AUTOMATED PROTOCOL

Identity Automation™ qPCR Setup Protocol for the PowerQuant® and Plexor® HY Systems on the Beckman Coulter Biomek® 4000 Workstation

Instructions for Use of Products
PQ5002, PQ5008, DC1001 and DC1000

EP062
Revised 11/16

Identity Automation™ qPCR Setup Protocol for the PowerQuant® and Plexor® HY Systems on the Beckman Coulter Biomek® 4000 Workstation

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Automated Protocol.
E-mail Promega Technical Services if you have questions on use of this system: Techserv@promega.com

1. Description

This document describes the automated protocol for the Identity Automation™ qPCR setup method for the PowerQuant®[a–c] and Plexor® HY Systems[a,d–g] on the Beckman Coulter Biomek® 4000 Laboratory Automation Workstation.

1. Description (continued)

The method allows use of a 4-point DNA standard curve (with the PowerQuant® System) or 7-point DNA standard curve (with the PowerQuant® or Plexor® HY System). We do not recommend using the 4-point DNA standard curve for the Plexor® HY System. The automated method prepares 96-well PCR plates for DNA quantitation by real-time quantitative PCR (qPCR) of up to 86 unknown samples per plate when using a 4-point DNA standard curve or up to 80 unknown samples per plate when using a 7-point DNA standard curve.

The automated method for DNA quantitation reaction setup is designed with flexibility to support the following:

- Setup of two amplification plates from a single sample plate to allow processing of duplicate quantitation reactions from up to 80 unknown samples using either a 7-point or 4-point DNA standard curve.
- Setup of one amplification plate from one sample plate to allow processing of single quantitation reaction from up to 86 samples or duplicate quantitation reactions from up to 43 unknown samples using a 4-point DNA standard curve or processing of single quantitation reaction from up to 80 samples or duplicate quantitation reactions from up to 40 unknown samples using a 7-point DNA standard curve.

The automated setup can be customized to meet the workflow needs of the forensics laboratory. You can: 1) specify the number of samples to be processed, 2) specify the sample volume to be transferred to each amplification reaction, 3) indicate the number of replicate qPCR amplifications for each sample, 4) choose a 4-point or a 7-point DNA standard curve (see Section 7), 5) choose whether DNA standard dilutions are prepared by the automated liquid handler or manually and 6) choose whether the PowerQuant® or Plexor® HY reaction mix is prepared by the automated liquid handler or manually.

Note: This automated method can be used with the PowerQuant® System or Plexor® HY System. The qPCR system is selected during Promega's installation service. Throughout this technical manual, "qPCR System" refers to the PowerQuant® System or Plexor® HY System, and "qPCR reagents" refers to the PowerQuant® reagents or Plexor® HY reagents.

For additional information about Identity Automation™ methods for human identification applications, visit: **www.promega.com/idautomation/**

For troubleshooting chemistry issues, refer to the appropriate qPCR Technical Manual: *PowerQuant® System Technical Manual* #TMD047 or *Plexor® HY System Technical Manual* #TM293, TM294 or TM296. All Promega Technical Manuals are available at: **www.promega.com/protocols/**

2. Product Requirements and Storage Conditions

PRODUCT	SIZE	CAT.#
PowerQuant® System	**200 reactions**	**PQ5002**
	800 reactions	**PQ5008**
Plexor® HY System	**200 reactions**	**DC1001**
	800 reactions	**DC1000**

Not for Medical Diagnostic Use.

Storage Conditions: See the *PowerQuant® System Technical Manual* #TMD047 or *Plexor® HY System Technical Manual* #TM293, TM294 or TM296 for detailed storage conditions.

3. Materials to Be Supplied By the User

- TE^{-4} buffer [10mM Tris (pH 8.0), 0.1mM EDTA]
- real-time PCR instrument compatible with the qPCR system
  Refer to the appropriate technical manual for a list of compatible instruments.
- analysis software compatible with the qPCR system
  Refer to the appropriate technical manual for more information.
- 96-well optical PCR plates and plate covers or sealing film
- 1.5ml microcentrifuge tubes
- two to six 8 strip tubes (e.g., Applied Biosystems Cat.# N801-0580) or an additional 96-well PCR plate (two to six columns will be used for DNA standard dilutions and amplification-grade water)
- 96-well plates or strip tubes containing unknown samples

Note: The PowerQuant® System and the Plexor® HY System are available in two formats: 200 reactions and 800 reactions. The 200-reaction system provides sufficient reagents to process two plates of 86 unknown samples plus 10 controls per plate when a 4-point DNA standard curve is selected or two plates of 80 unknown samples plus 16 controls when a 7-point curve is selected. The 800-reaction system provides sufficient reagents to process eight plates of 86 unknown samples plus 10 controls per plate when a 4-point standard curve is selected or eight plates of 80 unknown samples plus 16 controls when a 7-point surve is selected. The automated method will prompt you to confirm that a minimum volume of each component is available if reaction mix is prepared by the liquid handler.

4. Before You Begin

4.A. Sample Considerations

Unknown samples must be in 96-well format (plate or strip tubes). Unknown samples should be centrifuged briefly to remove any air bubbles that might be present. Air bubbles may interfere with sample aspiration.

4.B. Preparation of Reagents

Thaw the qPCR reagents thoroughly, and vortex well prior to use. The automated method allows you to use the liquid handler to prepare the qPCR reaction mix. If you choose to prepare the qPCR reaction mix manually, vortex the reaction mix thoroughly (several 5- to 10-second pulses) prior to placing the reaction mix on the deck.

Be sure that there are no bubbles in the reagents that are placed on the deck as these may adversely affect the preparation and dispensing of the qPCR reaction mix.

5. Automated Processing Requirements for the Beckman Coulter Biomek® 4000 Workstation

Confirm that you have the instrument and labware requirements listed in Sections 5.A and 5.B for use of the Identity Automation™ qPCR setup method for the PowerQuant® and Plexor® HY Systems on the Beckman Coulter Biomek® 4000 workstation. For automation of additional products, including full workflow automation, refer to Section 9 of this protocol and the appropriate automated protocol for the chemistry of interest.

5.A. Instrumentation Requirements

Minimum Installation Requirements

The following Beckman Coulter parts are required for the Identity Automation™ qPCR setup method for the PowerQuant® and Plexor® HY Systems on the Beckman Coulter Biomek® 4000 workstation.

Part Description	Quantity	Beckman Coulter Part#
Biomek® 4000 Basic Liquid Handling Package: Includes Biomek® 4000 Laboratory Automated Workstation, Biomek® Software Version 4.x[1] with Windows® 7 Automation Controller, Monitor and Mouse, P200L Single Channel Pipette Tool with LLS, MP200 Eight Channel Pipette Tool, Accu Frame Autoframing Tool, Tip Rack Holder (Qty 2), Labware Holder (Qty 3), Tool Holder, and Starter Kit with assorted BCI Labware, basic on-site training, basic application support and complete system installation	1	B22867
Biomek® 4000 Integration Deck	1	A95573
Module Accessory, Left Side	1	987264
Holder, Tip Rack	4[2]	391910
Holder, Labware, Gray	5[2]	609120
Tool Rack, Five Liquid-Handling Tools, One HDR Tool or One HDR Fan	1	609119[3]
Large Disposal Option, for BioWorks™ 2.0 and Later (optional but recommended)	1	609751[3]
Frame for Reservoirs (1 each)	1	372795

[1]Method developed for Beckman Coulter Biomek® Software, version 4.1.28.0, running on Windows® 7.

[2]These quantities represent the total number of items required. Two Holders, Tip Rack, and three Holders, Labware, Gray, are supplied with the Biomek® 4000 Workstation.

[3]The Gripper Tool Rack and Disposal Option are also available as part of the Gripper Tool System, Biomek® 3000/4000 (Beckman Coulter Part# 986129). The Gripper Tool System is not required for Identity Automation™ qPCR setup; it is required for full workflow automation (Section 9).

5.B. Additional Hardware, Labware and Consumables Required

The hardware and consumables below are required for the Identity Automation™ qPCR setup method for the PowerQuant® and Plexor® HY Systems on a Beckman Coulter Biomek® 4000 workstation. When automating additional products, refer to the appropriate Automated Protocol for a list of Promega items required for that kit and your platform.

Additional Hardware Required

Hardware Supplier	Cat.#	Description	Number Required
Promega	V1601	Four-Position Tube Holder	2
Promega	V8251	Plate Clamp 96 (for use with nonskirted plates and strip tubes)[1]	1–2 (optional)
Promega	V8261	Plate Stand (for use with nonskirted plates and strip tubes)[1]	1–2 (optional)

[1]The Plate Clamp 96 and Plate Stand are optional for securing nonskirted 96-well plates or MicroAmp® Strip Tubes on the deck. The Applied Biosystems MicroAmp® 96-Well Base (Cat.# N801-0531) or similar devices also may be suitable.

Consumables Required

Consumable Supplier	Cat.#	Description	Number Required Per Run
Beckman Coulter	717253	Biomek® AP96 P250 Tips, Pre-sterile with Barrier (case of 10 boxes)	<¼ box
Beckman Coulter	A21586	Biomek® P50 Tips, Pre-sterile with Barrier (case of 10 boxes)	1–3 boxes
Beckman Coulter	372788	Quarter Reservoir, Divided by Length (case of 48) (optional: for manual preparing of trough qPCR reaction mix at >1.45ml)	1[1]
User-selected	N/A	96-well optical PCR plate and plate cover (or sealing film) for qPCR amplification	1–2
User-selected	N/A	96-well PCR plate(s) or strip tubes containing unknown samples	1
User-selected	N/A	96-well plate or strip tubes	one plate or up to six 8-tube strips per run
User-selected	N/A	1.5ml microcentrifuge tubes	2 (one tube to prepare qPCR reaction mix at volumes <1.3ml[1] and one tube for DNA standard dilution buffer)

[1]Only one tube or reservoir type is required for qPCR reaction mix per run; the type depends on the qPCR reaction mix volume and user preference.

5.C. Beckman Coulter Biomek® 4000 Initial Deck Configuration

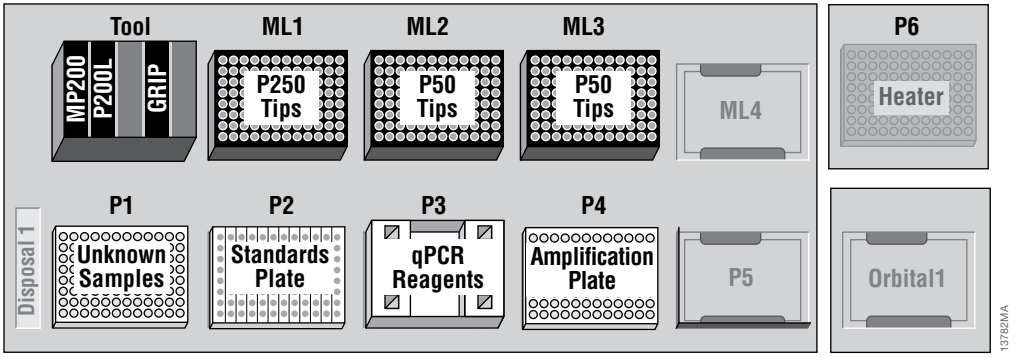

Figure 1. Beckman Coulter Biomek® 4000 initial deck configuration. Minimum requirements are shown for installation of the Identity Automation™ qPCR setup method for the PowerQuant® and Plexor® HY Systems on the Beckman Coulter Biomek® 4000 Workstation. This layout illustrates the processing of samples from one sample plate to one amplification plate and is shown as an example only; setup involving generation of two amplification plates will affect number and placement of plates and P50 tip racks in the empty positions on the deck.

Position Tool	Tool rack with, from left to right, MP200 tool, P200L tool, Gripper tool[1]
Position ML1	Holder, Tip Rack, with Biomek® AP96 P250 Tips
Position ML2	Holder, Tip Rack, with Biomek® P50 Tips
Position ML3	Holder, Tip Rack, with Biomek® P50 Tips (as required)
Position ML4	Holder, Tip Rack, with Biomek® P50 Tips (as required)
Position P6	Holder, Gray, Labware with V&P Scientific Heating Block[1], Deep Well Heat Transfer Block[1]
Disposal 1	Large Disposal Option (TipDisposal ALP is optional but recommended)
Position P1	Holder, Gray, Labware, with Strip tubes or 96-well plate containing unknown samples
Position P2	Holder, Gray, Labware, with Strip tubes or 96-well plate for preparation of DNA standard dilutions and amplification-grade water
Position P3	Holder, Gray, Labware, with Modular Reservoir Frame, Four-Position Tube Holders Frame and qPCR reagents (see Figure 2 for configuration)
Position P4	Holder, Gray, Labware, with empty 96-well optical qPCR amplification plate
Position P5	Empty (Holder, Gray, Labware, with empty qPCR amplification plate as needed)
Position P7	Orbital shaker[1]

[1]The Gripper tool (Tool), heating block with transfer block (P6) and orbital shaker (Orbital1) are not required for the Identity Automation™ qPCR setup method but may be left on the deck while the method is conducted. This extra hardware is required for the full Identity Automation™ workflow including the automated methods for the Differex™ System, DNA IQ™ System, DNA Normalization and PowerPlex® System on a Beckman Coulter Biomek® 4000 workstation. See Section 9 for details.

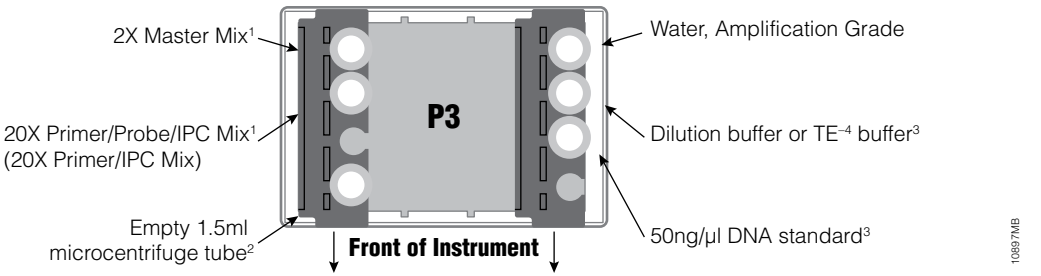

Figure 2. Configuration of PowerQuant® or Plexor® HY reagents and tubes in the Four-Position Tube Holders at deck position P3. Secure all open caps in the Four-Position Tube Holder so they do not interfere with pipetting steps. Orient the Four-Position Tube Holders with the cap-securing tabs positioned to the left. The minimum volume requirements for these reagents are determined by the number of samples processed. The liquid handler will prompt you to place tubes with the minimum volume of each reagent required.

[1]If manually preparing reaction mix, leave these positions empty.

[2]**Optional:** Manually prepared reaction mix.

[3]If manually preparing Standard DNA dilutions, these positions will be empty.

Note: If the qPCR reaction mix volume exceeds the capacity of a 1.5ml microcentrifuge (e.g., when two qPCR amplification plates are generated), the method will prompt you to prepare the reaction mix and place the reaction mix in a Quarter Reservoir Divided by Length in the left-most section of the Modular Reservoir Frame in place of the left Four-Position Tube Holder.

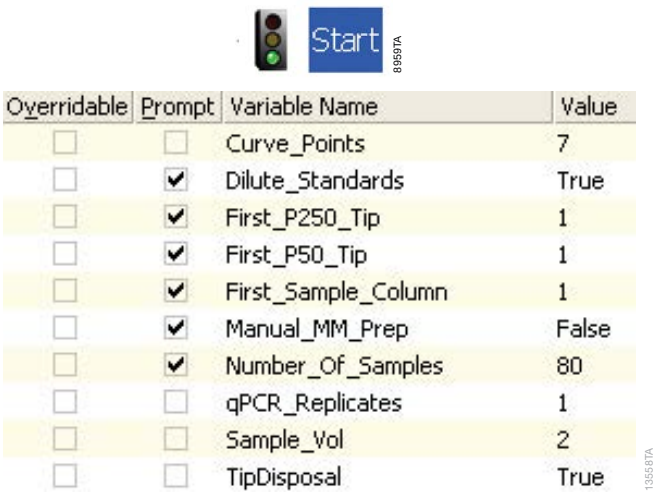

Figure 3. Variables for standard curve preparation, tip usage and qPCR reaction mix preparation.

5.C. Beckman Coulter Biomek® 4000 Initial Deck Configuration (continued)

Variable and Data Inputs

A series of variables is used to set the system and sample processing requirements at run time. These variables may be set and prompted based on specific laboratory needs. Declaration of these variables can be found by selecting the Start icon in the automated method script (Figure 3).

Curve_Points

The Curve_Points variable corresponds to the number of DNA standard dilution solutions to be transferred to the amplification plate. The automated method supports a 4-point DNA standard curve option and a 7-point DNA standard curve option. Values of 4 or 7 may be entered for this variable. For the PowerQuant® System, we recommend selecting either a 4-point or 7-point DNA standard curve. For the Plexor® HY System, we recommend a 7-point DNA standard curve.

Selection of a 4-point DNA standard curve when single qPCR amplifications are set up for samples (i.e., when qPCR_Replicates = 1) necessitates the use of a larger volume of the 50ng/µl Male DNA Standard. To conserve the volume of the 50ng/µl Male DNA Standard it is suggested that you select Curve_Points = 7 when qPCR_Replicates = 1.

Dilute_Standards

The Dilute_Standards variable determines whether a dilution series of the 50ng/µl DNA Standard will be prepared during the automated run (“True”) or if a previously or manually prepared dilution series will be used (“False”).

First_P250_Tip

The First_P250_Tip variable indicates the position of the first P250 tip in the box at position ML1, counting down each column, starting in the upper left corner. If a sufficient number of tips to perform this run are not available in a partial tip box, you will be prompted to replace the partial tip box with a full box of P250 tips.

First_P50_Tip

The First_P50_Tip variable indicates the position of the first P50 tip in the tip box at position ML2, counting down each column, starting in the upper left corner. Up to two additional boxes of P50 tips may be required for transfer of samples and standards to the amplification plate(s), and you will be prompted for their placement on the deck. If a sufficient number of tips to perform this run are not available in the partial P50 tip box plus two full P50 tip boxes, you will be prompted to replace the partial tip box with a full box of P50 tips.

First_Sample_Column

The First_Sample_Column variable indicates the first column of wells in the sample plate that contain unknown samples (e.g., if the first unknown sample is in well A2 then First_Sample_Column is set to a value of 2).

Note: Samples should always start in row A of the sample plate. This method does not recognize partial columns of samples that start in any row other than A.

Manual_MM_Prep

The Manual_MM_Prep variable determines whether the qPCR reaction mix will be prepared by the Beckman Coulter Biomek® 4000 workstation (”False”) or manually by the operator (“True”). When Manual_MM_Prep is set to “False”, you will be prompted to place the required reagents on the deck. When this variable is set to “True”, you will be prompted to manually prepare the qPCR reaction mix and place it on the deck.

Note: If the volume of the reaction mix required exceeds the volume that can be prepared by the Beckman Coulter Biomek® 4000 workstation, the automated method will default to manual preparation and prompt you accordingly regardless of the Manual_MM_Prep variable setting.

Number_Of_Samples

The Number_Of_Samples variable indicates the number of unknown samples to be processed from the sample plate in the current method run.

qPCR_Replicates

The qPCR_Replicates variable indicates the number of replicate qPCR amplifications to be prepared for each unknown sample. Replicates for each column of samples are arranged in adjacent columns on the qPCR amplification plate. The automated method supports single or duplicate reactions for all unknown samples processed in the current method run.

Sample_Vol

The Sample_Vol variable indicates the volume of each unknown sample to be transferred to the qPCR amplification plate in the current method run. To use the same liquid-handling steps for both unknown samples and DNA standard dilutions for optimal accuracy, the automated method transfers this same volume of DNA standard dilutions into the qPCR amplification plate. Values from 2 to 9 may be entered for the Sample_Vol variable.

TipDisposal

When a TipDisposal ALP is present, TipDisposal may be set to “True” so that used tips will be dropped at the TipDisposal ALP (Disposal 1 position, Figure 1). When TipDisposal is set to “False”, used tips will be placed back into tip boxes.

6. Description of the Identity Automation™ qPCR Setup Method for the PowerQuant® and Plexor® HY Systems

This overview describes the general liquid-handling steps required for the Identity Automation™ qPCR setup method for the PowerQuant® and Plexor® HY Systems on the Beckman Coulter Biomek® 4000 workstation.

1. **User-defined variable prompts:** When prompted, enter the number of unknown samples in the sample plate to be processed for amplification, the unknown sample volume (2–9µl) to process, and the number of replicate reactions to prepare for each unknown sample (1 or 2 replicates). Another prompt asks if the reaction mix will be prepared manually. A user prompt asks if the DNA standard will be serially diluted during the current method run or whether a manually or previously prepared standard dilution series will be used.

2. **Serial dilution of the PowerQuant® or Plexor® HY DNA Standard:** If you choose to use the liquid handler to prepare the serial dilution of the DNA standard, the liquid handler transfers the required volume of PowerQuant® Dilution Buffer or TE^{-4} buffer to columns 1, 2 or 3 (as required) of the strip tubes (or plate) at the standards plate position on the deck. The liquid handler then transfers the appropriate volume of the 50ng/µl DNA standard to the appropriate well position of the standards plate to prepare standard dilution 1, mixes (if required), transfers the appropriate volume of standard dilution 1 to the appropriate well to prepare standard dilution 2 and mixes. A serial transfer is repeated for standard dilution 3 through standard dilution 7 using a fresh tip for each transfer. The liquid handler also transfers Dilution Buffer or TE^{-4} buffer to the appropriate wells of the standards plate to serve as a no-template control. The volumes of 50ng/µl DNA standard, PowerQuant® Dilution Buffer or TE^{-4} buffer and serial dilution depend on the user-specified unknown sample volume (2–9µl) and the number of amplification plates generated during the method run. The well locations in the standards plate where each standard dilution and the no-template control (NTC) are prepared depend on the values of type of DNA standard curve selected (4- or 7-point curve) and the number of replicate qPCR amplifications being prepared for each unknown sample (1 or 2; see Section 7).

3. **Preparation of the PowerQuant® or Plexor® HY reaction mix:** If you choose to use the liquid handler to prepare the reaction mix, the liquid handler transfers the required volume of each component to a 1.5ml microcentrifuge tube and tip-mixes. The volumes are based on the total number of unknown samples, number of replicate qPCR amplifications to be conducted per unknown sample, required reagent dead volumes and an appropriate number of additional reactions for the DNA standard dilutions (e.g., 10 and 16 additional reactions for a 4-point and 7-point DNA standard curve, respectively). For volumes of reaction mix up to 1,300µl, the reaction mix can be prepared by the liquid handler in the empty 1.5ml tube. Larger volumes of reaction mix require manual preparation. Manually prepared qPCR amplification mix can be placed on the deck in a 1.5ml tube or a 19ml quarter-divided by length reservoir.

4. **Transfer of prepared PowerQuant® or Plexor® HY reaction mix to qPCR amplification plate:** The liquid handler transfers 11µl of reaction mix to the appropriate wells of the qPCR amplification plate(s).

5. **Transfer of unknown samples to the qPCR amplification plate:** The liquid handler transfers the user-specified volume (2–9µl) of each unknown sample from the unknown sample plate to the appropriate well of the qPCR amplification plate. The total reaction volume per well is 20µl. If duplicate qPCR amplifications are set up for each unknown sample, the replicates are placed in adjacent columns in the amplification plate (e.g., A1 and A2).

   For sample volumes less than 9µl, the liquid handler transfers amplification-grade water to columns 10, 11 and 12 (as required) of the standards plate. The liquid handler aspirates amplification-grade water from the standards plate and then aspirates the user-specified unknown sample volume (total volume transferred is always 9µl). The water and unknown sample then are dispensed to the appropriate well of the qPCR amplification plate.

6. **Transfer of PowerQuant® or Plexor® HY DNA standard dilutions to qPCR amplification plate:** The liquid handler transfers the user-specified volume of each DNA standard dilution to appropriate wells of columns 11 and 12 of the qPCR amplification plate (see Section 7). Duplicate qPCR amplifications are set up for the DNA standard dilutions and no-template control (NTC) on each amplification plate prepared. The total reaction volume per well is 20µl.

   For transfer volumes less than 9µl, the liquid handler first aspirates amplification-grade water from the standards plate followed by the user-specified volume of each DNA standard dilution (total volume transferred is always 9µl). The water and DNA standard dilution then are dispensed to the appropriate well of the qPCR amplification plate.

7. **End of Method:** The user seals the qPCR amplification plate with optical film or caps, centrifuges the plate and then immediately initiates thermal cycling. The total time for the automated method to process one full sample plate (86 samples) to one amplification plate (86 samples and 10 controls) is approximately 30 minutes.

Note: The automated method generates a report file with the concentrations of the DNA standard dilutions. These concentrations are required for setup of the qPCR instrument and analysis of the amplification data. The concentrations may vary from method run to method run based on the user-selected volume of unknown sample and DNA standard dilution transferred to the amplification plate. See Section 7 for details on the report file and location of standards in the amplification plate.

7. DNA Standard Curve

When assembling amplification reactions for the DNA standard curve, the method requires amplification of a volume of DNA standard that is equal to the volume of unknown sample chosen by the user (2–9µl) in the amplification plate. The Identity Automation™ qPCR setup method is programmed to perform all calculations and liquid-handling steps required to generate an appropriate DNA standard curve on each amplification plate. Each DNA standard is diluted such that the user-specified volume is added to each amplification and the final DNA concentration is within the linear range of the PowerQuant® or Plexor® HY System.

The automated method generates a file that reports the concentrations of DNA standard prepared during serial dilution of the 50ng/µl DNA standard so that you can input the correct concentrations when setting up the qPCR instrument and analyzing the data. The default file name and location is:
C:\Temp\LiquidHandlerOutput\IDAuto_qPCR_Report.csv

For the PowerQuant® System, we recommend using either a 4-point or 7-point DNA standard curve. For the Plexor® HY System, we recommend a 7-point DNA standard curve.

The Identity Automation™ qPCR setup method for the PowerQuant® and Plexor® HY Systems prepares a serial dilution of the provided 50ng/µl DNA standard using the PowerQuant® Dilution Buffer, TE^{-4} Buffer or other buffers supplied by the user. Irrespective of the user-supplied value for the Curve_Points variable, the method prepares seven DNA standard dilutions and the no-template control (NTC) in the standards plate on the deck of the Biomek® 4000 workstation. However, the placement of the standard dilutions in the standards plate and the volume of each standard made depends on the user-supplied values for the variables Curve_Points and qPCR_Replicates as shown in Figure 4 (Curve_Points = 7 and qPCR_Replicates = 1 or 2), Figure 5 (Curve_Points = 4 and qPCR_Replicates = 1) and Figure 6 (Curve_Points = 4 and qPCR_Replicates = 2). The Identity Automation™ qPCR setup method for the PowerQuant® and Plexor® HY Systems on the Beckman Coulter Biomek® 4000 Laboratory Automation Workstation employs these different modes of DNA standard dilution preparation to allow for both the time benefit of using of the MP200 8-tip tool to transfer samples and standards to the amplification plate and the increased sample throughput of the 4-point standard curve option.

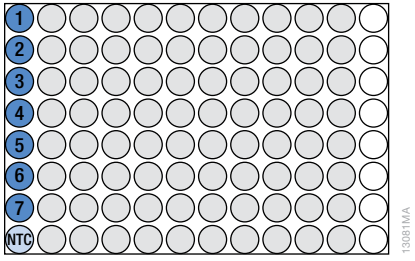

Figure 4. The standards plate for a 7-point DNA standard curve. During the serial dilution of the 50ng/µl DNA Standard to prepare a 7-point DNA standard curve, seven standard dilutions are prepared in column 1 (shown in dark blue and numbered from highest concentration 1 to lowest concentration 7). The no-template control (NTC; shown in light blue) is also placed in column 1, row H. Regardless of user-supplied value of qPCR_Replicates, the standard dilutions are prepared in the well locations as shown. The amplification-grade water (shown with white circles) is staged in column 12 (as required, see Section 6 steps 5 and 6).

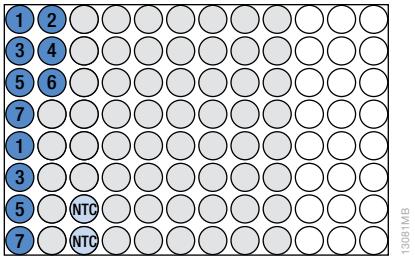

Figure 5. The standards plate for a 4-point DNA standard curve and single qPCR amplification setup for unknown samples. During the serial dilution of the 50ng/µl DNA Standard to prepare a 4-point DNA standard curve, seven standard dilutions are prepared in columns 1 and 2, rows A through D (shown in dark blue and numbered from highest concentration 1 to lowest concentration 7). After preparation, approximately half the volume of standard dilutions 1, 3, 5 and 7 are transferred to wells in column 1, rows E through H, so that duplicates of these standard dilutions can be transferred to the amplification plate using the MP200 8-tip tool as indicated in Figure 7, Panel B. The no-template control (NTC; shown in light blue) is also placed in duplicate in column 3, rows G and H. The amplification-grade water (shown with white circles) is staged in columns 10, 11 and 12 (as required, see Section 6, Steps 5 and 6).

Selection of a 4-point DNA standard curve when single qPCR amplifications are set up for samples (i.e., when qPCR_Replicates = 1) necessitates the use of a larger volume of the 50ng/µl Male DNA Standard.

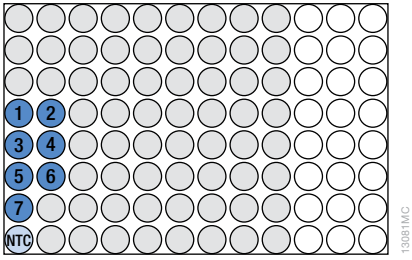

Figure 6. The standards plate for a 4-point DNA standard curve and duplicate qPCR amplification setup for unknown samples. During the serial dilution of the 50ng/µl DNA Standard to prepare a 4-point DNA standard curve, seven standard dilutions are prepared in columns 1 and 2, rows D through G (shown in dark blue and numbered from highest concentration 1 to lowest concentration 7). The no-template control (NTC; shown in light blue) is also placed in column 1, row H. The placement of standard dilutions 1, 3, 5 and 7 and the NTC in column 1 allows for these standard dilutions to be transferred to the amplification plate using the MP200 8-tip tool as indicated in Figure 7, Panel C. The amplification-grade water (shown with white circles) is staged in columns 10, 11 and 12 (as required, see Section 6, Steps 5 and 6).

7. DNA Standard Curve (continued)

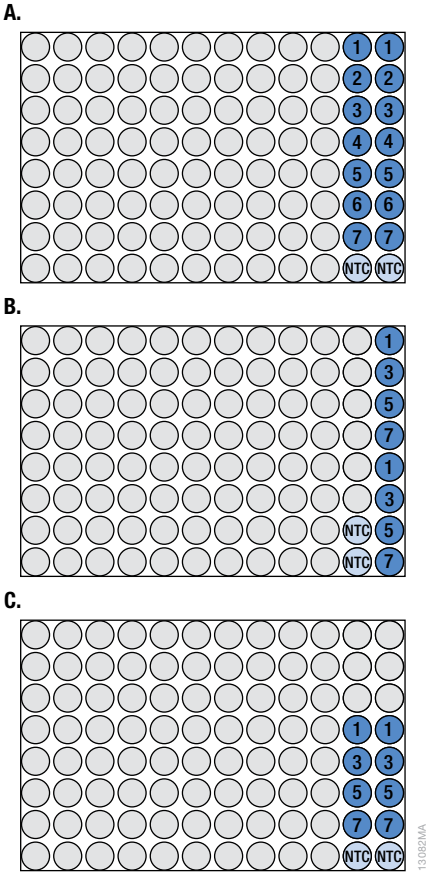

Figure 7. DNA standard curve and NTC placement on the qPCR amplification plate.
Panel A. Placement of a 7-point DNA standard curve and one or two replicate qPCR amplifications for each unknown sample. **Panel B.** Placement of a 4-point DNA standard curve and one replicate qPCR amplification for each unknown sample. **Panel C.** Placement of a 4-point DNA standard curve and two replicate qPCR amplifications for each unknown sample.

When a 7-point DNA standard curve is selected (Curve_Points equals 7), the Identity Automation™ qPCR setup method transfers all seven DNA standard dilutions and the NTC to each amplification plate shown in Figure 7, Panel A. When a 4-point DNA standard curve is selected (Curve_Points equals 4), the Identity Automation™ qPCR setup method transfers every other DNA standard dilution (i.e., dilutions 1, 3, 5 and 7 of the serial dilution series) and the NTC to each amplification plate. The exact position of the DNA standard dilutions on the amplification plate depends on whether you selected 1 or 2 replicate qPCR amplifications for each unknown sample (Figure 7, Panels B and C, respectively).

8. Important Considerations

1. Always use aerosol-resistant tips to minimize the risk of cross-contamination.
2. Thoroughly mix all qPCR system reagents by vortexing before placing reagents on the deck. This includes the manually prepared qPCR reaction mix; vigorous mixing will ensure homogeneity and will not harm performance.
3. Calculations for the qPCR reaction mix preparation include excess reagent to ensure that enough qPCR reaction mix is prepared for all qPCR amplification wells.
4. Pipetting techniques used must be calibrated to ensure accurate volume handling for both samples and amplification reagents. Calibration checks are performed as part of Promega standard installation service.
5. Aspirating and dispensing speeds, as well as pipetting heights, are critical to the success of this method. Aspiration of water with samples and standards as performed in this method (versus aspiration of sample alone) improves pipetting accuracy for low-volume transfers.
6. When defining labware, be certain to set well heights carefully at the well bottom of each labware present on the deck.
7. Keep dispensing tools clean and serviced to ensure accuracy and delivery of consistent volumes across the eight pipetting mandrels. It may be necessary to request maintenance from a robotic service representative if visible dispensing inaccuracies are noted or inconsistent results are obtained.

9. Automated Processing Requirements for Full Workflow on the Beckman Coulter Biomek® 4000 Workstation

The full workflow includes the Differex™ System, DNA IQ™ System, DNA Quantitation (PowerQuant® System or Plexor® HY System) and DNA Normalization and PowerPlex® System Setup.

Full Work-Flow Requirements

The following Beckman Coulter parts are required for the automated Differex™ System, DNA IQ™ System, DNA Quantitation (PowerQuant® System or Plexor® HY System) and DNA Normalization and PowerPlex® System methods on a Beckman Coulter Biomek® 4000 workstation.

Part Description	Quantity	Beckman Coulter Part#
Biomek® 4000 Basic Liquid Handling Package: Includes Biomek® 4000 Laboratory Automated Workstation, Biomek® Software Version 4.x with Windows® 7 Automation Controller, Monitor and Mouse, P200L Single Channel Pipette Tool with LLS, MP200 Eight Channel Pipette Tool, Accu Frame Autoframing tool, Tip Rack Holder (Qty 2), Labware Holder (Qty 3), Tool Holder, and Starter Kit with assorted BCI Labware, basic on-site training, basic application support and complete system installation	1	B22867
Biomek® 4000 Integration Deck	1	A95573
Module Accessory, Left Side	1	987264
Holder, Tip Rack	4[1]	391910
Holder, Labware, Gray	5[1]	609120
Gripper Tool System, Biomek® 3000/4000: Includes Gripper Tool, Gripper Tool Rack, Calibration Plate, Disposal Option, Disposal Bags and spare Gripper Pads	1	986129
Standard Single-Position ALP	1	719357
Orbital Shaker ALP	1	379448
Frame for Reservoirs (1 each)	1	372795

[1]These quantities represent the total number of items required. Two Holders, Tip Rack, and three Holders, Labware, Gray, are supplied with the Biomek® 4000 Workstation.

Additional Hardware, Labware and Consumables Required

The following additional items are required for Identity Automation™ full workflow processing on a Beckman Coulter Biomek® 4000 Workstation.

Additional Promega Hardware Required for Full Workflow: Differex™ System, DNA IQ™ System, DNA Quantitation (PowerQuant® System or Plexor® HY System) and DNA Normalization and PowerPlex® Setup

		Number Required for the Indicated Automated Method			
Cat.#	Description	Differex™ Method	DNA IQ™ Method	PowerQuant® or Plexor® HY Method	PowerPlex® Normalization Method
DG1820	STR Normalization Manager™				1
V6041	MagnaBot® Flat Top Magnetic Separation Device	1			
A2661	Heat Block Adapter	1			1[1]
V6761	V&P Scientific Heating Block (110V, for North America use only)		1		
V8151	MagnaBot® 96 Magnetic Separation Device		1		
Z3301	1/4 inch Foam Spacer		1		
V6741	Deep Well Heat Transfer Block		1		
V1601	Four-Position Tube Holder			2	2
V8251	Plate Clamp 96 (for use with nonskirted plates and strip tubes)			1–2 (optional)[2]	1–2 (optional)[2]
V8261	Plate Stand (for use with nonskirted plates and strip tubes)			1–2 (optional)[2]	1–2 (optional)[2]

[1]The Heat Block Adapter is only needed when conducting the Swab Protocol of the Identity Automation™ DNA Normalization and PowerPlex® Setup method on a Biomek® 4000 Workstation.

[2]The Plate Clamp 96 and Plate Stand are optional for securing nonskirted 96-well plates or MicroAmp® Strip Tubes on the deck. The Applied Biosystems MicroAmp® 96-Well Base (Cat.# N801-0531) or similar devices also may be suitable.

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Additional Consumables Required for Full Workflow Automation: Differex™ System, DNA IQ™ System, DNA Quantitation (PowerQuant® System or Plexor® HY System) and DNA Normalization and PowerPlex® Setup

			Number Required (Per Plate Processed) for the Indicated Automated Method			
Supplier	**Cat.#**	**Description**	**Differex™ Method**	**DNA IQ™ Method**	**PowerQuant® or Plexor® HY Method**	**PowerPlex® Normalization Method**
Beckman Coulter	717253	Biomek® AP96 P250 Tips, Pre-sterile with Barrier (case of 10 boxes)	1 box	2 boxes	<¼ box	<¼ box
Beckman Coulter	A21586	Biomek® P50 Tips, Pre-sterile with Barrier (case of 10 boxes)			1–3 boxes	1–3 boxes
Beckman Coulter	372786	Half Reservoir (case of 24)		1		1
Beckman Coulter	372788	Quarter Reservoir, Divided by Length (case of 48)	1	1	1[1]	1[1]
Beckman Coulter	372790	Quarter Reservoir (case of 48)	2	1		1[1]
Promega	V6771	1.2ml, Round-Bottom Deep Well Plate (case of 50)		2		
Promega	V6781	2.2ml, Square-Well Deep Well Plate (case of 50)	2–3	1[2]		
Promega	V1391	Slicprep™ 96 Device (pack of 10)	1	1[2]		
Promega	V6821	1.1ml, Square-Well, V-Bottom Deep Well Plate (case of 25)				2
User-selected		96-well PCR plate or strip tubes for PCR amplification				1
User-selected		96-well PCR plate for DNA Quantitation amplification			1–2 per run	
User-selected		96-well PCR plate or strip tubes for standard curve preparation			one plate or up to six 8-tube strips per run	
User-selected		96-well PCR plate or strip tubes for purified unknown DNA samples		1		
User-selected		1.5ml microcentrifuge tube			2[3]	1[1]

[1]Only one tube or reservoir type is required per run; the type depends on user preference and the volume of qPCR reaction mix or PowerPlex® PCR amplification mix.
[2]The 2.2ml, Square Well Plate, or Slicprep™ 96 Device can be used for samples; both are not required for the DNA IQ™ method. Samples processed using the Differex™ System do not require an additional plate for DNA purification using the DNA IQ™ System.
[3]One tube[1] is to prepare qPCR reaction mix at volumes <1.3ml, and one tube is for DNA standard dilution buffer.

10. Summary of Change

The following change was made to the 11/16 revision of this document:

1. Updated to include the protocol for using the PowerQuant® System in the Beckman Coulter Biomek® 4000 Workstation.

[a]U.S. Pat. No. 6,242,235, Australian Pat. No. 761757, Canadian Pat. No. 2,335,153, Chinese Pat. No. ZL99808861.7, Hong Kong Pat. No. HK 1040262, Japanese Pat. No. 3673175, European Pat. No. 1088060 and other patents pending.

[b]Dye compounds in this product are sold under license from Biosearch Technologies, Inc., and protected by U.S. and worldwide patents either issued or in application. The license does not include rights for human IVD use.

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[d]This product is sold under licensing arrangements with Stratagene. The purchase price of this product includes limited, nontransferable rights under U.S. Pat. Nos. 5,449,603, 5,605,824, 5,646,019 and 5,773,257 owned by Stratagene to use only this amount of the product to practice the claims in said patent solely for activities of end users within the fields of life science research and forensic analysis of genetic material relating to, or obtained as the result of, criminal investigations or disaster sites conducted either by or for a governmental entity, or for use in or preparation for legal proceedings, as well as the compilation and indexing of the results of such analysis, and also analysis for parentage determination (the "Forensic and Genetic Identity Applications Field"). The Forensic and Genetic Identity Applications Field specifically excludes tissue typing related to transplantation or other medical procedures. Further licensing information may be obtained by contacting the Business Development Department, Stratagene California, 11011 North Torrey Pines Road, La Jolla, CA 92037.

[e]This product is sold under licensing arrangements with the USB Corporation. The purchase price of this product includes limited, nontransferable rights under U.S. Patent Application Serial Number 11/171,008 owned by the USB Corporation to use only this amount of the product to practice the claims in said patent solely for activities of end users within the fields of life science research and forensic analysis of genetic material relating to, or obtained as the result of, criminal investigations or disaster sites conducted either by or for a governmental entity, or for use in or preparation for legal proceedings, as well as the compilation and indexing of the results of such analysis, and also analysis for parentage determination (the "Forensic and Genetic Identity Applications Field"). The Forensic and Genetic Identity Applications Field specifically excludes tissue typing related to transplantation or other medical procedures. Further licensing information may be obtained by contacting the USB Corporation, 26111 Miles Road, Cleveland, OH 44128.

[f]CAL Fluor® technology is the subject of pending patents and is licensed and sold under agreement with Biosearch Technologies, Inc., for research and development and forensic and paternity testing. These products are sold for use by the end-user only and may not be resold, distributed or repackaged.

[g]Use of this product for basic PCR is outside of any valid US or European patents assigned to Hoffman La-Roche or Applera. This product can be used for basic PCR in research, commercial or diagnostic applications without any license or royalty fees.

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