

AUTOMATED PROTOCOL

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

Instructions for Use of Products

PQ5002, PQ5008, DC1001 and DC1000



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: genetic@promega.com

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1. Description

This document describes the automated protocol for the Identity Automation™ qPCR setup method for the PowerQuant® System^(a) and Plexor® HY System^(b-e) on the Tecan Freedom EVO® automated liquid-handling workstation. The automated method for DNA quantitation reaction setup is designed with the flexibility to support the following:

- Using the PowerQuant® System and Plexor® HY System.
- Unknown samples in 96-well format (96-well plate or strip tubes) or tube format (1.5ml or 2.0ml tubes).
- Tracking sample identifiers via import of an ordered list, manual entry (hand-held scanner or keyboard), or the Tecan PosID™ Positive Identification System.
- Processing volumes from 2µl to 9µl.
- Amplifying extracted DNA.
- Amplifying lysates generated by preprocessing with Casework Direct System.
- Processing up to four control samples in tube format (1.5ml or 2.0ml tubes).
- Setting up replicate qPCR amplifications for each unknown sample and control sample (1 to 10 replicates).
- Setting up a four-point (PowerQuant® System) or seven-point (PowerQuant® or Plexor® HY System) DNA standard curve.
- Robotically or manually preparing standard dilutions in 96-well format (96-well plate or 8-well strip tubes) or manually preparing standard dilutions in tube format (1.5ml or 2.0ml tubes).
- Setting up one or two amplification plates.

On-site method installation by Promega includes training, installation and initial set up of the method and its graphical user interface to meet the workflow needs for your laboratory.

For information on the PowerPlex® Systems and STR amplification-related products, visit:

www.promega.com/products/forensic-dna-analysis-ce/str-amplification/

Note: Throughout this technical manual, “qPCR System” refers to the PowerQuant® System or Plexor® HY System, “qPCR reagents” refers to the PowerQuant® or Plexor® HY reagents, “Unknowns” refer to unknown samples, “Controls” refer to control samples, and “Standards” refer to standard DNA dilutions and the no-template control associated with the standard curve.

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: Refer to the appropriate Technical Manual (*PowerQuant® System Technical Manual* #TMD047 or *Plexor® HY System Technical Manual* #TM293, TM294 or TM296) for information related to product storage, equipment, consumables and software required for amplification and data analysis as well as for troubleshooting PowerQuant® or Plexor® HY chemistry issues. All these Technical Manuals are available at: www.promega.com/protocols/

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 Unknowns and Controls plus 10 Standards per plate when a four-point DNA standard curve is selected or two plates of 80 Unknowns and Controls plus 16 Standards when a seven-point curve is selected. The 800-reaction system provides sufficient reagents to process eight plates of 86 Unknowns and Controls plus 10 Standards per plate when a four-point standard curve is selected or eight plates of 80 Unknowns and Controls plus 16 Standards when a seven-point curve is selected. The automated method will prompt you to confirm that a minimum volume of each component is available if the amplification mix is prepared by the liquid handler.

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. Find a list of compatible instruments in the appropriate technical manual.
- analysis software compatible with the qPCR System. See the appropriate qPCR system technical manual for more information.
- centrifuge compatible with tubes (if unknown samples or control samples are supplied in 1.5ml or 2.0ml tubes)
- See Sections 5.A and 5.B for instrumentation and labware requirements, respectively.
- **Optional:** Casework Direct System (Cat.# DC4560, DC4561), for treating Casework Samples (swabs/fabric cuttings)
- **Optional:** 5X AmpSolution™ Reagent (Cat.# DM1231) for quantifying Casework Samples with the Plexor® HY System.
- **Optional:** SwabSolution™ Reagent (Cat.# DC8271) for treating buccal swabs and quantifying with the Plexor® HY System. See the *SwabSolution™ Kit Technical Manual* #TMD037 for more information.

4. Before You Begin

4.A. Unknown and Control Sample Considerations

Unknowns can be in either 96-well format (96-well plate or 8-well strip tubes) or tube format (1.5ml or 2.0ml tubes). Within a run, all Unknowns must be in the same labware format (i.e., all Unknowns in tubes or all Unknowns in up to three 96-well plates). Unknowns in tubes may be placed in up to twelve 16-position tube racks.

Up to four Controls may be in tube format (1.5ml or 2.0ml tubes).

Amplification of Extracted DNA or Lysates Prepared Using the Casework Direct System


Centrifuge Unknowns and Controls briefly to remove any air bubbles that might be present because air bubbles can interfere with aspiration. The automated method pipets Unknowns and Controls in the same manner. Within a run, all Unknowns and Controls must be of the same type; extracted DNA or Casework Direct Lysates.

Direct Amplification of DNA from Swab Preprocessed with SwabSolution™ Reagent

If swab heads are present in the deep-well plate or tube during automated transfer of the Unknown or Control, completely cover the substrate. The liquid handler will aspirate a small volume of swab extract from near the top of the liquid. If the disposable tips strike the swab head, the tips may fail to aspirate or become bent, causing the tips to crash into the amplification plate or cross-contaminate nontarget sources. Sticks from whole swabs should be cut or snapped off uniformly and close to the head to prevent these issues.


4.B. Preparation of Reagents

Thaw the qPCR reagents (primer pair mix, master mix, etc.) thoroughly, and vortex well prior to use. The automated method uses the liquid handler to prepare the qPCR amplification mix at volumes <1.3ml in a 1.5ml microcentrifuge tube or <1.85ml in a 5ml tube. Larger volumes of PCR amplification mix must be prepared manually and placed into an appropriate tube or trough. If the qPCR amplification mix is prepared manually, vortex the amplification mix thoroughly (several 5- to 10-second pulses) prior to placing the mix on the worktable.

 Be sure there are no bubbles in the reagents placed on the worktable as this can adversely affect the preparing or dispensing of the qPCR amplification 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 10 of this protocol and the appropriate automated protocol for the chemistry of interest.

 The computer connected the Tecan Freedom EVO® Workstation must be installed with the Windows® 7 Operating System, Windows® PowerShell 2.0 compatibility, and the Freedom EVOware® Standard software, version 2.4 or newer.

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. For information related to the Tecan PosID™ Positive Identification System, contact your local Tecan representative.

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, 100ml 3-Position ²	1	10650037
DiTi Carrier, 3-Position ³	1–2	10613022
Microplate Carrier, 3 Position, Landscape (MP 3Pos) ⁴ Note: If samples are supplied in tube format, only one carrier is required.	1–2	10612604 or 10612624
Tube Carrier, 1.5ml or 2.0ml Microcentrifuge with Hinged Cap, 16-Position ⁵ Note: If samples are supplied in a 96-well format, this carrier is not required.	6	10613035
Tube Carrier, 16mm, 16-Position (1 × 16) ⁶	1	30019987

¹The method was developed for Freedom EVOware® Standard software, version 2.6, running Windows® 7 using a liquid LiHa configured with 500µl syringes. The method is compatible Freedom EVOware® Standard software, version 2.4. The method volumes are also fully compatible with 250µl syringes. A Tecan Freedom EVO® instrument configured with an Air LiHa arm also may be used.

²If an Air LiHa arm is used, a Trough Carrier, 3-Position (10650020 or 10613020), and a DiTi Waste Chute are required instead of the Wash Station listed above. For runs involving the setup of Controls and or manually prepared Standards in tube format (1.5ml or 2ml), an additional Trough Carrier, 3-Position (Part# 10650020 or 10613020) is needed.

³For walk-away functionality, the method requires at least 4 tip rack positions, 3 positions for 50µl tips and 1 position for 200µl tips. If only one DiTi Carrier, 3-Position, is present, the EVOware® software will prompt you to place more tips on the worktable when required during a run.

⁴One Microplate Carrier, 3-Position Landscape, is required to process one plate of Unknowns or Unknowns in tubes to one amplification plate. Automated runs involving multiple plates of Unknowns or multiple amplification plates will require an additional Microplate Carrier. The number of worktable positions required for labware varies based on the number of plates of Unknowns or tubes of Unknowns processed (Tables 1 and 2). Be sure to install enough labware carriers to support the maximum number of plates per automated method run.

⁵The maximum number allowed on a Freedom EVO® 100 worktable is 6. For Freedom EVO® 150 and 200 worktables, the number required (6 or 12) varies based on the protocol and the number of Unknowns in tubes processed (Table 2). Be sure to install enough labware carriers to support the maximum number of Unknowns in tubes processed per automated method run (6 carriers for up to 96 Unknowns and 12 for up to 192).

⁶**Optional:** For robotically prepared or manually prepared single-tube qPCR amplification mix volumes of 1.3–1.85ml or 1.3–5.0ml, respectively. Alternatively, the following reagent blocks may also be used: Reagent Block AB Quantifier (Part# 30019975) and AB STR-PCR (Part# 30019974).

5.A. Instrumentation Requirements (continued)

Table 1. 96-Well Plate Positions Required on the Worktable Per Number of Unknown Sample Plates.

Plates of Unknowns that can be Processed	Plate Positions Required ¹
1–3	3–6

¹Includes positions for plate(s) for Unknowns, amplification plate(s) and the plate position for standard curve preparation.

Table 2. Labware Positions Required on the Worktable by Number of Unknown Sample Tubes.

Number of Unknowns in Tubes that can be Processed	Number of Tube Carriers (Tube Rack Positions)	Plate Positions Required ¹
1–96	6	2–3
97–192	12	2–3

¹Includes positions for amplification plate(s) and the plate position for standard curve preparation.

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.

Hardware Required

Supplier	Cat. #	Description	Number Required
Promega	V1601	Four-Position Tube Holder	2–5 ¹
User-selected		Plate stand for 96-well PCR plate or strip tubes	1–5 ²

¹A minimum of two (2) Four-Position Tube Holders are required for all installations of the automated method. **Optional:** One additional Four-Position Tube Holder is required if you choose to set up Control amplifications. Two additional Four-Position Tube Holders are required if you choose to present manually prepared Standards in tube format (1.5ml or 2ml). Three additional Four-Position Tube Holders are required if you choose to both set up Control amplifications and use manually prepared Standards in tube format (1.5ml or 2ml).

²The plate stand is required if Unknowns are supplied in 96-well format (e.g., 96-well PCR plate or strip tubes), for standard curve preparation, and for the amplification plates. The Promega Plate Clamp 96 (Cat.# V8251) and Plate Stand (Cat.# V8261) can be used to secure nonskirted 96-well plates or MicroAmp® Strip Tubes on the worktable. The Applied Biosystems MicroAmp® 96-Well Base (Cat.# N801-0531), MicroAmp® Splash-Free 96-Well Base (Cat.# 4312063) or Tecan Plate Adapter PCR (Cat.# 30032860) also may be suitable.

Consumables Required

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
Tecan	30055743	Disposable Trough, 25ml, Low Dead Volume (optional: for manually preparing qPCR amplification mix at volumes >1.45ml)	1 ¹
Tecan	10613048 or 10613049	Trough, Disposable, 100ml, Polypropylene Natural or Trough, Disposable, 100ml, Polypropylene Gray	2–6 ²
User-selected (e.g., VWR Cat.# 89005-596 or Evergreen Scientific Cat.# 222-3007-080)		5ml graduated self-standing tube (optional: for robotically preparing single-tube ≤1.85ml of qPCR amplification mix or manually preparing ≤5.0ml)	1 ¹
User-selected		1.5ml microcentrifuge tube (one tube for robotically preparing single-tube ≤1.3ml of PCR amplification mix or manually preparing ≤1.45ml and one tube of buffer for DNA standard dilution)	2 ¹
User-selected		1.5ml or 2.0ml microcentrifuge tubes containing Unknowns	≤192 ³
User-selected		1.5ml or 2.0ml microcentrifuge tubes (optional: for Controls)	≤4
User-selected		1.5ml or 2.0ml microcentrifuge tubes (optional: for manually prepared Standards)	≤8
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 8-well strip tubes for Unknowns	1–3 ³
User-selected		96-well plate or strip tubes for preparing Standards and staging Water, Amplification Grade	One plate or two 8-tube strips per run

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

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

³The microcentrifuge tubes are not required if Unknowns are in 96-well format. The 96-well plate or strip tubes are not required if Unknowns are in tube format.

5.C. Tecan Freedom EVO® Initial Worktable Configuration

Minimum Worktable Requirements

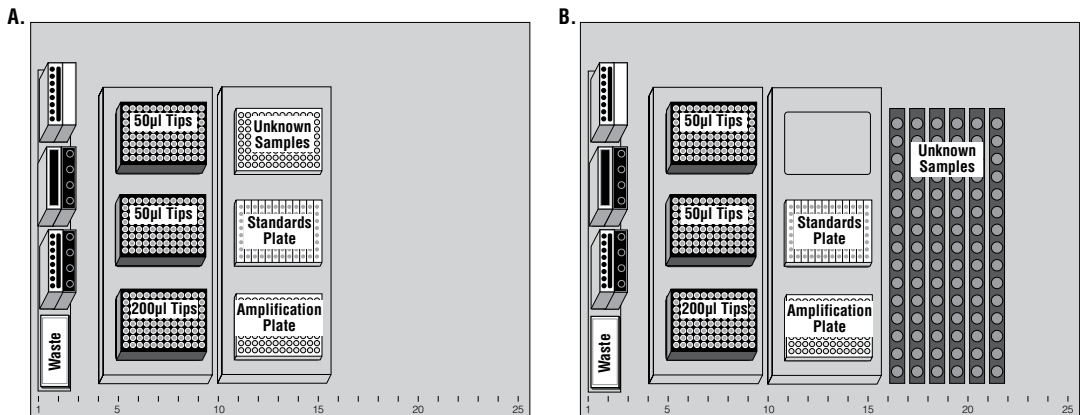


Figure 1. Tecan Freedom EVO® initial worktable configuration. Panel A. Unknowns in a 96-well plate. **Panel B.** Unknowns in 1.5ml tubes. Shown are the minimum requirements for installation of the Identity Automation™ qPCR Setup Method for the PowerQuant® and Plexor® HY Systems on the Tecan Freedom EVO® workstation. This example layout shown supports processing of Unknowns from one plate (**Panel A**) or one set of tube racks (**Panel B**) to one amplification plate. The automated method can be adapted to other worktable layouts provided these hardware (or equivalent) are present. Additional labware positions are required to run the maximum number of plates of Unknowns, tube racks or amplification plates. Additional carriers and labware are required to process Controls in 1.5ml tubes or manually prepared Standards in 1.5ml tubes. For walk-away functionality, the method requires 4 tip rack positions, 3 positions for 50µl tips and 1 for 200µl tips. As shown with one DiTi Carrier, 3-Position, the EVOware® software will prompt for additional tips when required during the run.

Grid 1 Wash Station, LiHa, with DiTi Waste Chute and Trough Carrier, 100ml, 3-Position

Position 1–3 (left)	Wash and Waste
Position 4 (rear right)	Trough, Disposable, 100ml, with 25ml Low Dead Volume Disposable Trough (when required)
Position 5	Trough, Disposable, 100ml, with Promega Four-Position Tube Holder
Position 6 (front right)	Trough, Disposable, 100ml, with Promega Four-Position Tube Holder
Position 7	Tip Waste Chute

Grid 4 DiTi Carrier, 3-Position

Position 1 (rear)	50µl LiHa Disposable Tips with Filter
Position 2	50µl LiHa Disposable Tips with Filter (as needed)
Position 3 (front)	200µl LiHa Disposable Tips with Filter

Grid 10 Microplate Carrier, 3 Position

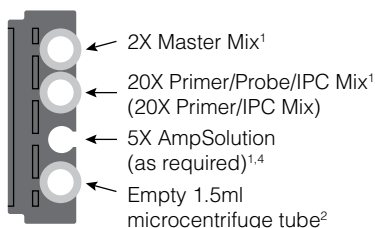
- Position 1 (rear) 8-well strip tubes or 96-well plate containing Unknowns (Unknowns in plates); empty if Unknowns are in tube format
- Position 2 8-well strip tubes or 96-well plate for Standard preparation and Water, Amplification Grade, staging
- Position 3 (front) qPCR amplification plate

Grid 16–21 Tube Carriers, 1.5ml or 2.0ml Microcentrifuge with Hinged Cap, 16-Position (Samples supplied in tube format)

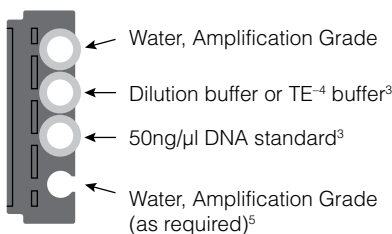
Positions 1–16 of each tube rack 1.5ml or 2.0ml tubes containing Unknowns

Note: Be sure to turn all open caps so that the caps do not interfere with pipetting steps.

Tube holder at Grid 1, Position 5:



Tube holder at Grid 1, Position 6:



¹If manually preparing PCR amplification mix, these positions will be empty.

²**Optional:** Manually prepared PCR amplification mix. If a 5ml tube or 25ml trough is used for the PCR amplification mix, this position in the tube holder is empty.

³If manually preparing Standards, these positions will be empty.

⁴If Unknowns and Controls are Casework Direct Lysates and quantitated with the Plexor® HY System, the graphical user interface will prompt you to place a tube of 5X AmpSolution™ Reagent at this position.

⁵The graphical user interface may prompt you to place a tube of Water, Amplification Grade, at this position for setting up two amplification plates.

Figure 2. Configuration of PowerQuant® or Plexor® HY reagents and tubes in the Four-Position Tube Holders at Grid 1, Positions 5 and 6. 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 worktable in Figure 1 requires the cap-securing tabs to be positioned to the right.) 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.

6. Graphical User Interface

The Identity Automation™ qPCR setup method for the PowerQuant® and Plexor® HY Systems on a Tecan Freedom EVO® workstation is integrated with an easy-to-use graphical user interface that:

- Allows you to choose the qPCR System and the qPCR amplification mix preparation style (e.g., by the automated liquid handler or manually).
- Allows you to select method options at run time that set the processing requirements for Unknowns and Controls (e.g., type, input labware for Unknowns, processing volume, number of replicates to process and number of Controls).
- Allows you to select method options at run time that set standard curve requirements (e.g., number of curve points, preparation style and input labware type for manually prepared Standards).
- Allows you to enter information for Unknowns (e.g., location in tubes or plates, identifiers and bar codes) manually (via a keyboard or hand-held scanner), via the import of an ordered list or in the case of Unknowns presented in tubes using the Tecan PosID™ Positive Identification System.
- Guides you through the physical setup of the Tecan Freedom EVO® workstation (e.g., placement of Unknowns, Controls, reagents, and labware on the worktable of the liquid handler).

During the on-site installation of the method, default values for the method options are set based on specific laboratory needs. At the start of each run, you can accept the defaults (indicated with either a ✓ or a numerical value) or make run-dependent selections. The options displayed in each window are dependent on the values selected; some of these dependencies are provided below. In addition, the graphical user interface can be customized for your preferred language.

6.A. Before You Start the Graphical User Interface

During the on-site installation of the Identity Automation™ qPCR setup method for the PowerQuant® System and Plexor® HY System, the method can be configured to handle disposable tips in one of two ways.

Promega tip handling within the Promega user interface. If the instrument worktable is configured with at least 3 sites for 50µl tips and one site for 200µl tips, Promega tip handling supports walk-away runs for set up of a single amplification plate. The user interface will prompt you to supply the location of the first 50µl tip and the first 200µl tip on the worktable. The method is programmed to calculate the number of tips required and will signal whether there are sufficient tips to complete the run; the user interface allows you to substitute a full tip rack for a partial tip rack when there is not a sufficient number of tips. The information collected by the user interface is used within the EVOware® script to set the first usable DiTi Position for each type of tip rack. For more information, contact Promega.

Standard tip handling provided by the Tecan EVOware® software. Standard tip handling requires that you manually set the first usable tip position for each type of tip rack before you start the Identity Automation™ qPCR setup method for the PowerQuant® System and Plexor® HY System. If the tips are depleted during a run, the EVOware® Software will pause the method and prompt you for additional tips. Standard tip handling option is not compatible with 4-position tip carriers.

After logging into EVOware® software, use the **Set DiTi position** command in the EVOware® software to set the positions of the first usable 50µl tip and the first usable 200µl tip on the worktable of the instrument. For details on the use of the **Set DiTi position** command, consult the Tecan EVOware® User Manual.

6.B. Example Data Inputs Windows

After logging into the EVOware® software, setting the first usable tip positions (if required), choosing the script and selecting the **Run** button, the following windows will appear (Figure 3):

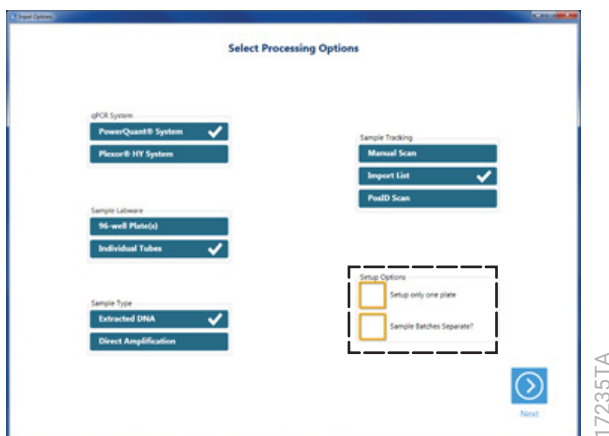


Figure 3. Example ‘Select Processing Options’ window. Select the qPCR System, the Sample Labware, Sample Type and the Sample Tracking types for the run. Depending on the instrument configuration, additional Setup Options may be displayed (see insert in black dashed box). In the user interface, “Sample” refers to Unknown Samples.

qPCR System

The qPCR System option allows you to choose to use either the PowerQuant® System or Plexor® HY System.

Sample Labware

The Sample Labware option allows you to choose the input labware for the Unknowns; 96-well plate(s) or individual tubes (1.5ml or 2.0ml). Within a run, all Unknowns must be supplied in the same labware type.

Sample Type

The Sample Type option allows you to choose to process either “Extracted DNA” Unknowns or “Direct Amplification” Unknowns. “Extracted DNA” Unknowns are purified and aqueous in nature (i.e., DNA in TE⁻⁴ buffer). “Direct Amplification” Unknowns are DNA-containing lysates (e.g., lysates generated by preprocessing swabs or stained fabric using the Casework Direct System or lysates generated by preprocessing buccal swabs using SwabSolution™ Reagent) and contain detergent. The automated method pipets Unknowns and Controls in the same manner. Within a run, all Unknowns and Controls must be of the same type; “Extracted DNA” or “Direct Amplification”.

6.B. Example Data Inputs Windows (continued)

Sample Tracking

The Sample Tracking option allows you to choose the way identifier and position information are entered for Unknowns. Tracking information may be provided manually (with a keyboard or hand-held scanner), by importing an ordered list or using the Tecan PosID™ Positive Identification System (for Unknowns presented in tubes only). For more information on how the user interface allows you to enter the information, see Sections 6.C and 11.

Setup Options

For instrument configurations that allow set up of two amplification plates, the “Setup only one plate” checkbox is displayed to limit the method at run time for setting up only one amplification plate. For instrument configured to allow more than 1 plate of Unknowns or greater than 6 tube racks for Unknowns in tubes, the “Sample Batches Separate?” checkbox forces Unknowns from different batches to be processed to different amplification plates. For Unknowns in plates, each plate is considered a batch. For instruments configured to allow 12 tube racks, Unknowns in the six leftmost tube racks are considered a batch and Unknowns in the six rightmost tube racks are considered a batch.

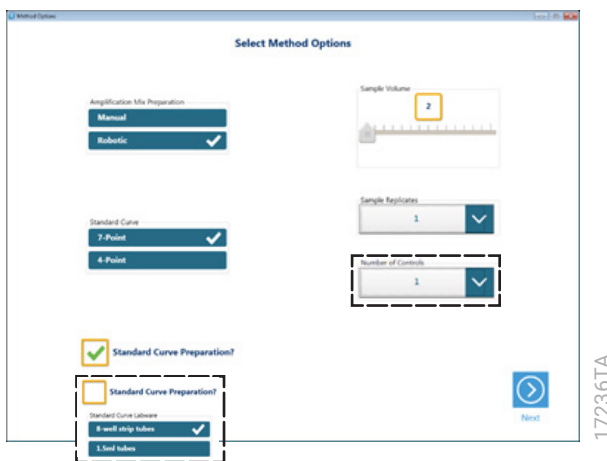


Figure 4. Example ‘Select Method Options’ window. Select the style of Amplification Mix Preparation, the type of Standard Curve, whether Standard Curve Preparation is conducted robotically at run time, the volume of each Unknown (and Control) to process, the number of replicates to process for each Unknown (and Control), and the Number of Controls. The exact options displayed in the ‘Select Method Options’ window depends on the values selected for qPCR System and Sample Type in the ‘Select Processing Options’ window (Figure 3) as well as method options set during on-site installation. The Standard Curve Labware option (see left dashed black box) is only displayed when the “Standard Curve Preparation” checkbox is not checked and you chose to present manually prepared Standards in 1.5 ml (or 2ml) tubes. The Number of Controls option (see right dashed black box) is displayed when the Controls are presented in 1.5 ml (or 2ml) tubes.

Amplification Mix Preparation

The Amplification Mix Preparation option allows you to choose whether the qPCR amplification mix will be prepared by the Tecan Freedom EVO[®] workstation (**Robotic**) or manually by you (**Manual**). When this variable is set to **Robotic**, you will be prompted to place the required reagents on the worktable. When this variable is set to **Manual**, you will be prompted to manually prepare the qPCR amplification mix and place it on the worktable. You can robotically prepare qPCR amplification mix volumes of up to 1.3ml in a 1.5ml tube and up to approximately 1.85ml in a 5ml tube; larger volumes require manual preparation. When the volume of qPCR amplification mix required for the run exceeds the volume that can be prepared robotically or requires more than one vial of any reagent (e.g., 2 vials of 2X Master Mix), the method will prompt you to manually prepare the qPCR amplification mix regardless of the selection entered into the user interface.

Standard Curve

The Standard Curve option allows you to choose the number of Standards to be transferred to the qPCR amplification plate. The automated method supports a 4-point DNA standard curve option and a seven-point DNA standard curve option when the PowerQuant[®] System is selected. The automated method supports a seven-point DNA standard curve option when the Plexor[®] HY System is selected. A seven-point curve consists of seven dilutions of the 50ng/μl DNA standard and one no-template control (NTC; i.e., a total of eight Standards). A four-point curve consists of four dilutions of the 50ng/μl DNA standard and one no-template control (NTC; i.e., a total of five Standards).

Standard Curve Preparation and Standard Curve Labware

The “Standard Curve Preparation” checkbox allows you to choose whether a dilution series of the 50ng/μl DNA standard will be prepared by the Tecan Freedom EVO[®] workstation (checkbox is selected) or if a previously or manually prepared dilution series will be used (checkbox empty). When the Tecan Freedom EVO[®] workstation prepares the dilution series, you will be prompted to place the 50ng/μl DNA standard and the appropriate dilution buffer on the worktable. When a previously or manually prepared dilution series will be used, you will be prompted to place standard dilutions at specific positions on the worktable at concentrations and volumes that are appropriate for the selected values of the Standard Curve, Sample Volume and Sample Replicates options and sufficient for the number of amplification plates generated.

Sample Volume

The Sample Volume slider allows you to select the volume of each Unknown to be transferred to the qPCR amplification plate. For optimal accuracy, the automated method uses the selected volume to transfer Unknowns, Controls and all Standards into the qPCR amplification plate. The method is programmed to limit the allowed processing volumes based on the value selected for Sample Type in the ‘Select Processing Options’ window (Figure 3). For example, when **Extracted DNA** is selected, you can select 2μl to 9μl in 0.5μl increments whereas when **Direct Amplification** is selected, only 2μl is allowed).

Sample Replicates

The Sample Replicates dropdown menu allows you to select the number of replicate qPCR amplifications to prepare for each Unknown and Control. Replicates are arranged in adjacent columns on the qPCR amplification plate. The automated method supports one to ten replicate reactions for all Unknowns and Controls. When more than two replicates are selected, the method is programmed to automatically limit the run to set up of one amplification plate.

6.B. Example Data Inputs Windows (continued)

Number of Controls

The Number of Controls dropdown menu allows you to process 0–4 Controls. The no-template control (NTC) is not a Control; the NTC is handled as the final Standard of the selected standard curve. When one or more Controls are selected, you will be prompted to place the Control(s) on the worktable in 1.5 ml (or 2 ml) tube(s). The Controls are expected to be the same Sample Type (i.e., **Extracted DNA** or **Direct Amplification**) as the Unknowns and are processed with the selected Sample Volume and Sample Replicates. Reaction wells for the Controls are set up in the amplification plate(s) based on method options you select during method installation. The Control reactions can be placed in the amplification plate before the reactions for the Unknowns (i.e., starting at well A1), immediately after the reactions for the Unknowns or at four user-defined positions. When the Controls are placed at user-defined positions, the method is programmed to limit the number of replicate qPCR amplifications set up for all Unknowns and Controls to one. User-defined Control positions must not interfere with the placement of Standards in the qPCR amplification plate (see Section 8, Figure 18). When two amplification plates are generated, Controls are set up in both plates.

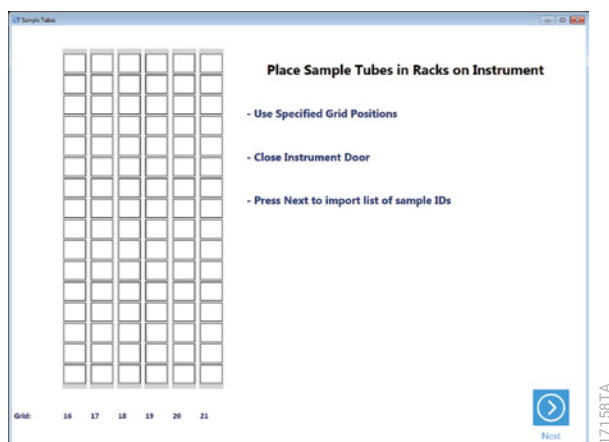


Figure 5. Example ‘Sample Tubes’ window. When the **Individual Tubes** button is chosen in the ‘Select Processing Options’ window (Figure 3), a ‘Sample Tubes’ window directs you to place the tubes of Unknowns in tube racks at specific grid positions on the worktable of the liquid handler. The example ‘Sample Tubes’ window shown supports processing of Unknowns from six tubes racks with sample tracking information entered via list import on the worktable configuration shown in Figure 1, Panel B; the exact window displayed depends on the sample tracking types selected and the specific configuration of the Tecan Freedom EVO® workstation (i.e., the placement of carriers on the worktable and the specific number of tube racks on the worktable; 6 or 12).

Note: Controls are not placed in these tubes racks. If applicable, a ‘Worktable Setup’ window (see Section 6.D, Figure 16, Site 1) will prompt for Controls placement and entry of related information.

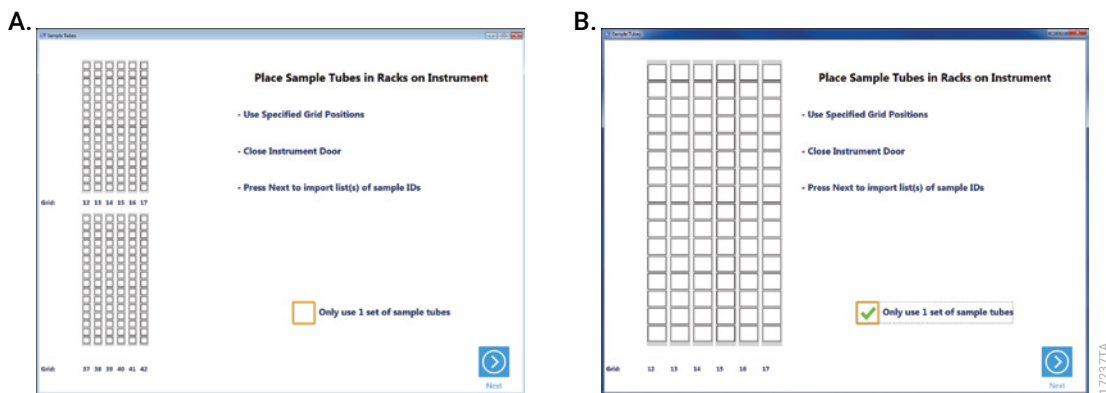


Figure 6. Example ‘Sample Tubes’ window for an EVO® 200 instrument configured for 12 tube racks of Unknowns. Panel A. Display of grid positions for 12 tube racks. **Panel B.** Functionality of the “Only use 1 set of sample tubes” checkbox; display of grid positions for first batch of Unknowns in tubes.

Important points to note for workstations that have 12 tube racks:

- When you have sufficient worktable space and hardware for 12 tube racks, the ‘Sample Tubes’ window will display both sets of tube racks and their respective grid positions (Figure 6; Panel A).
- If two sets of six tube racks are displayed, Unknowns in the first set of six tube racks (six leftmost racks on worktable; grids 12–17 in Figure 6, Panel A) are processed as the first batch of Unknowns and the second set of six tube racks (six rightmost racks on the worktable; grids 37–42 in Figure 6, Panel A) are processed as the second batch of Unknowns. Processing of the batches is dependent on whether the “Sample Batches Separate?” checkbox is checked or not (Figure 3; ‘Select Processing Options’ window).
- If the Unknowns are being scanned using the PosID™ system, a checkbox is available for selecting only one set of tubes when running ≤ 96 Unknowns (“Only use 1 set of sample tubes” checkbox; Figure 6, Panel B). Checking the checkbox indicates to the method that all Unknowns will be placed in the first set of six tube racks (grids 12–17) and to only scan the first six tube racks with the PosID™ reader, saving significant method time.

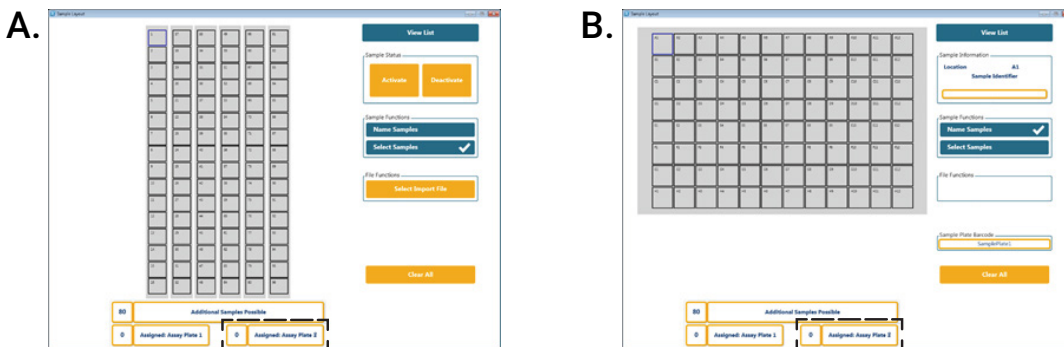


Figure 7. Example ‘Sample Layout’ windows. The exact window displayed depends on the Sample Labware and the Sample Tracking values selected in the ‘Select Processing Options’ window (Figure 3). The “Additional Samples Possible” box displays the number of Unknowns that may be placed in the amplification plate(s); the number depends on the instrument configuration and the values for the Standard Curve type, Samples Replicates and Number of Controls selected in the ‘Select Method Options’ window (Figure 4). In these examples, the instrument is configured for one amplification plate (96 assay wells), the method settings don’t allow Controls, and the values selected are seven-point Standard Curve (curve requires 16 assay wells) and 1 Sample Replicate ($96 - 16 = 80$ single Unknown qPCR amplifications). The “Assigned: Assay Plate 1” box provides the number of Unknowns currently saved to amplification plate for processing. When an instrument is configured to allow two amplification plates, an “Assigned: Assay Plate 2” box is also displayed (see black dashed box). Additional features of the ‘Sample Layout’ window are described in Section 6.C. **Panel A.** The example layout window shown supports processing of Unknowns from tubes with tracking information entered via list import. Choose the **Select Import File** button to import a file containing identifier information. For information on import files, see Section 11. **Panel B.** The example layout window shown supports processing of Unknowns from a 96-well plate with tracking information entered manually. Manually enter the identifier information in the “Sample Identifier” input box. Press the **Tab** key to set the entered information and move to the next position.

Note: Information for Controls is not entered via this window. If applicable, a ‘Worktable Setup’ window will prompt for Controls placement and entry of related information (refer to Section 6.D, Figure 16 Site 1).

6.C. Example ‘Sample Layout’ Windows

The automated Identity Automation™ qPCR setup method for the PowerQuant® System and Plexor® HY System allows Unknowns to be presented in 96-well plates or in individual tubes (1.5ml or 2.0ml). You must supply the positions of all the Unknowns in the plates or tubes using the ‘Sample Layout’ window. Information for the Unknowns can be entered manually (via keyboard or a hand-held scanner), by importing an ordered list of identifiers and locations or using the Tecan PosID™ Positive Identification System (for Unknowns presented in tubes only).

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Features of the ‘Sample Layout’ Window.

Pressing the **View List/View Map** toggle button switches the view between a pictorial map of the Unknowns in six 16-position tube racks or in a single 96-well plate (Map View) and a single column list of Unknowns (List View). When Unknowns are supplied in tubes, numbering starts at the top position of the leftmost rack as tube 1, is counted down the leftmost rack and continues at the top of the next tube rack (i.e., 1 through 96). When Unknowns are supplied in a 96-well plate, numbering is based the well position (i.e., A1, B1, C1...F12, G12, H12). In the List View, a “Hide Empty” checkbox allows you to hide any empty Unknown positions. Whether viewed in List or Map View, the selection of Unknowns may be performed one at a time or by pressing the **Ctrl** key and selecting multiple Unknowns. To navigate through the Unknown positions in either the List or Map View, use the mouse or **Tab** key. After adding, removing or modifying identifier information, press the **Tab** key to accept the change and update the window.

Color coding is used to indicate the status of the Unknowns.

- **White:** Unknowns that are both active and have unique identifiers (or bar codes).
- **Gray:** Deactivated Unknowns and empty Unknown positions.
- **Salmon:** Unknowns having replicate identifiers. When multiple Unknowns have the same identifier, the replicate Unknowns must be deactivated or replicate identifiers need to be changed. When using the PosID™ System to scan Unknown tube bar codes, a tube with a scan error will be displayed in salmon as “***”; you must manually provide the bar code or deactivate the Unknown.

When selected, the **Select Samples** button causes a “Sample Status” box to appear that contains an **Activate** button and a **Deactivate** button. The **Deactivate** button will deactivate any Unknown that is selected in the Map View or List view. The **Activate** button will activate any Unknown with a unique identifier that is selected in the Map View or List View.

When selected, the **Name Samples** button causes a “Sample Information” box to appear that contains the Location of the Unknown (i.e., tubes, 1–96; plate, A1–H12) and the “Sample Identifier” input box. When the cursor is placed in the “Sample Identifier” input box, you can manually enter a unique identifier (or bar code) to an empty location or modify/remove an existing identifier. After adding, modifying or removing the identifier, press the **Tab** key to accept the change and update the window. To view the “Sample Information” box of a specific Unknown, select the location of the Unknown in the Map View or List View.



When manually adding, modifying, or removing an identifier, you must press the **Tab** key to accept the action. If the **Tab** key is not pressed and you navigate to a different Unknown using the mouse, any changes to the identifier may be lost.



Be sure the cursor is placed after the final character of the identifier before pressing the **Tab** key.

Pressing the **Save Sample Set** button assigns each active Unknown to the appropriate position(s) in the amplification plate.

6.C. Example ‘Sample Layout’ Windows (continued)

Important points about how the method saves or assigns Unknowns to position in the amplification plate:


- Only active Unknowns are assigned to positions in an amplification plate.
- When more than one replicate qPCR amplification is selected, the replicates for each Unknown are assigned to positions in adjacent columns of the amplification plate. All replicates for an Unknown are assigned to positions on the same amplification plate (i.e., replicates for the same Unknown will not be assigned across two amplification plates when the instrument is configured for two amplification plates).
- Prior to assigning the positions to the Unknowns, the method assigns the appropriate wells for the user-selected Standard Curve (refer to See Section 8, Figure 18), the Controls when set to be placed before the Unknowns reactions (i.e., starting at well A1), and the Controls to be placed at user-defined positions.
- Prior to assigning the positions to the Unknowns, the method reserves the correct number of Control positions to be placed after the Unknown reactions.
- Skipping any empty positions or positions containing a deactivated Unknown, the method processes active Unknowns consecutively starting from position 1 (i.e., 1, 2, 3...96) in tube racks or A1 (i.e., A1, B1, C1...H12) in plates containing Unknowns. Unknowns are processed to the first unassigned position in an amplification plate.
- For instruments configured for more than one plate of Unknowns or two sets of six tube racks containing Unknowns, each plate of Unknowns and each set of six tube racks is considered a batch. The ‘Sample Layout’ window allows import or entry of each single batch of Unknowns at a time. The method assigns Unknowns in the batch to an amplification plate based on the value of the “Sample Batches Separate?” checkbox (‘Select Processing Options’ window, Figure 3). If the “Sample Batches Separate?” checkbox is checked, the method will force Unknowns from different batches to be processed in different amplification plates. If the “Sample Batches Separate?” checkbox is not checked, the method will assign Unknowns to the first unassigned well in the amplification plates, processing Unknowns from different batches in the same amplification plate or Unknowns for a single batch across both amplification plates.

Note: Each plate of Unknowns is considered a batch. For instruments configured to allow 12 tube racks, Unknowns in tubes placed in the six leftmost tube racks are considered a batch and Unknowns in tubes placed in the six rightmost tube racks are considered a batch.

- When setting up amplification plates in a single automated run from up to 3 plates of Unknowns or two sets of Unknowns in tubes, you may repeat one of the following processes before selecting the **Next** button:
 1. Select **Import File**, make modifications as required and choose **Save Sample Set**.
 2. Manually enter the identifier information for the Unknowns and select **Save Sample Set**.
 3. Select **Import PosID Scan**, make modifications as required and choose **Save Sample Set**.
- Once an Unknown is assigned (i.e., once **Save Sample Set** is selected), you are unable deactivate or remove the Unknown without aborting and restarting the run.

Pressing the **Select Import File** button allows you to select and open an ordered list containing identifiers and locations for Unknowns in a plate (well location) or in tubes (tube rack and tube position information). The file format will be configured based on your specifications during the on-site installation. For information about accepted file formats, refer to Section 11.

Selecting the **Clear All** button removes the identifier information for all Unknowns in the current ‘Sample Layout’ window.

 The **Clear All** button does not clear any Unknown that have already assigned using the **Save Sample Set** button. To clear Unknowns that have already been assigned, abort the method and restart the run.

When the instrument is configured for two amplification plates and Unknowns have been assigned to the second amplification plate, an “Only Assay Plate 1” checkbox will be displayed. This checkbox can cancel the setup of the second amplification plate.

When checked, the “Hide Empty” checkbox hides any empty Unknown positions when the Unknowns are viewed in a List View.

When the last set (batch) of Unknowns has been assigned, select the **Next** button to close the ‘Sample Layout’ window and proceed to the ‘Worktable Layout’ windows (Section 6.D).

Example ‘Sample Layout’ Windows

The ‘Sample Layout’ window displayed depends on the Sample Labware, Sample Tracking and Setup options selected in the ‘Select Processing Options’ window (Figure 3) and whether the instrument is configured for more than one batch of Unknowns or the setup more than one amplification plate. The example windows provided illustrate the features of the ‘Sample Layout’ window; the windows shown support runs in which the Unknowns are supplied in individual tubes and the Unknown information (i.e., identifiers and location) are imported via an ordered list. In these examples, the instrument is configured for set up of either one amplification plate from Unknowns in tubes placed in up to six tube racks (Figures 8 through 11) or two amplification plates from Unknowns in tubes placed in up to 12 tube racks (Figures 12).

6.C. Example ‘Sample Layout’ Windows (continued)

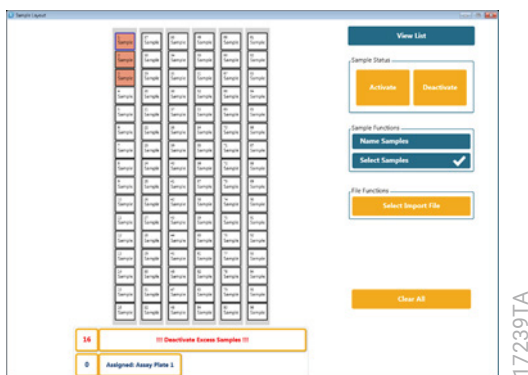


Figure 8. Example Map View of Sample Information Imported from a List. Map View and “Sample Status” box. Choose the **Select Import File** button (initial window as shown in Figure 7, Panel A), navigate to and load an import list; the results of the actions are shown. Unknowns with replicate identifiers (positions 1, 2 and 3) are salmon in color. When there are more active Unknowns than will fit in the amplification plate, a “Deactivate Excess Samples” warning is displayed.

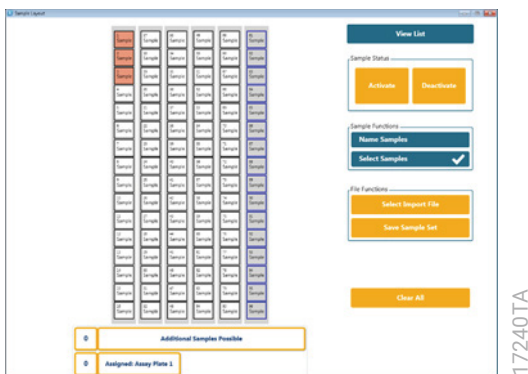
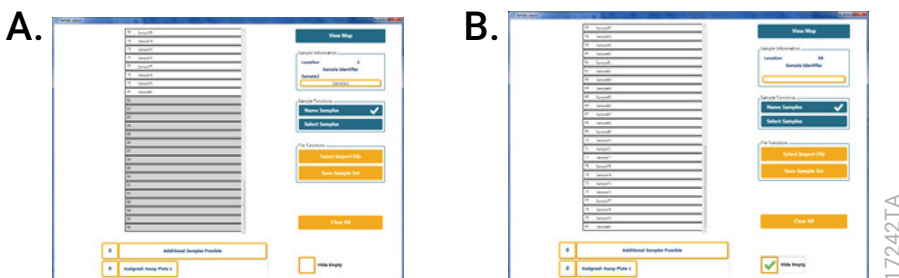


Figure 9. Example Map View of Sample Information Imported from a List. Map View and Deactivation of Unknowns. Highlight the Unknowns that you do not want to process (Unknowns at positions 81–96) and select the **Deactivate** button; the Unknowns will change color from white to gray (i.e., no longer active). Selecting Unknowns can be performed one at a time or by pressing the **Ctrl** key and selecting multiple Unknowns. When a sufficient number of Unknowns are deactivated, the ‘Deactivate Excess Samples’ warning is no longer displayed.



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Figure 10. Example List View of Sample Information Imported from a List. List View, “Sample Information” box, Handling of Unknowns with Replicate Identifiers, Modification of Identifiers and Deletion of Identifiers. Press the **View List/View Map** toggle button to display the Unknown information in list format. Choose the **Name Samples** button to toggle from the “Sample Status” box to the “Sample Information” box (see insert in black box; right). Modify the identifier information using the “Sample Identifier” input box. Press the **Tab** key to accept the modified identifier information. The Unknown will change from salmon to white in color (i.e., active) (see insert in black box; left). To delete identifier information, highlight the Unknown, select within the “Sample Identifier” input box, delete the identifier information, and press the **Tab** key. The position will be empty and gray in color (not shown).



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Figure 11. Example List View of Sample Information Imported from a List, including List View, “Sample Information” box, Hide Empty and Save Sample Set. Select the **View List/View Map** toggle button. Scroll to the end of the Unknowns list, and check the “Hide Empty” checkbox. The empty positions 81–96 (**Panel A**) are no longer displayed (**Panel B**). When all of the identifier information for the current batch of Unknowns has been entered into the ‘Sample Layout’ window and modified as appropriate, select the **Save Sample Set** button to assign the Unknowns to positions in the amplification plate.

6.C. Example ‘Sample Layout’ Windows (continued)

In the example setup shown in Figure 11, the ‘Sample Layout’ window closes and the ‘Worktable Setup’ windows (Section 6.D) begin when the **Save Sample Set** button is pressed. The method closes the ‘Sample Layout’ window automatically as the program recognizes that there are no further actions in the ‘Sample Layout’ window for the instrument configuration (i.e., the configuration allows only one batch of tubes containing Unknowns and the setup of only one amplification plate). For instrument configurations that allow set up of two amplification plates, the “Assay Only Plate 1” checkbox and the **Next** button may be displayed (Figure 12).

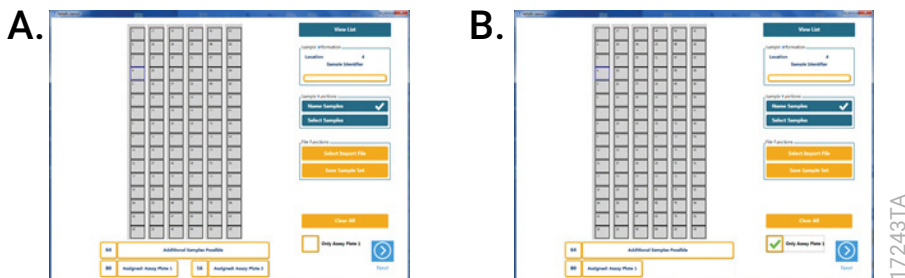


Figure 12. “Only Assay Plate 1” Checkbox and Next Button. The example shown corresponds to an instrument configuration that allows Unknowns from tubes in 2 batches of tube racks and allows set up of two amplification plates. All 96 Unknowns from the first batch of tube racks have been assigned. The ‘Sample Layout’ windows indicates that 80 of the Unknowns are assigned to the first amplification plate and 16 are assigned to the second amplification plate. **Panel A.** To set up both amplification plates, leave the “Only Assay Plate 1” unchecked and choose the **Next** button. **Panel B.** To set up only the first amplification plate, check the “Only Assay Plate 1” checkbox and then choose the **Next** button. When the **Next** button is selected, the ‘Sample Layout’ window will automatically close and the ‘Worktable Layout’ windows (Section 6.D) will commence.

6.D. Example ‘Worktable Setup’ Windows

The automated Identity Automation™ qPCR setup method for the PowerQuant® and Plexor® HY Systems guides you through the placement of labware and reagents on the worktable of the liquid handler. The exact worktable setup instructions displayed depend on the selections made in the ‘Select Processing Options’ and ‘Select Method Options’ windows (Figure 3 and 4, respectively), the physical configuration of the Tecan Freedom EVO® workstation, and the number of Unknowns processed. The user interface will prompt for the minimum reagent volumes required. The example ‘Worktable Setup’ windows shown below support processing of Unknowns in tubes and use the worktable configuration shown in Figure 1. Use the **Next** and **Prev** buttons located at the bottom right and left corners of the windows to navigate through the various windows.

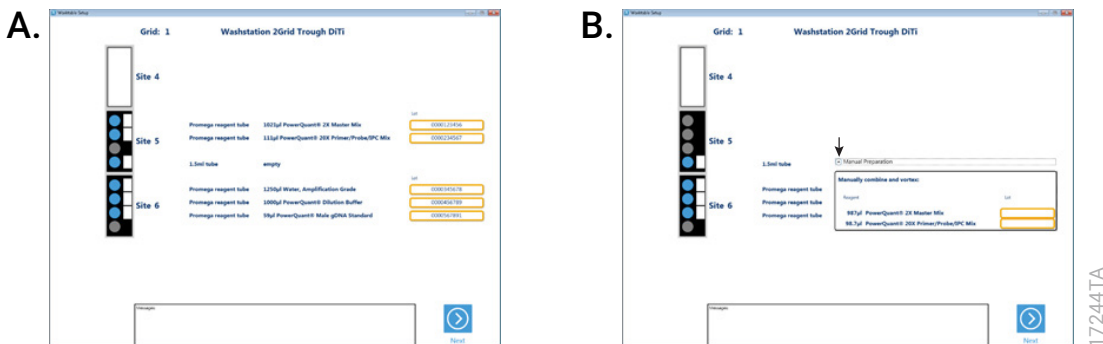


Figure 13. Example Setup of Reagent Tube: Master Mix, Primer Mix, Dilution Buffer and gDNA Standard. Panel A. Robotic Preparation of the qPCR Amplification Mix and Standard Curve. **Optional:** Reagent lot information may be supplied manually in the boxes on the right side of the window. **Panel B.** Manual Preparation of the qPCR Amplification Mix. To display the instructions for manual preparation, select the **Manual Preparation** expander button (see arrow). **Optional:** Reagent lot information may be supplied manually in the boxes on the right side of the window.

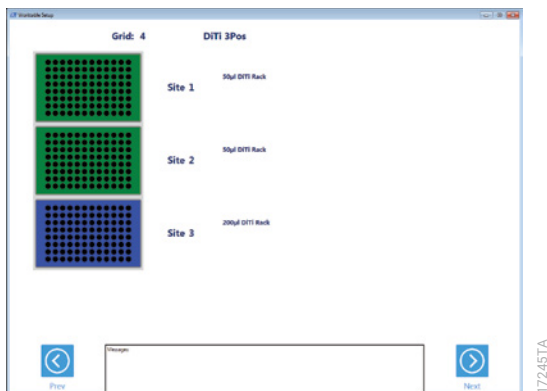


Figure 14. Example Setup of Disposable Tips. The example 'Worktable Setup' window shown supports the standard tip handling option.

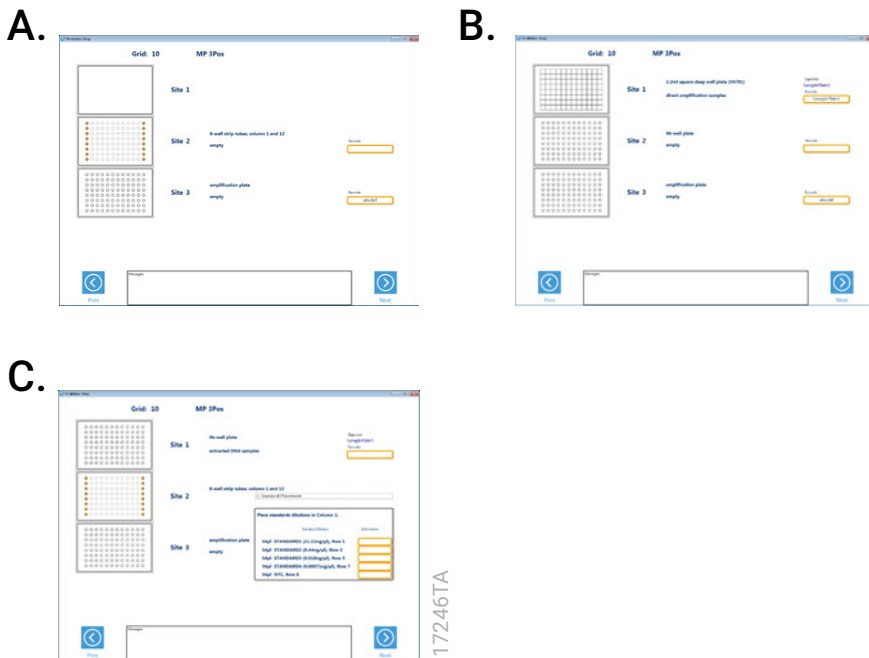


Figure 15. Example Setup of Standard Curve Dilution Position and an Amplification Plate. Panel A. The example ‘Worktable Setup’ window shown supports robotic preparation of the standard curve in an 8-well strip tube (column 1 of Site 2), set up of one amplification plate (site 3) and Unknowns supplied in tubes (placement of tubes containing Unknowns are shown in Figure 5). The exact window displayed depends the Sample Labware value selected in the ‘Select Processing Options’ window (Figure 3) and the standard curve labware chosen during the installation of the method. **Panel B.** The example window shown supports Unknowns supplied in a plate (Site 1), robotic preparation of the standard curve in a 96-well plate (Site 2), and set up of one amplification plate (site 3). **Panel C.** The example window shown supports Unknowns supplied in a plate (Site 1), placement of previously prepared Standards in an 8-well strip tube (column 1 Site 2; four-point curve), and set up of one amplification plate (site 3). **Optional:** Bar code information for labware may be supplied manually in the boxes on the right side of the window.

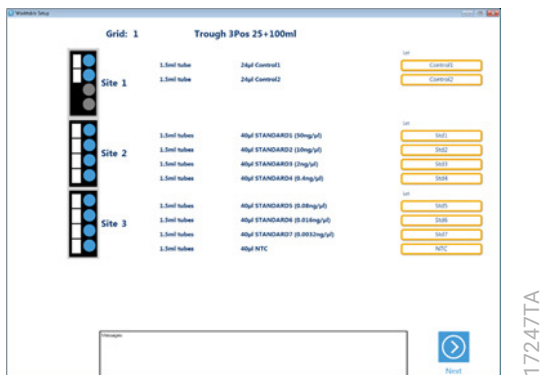


Figure 16. Example Setup of Controls and Manually Prepared Standards in 1.5ml tubes. The exact window displayed depends on the Standard Curve, Standard Curve Labware, Sample Volume, Sample Replicates and Number of Controls values selected in the ‘Select Method Options’ window (Figure 4). The example window shown supports processing of two Controls (Site 1) and use of manually prepared Standards in 1.5ml tubes (Site 2 and 3). This example is based on the worktable (i.e., the hardware and worktable positions) shown in Figure 19.

7. 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. **EVOWare® Software:** Within EVOWare® set the positions of the first usable 50µl tip and first usable 200µl tip (if required; see Section 6.A) and start the Identity Automation™ qPCR Setup Method.
2. **Graphical User Interface:** Select the qPCR System, Sample Labware, Sample Type, Setup Options, Standard Curve Labware (if applicable), Sample Volume, Sample Replicates, Number of Controls (if applicable) as well as the Sample Tracking, Amplification Mix and Standard Curve Preparation options. Through the ‘Sample Layout’ window, provide the tracking information (identifier and position) for all Unknowns. Set up the worktable of the Tecan Freedom EVO® workstation as directed.
3. **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 (or 96-well plate) 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 (NTC). The volumes of 50ng/µl DNA standard, PowerQuant® Dilution Buffer or TE⁻⁴ buffer, and serial dilution depend on the user-specified Sample Volume (2–9µl) and the number of amplification plates generated during the method run. Refer to Section 8 of this protocol for information related to the concentrations of the Standards generated during the serial dilution.

7. **Description of the Identity Automation™ qPCR Setup Method for the PowerQuant® and Plexor® HY Systems (continued)**

4. **Preparation of the PowerQuant® or Plexor® HY amplification mix:** If you choose to use the liquid handler to prepare the amplification 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 Unknowns and Controls, the number of replicate qPCR amplifications to be conducted per Unknown and Control, the required reagent excess volumes and an appropriate number of additional reactions for the Standards (e.g., 10 and 16 additional reactions for a four-point and seven-point DNA standard curve, respectively). For volumes of amplification mix up to 1,300µl, the amplification mix can be prepared by the liquid handler in an empty 1.5ml tube. For volumes of amplification mix up to approximately 1,850µl, the amplification mix can be prepared by the liquid handler in an empty 5ml tube. Larger volumes of amplification mix require manual preparation. Manually prepared qPCR amplification mix can be placed on the worktable in a 1.5ml tube, a 5ml tube or a 25ml low-dead-volume trough.

Note: Preparing the amplification mix involves 2X Master Mix, 20X Primer Mix and in some cases Water, Amplification Grade, or 5X AmpSolution™ Reagent or both. Whether Water, Amplification Grade, 5X AmpSolution™ Reagent or both is included in the amplification mix depends on the values for qPCR System, Sample Type and Sample Volume selected ('Select Processing Options' and 'Select Method Options' windows; Figures 3 and 4) as well as settings applied during the on-site installation of the method and user interface.

5. **Transfer of PowerQuant® or Plexor® HY amplification mix to the qPCR amplification plate:** The liquid handler transfers the amplification mix to the appropriate wells of the qPCR amplification plate(s).
6. **Transfer of Unknowns and Controls to the qPCR amplification plate:** The liquid handler transfers the user-specified volume (2–9µl) of each Unknown and Control to the appropriate well of the qPCR amplification plate. The total reaction volume per well is 20µl. If more than 1 replicate qPCR amplifications are set up for each Unknown and Control, the replicates are placed in adjacent columns in the amplification plate (e.g., wells A1 and A2 when 2 replicates are selected).

For processing 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 processing volume of an Unknown (or Control). The water and Unknown (or Control) are dispensed to the appropriate well of the qPCR amplification plate.

7. **Transfer of PowerQuant® or Plexor® HY DNA standard dilutions to the qPCR amplification plate:** The liquid handler transfers the user-specified volume (2–9µl) of each DNA standard dilution to the appropriate wells of columns 11 and 12 of the qPCR amplification plate (refer to Section 8). 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 (or NTC). The water and DNA standard dilution (or NTC) then are dispensed to the appropriate well of the qPCR amplification plate.

- 8 **End of Method:** Seal the qPCR amplification plate, centrifuge the plate(s) and initiate thermal cycling. Not including the time required to enter information into the user interface and set up the worktable, the total time for the automated method to process one full plate of Unknowns (86 Unknowns) to one amplification plate (86 Unknowns and 10 Standards) is approximately 30 minutes.

Note: The automated method may be configured during the on-site method installation to generate a customized report file for import of the qPCR amplification plate layout into many qPCR instruments. This file can include the well locations and concentrations of the DNA standard dilutions as well as the well locations and information for Unknowns and Controls. For more information about the report(s) that the automated method can generate, refer to Section 12.

8. DNA Standard Curve Considerations

When assembling qPCR amplification reactions for the DNA standard curve, we recommend amplifying a volume of DNA standard that is equal to the volume of Unknown chosen by the user (2–9 μ l). 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 qPCR amplification reaction is within the linear range of the PowerQuant® or Plexor® HY System.

For the PowerQuant® System, we recommend using either a four-point or seven-point DNA standard curve. For the Plexor® HY System, we recommend a seven-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/ μ l DNA standard using the PowerQuant® Dilution Buffer, TE⁻⁴ Buffer or other buffers supplied by the user. Irrespective of the Standard Curve type selected in the user interface, the method prepares seven DNA standard dilutions and one no-template control (NTC) in column 1 of the standards plate on the worktable (Figure 17).

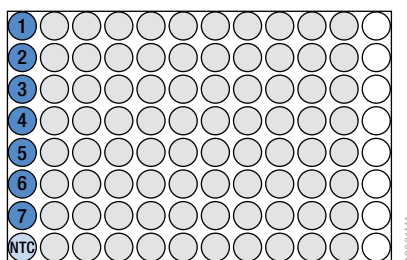


Figure 17. The standards plate. During serial dilution of the 50ng/ μ l DNA standard, seven standard dilutions are prepared in column 1 (shown in dark blue and numbered from highest concentration 1 to lowest concentration 7). The no-template control (NTC; shown in light blue) is placed in column 1, row H. The Water, Amplification Grade (shown with white circles), is staged in column 12 (if required, see Section 7, Steps 6 and 7).

8. DNA Standard Curve Considerations (continued)

When a seven-point DNA standard curve is selected, the Identity Automation™ qPCR setup method transfers all seven DNA standard dilutions and the NTC in duplicate to each amplification plate as shown in Figure 18, Panel A. When a four-point DNA standard curve is selected, 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 in duplicate to each amplification plate. The exact position of the DNA standard dilutions on the amplification plate depends on the number of replicate amplifications for each unknown sample that you selected (Figure 18, Panels B and C).

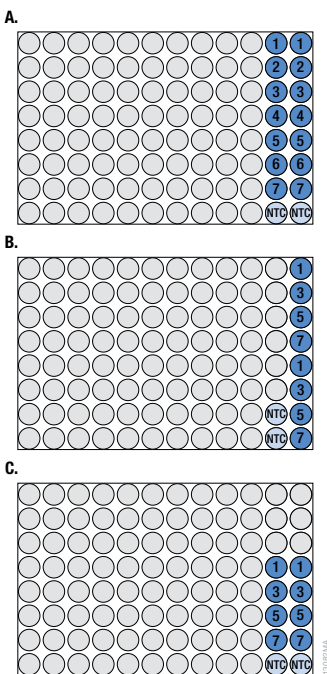


Figure 18. DNA standard curve and NTC placement on the qPCR amplification plate. Panel A.

Placement of duplicate seven-point DNA standard curves with from one to ten replicate qPCR amplifications for each Unknown and Control. **Panel B.** Placement of duplicate four-point DNA standard curves with one replicate qPCR amplification for each Unknown and Control. **Panel C.** Placement of duplicate four-point DNA standard curves with two to ten replicate qPCR amplifications for each Unknown and Control.

The concentrations of the Standards prepared during serial dilution of the 50ng/μl DNA standard and the final position of each Standard in the amplification plate depends on the values of Standard Curve, Sample Volume and Sample Replicates chosen in the ‘Select Method Options’ window (Figure 4). To facilitate set up of the qPCR instrument, the automated method can be installed to create one or more report files that simply provide the concentrations of the Standards or provide plate layout information for import into your qPCR instrument. For more information about the report(s) that the automated method can generate, refer to Section 12.

9. 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 worktable. This includes the manually prepared qPCR amplification mix; vigorous mixing will ensure homogeneity and will not harm performance.
3. Calculations for the qPCR amplification mix preparation include excess reagent to ensure that enough qPCR amplification 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 during the on-site installation service.
5. Aspiration and dispensing speeds, as well as pipetting heights, are critical to the success of this method. Aspiration of water with Unknowns, Controls and Standards as recommended and performed by default in this method (versus aspiration without water) 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.
7. Identifier information should be alphanumeric; avoid special characters, spaces and tabs. The method is not case sensitive (i.e., the method can't differentiate the identifier 123abc from the identifier 123ABC).

10. 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. For information related to the Tecan Freedom EVO® workstation or the Tecan PosID™ Positive Identification System, contact your local Tecan representative.

Part Description	Minimum 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) ^{1,2}	1	Contact Tecan
Wash Station, LiHa, with DiTi Waste Chute and Trough Carrier ³	1	10650037
DiTi Carrier, 3-Position ⁴	1–3	10613022
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 ⁵	1–2	10612604 or 0612624
Trough Carrier, 3-Position	1	10650020 or 10613020
Tube Carrier, 16mm, 16-Position (1 × 16) ⁶	1	30019987
Tube Carrier, 1.5ml or 2.0ml Microcentrifuge with Hinged Cap, 16-Position ⁷	6	10613035
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	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) ⁸	1	30034627
Microplate Adaptor, Torrey Pines Scientific, IC20/IC22/IC25 ⁸	1	30034628
Mounting Plate, Torrey Pines Scientific EchoTherm Dry Bath, IC20/IC22/IC25 ⁸	1	30034624

¹The full workflow of Identity Automation™ methods were developed for Freedom EVOware® Standard software, version 2.6, running Windows® 7 using a liquid LiHa configured with 500µl syringes. The DNA IQ™ System method volumes are fully compatible with 1ml syringes whereas the DNA Quantitation (PowerQuant® System or Plexor® HY System) and DNA Normalization and PowerPlex® System methods are fully compatible with 250µl syringes. A Tecan Freedom EVO® instrument configured with an Air LiHa arm also may be used.

Footnotes continued on next page.

²The computer connected the Tecan Freedom EVO® Workstation must be installed with the Windows® 7 Operating System, Windows® PowerShell 2.0 compatibility, and the Freedom EVOware® Standard software, version 2.4 or newer.

³If an Air LiHa arm is used, a Trough Carrier, 3-Position (Cat.# 10650020 or 10613020), and a DiTi Waste Chute are required instead of the Wash Station listed above.

⁴For Tecan Freedom EVO® 150 or 200 instruments, multiple DiTi Carriers, 3-Position may be used. Three DiTi Carriers, 3-Position are required for walk-away running of the DNA IQ™ System method. Two DiTi Carriers, 3-Position are required for walk-away set up of a single amplification plate using the Identity Automation™ qPCR setup and the Identity Automation™ DNA Normalization and PowerPlex® Setup methods.

⁵For the Identity Automation™ qPCR setup method (PowerQuant® and Plexor® HY Systems), one Microplate Carrier, 3-Position Landscape, is required to process one plate of Unknowns or Unknowns in tubes to one amplification plate. Automated runs involving multiple plates of Unknowns or multiple amplification plates will require an additional Microplate Carrier (refer to Table 1). For the Identity Automation™ DNA Normalization and PowerPlex® Setup method, the number of worktable positions required for labware varies based on the protocol and the number of sample plates or sample tubes processed (refer to the *Identity Automation™ DNA Normalization and PowerPlex® Setup Protocol for the Tecan Freedom EVO® Workstation Automated Protocol #EP042*). Be sure to install enough labware carriers to support the maximum number of plates per automated method run.

⁶**Optional:** For the Identity Automation™ qPCR setup method (PowerQuant® and Plexor® HY Systems) and the Identity Automation™ DNA Normalization and PowerPlex® Setup method, this carrier may be used for the robotic or manual preparation of single-tube qPCR or PCR amplification mix volumes from 1.3ml to 5.0ml. Alternatively, the following reagent blocks may also be used: Reagent Block AB Quantifiler (Part# 30019975) and AB STR-PCR (Part# 30019974).

⁷The maximum number of Tube Carriers allowed on a Freedom EVO® 100 worktable is 6. For Freedom EVO® 150 and 200 worktables, up to 12 Tube Carriers may be used. Regardless of instrument size, the Identity Automation™ DNA IQ™ System method can be installed to supply samples in tube format and elute DNA into tube format using only 6 Tube Carriers (refer to the *Identity Automation™ DNA IQ™ System Protocol for the Tecan Freedom EVO® Workstation Automated Protocol #EP039*). For the Identity Automation™ qPCR setup method (PowerQuant® and Plexor® HY Systems) on the Freedom EVO® 150 and 200 worktables, install enough labware carriers to support the maximum number of Unknowns in tubes processed per automated method run (six carriers for up to 96 Unknowns and 12 for up to 192; refer to Table 2). Likewise, for the Identity Automation™ DNA Normalization and PowerPlex® Setup method on the Freedom EVO® 150 and 200 worktables, install enough labware carriers to support the maximum number of amplification plates set up per automated method run (six carriers for set up of one amplification plate and 12 for two) (refer to the *Identity Automation™ DNA Normalization and PowerPlex® Setup Protocol for the Tecan Freedom EVO® Workstation Automated Protocol #EP042*).

⁸Contact Tecan for information regarding compatibility of the method with other heater options.

10. Automated Processing Requirements for Full Workflow on the Tecan Freedom EVO® Workstation (continued)

Additional Software, Hardware, Labware and Consumables Required

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

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

Supplier	Cat.#	Description	Number Required for the Indicated Automated Method		
			DNA IQ™ Method	PowerQuant® or Plexor® HY Method	PowerPlex® Normalization Method
Promega	DG1820	STR Normalization Manager™			1
Promega	A2661	Heat Block Adapter			1 (Swab Protocol) ¹
Promega	V8151	MagnaBot® 96 Magnetic Separation Device	1		
Promega	Z3301	1/4 inch Foam Spacer	1		
Promega	V6741	Deep Well Heat Transfer Block	1		
Promega	V1601	Four-Position Tube Holder		2–5 ²	2
User selected		Plate stand for 96-well PCR plate or strip tubes		1–5 ³	1–4 ³

¹Required only for off-instrument preprocessing of swabs in SwabSolution™ Reagent when swabs are preprocessed in plates.

²A minimum of two (2) Four-Position Tube Holders are required for all installations of the automated method. For each Four-Position Tube Holder, one Trough, Disposable, 100ml is required (Tecan 10613048 or 10613049); these 100ml troughs may be reused.

Optional: For the Identity Automation™ qPCR setup method (PowerQuant® and Plexor® HY Systems), one additional Four-Position Tube Holder is required if you choose to set up amplifications from Controls. Two additional Four-Position Tube Holders are required if you choose to present manually prepared Standards in tube format (1.5ml or 2ml). Three additional Four-Position Tube Holders are required if you choose to both set up amplifications from Controls and present manually prepared Standards in tube format (1.5ml or 2ml).

³The Promega Plate Clamp 96 (Cat.# V8251) and Plate Stand (Cat.# V8261) are for securing nonskirted 96-well plates or MicroAmp® Strip Tubes on the worktable. The Thermo Fisher Scientific MicroAmp® 96-Well Base (Cat.# N8010531), MicroAmp® Splash-Free 96-Well Base (Cat.# 4312063) or the Tecan Plate Adapter PCR (Cat.# 30032860) also may be suitable. For DNA IQ™ Method, the plate stand is required if DNA is eluted into 96-well format (e.g., 96-well PCR plate or strip tubes). For the PowerQuant® or Plexor® HY System Method, the plate stands are required if Unknowns are supplied in 96-well format (e.g., 96-well PCR plate or strip tubes), for standard curve preparation, and for the amplification plates. For the PowerPlex® Normalization Method, the plate stands are required if samples are supplied in a 96-well format (e.g., 96-well PCR plate or strip tubes) and for the amplification plates.

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

Supplier	Cat. #	Description	Number Required for the Indicated Automated Method		
			DNA IQ™ Method (per run)	PowerQuant® or Plexor® HY Method (per run)	PowerPlex® Normalization Method (per amplification plate)
Tecan	30000631 or 10612513	1,000µl LiHa Disposable Tips with Filter	<1/2 rack		
Tecan	10612553 or 30000629	200µl LiHa Disposable Tips with Filter	<8 racks	<1/4 rack	<1/4 rack
Tecan	30032114	50µl LiHa Disposable Tips with Filter		1–3 racks	1–3 racks
Tecan	10613049 or 10613048	Trough, Disposable, 100ml, Polypropylene Gray, or Trough, Disposable, 100ml, Polypropylene Natural	3	2–6 ¹	1–3 per run ¹
Tecan	30055743	Disposable Trough, 25ml, Low Dead Volume (Optional: To prepare ≤20ml PCR or qPCR amplification mix)		1 ²	1 per run ²
VWR	47743-968	Axygen® Multiple Well Reagent Reservoir with 12-Channel Trough, High Profile, Nonsterile (Corning Cat. # RES-MW12-HP)	1		
Promega	V6771	1.2ml, Round-Bottom Deep Well Plate	2		
Promega	V6781	2.2ml, Square-Well Deep Well Plate	1 ³		1 ⁴
Promega	V1391	Slicprep™ 96 Device	1 ³		
Promega	V6821	1.1ml, Square-Well, V-Bottom Deep Well Plate			2
User-selected		96-well PCR plate(s) or strip tubes for purified DNA samples	1 plate ⁵	1–3 plates ⁵	1 ⁵

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

Supplier	Cat.#	Description	Number Required for the Indicated Automated Method		
			DNA IQ™ Method (per run)	PowerQuant® or Plexor® HY Method (per run)	PowerPlex® Normalization Method (per amplification plate)
User-selected		1.5ml or 2.0ml microcentrifuge tubes containing samples	≤96 ⁶	≤192 ⁵	≤96 ^{4,5}
User-selected		1.5ml or 2.0ml microcentrifuge tubes, empty (for eluting purified DNA)	≤96 ⁵		
User-selected		1.5ml or 2.0ml microcentrifuge tubes (Optional: for Controls in tube format)		≤4	≤4 per run
User-selected		1.5ml or 2.0ml microcentrifuge tubes (Optional: for manually prepared standard dilutions in tube format)		≤8	
User-selected		96-well PCR plate or strip tubes for PCR amplification			1
User-selected		96-well optical PCR plate and plate cover (or sealing film) for DNA quantitation		1–2	
User-selected		96-well plate or 8-well strip tubes for standard dilution preparation and staging of Water, Amplification Grade		One plate or two 8-well strip tubes	

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

Supplier	Cat.#	Description	Number Required for the Indicated Automated Method		
			DNA IQ™ Method (per run)	PowerQuant® or Plexor® HY Method (per run)	PowerPlex® Normalization Method (per amplification plate)
User-selected		1.5ml microcentrifuge tube (to prepare PCR or qPCR amplification mix at <1.3ml robotically or <1.45ml manually)		Two tubes; one for preparing qPCR amplification mix ² and one for dilution buffer	1 per run ²
User-selected		5ml graduated self-standing tube (Optional: To prepare single-tube 1.3–5.0ml PCR or qPCR amplification mix; e.g., VWR Cat.# 89005-596 or Evergreen Scientific Cat.# 222-3007-080)		1 ²	1 per run ²

¹A 100ml trough is required to hold each Four-Position Tube Holder and a Disposable Trough, 25ml, Low Dead Volume (if required). These 100ml troughs may be reused. For the PowerPlex® Normalization Method, one 100ml trough may be used for dilution buffer.

²Only one tube or reservoir type is required per run; the type depends on user preference and the volume of qPCR or PCR amplification mix.

³The 2.2ml, Square-Well Deep Well Plate or SlicPrep™ 96 Device can be used for presenting samples on the worktable of the liquid handler.

⁴Labware may be used for pretreatment of swabs with SwabSolution™ Reagent when conducting the Swab Protocol. The 2.2ml, Square-Well Deep Well Plate is not required if samples are presented in tube format; the tubes are not required if the samples are presented in plate format.

⁵DNA IQ™ System Method: The 96-well PCR plate(s) or 8-well strip tubes are not required if the purified DNA is eluted into 1.5ml or 2.0ml tubes; the tubes are not required if the DNA is eluted into plate format.

PowerQuant® or Plexor® HY Method and PowerPlex® Normalization Method: The 96-well PCR plate(s) or 8-well strip tubes are not required if the purified DNA samples are in microcentrifuge tubes; the 1.5 or 2.0ml tubes are not required if purified DNA samples are in 96-well plate format.

⁶Up to 96 tubes are only required if samples are supplied in tube format.

10. Automated Processing Requirements for Full Workflow on the Tecan Freedom EVO® Workstation (continued)

Full Workflow Worktable Configuration

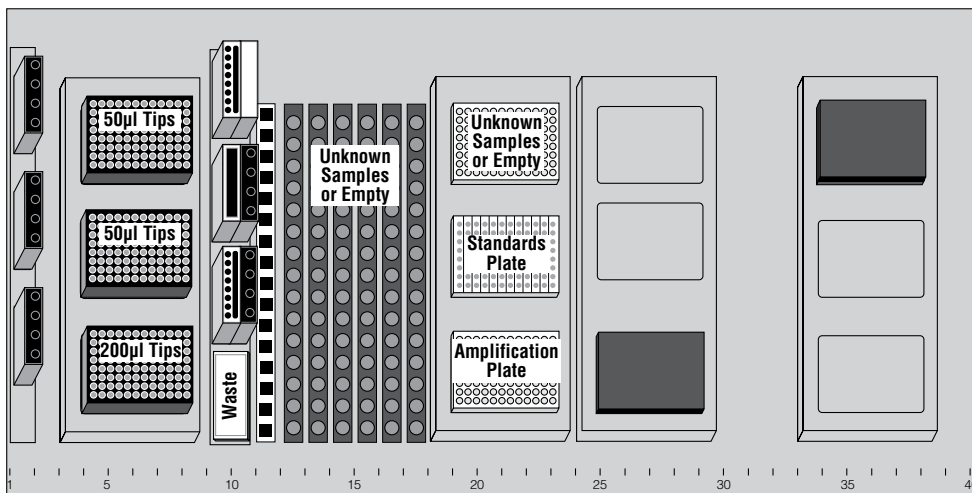


Figure 19. Tecan Freedom EVO® initial worktable configuration. Shown is a worktable that supports the full workflow of Promega automated methods for the Tecan Freedom EVO® workstation, including the DNA IQ™ System, DNA Quantitation (PowerQuant® System or Plexor® HY System) and DNA Normalization and PowerPlex® Setup. The labware shown supports processing of Unknowns from one plate or set of tubes to one amplification plate using the Identity Automation™ qPCR setup method for the PowerQuant® System and Plexor® HY System. This worktable is shown as an example only; the automated method can be adapted to any worktable layout provided these hardware (or equivalent) are present. For walk-away single amplification set up, the method requires four tip rack positions, three positions for 50µl tips and one for 200µl tips. As shown with one DiTi Carrier, 3-Position, the EVOware® software will prompt for additional tips when required during the run.

Grid 1 Trough Carrier, 100ml, 3-Position

Position 1 (rear) Trough, Disposable, 100ml, with Four-Position Tube Holder for Control in tubes (when required)

Position 2 Trough, Disposable, 100ml, with Four-Position Tube Holder for manually prepared Standards in tubes (when required)

Position 3 (front) Trough, Disposable, 100ml, with Four-Position Tube Holder for manually prepared Standards in tubes (when required)

Grid 3 DiTi Carrier, 3-Position

Position 1 (rear) 50µl LiHa Disposable Tips with Filter

Position 2 50µl LiHa Disposable Tips with Filter (as needed)

Position 3 (front) 200µl LiHa Disposable Tips with Filter

Grid 5 Shelf, 16-Position (back of worktable) (used with the DNA IQ™ System only)

Grid 9 Wash Station, LiHa, with DiTi Waste Chute and Trough Carrier, 100ml, 3-Position

Positions 1–3 (left) Wash and Waste

Position 4 (rear right) Trough, Disposable, 100ml with 25ml Low Dead Volume Disposable Trough (when required)

Position 5 Trough, Disposable, 100ml, with Four-Position Tube Holder containing reagents as described in Figure 2, Tube Holder Position 5

Position 6 (front right) Trough, Disposable, 100ml, with Four-Position Tube Holder containing reagents as described in Figure 2, Tube Holder Position 6

Position 7 Tip Waste Chute

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

Grid 12–17 Tube Carriers, 1.5ml or 2.0ml Microcentrifuge with Hinged Cap, 16-Position (Unknowns supplied in tube format)

Positions 1–16 1.5ml or 2.0ml tubes containing Unknowns (empty if Unknowns are supplied in tube format)

Note: Turn all open caps so that the caps do not interfere with pipetting steps.

Grid 18 Microplate Carrier, 3-Position

Position 1 (rear) 8-well strip tubes or 96-well plate containing Unknowns (empty if Unknowns are in tube format)

Position 2 8-well strip tubes (two 8-tube strips per run) or 96-well plate for DNA standards or staging Water, Amplification Grade, or both (columns 1 and 12)

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)

11. Identifier Import File Format for Unknown Samples: Minimum File Requirements

The Identity Automation™ qPCR setup method for the PowerQuant® System and Plexor® HY System on the Tecan Freedom EVO® automated liquid-handling workstation can import a file containing an ordered list of identifiers and locations in the plate of Unknowns (well location) or for the tubes of Unknowns (tube rack and tube position information). The graphical user interface is compatible with various file formats [e.g., comma, tab or semicolon delimited files, files with and without header lines, well location styles (1, A1 or A01)]; the format will be configured based on laboratory specifications during the on-site installation.

Identifier Import File: Minimum File Requirements

Sample Identifier	Sample Location
sampleID, sample name, or sample tube bar code	Samples supplied in 96-well plate format: Well location (e.g, A1, A2...H12 or 1, 2...96)
	Samples supplied in tube format: Tube rack name and tube position in tube rack


```

Report Title          Identity Automation qPCR Setup
RunDate              201807081155
User                 No log in
Instrument Information
Script               Promega_qPCR_V2_0_0
Script               qPCR_ExtractedDNALayout_V2_0_0

Run Options
Kit Type             PowerQuant
Sample Type          ExtractedDNA
Sample Input Labware Tubes
SampleID Input Type Import
Sample Batch Separation FALSE
Sample Volume        2
Sample Replicates    1

Robotic Standard Preparation TRUE
Curve Points         7

Control Sample Number 0

Setup Details
Sample Assigned Wells 80
Control Assigned Wells 0
Total Number Assays   96
Number Sample Sets    1
Number Amplification Plates 1

Amplification Reaction Mix Components
Buffer Volume         10
Primer Volume         1
AmpSolution Volume    0
Reaction Mix Water Volume 0
Total Reaction Volume 20

Other Reagents       Water, Dilution Buffer and Standard
Water Tube 1 Volume 1250
Dilution Buffer Tube Volume 1000
50ng/ul Standard Tube Volume 50
Water Tube 2 Volume 0
Reaction Mix         Preparation Settings
Labware              RM/Vessel:1
Total Volume Prepared 1111.99
Manual Preparation   FALSE
Dispense Tip Size    DIT150

Reagent Lots/Barcodes/Information
Buffer               123456
Primer               254567
AmpSolution
Water (Position1)   845678
Dilution Buffer     456789
50ng/ul Standard   567891
Water (Position 4)
Control Tube1
Control Tube2
Control Tube3
Control Tube4
Standard Position 1
Standard Position 2
Standard Position 3
Standard Position 4
Standard Position 5
Standard Position 6
Standard Position 7
Standard Position 8

AmpPlateNumber      AmpPlateWellNumber
1                    1
1                    2
1                    3
1                    4
1                    5
1                    6
1                    7
1                    8
1                    9
1                    10
1                    11
1                    12
1                    13

```

17249TA

Figure 21. Example Report File. The example text file shown is a timestamped record of run parameters. This type of report file can also be configured to contain information for each qPCR assay that is set up in the qPCR amplification plate (e.g., source and destination information, identifier information, processing volume, source plate bar code information and qPCR amplification plate bar code information).



Promega

13. Summary of Changes

The following changes were made to the 1/21 revision of this document:

1. Updated to include ability of method to process Unknowns and Controls supplied in 1.5ml or 2.0ml tubes.
2. Added new Sections 6, 11 and 12.
3. Updated disclaimers.

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