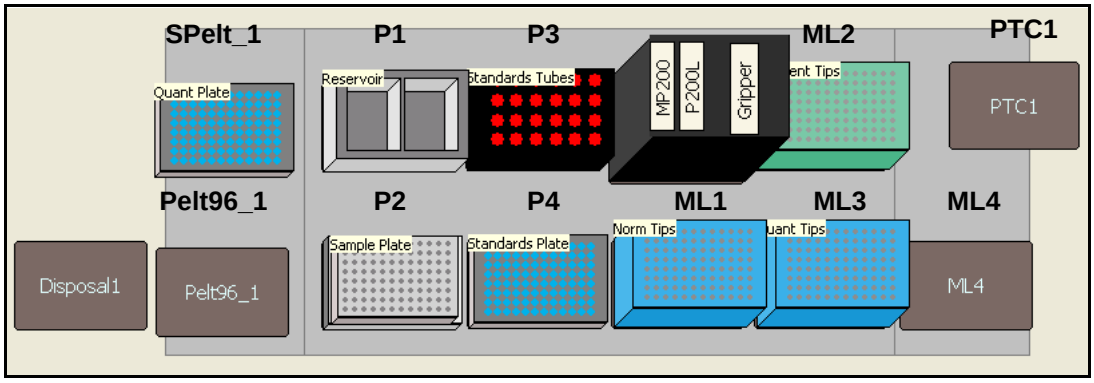
    b. If using fluorescence, add the volume specified of Quant-iT reagent to the second column of the modular reservoir. Otherwise, the second column is left empty.

> **Note:** The Quant-iT reagent is light sensitive and should be kept protected from light and added just prior to use. For more detailed information on preparing Quant-iT reagents, refer to the appropriate Quant-iT protocol.

15. If using fluorescence, prepare the 24-position tube rack:
    a. Place the tube with the RNA or DNA standard in position A1 of the 24-position microfuge tube holder plate.
    b. Add the volume specified of 1X TE buffer to a 1.5 mL microfuge tube, and place the tube in position A2 of the 24-position microfuge tube holder plate.
    c. If running DNA samples, place an empty 1.5 mL microfuge tube in position B1 of the 24-position microfuge tube holder plate for use during the method.

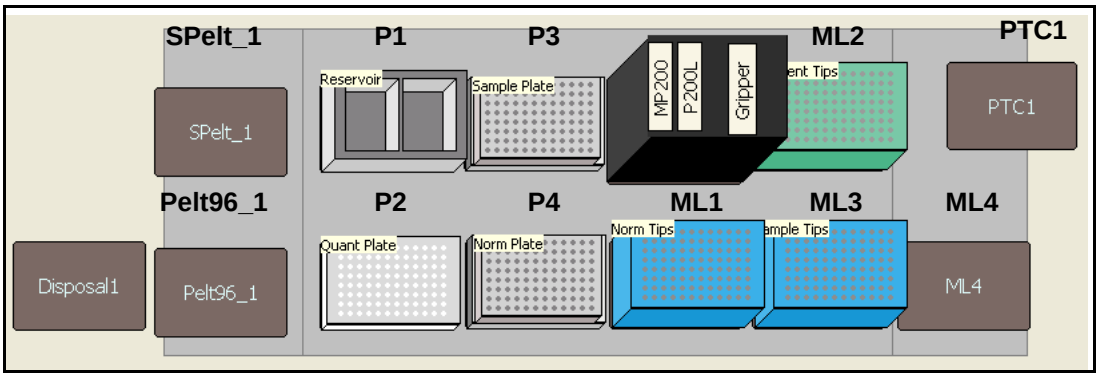
16. Load the labware and tips on the deck of the Biomek 3000 workstation according to the deck layout shown in Figure 1-3 below for fluorescence or Figure 1-4 for absorbance.

Note: The deck layouts shown below are with an integrated thermocycler. However, labware is placed in the same positions when running on a deck without an integrated thermocycler.



Position	Labware Name	Labware Type
Tool Rack	MP200 Tool P200L Tool Gripper Tool	
Pelt96_1 (Static Peltier)	Quantitation plate (Quant Plate)	Greiner UV Star (GLx_Greiner96Flat)
P1	Reagent reservoir (Reservoir)	Half Modular Reservoir (GLx_ModularReservoir_Half)
P2	Sample Plate stacked on Plate Holder	BCI V-Bottom Thermo Plate on Costar Round Bottom Plate (GLx_BCI_V_thermo96 on GLx_Costar96Roundps)
P3	Standards Tubes	Microfuge tube rack (GLx_SmallTuberack_Microfuge)
P4	Standards Plate	Greiner UV Star (GLx_Greiner96Flat)
ML1	Normalization tips (Norm Tips)	AP96 P20 tip box (GLx_AP96_20uL)
ML2	Reagent Tips	AP96 P200 tip box (GLx_AP96_200uL)
ML3	Quantitation tips (Quant Tips)	AP96 P20 tip box (GLx_AP96_20uL)

Figure 1-3. Deck layout for Quant and Norm method (fluorescence)



Position	Labware Name	Labware Type
Tool Rack	MP200 Tool P200L Tool Gripper Tool	
P1	Reagent reservoir (Reservoir)	Half Modular Reservoir (GLx_ModularReservoir_Half)
P2	Quantitation plate (Quant Plate)	Greiner UV Star (GLx_Greiner96Flat) OR Corning Half Area UV Plate (GLx_CorningUV_HalfWell96)
P3	Sample Plate stacked on Plate Holder	BCI V-Bottom Thermo Plate on Costar Round Bottom Plate (GLx_BCI_V_thermo96 on GLx_Costar96Roundps)
P4	Normalization plate (Norm Plate) stacked on Plate Holder	BCI V-Bottom Thermo Plate on Costar Round Bottom Plate (GLx_BCI_V_thermo96 on GLx_Costar96Roundps)
ML1	Normalization tips (Norm Tips)	AP96 P20 tip box (GLx_AP96_20uL)
ML2	Reagent Tips	AP96 P200 tip box (GLx_AP96_200uL)
ML3	Quantitation tips (Quant Tips)	AP96 P20 tip box (GLx_AP96_20uL)

Figure 1-4. Deck layout for Quant and Norm method (absorbance)

17. Click **Run** on the toolbar to start the method. The GenomeLab splash screen displays briefly, then Deck Confirmation appears (Figure 1-5).

Note: The Deck Confirmation prompt may differ from the one shown below, depending on the options selected in the Quant and Norm User Interface step.

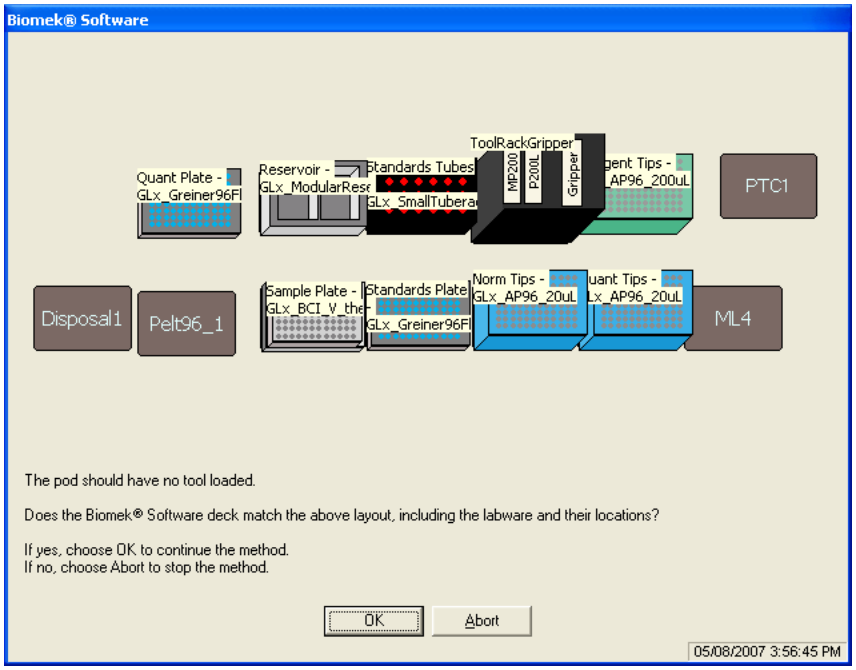


Figure 1-5. Deck confirmation prompt for Quant and Norm method

18. Confirm that the deck and labware are set up as shown in Deck Confirmation.
19. Choose **OK** to accept the deck confirmation and continue with the method.

Note: If the physical deck does not match the deck shown in Deck Confirmation exactly, either modify the physical deck to match the deck shown in the prompt, or choose **Abort** in Deck Confirmation.

20. The method runs until the quantitation is complete. At the completion of quantitation, a Pause message is displayed with instructions to refresh the labware and reagents on the deck and to read the quantiation plate at an off-deck DTX 880 Multimode Detector.

Note: Quantitation takes approximately 18 minutes for a full plate using fluorescence, or 8 minutes for a full plate using absorbance.

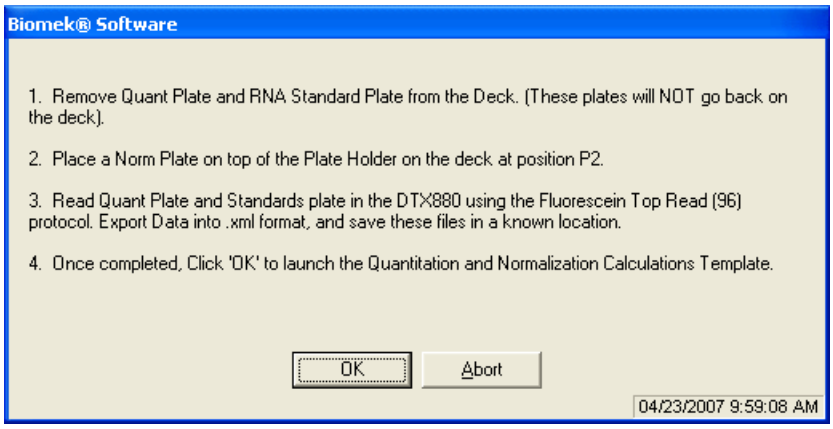


Figure 1-6. Pause message to read plates (fluorescence)

21. Remove the Quantitation Plate and Standards Plate (for fluorescence) from the deck. These plates will *not* be placed back on the deck.
22. If using fluorescence, place a Normalization Plate (96-Well Beckman Thermocycler Plate) on top of the Plate Holder at position P2.
23. Read the Quantitation Plate and Standards Plate (for fluorescence) with the DTX Multimode Detector and Multimode Software, using the appropriate protocol:
    - fluorescence—Fluorescein Top Read (96)
    - absorbance—Basic Absorbance DNA Quantitation (96)

Note: If necessary, tap the plate to collect any liquid on the side of the wells, and use a clean pipette tip to remove any lint or bubbles from the wells of the plate.

Note: If using a plate reader other than the DTX 880 Multimode Detector, use the **Quant Plate.xml** file located in the GeXP Common Files folder on the desktop to convert raw data to the appropriate format.

24. In Multimode Software, export the data from the measurement(s) to an XML file and save in a known location.

25. Once the XML files are saved, close them and choose **OK** in the Biomek Software prompt to launch the Quantitation and Normalization Calculations Template. The template opens to the Data Import tab (Figure 1-7). The Data Import tab includes areas for quantitation plate and standard curve data, input for normalization volume and concentration, and a list of Biomek variables

Note: Make sure the XML files are closed before choosing OK, or an error occurs in the method.

Note: Depending on the macro security settings in Microsoft Excel, a security prompt may appear. Select **Enable Macros** and choose **Yes** when asked to open as a read-only file.

Note: The Biomek Software method pauses until a normalization file is exported. A reminder prompt appears periodically in Biomek Software.

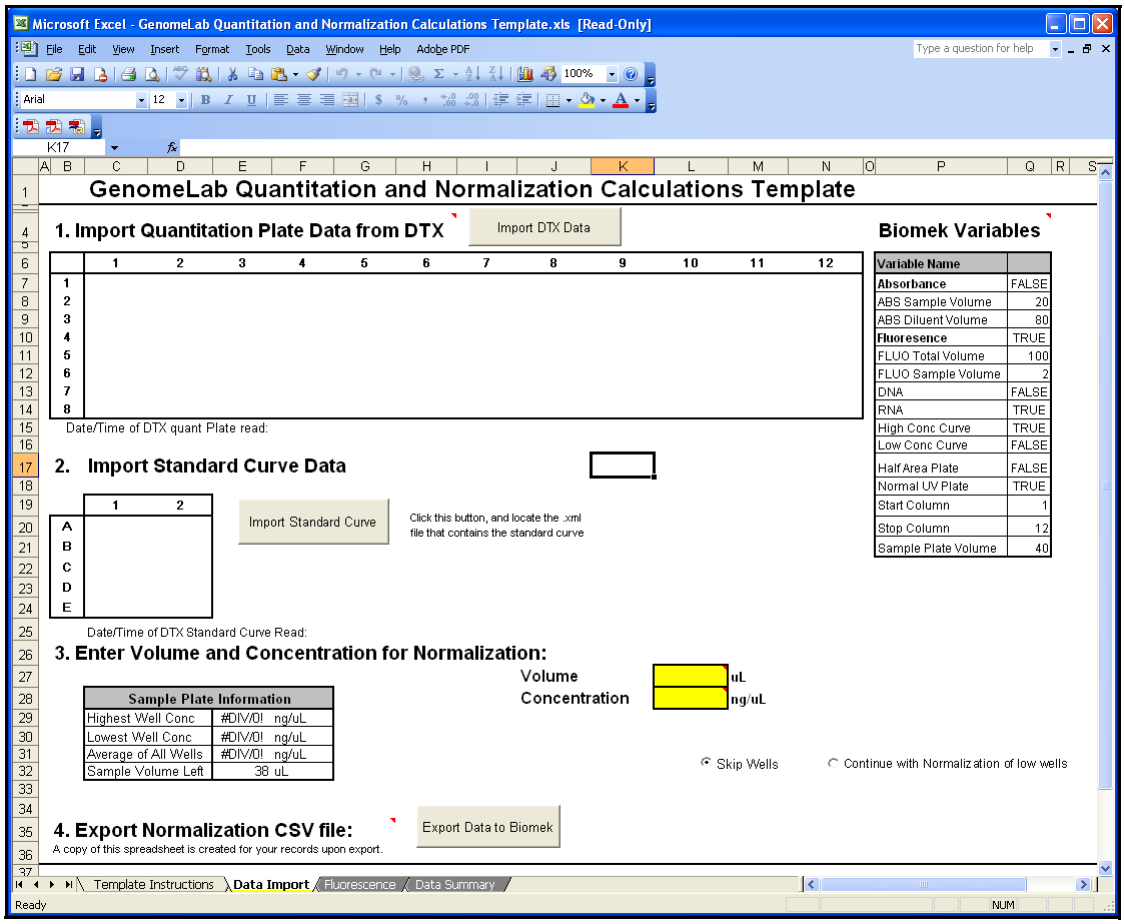


Figure 1-7. Quantitation and Normalization Calculations Template

26. In Import Quantitation Plate Data from DTX on the calculations template, choose **Import DTX Data**. Open appears.

27. Browse to and select the XML file containing the data from the quantitation plate and choose **Open**. The values from the quantitation plate data file fill in the plate values in the calculations template.

28. If using fluorescence, in Import Standard Curve Data, choose **Import Standard Curve**. Open appears.
29. Browse to and select the XML file containing the data from the standards plate and choose **Open**. The values from the standard curve data file fill in the values for the standard curve in the calculations template.
30. If using absorbance, in A260 Blank, enter the average value of blank wells read using the same microplate type.

    Note: An A260 blank can be determined by reading at least one column of water in a microplate of the same type used for the quantitation plate.

31. In Volume, enter the desired volume for normalization.
32. In Concentration, enter the desired concentration for normalization.

    Note: Use the data provided in Sample Plate Information to help choose appropriate values for the normalization volume and concentration. More complete data can be viewed under the Fluorescence or Absorbance tabs in the workbook. If using Absorbance, the A280 can be imported to determine A260/A280 ratios of the samples.

33. Specify how to deal with wells in which there is insufficient volume remaining in the sample plate or the concentration is too low:
    - **Skip Wells** — do not transfer any sample or diluent to the normalization well for that sample
    - **Continue with Normalization of Low Wells** — transfer what is available of the sample and normalize to the highest concentration and volume possible
34. In Export Normalization CSV File, choose **Export Data to Biomek**. A prompt to replace normalization.csv appears.
35. Choose **Yes** in the prompt asking to replace normalization.csv. Save As appears.
36. Browse to the desired location to save the file and enter a name for the file.
37. The file is saved and Biomek Software continues with the method. A Deck Confirmation prompt appears.
38. Confirm that the deck and labware are set up as shown in Deck Confirmation.
39. Choose **OK** to accept the deck confirmation and continue with the method.

    Note: If the physical deck does not match the deck shown in Deck Confirmation exactly, either modify the physical deck to match the deck shown in the prompt, or choose **Abort** in Deck Confirmation.

1.3 Reaction Setup Method

The Reaction Setup method generates a partial or full reaction plate containing master mix, primers and nucleic acids. Additionally, the method has default settings for producing an assay plate using the GenomeLab™ GeXP Kit protocol and reagents. Sample RNA concentrations between 5-20ng/µl (total RNA should be 25-100ng) are recommended for this assay and are dependent upon the gene expression levels present in the sample. Control template should be diluted to 5ng/µl.

This section of the quick-start guide is divided into the following sections and appendices.

- *Materials Required* (Section 1.2.1)
- *Quantitation and Normalization Method Procedure* (Section 1.2.2)

1.3.1 Materials Required

Performing the Reaction Setup method on the Biomek 3000 Workstation requires the following equipment, reagents, labware, and other supplies.

Note: Part numbers are listed in the following sections for convenience. Third party vendors may change part numbers. Confirm part numbers with the specified vendor before ordering.

1.3.1.1 Equipment

Running the Reaction Setup method requires the following instruments:

- Biomek 3000 Laboratory Automation Workstation with integrated:
  - Static Peltier Device
  - Shaking Peltier Device
  - DNA Engine (PTC-200) Thermocycler (optional)
  - MP200 Tool
  - P200L Tool
  - Gripper Tool
- GenomeLab™ GeXP Genetic Analysis System
- DNA Engine Thermocycler (PTC-200) or equivalent (if not on the deck)

1.3.1.2 Reagents

The following reagents are used in the Reaction Setup method. The volume of reagent required varies depending on the number of samples processed:

Table 1-3. Reagent Requirements

Reagent	Ordering Information
GenomeLab GeXP Kit	
Thermo-Start DNA Polymerase	ABgene, PN AB-0908/A (with separate MgCl2)
Nuclease-Free Water, non-DEPC treated	Invitrogen, PN 10977-015

1.3.1.3 Tips and Labware

The following tips and labware are used in the Reaction Setup method. The quantity of each required depends on the number of samples processed.

Table 1-4. Tip and Labware Requirements

Tips and Labware	Ordering Information
Biomek AP96 P250 Pipette Tips, presterile with barrier	Beckman Coulter, PN 717253
96-Well Sample Microtiter V-Bottom Thermocycler-Compatible Polypropylene Plate	Beckman Coulter, PN 609801
24-Position Tube Rack	Beckman Coulter, PN 373661
Diameter Insert, 11mm, White, for 1.5 mL Microfuge Tubes	Beckman Coulter, PN 373696
Polypropylene Tubes with Snap-On Caps, 1.5 mL	Beckman Coulter, PN 356090
Arched Auto-Sealing Microplate Lid	Biorad, PN MSL-2022
Hard-Shell Thin-Wall 96-Well Skirted PCR Plate	Biorad, PN HSP-9601
Costar 96-Well Clear Round Bottom Polystyrene Plate	Corning, PN 3795
Costar Thermowell Cap Strips	Corning, PN 6556

1.3.1.4 Other Supplies

The following additional supplies are used in the Reaction Setup method.

- Calibrated pipettors—P20, P200, and P1000.
- Nuclease-free barrier pipette tips.
- Pipette-aid.
- Nuclease-free 2 mL Eppendorf Genomic Micro Centrifgue Tubes (VWR 80077-234 or equivalent)
- RNase ZAP solution, 250 mL bottle (Ambion 9780).
- RNase ZAP Wipes (Ambion 9786)
- DNA Away (Molecular BioProbes 7010)

1.3.2 Reaction Setup Method Procedure

The Reaction Setup method is set up specifically for use with Beckman Coulter's GenomeLab GeXP kits. However, it is flexible enough to provide support for use with other applications.

The Reaction Setup method can be set up in two ways:

- for Gene Expression kits using default settings.
- for custom applications using custom settings.

To set up the deck and run the Reaction Setup method:

Note: Prior to the setting up and running the method, wipe down the instrument, deck, and tools with RNase zap or DNA Away to ensure work is done in a nuclease-free environment.

1. Turn on the Biomek 3000 instrument and open **Biomek Software**.
2. Turn on power to the Shaking Peltier Device and the Static Peltier Device.
3. Turn on power to the thermocycler, if integrated with the Biomek 3000 instrument.
4. From the Instrument menu, select **Home All Axes**.
5. Remove the required reagents from the GenomeLab GeXP Kit and thaw on ice until completely melted.
6. Open the Reaction Setup method in Biomek Software.
7. Select the **Reaction Setup User Interface** step (Figure 1-8).

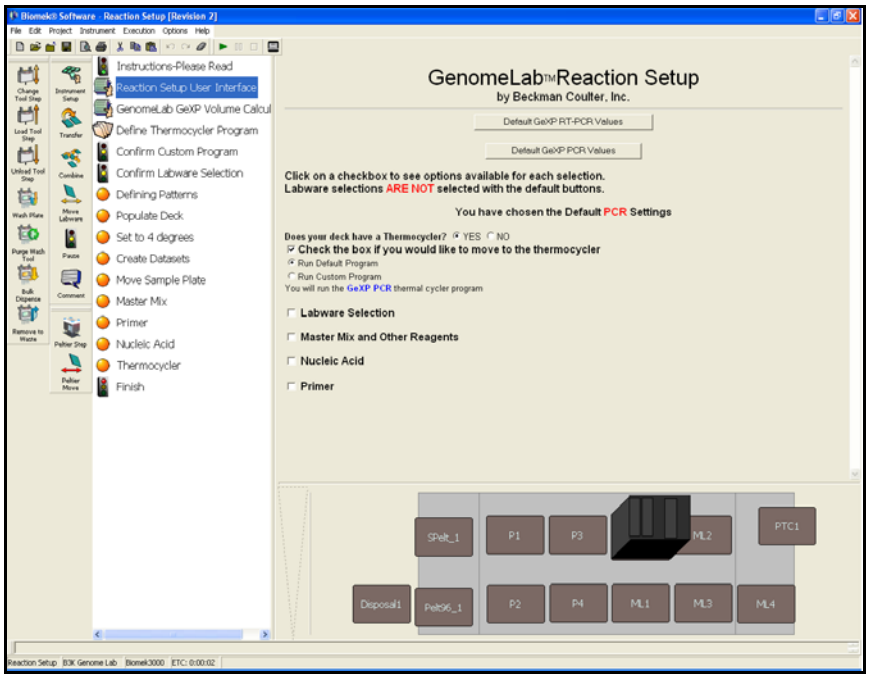


Figure 1-8. Quant and Norm User Interface step

8. Select **Default GeXP RT-PCR Values** to configure the method to process a full plate of samples using the GenomeLab GeXP protocol for RT-PCR.

   OR

   Select **Default GeXP PCR Values** to configure the method to process a full plate of samples using the GenomeLab GeXP protocol for PCR.

9. In Does your deck have a Thermocycler?, select the appropriate option: **YES** if a thermocycler is integrated on the deck, or **NO** if the thermocycler is off-deck.
10. If the thermocycler is on deck, select **Check the box if you would like to move to the thermocycler** to move the reaction plate to the thermocycler during the method.

11. If moving to the thermocycler, select whether to **Run Default Program** or **Run Custom Program**. Run Default Program runs the default thermocycler program as specified in the GeXP RT-PCR or GeXP PCR protocol.

Note: If Run Custom Protocol is selected, configure the thermocycler program by selecting the **Configure Custom Program Here** step, located inside the Define Thermocycler Program step (Figure 1-9). Refer to the *Biomek 3000 Thermocycler Device Integration Manual* for information on configuring custom protocols.

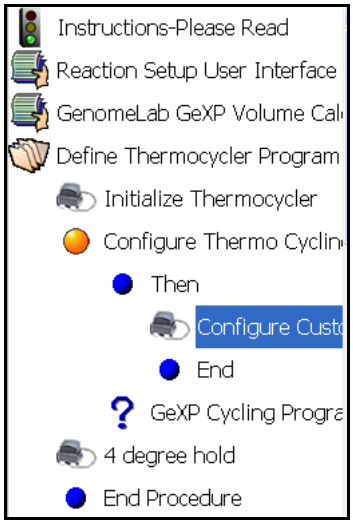


Figure 1-9. Location of Configure Custom Thermocycler Program Here step

12. Choose **Labware selection** to view the labware selection options for the reaction plate and nucleic acid source plate (Figure 1-10).

Note: Choose **Labware selection** again to hide the labware options. The configuration is maintained, but the dispaly is hidden to conserve space.

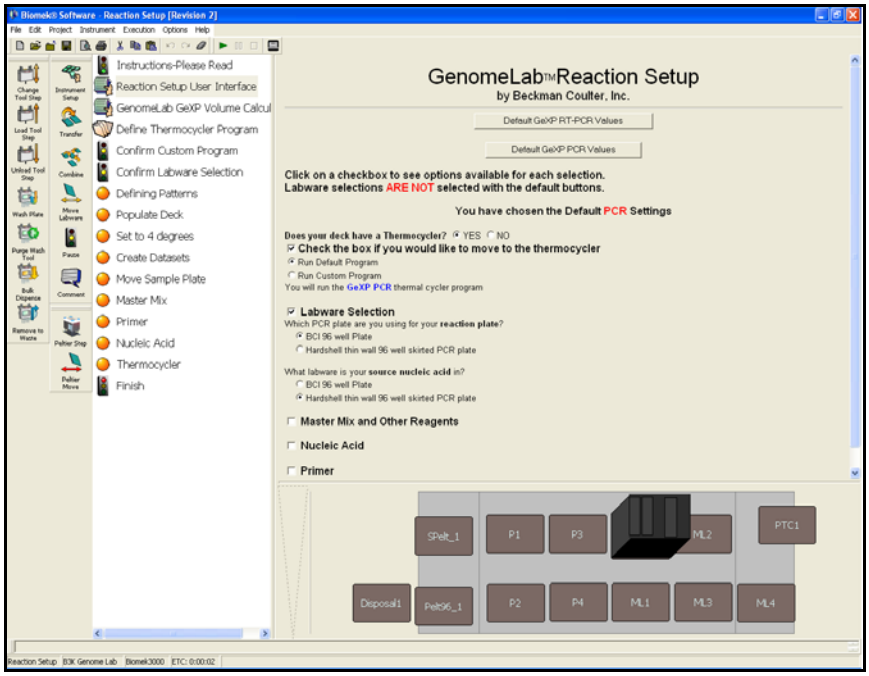


Figure 1-10. Reaction Setup User Interface step with Labware options displayed

13. In Which PCR plate are you using for your reaction plate?, select the labware type used for the reaction plate:**BCI 96-well plate** or **Hardshell thin wall 96-well skirted PCR plate**.

- The BCI 96-well plate is a V-bottom microplate and requires that a Costar U-bottom plate is used as a plate holder. It also must be manually sealed before moving to an integrated thermocycler.
- The Hardshell thin wall 96-well skirted PCR plate may be moved directly to an integrated thermocycler and does not require a plate holder.

14. In What labware is your source nucleic acid in?, select the labware type used for source nucleic acid: BCI 96-well plate or Hardshell thin wall 96-well skirted PCR plate.

15. If running a custom application, continue configuring options in the Reaction Setup User Interface step. Refer to Section 1.3.2.1, Setting Up a Custom Application.

    OR

    If running a Gene Expression GeXP protocol, select the GenomeLab GeXP Volume Calculator step. Refer to Section 1.3.2.2, Preparing Reagents Using the Volume Calculator, for information about using the volume calculator.

1.3.2.1 Setting Up a Custom Application

To configure the Reaction Setup method to run a custom application (any application that is NOT Gene Expression):

Note: If any of the following options are changed, the Reaction Setup method is no longer configured for the Gene Expression protocol.

1. Select **Master Mix and Other Reagents** to display the master mix options (Figure 1-11).

   Note: Choose **Master Mix and Other Reagents** again to hide the master mix options. The configuration is maintained, but the disaply is hidden to conserve space.

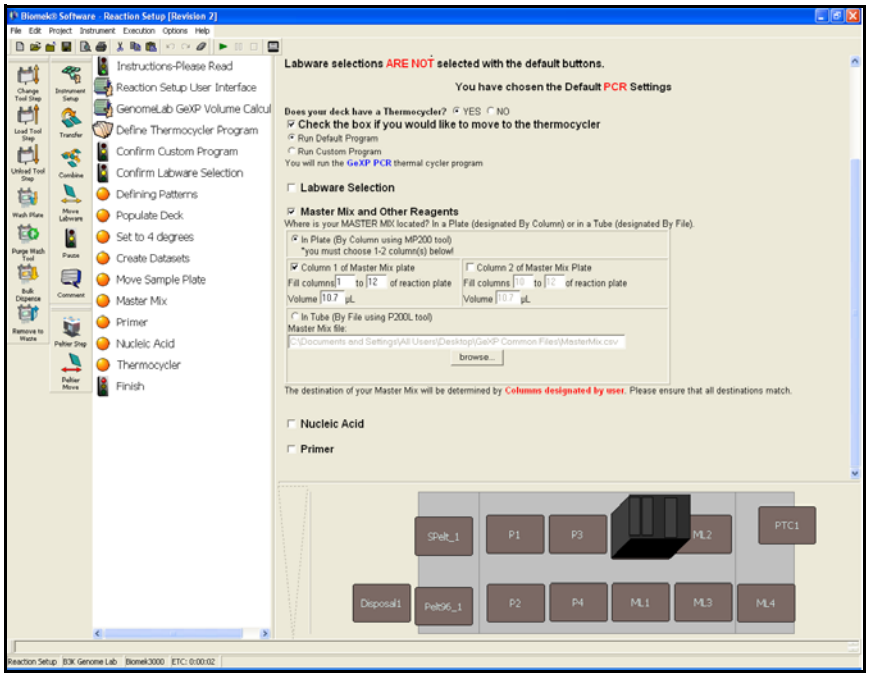
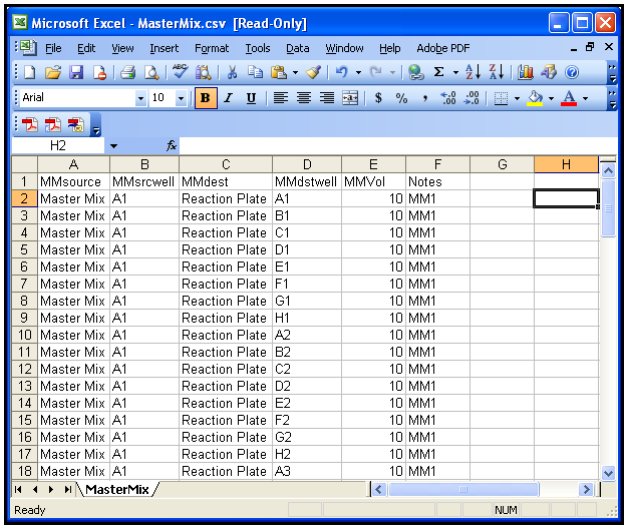


Figure 1-11. Reaction Setup User Interface step with Master Mix options displayed

2. Select the desired option for master mix:

   - **In Plate (By Column using MP200 tool)** — up to two master mix solutions are located in the first two columns of a microplate. Configure Column 1 and Column 2 to specify which columns of the reaction plate the master mix should be added and the Volume of master mix to to transfer to each well.
   - **In Tube (By File using P200L tool)** — up to 24 master mix solutions are located in tubes placed in a 24-position tube rack holder. A CSV file is used to specify the source position of the master mix, destination well on the reaction plate, and transfer volume (see Figure 1-12 for an example file). Choose **Browse** to browse to and select the CSV file.

Note: Each master mix tube requires an additional 20 µL of dead volume. Using tubes also lengthens the method, as a single-probe tool is used for master mix transfers instead of an 8-probe tool.



	A	B	C	D	E	F	G	H
1	MMsource	MMsrcwell	MMdest	MMdstwell	MMVol	Notes		
2	Master Mix	A1	Reaction Plate	A1	10	MM1		
3	Master Mix	A1	Reaction Plate	B1	10	MM1		
4	Master Mix	A1	Reaction Plate	C1	10	MM1		
5	Master Mix	A1	Reaction Plate	D1	10	MM1		
6	Master Mix	A1	Reaction Plate	E1	10	MM1		
7	Master Mix	A1	Reaction Plate	F1	10	MM1		
8	Master Mix	A1	Reaction Plate	G1	10	MM1		
9	Master Mix	A1	Reaction Plate	H1	10	MM1		
10	Master Mix	A1	Reaction Plate	A2	10	MM1		
11	Master Mix	A1	Reaction Plate	B2	10	MM1		
12	Master Mix	A1	Reaction Plate	C2	10	MM1		
13	Master Mix	A1	Reaction Plate	D2	10	MM1		
14	Master Mix	A1	Reaction Plate	E2	10	MM1		
15	Master Mix	A1	Reaction Plate	F2	10	MM1		
16	Master Mix	A1	Reaction Plate	G2	10	MM1		
17	Master Mix	A1	Reaction Plate	H2	10	MM1		
18	Master Mix	A1	Reaction Plate	A3	10	MM1		

Figure 1-12. Example .csv file for master mix in tubes

3. Choose **Nucleic Acid** to display the nucleic acid options (Figure 1-13).

Note: Choose **Nucleic Acid** again to hide the nucleic acid options. The configuration is maintained, but the display is hidden to conserve space.

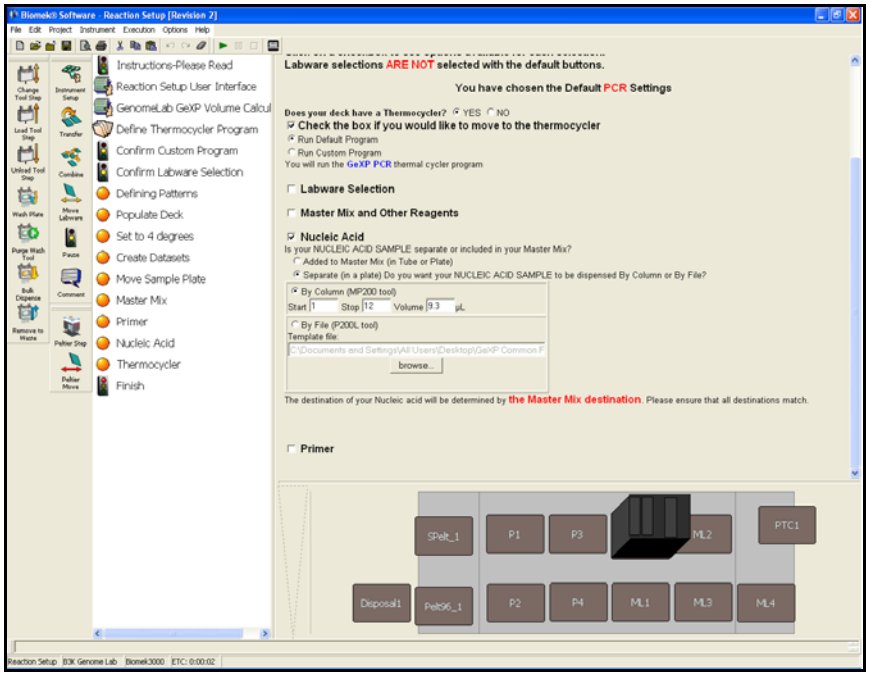


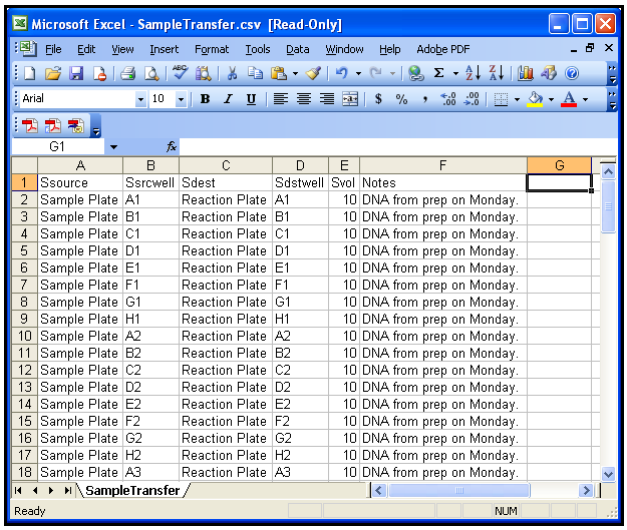
Figure 1-13. Reaction Setup User Interface step with Nucleic Acid options displayed

4. In Is your NUCLEIC ACID SAMPLE separate or included in your Master Mix?, select the appropriate option:

   - **Added to Master Mix (in Tube or Plate)** — nucleic acid samples are included in the master mix solution.
   - **Separate (in a plate)** — nucleic acid samples are stored separately in a microplate.

5. If nucleic acid samples are Separate, specify whether samples should be dispensed **By Column** or **By File**:

   - By Column (MP200 tool) — transfer nucleic acid samples by column using the MP200 tool. Specify the Start and Stop columns on the sample plate and Volume to transfer. Nucleic acid samples are transferred to all columns on the reaction plate which received master mix.
   - By File (P200L tool) — transfer nucleic acid samples to the reaction plate based on configuration information in a CSV file. A CSV file is used to specify the source well on the nucleic acid sample plate, destination well on the reaction plate, and transfer volume (see Figure 1-14 for an example file). Choose **Browse** to browse to and select the CSV file. Nucleic acid samples are transferred to all wells specified in the CSV file.



	A	B	C	D	E	F	G
1	Ssource	Ssrcwell	Sdest	Sdstwell	Svol	Notes	
2	Sample Plate	A1	Reaction Plate	A1	10	DNA from prep on Monday.	
3	Sample Plate	B1	Reaction Plate	B1	10	DNA from prep on Monday.	
4	Sample Plate	C1	Reaction Plate	C1	10	DNA from prep on Monday.	
5	Sample Plate	D1	Reaction Plate	D1	10	DNA from prep on Monday.	
6	Sample Plate	E1	Reaction Plate	E1	10	DNA from prep on Monday.	
7	Sample Plate	F1	Reaction Plate	F1	10	DNA from prep on Monday.	
8	Sample Plate	G1	Reaction Plate	G1	10	DNA from prep on Monday.	
9	Sample Plate	H1	Reaction Plate	H1	10	DNA from prep on Monday.	
10	Sample Plate	A2	Reaction Plate	A2	10	DNA from prep on Monday.	
11	Sample Plate	B2	Reaction Plate	B2	10	DNA from prep on Monday.	
12	Sample Plate	C2	Reaction Plate	C2	10	DNA from prep on Monday.	
13	Sample Plate	D2	Reaction Plate	D2	10	DNA from prep on Monday.	
14	Sample Plate	E2	Reaction Plate	E2	10	DNA from prep on Monday.	
15	Sample Plate	F2	Reaction Plate	F2	10	DNA from prep on Monday.	
16	Sample Plate	G2	Reaction Plate	G2	10	DNA from prep on Monday.	
17	Sample Plate	H2	Reaction Plate	H2	10	DNA from prep on Monday.	
18	Sample Plate	A3	Reaction Plate	A3	10	DNA from prep on Monday.	

Figure 1-14. Example .csv file for dispensing nucleic acids by file

6. Choose **Primer** to display the primer options (Figure 1-15).

Note: Choose **Primer** again to hide the primer options. The configuration is maintained, but the display is hidden to conserve space.

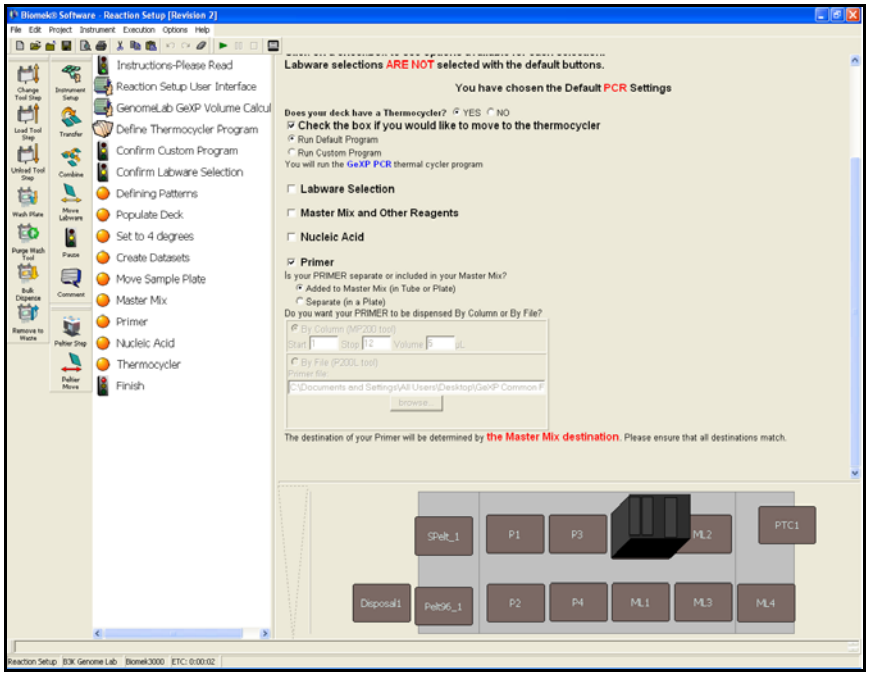
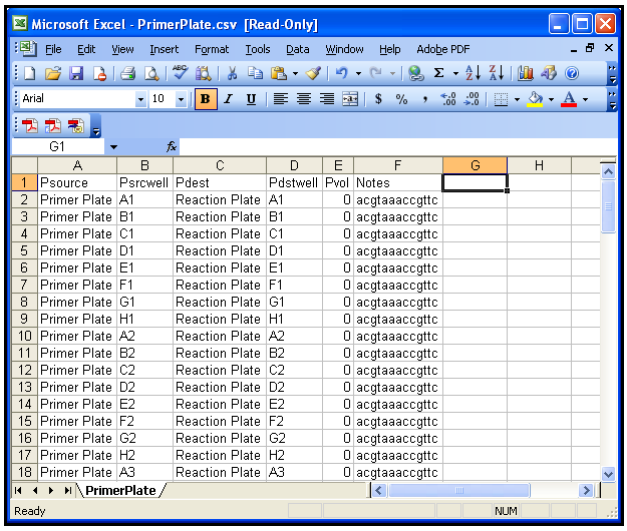


Figure 1-15. Reaction Setup User Interface step with Primer options displayed

7. In Is your PRIMER separate or included in your Master Mix?, select the appropriate option:
   - **Added to Master Mix (in Tube or Plate)** — primers are included in the master mix solution.
   - **Separate (in a plate)** — primers are stored separately in a microplate.

8. If primers are Separate, specify whether primers should be dispensed **By Column** or **By File**:

   - By Column (MP200 tool) — transfer primers by column using the MP200 tool. Specify the Start and Stop columns on the primer plate and Volume to transfer. Primers are transferred to all columns on the reaction plate which received master mix.
   - By File (P200L tool) — transfer primers to the reaction plate based on configuration information in a CSV file. The CSV file is used to specify the source well on the primer plate, destination well on the reaction plate, and transfer volume (see Figure 1-16 for an example file). Choose **Browse** to browse to and select the CSV file. Primers are transferred to all wells specified in the CSV file.



	A	B	C	D	E	F	G	H
1	Psource	Psrcwell	Pdest	Pdstwell	Pvol	Notes		
2	Primer Plate	A1	Reaction Plate	A1	0	acgtaaaccgttc		
3	Primer Plate	B1	Reaction Plate	B1	0	acgtaaaccgttc		
4	Primer Plate	C1	Reaction Plate	C1	0	acgtaaaccgttc		
5	Primer Plate	D1	Reaction Plate	D1	0	acgtaaaccgttc		
6	Primer Plate	E1	Reaction Plate	E1	0	acgtaaaccgttc		
7	Primer Plate	F1	Reaction Plate	F1	0	acgtaaaccgttc		
8	Primer Plate	G1	Reaction Plate	G1	0	acgtaaaccgttc		
9	Primer Plate	H1	Reaction Plate	H1	0	acgtaaaccgttc		
10	Primer Plate	A2	Reaction Plate	A2	0	acgtaaaccgttc		
11	Primer Plate	B2	Reaction Plate	B2	0	acgtaaaccgttc		
12	Primer Plate	C2	Reaction Plate	C2	0	acgtaaaccgttc		
13	Primer Plate	D2	Reaction Plate	D2	0	acgtaaaccgttc		
14	Primer Plate	E2	Reaction Plate	E2	0	acgtaaaccgttc		
15	Primer Plate	F2	Reaction Plate	F2	0	acgtaaaccgttc		
16	Primer Plate	G2	Reaction Plate	G2	0	acgtaaaccgttc		
17	Primer Plate	H2	Reaction Plate	H2	0	acgtaaaccgttc		
18	Primer Plate	A3	Reaction Plate	A3	0	acgtaaaccgttc		

Figure 1-16. Example .csv file for dispensing primers by file

9. Prepare to run the Reaction Setup method. Refer to Section 1.3.2.3, Running the Reaction Setup Method

1.3.2.2 Preparing Reagents Using the Volume Calculator

When using the Reaction Setup method to run a Gene Expression GeXP protocol, the Volume Calculator step provides useful information to assist in preparing reagents for the protocol.

To use the GenomeLab GeXP Volume Calculator step:

1. Select the **GenomeLab GeXP Volume Calculator** step (Figure 1-17). The GenomeLab GeXP Volume Calculator displays the required reagent volumes (including dead volumes) for the specified number of samples to prepare the master mix solutions.

Note: The **GenomeLab GeXP Volume Calculator** step is a tool to help prepare the master mix when running the default GeXP RT-PCR or GeXP PCR protocol.

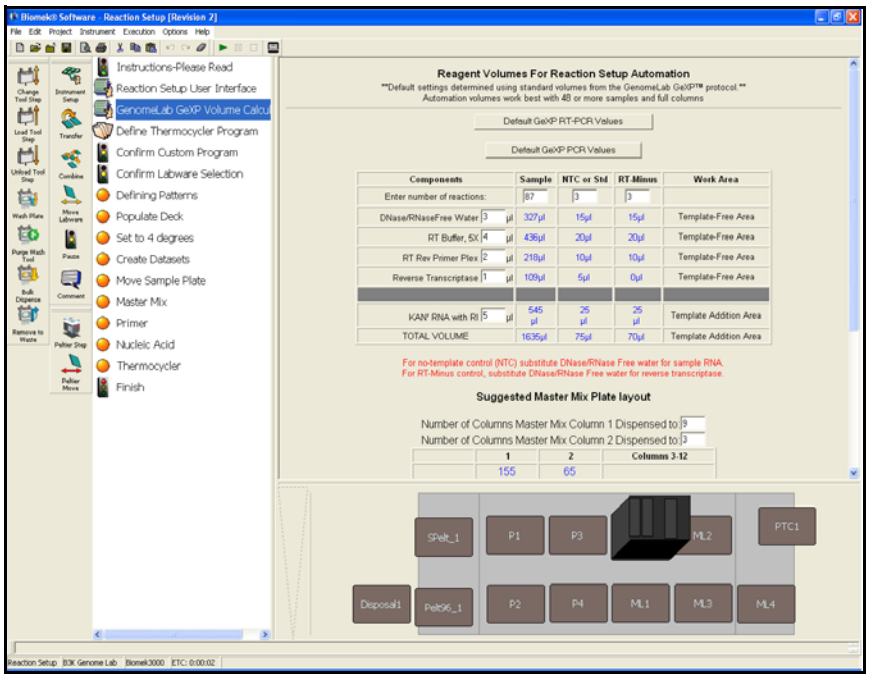


Figure 1-17. GenomeLab GeXP Volume Calculator

2. Select either **Default GeXP RT-PCR Values** or **Default GeXP PCR Values** to set the volumes for the GeXP RT-PCR or GeXP PCR protocol.

Note: Volumes are set for the default values required to run a full plate for RT-PCR or PCR using the GeXP protocol. To run a partial plate, change the number of reactions. To use a different protocol, change the volumes as specified by the protocol.

---

3. Prepare the master mix by adding the reagents and volumes specified in the **GenomeLab GeXP Volume Calculator** step.

Note: Add reagents in the appropriate Work Area, as specified in the table. Template-Free Area refers to an area that has been completely cleaned of any contaminating DNA/RNA or RNase/DNase; Template Addition Area refers to an area that has been cleaned of any RNase/DNase.

---

4. If Master Mix is stored in a plate using the default settings, add the specifed volume of the master mix solutions to each well of the columns specified on the Master Mix plate.

   Note: If using tubes or custom settings for the master mix, the information provided in Suggested Master Mix Plate layout is invalid. Determine the appropriate volumes manually based on the volume per sample and number of reactions to which the master mix is added, including a 20 µL dead volume, and add to the appropriate labware.

5. Prepare the sample plate according to the Suggested Full Sample Plate layout. Note the inclusion of Standard Controls (STD), Non-Template Controls (NTC), and without Reverse Transcriptase (RT-) wells in rows C, D, and E. It is recommended that these controls are run in triplicate and included in the same row for every sequencing run so they are processed with the same capillary array each time.

6. Prepare to run the Reaction Setup method. Refer to Section 1.3.2.3, Running the Reaction Setup Method

1.3.2.3 Running the Reaction Setup Method

Once the Reaction Setup method is configured as desired, the method can be run:

1. Click **Run** on the toolbar to start the method.

2. If the thermocycler is configured to use a custom program, a prompt appears to confirm that the custom thermocycling program has been configured. Choose **OK** if the program is configured, or **Abort** to stop the method. Return to the User Interface step and configure it to use the default thermocycler program or deselect the Move to Thermocycler option, or select the Configure Custom Program Here step, located inside the Define Thermocycler Program step.

3. A prompt appears to confirm the reaction plate and sample plate labware type. Choose **OK** if the labware types indicated in the prompt match the labware used for the reaction plate and sample plate, or choose **Abort** to stop the method. Return to the User Interface and select the appropriate labware type in Labware Selection.

4. The GeXP splash screen appears, followed by Deck Confirmation (Figure 1-18).

Note: The Deck Confirmation prompt may differ from the one shown below, depending on the options selected in the Reaction Setup User Interface step.

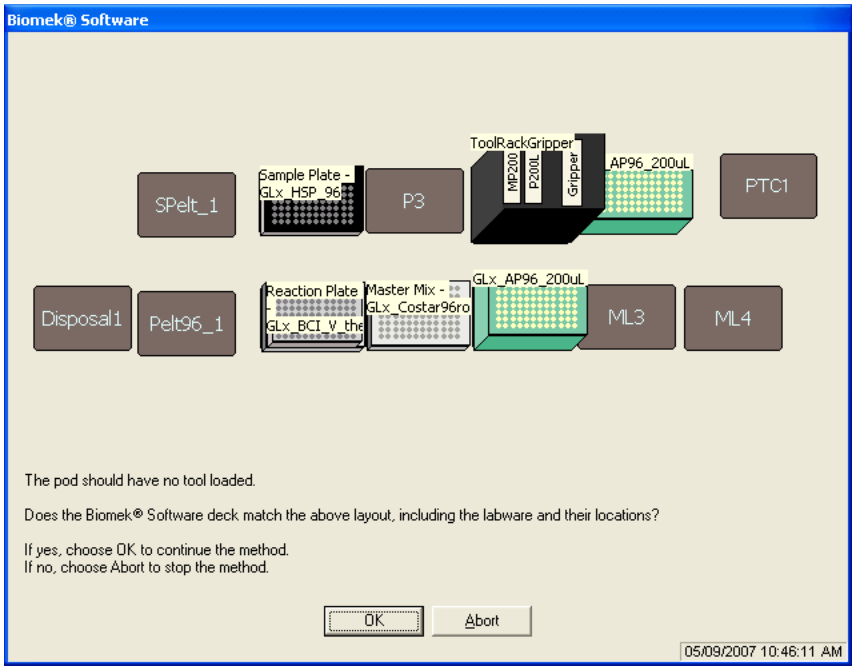


Figure 1-18. Deck confirmation prompt for Reaction Setup method

5. Load the labware and tips on the deck of the Biomek 3000 workstation according to the deck layout shown in the deck confirmation prompt.
6. Confirm that the deck and labware are set up as shown in Deck Confirmation.
7. Choose **OK** to accept the deck confirmation and continue with the method.

Note: If the physical deck does not match the deck shown in Deck Confirmation exactly, either modify the physical deck to match the deck shown in the prompt, or choose **Abort** in Deck Confirmation.

8. If running Reaction Setup with the default settings for a Gene Expression GeXP protocol, the reaction setup method takes approximately 12 minutes to complete. Run time varies for custom applications. If moving to the thermocycler, the appropriate default thermocycler program or custom program is run.

   Note: If moving to the thermocycler and the reaction plate is a BCI 96-well plate, a Pause message appears with instructions to manually seal the plate with Costar Thermowell Cap Strips and place the microplate in the PTC-200 thermocycler. Choose **OK** once the plate is placed in the thermocycler.

9. The thermocycler program includes a hold at the conclusion of the incubation which holds the plate at 4ºC indefinitely. To end the hold and retrieve the plate:

   a. From the Instrument menu, select **Device Editor**.

   b. Choose **PTC200_1** from the list and select **Action Commands**.

   c. Select **Initialize** and press the **Initialize** button. This ends the hold.

   d. Select **Open** and press the **Open Lid** button to open the lid.

   e. Remove the reaction plate from the thermocycler.

1.4 Plate Preparation Method

The Plate Preparation method generates a partial or full buffer plate, dilution plate, and GenomeLab sample plate.

This section of the quick-start guide is divided into the following sections and appendices.

- *Materials Required* (Section 1.2.1)
- *Quantitation and Normalization Method Procedure* (Section 1.2.2)

1.4.1 Materials Required

Performing the Plate Preparation method on the Biomek 3000 Workstation requires the following equipment, reagents, labware, and other supplies.

Note: Part numbers are listed in the following sections for convenience. Third party vendors may change part numbers. Confirm part numbers with the specified vendor before ordering.

1.4.1.1 Equipment

Running the Plate Preparation method requires the following instruments:

- Biomek 3000 Laboratory Automation Workstation with integrated:
  - Static Peltier Device
  - Shaking Peltier Device
  - DNA Engine (PTC-200) Thermocycler (optional)
  - MP200 Tool
  - P200L Tool
  - Gripper Tool
- GenomeLab™ GeXP Genetic Analysis System
- DNA Engine Thermocycler (PTC-200) or equivalent (if not on the deck)

1.4.1.2 Reagents

The following reagents are used in the Plate Preparation method. The volume of reagent required varies depending on the number of samples processed:

Table 1-5. Reagent Requirements

Reagent	Ordering Information
GenomeLab GeXP Kit	
GenomeLab Separation Buffer	Beckman Coulter, PN 608012
1 M Tris-HCl pH 8.0	USB, PN 22638
Nuclease-Free Water, non-DEPC treated	Invitrogen, PN 10977-015

1.4.1.3 Tips and Labware

The following tips and labware are used in the Plate Preparation method. The quantity of each required depends on the number of samples processed.

Table 1-6. Tip and Labware Requirements

Tips and Labware	Ordering Information
Biomek AP96 P250 Pipette Tips, presterile	Beckman Coulter, PN 717252
Biomek AP96 P20 Tips, presterile with Barrier	Beckman Coulter, PN 717256
96-Well Sample Microtiter V-Bottom Thermocycler-Compatible Polypropylene Plate	Beckman Coulter, PN 609801
Quarter Module Reservoir	Beckman Coulter, PN 372790
Modular Reservoir Frame	Beckman Coulter, PN 372795
96-Well Buffer Microplate	Beckman Coulter, PN 609844
Hard-Shell Thin-Wall 96-Well Skirted PCR Plate	Biorad, PN HSP-9601
Costar 96-Well Clear Round Bottom Polystyrene Plate	Corning, PN 3795

1.4.1.4 Other Supplies

The following additional supplies are used in the RNAdvance method.

- Calibrated pipettors—P20, P200, and P1000.
- Nuclease-free barrier pipette tips.
- Pipette-aid.
- Nuclease-free 2 mL Eppendorf Genomic Micro Centrifgue Tubes (VWR 80077-234 or equivalent)
- RNase ZAP solution, 250 mL bottle (Ambion 9780).
- RNase ZAP Wipes (Ambion 9786)
- DNA Away (Molecular BioProbes 7010)
- GenomeLab Separation Capillary Array (Beckman Coulter, PN 608087)
- GenomeLab Separation Gel (Beckman Coulter, PN 391438)
- GenomeLab User's Guide (Beckman Coulter, PN A29142)

1.4.2 Plate Preparation Method Procedure

To set up the deck and run the Plate Preparation method:

Note: Prior to the setting up and running the method, wipe down the instrument, deck, and tools with RNase zap or DNA Away to ensure work is done in a nuclease-free environment.

1. Turn on the Biomek 3000 instrument and open **Biomek Software**.
2. Turn on power to the Shaking Peltier Device and the Static Peltier Device.
3. From the Instrument menu, select **Home All Axes**.

4. Remove the required reagents from the GenomeLab GeXP Kit and thaw completely on ice.
5. Open the Plate Preparation method in Biomek Software.
6. Select the Plate Preparation **User Interface** step (Figure 1-19).

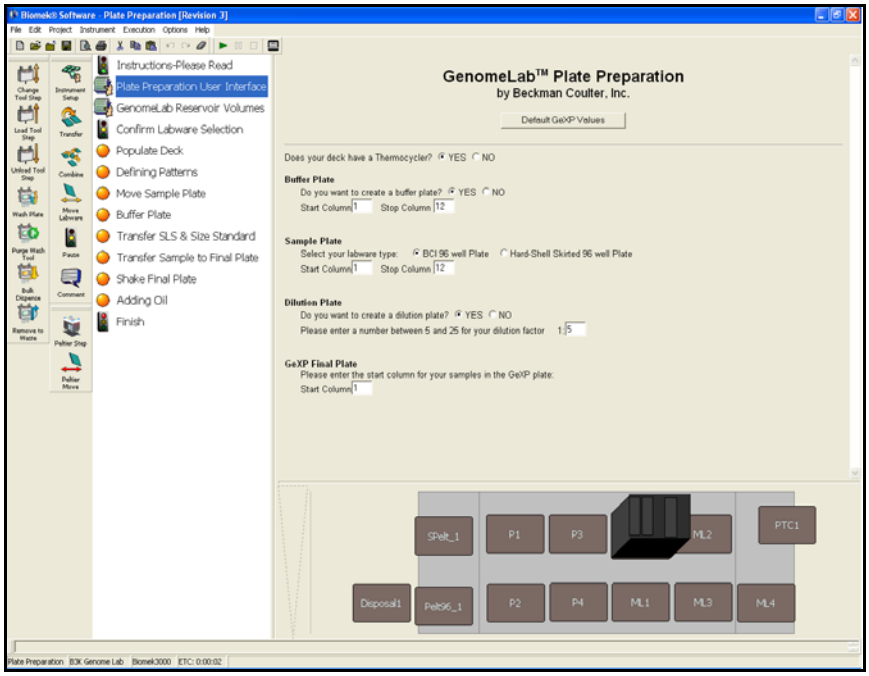


Figure 1-19. Plate Preparation User Interface step

7. In Does your deck have a thermocycler?, select the appropriate option: **Yes** if a thermocycler is integrated with the deck, or **No** if there is not a thermocycler integrated with the deck.
8. In Do you want to create a buffer plate?, select the desired option: **Yes** or **No**.
9. If creating a buffer plate, enter the **Start** and **Stop** columns. There must be separation buffer in every column that the corresponding column of the final plate will have samples in.
10. In Sample plate, select the labware type that samples are stored in: **BCI 96 well Plate** or **Hard-Shell Skirted 96 well Plate**.
11. In Start column, enter the first column of the sample plate that contains samples.
12. In End column, enter the last column of the sample plate that contains samples.
13. In Do you want to create a dilution plate?, select the desired option: **Yes** or **No**.
14. If creating a dilution plate, specify a dilution factor between 5 and 25.

Note: Many GeXP kits recommend a 1:5 dilution. The method always uses 4 µL of sample and automatically adjusts the amount of Tris-HCl added to achieve the desired dilution.

15. In GeXP Final Plate, enter the first column of the final plate to which to add samples.

Note: Account for conditioning runs by leaving the first columns empty and setting the start column to the first available column after the conditioning samples. The stop column is automatically determined by the number of sample columns used and the start column on the final plate. If more columns from the sample plate are being used than what is available in the assay plate, the method will fail to validate.

16. Select the GenomeLab Reservoir Volumes step (Figure 1-20).

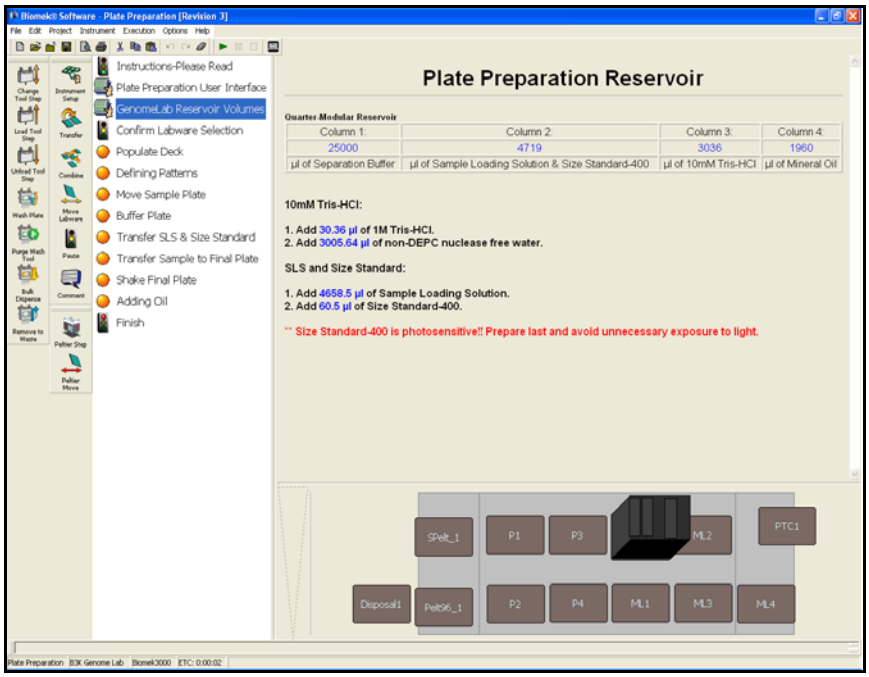


Figure 1-20. GenomeLab Reservoir Volumes step

17. Place the appropriate reagents in the appropriate locations on the modular reservoir as described in GenomeLab Reservoir Volumes.

Note: Following the table are instructions for preparing the solutions for the second and third quarter modules. Prepare the solutions as instructed. Size Standard-400 is photosensitive and should be prepared last and kept away from light as much as possible.

18. Click **Run** on the toolbar to start the method.
19. A prompt appears to confirm the sample plate labware type. Choose **OK** if the labware type indicated in the prompt matches the labware used for the sample plate, or choose **Abort** to stop the method. Return to the User Interface and select the appropriate labware type in Sample Plate.

20. The GeXP splash screen appears, followed by Deck Confirmation (Figure 1-21).

Note: The Deck Confirmation prompt may differ from the one shown below, depending on the options selected in the Plate Preparation User Interface step.

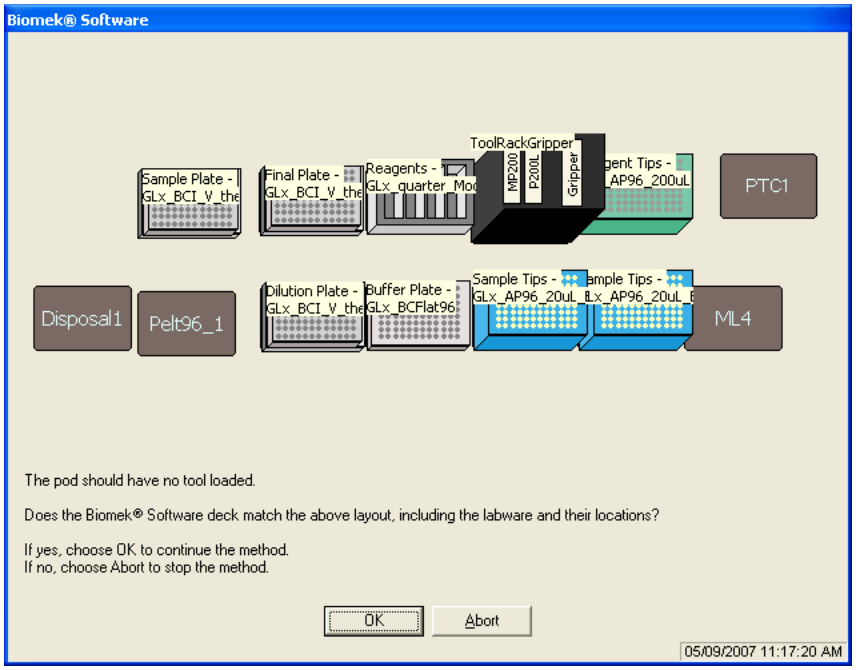


Figure 1-21. Deck confirmation prompt for Reaction Setup method

21. Load the labware and tips on the deck of the Biomek 3000 workstation according to the deck layout shown in the Deck Confirmation prompt.
22. Confirm that the deck and labware are set up as shown in Deck Confirmation.
23. Choose **OK** to accept the deck confirmation and continue with the method.

Note: If the physical deck does not match the deck shown in Deck Confirmation exactly, either modify the physical deck to match the deck shown in the prompt, or choose **Abort** in Deck Confirmation.

24. Once the final sample plate and buffer plate have been made, they are ready to load into the GenomeLab™ GeXP Genetic Analysis System for analysis. Refer to the *GenomeLab User's Guide* (A29142) for detailed instructions on running samples with the GenomeLab GeXP System, using the Fragment Analysis software module, the eXpress Profiler software, the eXpress Analysis software module and for performing GeXP gene expression profiling analysis.