AUTOMATED PROTOCOL

Identity Automation™ qPCR Setup Protocol for the PowerQuant® and Plexor® HY Systems on the Tecan Freedom EVO® Workstation

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

Revised 3/18
EP047

Identity Automation™ qPCR Setup Protocol for the PowerQuant® and Plexor® HY Systems on the Tecan Freedom EVO® 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® System [a,c] and Plexor® HY System [a,b,d–g] on the Tecan Freedom EVO® automated liquid-handling workstation.

The automated method is used with the PowerQuant® System and Plexor® HY System. The method allows use of a 4-point standard curve (with the PowerQuant® System) or 7-point DNA standard curve (with the PowerQuant® or Plexor® HY System). We do not recommend using a 4-point 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 the flexibility to support the following:

- Setup of two amplification plates from a single sample plate to allow processing of single quantitation reactions from up to 96 unknown samples or duplicate quantitation reactions from up to 86 unknown samples.
- Setup of two amplification plates from two sample plates to allow processing of single quantitation reactions from up to 86 unknown samples per sample plate (172 total samples) or duplicate quantitation reactions from up to 43 unknown samples per sample plate (86 total samples).
- Setup of one amplification plate from 1 full sample plate or 1–3 partial sample plates to allow processing of single quantitation reactions from up to 86 samples or duplicate quantitation reactions from up to 43 unknown samples.

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 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
- 2 × 8 strip tubes (e.g., Applied Biosystems Cat.# N801-0580) or an additional 96-well PCR plate (two columns will be used for DNA standard dilutions and amplification-grade water)
- 96-well plates or strip tubes containing unknown samples
- graduated self-standing 5ml tube (optional; for preparing single-tube PCR amplification mix volumes of 1.3–5.0ml) (e.g. VWR Cat.# 89005-596 or Evergreen Scientific Cat.# 222-3007-080)

Note: The PowerQuant® System and 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 curve is selected. The automated method will prompt you to confirm that a minimum volume of each component is available if the reaction mix is prepared by the liquid handler.

4. Before You Begin

4.A. Sample Considerations

Unknown samples must be in a 96-well format (plate or strip tubes). Unknown samples should be centrifuged briefly to remove any air bubbles that might be present as 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 this may adversely affect the preparation and/or dispense of the qPCR reaction mix.

5. Automated Processing Requirements for the Tecan Freedom EVO® Workstation

Confirm that you have the required instrumentation and labware 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 Tecan Freedom EVO® 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 Tecan parts are required for the Identity Automation™ qPCR setup method for the PowerQuant® and Plexor® HY Systems on the Tecan Freedom EVO® workstation.

Part Description	Quantity	Tecan Part#
Tecan Freedom EVO® 100 instrument (or larger) configured with 4 or 8 × 500µl syringes and Disposable Tip LiHa Arm (Freedom EVOware® Standard software)[1]	1	Contact Tecan
Wash Station, LiHa, with DiTi Waste Chute and Trough Carrier	1	10650037
DiTi Carrier, 4-Position[2]	1	30019579
Microplate Carrier, 3 Position, Landscape (MP 3Pos)[3]	1–2	10612604
Tube Carrier, 16mm, 16-Position (1 × 16)[4]	1	30019987

[1]The method was developed for Freedom EVOware® Standard software, version 2.6, running Windows® 7 using a liquid LiHa configured with 500µl syringes. Contact Promega for information regarding compatibility with earlier versions of Freedom EVOware® Standard software and different syringe sizes.

[2]Alternatively, two DiTi Carriers, 3-Position (Cat.# 10613022), can be used; at least 4 tip rack positions are required.

[3]One Microplate Carrier, 3-Position Landscape, is required to process one plate of unknown samples to one amplification plate. Automated runs involving multiple unknown sample plates or multiple amplification plates will require an additional Microplate Carrier.

[4]**Optional:** For robotically prepared or manually prepared single-tube qPCR reaction mix volumes of 1.3–1.85ml or 1.3–5.0ml, respectively.

5.B. Labware and Consumables Required

The following additional items are required for the Identity Automation™ qPCR setup method for the PowerQuant® and Plexor® HY Systems on a Tecan Freedom EVO® 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 or strip tubes)[1]	1–2 (optional)
Promega	V8261	Plate Stand (for use with nonskirted plates or strip tubes)[1]	1–2 (optional)
Tecan	10613048 or 10613049	Trough, Disposable, 100ml, Polypropylene Natural or Trough, Disposable, 100ml, Polypropylene Gray	2–3[2]

[1]The Plate Clamp 96 and Plate Stand are optional for securing nonskirted 96-well plates or MicroAmp® Strip Tubes on the worktable. The Applied Biosystems MicroAmp® 96-Well Base (Cat.# N801-0531) or Tecan Plate Adapter PCR (Cat.# 30032860) also may be suitable.

[2]A 100ml trough is required to hold each Four-Position Tube Holder and the Disposable Trough, 25 ml, Low Dead Volume (if required). These 100ml troughs may be reused.

5.B. Labware and Consumables Required (continued)

Consumables Required

Consumable Supplier	Cat.#	Description	Number Required per Run
Tecan	10612553 or 30000629	200µl LiHa disposable tips with filter	<¼ rack
Tecan	30032114	50µl LiHa disposable tips with filter	1–3 racks
User-selected		96-well optical PCR plate and plate cover (or sealing film) for qPCR amplification	1–2
User-selected		96-well PCR plate(s) or strip tubes for unknown samples	1–3
User-selected		96-well plate or strip tubes	One plate or two 8-tube strips per run
User-selected		1.5ml microcentrifuge tube	One tube to prepare qPCR reaction mix at volumes <1.3ml[1] and one tube for DNA standard dilution buffer
User-selected		5ml graduated self-standing tube (optional; for preparing single-tube qPCR reaction mix volumes of 1.3–5.0ml)	1[1]
Tecan	30055743	Disposable Trough, 25ml, Low Dead Volume (optional; for preparing qPCR reaction mix at volumes >1.45ml)	1[1]

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

5.C. Tecan Freedom EVO® Initial Deck Configuration

Minimum Worktable Requirements

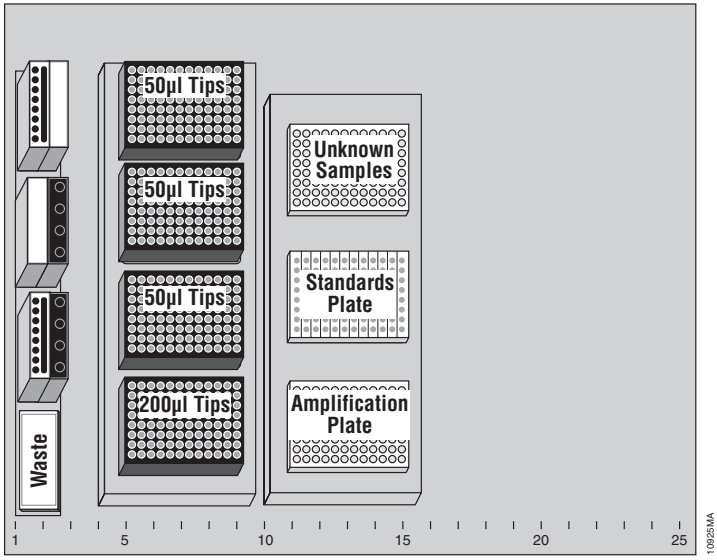

Figure 1. Tecan Freedom EVO® initial deck configuration. Shown are the minimum requirements for installation of the Identity Automation™ qPCR Setup Method for the PowerQuant® and Plexor® HY Systems on the Tecan Freedom EVO® workstation. This layout supports processing of samples from one sample plate to one amplification plate and is shown as an example only; the automated method can be adapted to any worktable layout as long as this hardware (or equivalent) is present. Note that additional labware positions are required to run the maximum number of sample plates or amplification plates.

Grid 1	Wash Station, LiHa, with DiTi Waste Chute and Trough Carrier	
	Position 1 (rear)	Trough, Disposable, 100ml , with 25ml Low Dead Volume Disposable Trough (when required)
	Position 2	Trough, Disposable, 100ml, with Promega Four-Position Tube Holder
	Position 3 (front)	Trough, Disposable, 100ml, with Promega Four-Position Tube Holder
Grid 4	DiTi Carrier, 4-Position	
	Position 1 (rear)	50µl LiHa Disposable Tips with Filter
	Position 2	50µl LiHa Disposable Tips with Filter (as needed)
	Position 3	50µl LiHa Disposable Tips with Filter (as needed)
	Position 4 (front)	200µl LiHa Disposable Tips with Filter
Grid 10	Microplate Carrier, 3 Position	
	Position 1 (rear)	Strip tubes or 96-well plate containing unknown samples
	Position 2	Strip tubes or 96-well plate for preparation of DNA standard dilutions
	Position 3 (front)	qPCR amplification plate

5.C. Tecan Freedom EVO® Initial Deck Configuration (continued)

Tube holder at Grid 1, Position 2: **Tube holder at Grid 1, Position 3:**

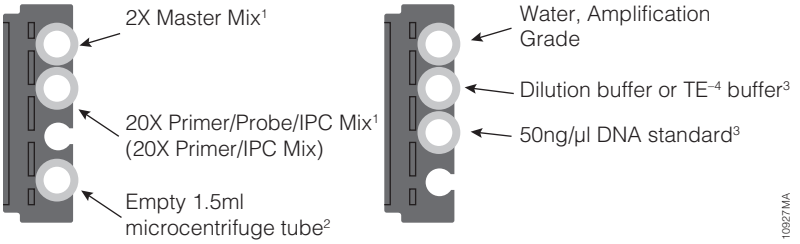

Figure 2. Configuration of PowerQuant® or Plexor® HY reagents and tubes in the Four-Position Tube Holders at Grid 1, Positions 2 and 3. Secure all open caps in the Four-Position Tube Holder so that they do not interfere with pipetting steps. The orientation of the Four-Position Tube Holders (e.g., cap-securing tabs positioned to the left or right) depends on the worktable layout and is shown to the left as an example only. 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 qPCR reaction mix, these positions will be empty.

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

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

Variable and Data Inputs

NumberSamplePlates

The NumberSamplePlates variable corresponds to the number of sample plates containing unknown samples to be processed in the current method run.

SamplesInPlate1 (SamplesInPlate2, SamplesInPlate3)

The SamplesInPlate1 variable indicates the number of unknown samples to be processed from the first sample plate in the current method run. The SamplesInPlate2 variable indicates the number of unknown samples to be processed from the second sample plate. The SamplesInPlate3 variable indicates the number of unknown samples to be processed from the third sample plate.

FirstWellPlate1 (FirstWellPlate2, FirstWellPlate3)

The FirstWellPlate1 variable indicates the position of the first unknown sample in the first sample plate to be processed. Well numbering starts at well A1 as well number 1 and is counted down the leftmost column and continues at the top of the next column (e.g., A2 is well number 9). Samples will be processed consecutively from the first well specified for a plate to the maximum sample number for that plate (e.g., if 32 samples are present in plate 1 starting at well 2, then wells 2 through 33 will be processed).

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.

Curve_Points

The Curve_Points variable indicates the number of DNA standards to be transferred to the qPCR 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 using either a 4-point or 7-point DNA standard curve. For the Plexor® HY System, we recommend a 7-point DNA standard curve.

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”). When this variable is set to “False”, you will be prompted to place standard dilutions at specific positions on the deck at concentrations and volumes that are appropriate for the selected values of Curve_Points and Sample_Vol and that are sufficient for the number of amplification plates generated.

Manual_MM_Prep

The Manual_MM_Prep variable determines whether the qPCR reaction mix will be prepared by the Tecan Freedom EVO® 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 Tecan Freedom EVO® workstation, the automated method will default to manual preparation and prompt you accordingly regardless of the Manual_MM_Prep variable setting.

Used Tip Variables: Used_DiTi200s, Used_DiTi50s

You will be prompted to enter the number of tips that have been used from the DiTi 200 and DiTi 50 tip racks at the start of the automated method. These variables are used within the EVOware® script to set the first usable DiTi Positions for these racks.

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 Tecan Freedom EVO® workstation.

1. **User-defined variable prompts:** When prompted, enter the number of unknown samples per sample plate to be processed for amplification, unknown sample volume (2–9µl) to process and 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 column 1 of the strip tubes at the standards plate position on the worktable. The liquid handler then transfers the appropriate volume of 50ng/µl DNA standard to well A1, mixes (if required), transfers the appropriate volume from well A1 to well B1 and mixes well B1. A serial transfer is repeated for wells C1 through G1 using a fresh tip for each transfer. Well H1 contains only PowerQuant® Dilution Buffer or TE^{-4} buffer and serves 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.
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 or 5ml tube and then 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. For volumes of reaction mix up to approximately 1,850µl, the reaction mix can be prepared by the liquid handler in the empty 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, a 5ml tube or a 25ml low-dead-volume trough.
4. **Transfer of prepared PowerQuant® or Plexor® HY reaction mix to the 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., wells A1 and A2).

   For sample volumes less than 9µl, the liquid handler transfers amplification-grade water to column 12 of the standards plate. The liquid handler aspirates amplification-grade water from the standards plate and then aspirates the user-specified unknown sample volume (the 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 the qPCR amplification plate:** The liquid handler transfers the user-specified volume of each DNA standard dilution to the 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. 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 Considerations

When assembling amplification reactions for the DNA standard curve, we recommend amplifying 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 in the amplification reaction is within the linear range of the PowerQuant® or Plexor® HY System.

The automated method generates a file that reports the concentrations of DNA standards 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 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 one no-template control (NTC) in column 1 of the standards plate on the worktable (Figure 3).

7. DNA Standard Curve Considerations (continued)

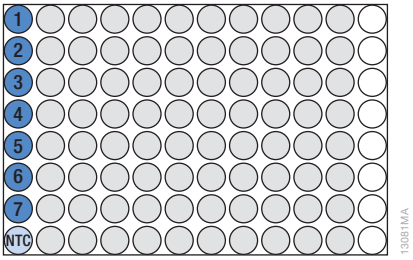

Figure 3. The standards plate. During serial dilution of the 50ng/µl DNA standard, seven standard dilutions are prepared in column 1 (shown in dark blue and numbered from highest concentration 1 to lowest concentration 7). The no-template control (NTC; shown in light blue) is placed in column 1, row H. The amplification-grade water (shown with white circles) is staged in column 12 (if required, see Section 6, Steps 5 and 6).

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 as shown in Figure 4, 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 positon of the DNA standard dilutions on the amplification plate depends on whether you selected 1 or 2 replicate amplifications for each unknown sample (Figure 4, Panels B and C, respectively).

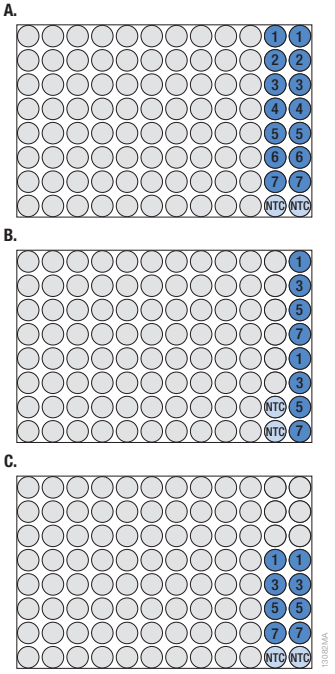

Figure 4. DNA standard curve and NTC placement on the qPCR amplification plate. Panel A. Placement of a 7-point DNA standard curve and one or two replicate qPCR amplifications for each unknown sample. **Panel B.** Placement of a 4-point DNA standard curve and one replicate qPCR amplification for each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

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 and liquid classes used must be calibrated to ensure accurate volume handling for both samples and amplification reagents. Calibration checks are performed as part of the Promega standard installation service.
5. Aspiration 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 Z-max heights carefully at the well bottom of each labware present on the worktable.

9. Automated Processing Requirements for Full Workflow on the Tecan Freedom EVO® Workstation

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

Full Workflow Requirements

The following Tecan parts are required for the automated DNA IQ™ System, DNA Quantitation (PowerQuant® System or Plexor® HY System) and DNA Normalization and PowerPlex® System methods on a Tecan Freedom EVO® workstation. A Freedom EVO® 100 or 150 worktable is required for full workflow automation; contact your local Promega representative for more information.

Part Description	Quantity	Tecan Part#
Tecan Freedom EVO® 100 instrument (or larger) configured with 4 or 8 × 500µl syringes and Disposable Tip LiHa Arm and ROMA Gripper (Freedom EVOware® Standard software)	1	Contact Tecan
Wash Station, LiHa, with DiTi Waste Chute and Trough Carrier	1	10650037
DiTi Carrier, 4-Position (for reracking DiTi Tips)	1	30019579
Shelf, 16-Position (four sites with four shelves per site)	1	10650627
Microplate Carrier, 3-Position, Landscape (MP 3Pos) or alternative carrier(s) to provide up to nine microplate positions	1	10612604
Tube Carrier, 16mm, 16-Position (1 × 16)	1	30019987
Trough/DiTi Carrier, 100ml, 3-Position, with three dedicated positions for reusing disposable tips	1	30015508
Te-Shake™ Microplate Shaker Unit	1	10760723
Microplate Nest, 1-Position, with Hold Down, Te-Shake™ Shaker	1	10760724
Mounting Plate, Te-Shake™ 1-Position with two additional Microplate Positions[1]	1	30015506
EchoTherm Dry Bath, Torrey Pines Scientific, IC20, Electronic Chilling/Heating (or equivalent; contact Tecan for additional options for heating units, adaptors and mounting hardware)[1]	1	30034627
Microplate Adaptor, Torrey Pines Scientific, IC20/IC22/IC25	1	30034628
Mounting Plate, Torrey Pines Scientific EchoTherm Dry Bath, IC20/IC22/IC25	1	30034624

[1]These parts are included in the Tecan Package #1006.

Additional Hardware, Labware and Consumables Required

The following additional items are required for Identity Automation™ full workflow processing on a Tecan Freedom EVO® workstation.

Additional Promega Hardware Required for Full Workflow: 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	DNA IQ™ Method	PowerQuant® or Plexor® HY Method	PowerPlex® Normalization Method
DG1820	STR Normalization Manager™			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)[1]	1–2 (optional)[1]
V8261	Plate Stand (for use with nonskirted plates and strip tubes)		1–2 (optional)[1]	1–2 (optional)[1]

[1]The Plate Clamp 96 and Plate Stand are optional for securing nonskirted 96-well plates or MicroAmp® Strip Tubes on the worktable. The Applied Biosystems MicroAmp® 96-Well Base (Cat.# N801-0531) or Tecan Plate Adapter PCR (Cat.# 30032860) also may be suitable.

Additional Consumables Required for Full Workflow Automation: 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	DNA IQ™ Method	PowerQuant® or Plexor® HY Method	PowerPlex® Normalization Method
Tecan	30000631 or 10612513	1000µl LiHa Disposable Tips with Filter	<¼ rack		
Tecan	10612553 or 30000629	200µl LiHa Disposable Tips with Filter	2 racks	<¼ rack	<¼ rack
Tecan	30032114	50µl LiHa Disposable Tips with Filter		1–3 racks	1–3 racks

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

			Number Required (Per Plate Processed) for the Indicated Automated Method		
Supplier	Cat.#	Description	DNA IQ™ Method	PowerQuant® or Plexor® HY Method	PowerPlex® Normalization Method
Tecan	10613049 or 10613048	Trough, Disposable, 100ml, Polypropylene Gray, or Trough, Disposable, 100ml, Polypropylene Natural	3		1
Tecan	30055743	Disposable Trough, 25 ml, Low Dead Volume		1[1]	1[1]
Promega	V6791	Pyramid-Bottom Reservoir, 12 Column	1		
Promega	V6771	1.2ml, Round-Bottom Deep Well Plate	2		
Promega	V6781	2.2ml, Square-Well Deep Well Plate	3[2]		
Promega	V1391	Slicprep™ 96 Device	1[2]		
Promega	V6821	1.1ml, Square-Well, V-Bottom Deep Well Plate			2
User-selected		96-well PCR plate or strip tubes for amplification			1
User-selected		96-well optical PCR plate or strip tubes for DNA quantitation amplifications		1–2 per run	
User-selected		96-well PCR plate(s) or strip tubes for standard curve preparation		1	
User-selected		96-well PCR plate or strip tubes for unknown samples		1–3 per run	1
User-selected		1.5ml microcentrifuge tube		1[1]	1[1]
User-selected		5ml graduated self-standing tube (optional; to prepare single-tube PCR amplification mix volumes of 1.3–5.0ml)		1[1]	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; two plates are required under the hanging 200µl pipette tips.

Full Workflow Requirements

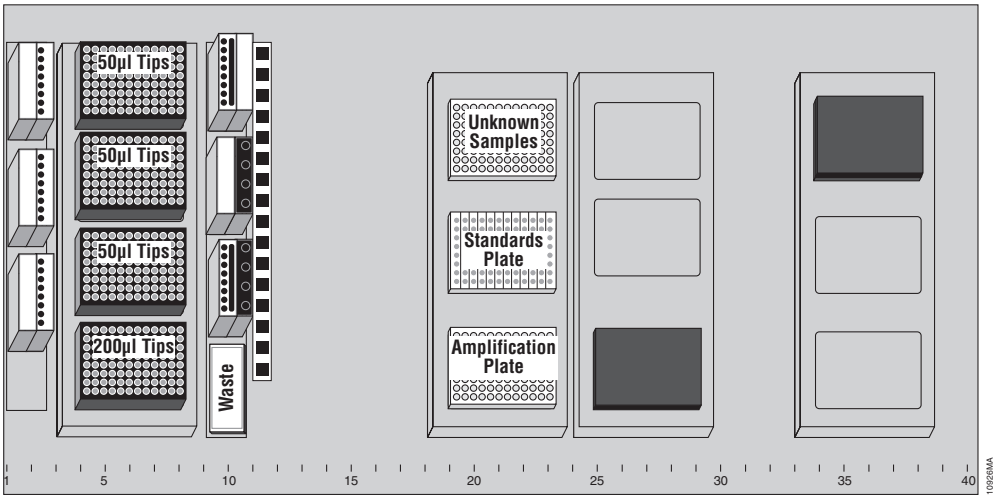

Figure 5. Tecan Freedom EVO® initial deck configuration. This worktable supports the full suite of Promega automated methods for the Tecan Freedom EVO® workstation, including the DNA IQ™ System, DNA Quantitation (PowerQuant® System or Plexor® HY System) and DNA Normalization and PowerPlex® Setup. The labware shown supports processing of samples from one sample plate to one amplification plate and is shown as an example only; the automated method can be adapted to any worktable layout as long as these hardware (or equivalent) are present.

Grid 1 Trough/DiTi Carrier, 100ml, 3-Position, with associated tip rack positions (troughs and tip rack positions used during the DNA Normalization and PowerPlex® Setup and/or the DNA IQ™ System)

Grid 3 DiTi Carrier, 4-Position
Position 1 (rear) 50µl LiHa Disposable Tips with Filter
Position 2 50µl LiHa Disposable Tips with Filter (as needed)
Position 3 50µl LiHa Disposable Tips with Filter (as needed)
Position 4 (front) 200µl LiHa Disposable Tips with Filter

Grid 9 Wash Station, LiHa, with DiTi Waste Chute and Trough Carrier Grid
Position 1 (rear) Trough, Disposable, 100ml with 25ml Low Dead Volume Disposable Trough (when required)
Position 2 Trough, Disposable, 100ml, with Four-Position Tube Holder
Position 3 (front) Trough, Disposable, 100ml, with Four-Position Tube Holder

Grid 11 Tube Carrier, 16mm, 16-Position (used with optional 5ml tube in amplification mix preparation)

Grid 18 Microplate Carrier, 3-Position
Position 1 (rear) Strip tubes or 96-well plate containing samples
Position 2 Strip tubes (two 8-tube strips per run) or 96-well plate for DNA standard dilutions
Position 3 (front) qPCR amplification plate

Grid 24 Mounting Plate, Te-Shake™ 1-Position with two additional Microplate Positions and Te-Shake™ Microplate Shaker Unit (used with the DNA IQ™ System only)

Grid 33 Torrey Pines Heater, Promega Deep-Well Heat Transfer Block (used with the DNA IQ™ System only)

10. Summary of Change

The following change was made to the 3/18 revision of this document:

PowerQuant™ was updated to PowerQuant®.

[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] The purchase of this product conveys to the buyer the limited, nonexclusive, nontransferable right (without the right to resell, repackage, or further sublicense) under U.S. Pat. Nos. 7,422,850, 7,517,651 and 7,541,147 to use the product. No other license is granted to the buyer whether expressly, by implication, by estoppel or otherwise. In particular, the purchase of this product does not include or carry any right or license to sell this product. For information on purchasing a license for other uses, please contact Promega Corporation, Business Development, 2800 Woods Hollow Road, Madison, WI 53711, or EraGen Biosciences, Corporate Licensing, 918 Deming Way, Suite 201, Madison, WI 53717. Phone (608) 662-9000; Fax (608) 662-9003.

[c] 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.

[d] 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.

[e] 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.

[f] 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.

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