

AUTOMATED PROTOCOL

Identity Automation™ DNA IQ™ System Protocol for the Tecan Freedom EVO® Workstation

Instructions for Use of Product
DC6701 and DC6700



Revised 12/20
EP039

Identity Automation™ DNA IQ™ System Protocol for 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™ DNA IQ™ System on the Tecan Freedom EVO® automated liquid-handling workstation. The automated method for DNA isolation is designed with the flexibility to support the following:

- Use of the DNA IQ™ System.
- Samples in 96-well format (96-well plate) or tube format (1.5ml or 2.0ml tubes).
- Tracking of sample identifiers via import of an ordered list, manual entry (handheld scanner or keyboard), or the Tecan PosID™ Positive Identification System.
- Processing aqueous samples with volumes of 25–400µl.
- Processing samples containing DNA IQ™ Lysis Buffer (“lysis samples”) volumes of 25–800µl.
- Processing up to 96 samples.
- Eluting DNA into 96-well format (96-well plate) or tube format (1.5ml or 2.0ml tubes).
- Elution volumes of 30–100µl.
- Processing with DNA IQ™ Resin volumes of 7–21µl.

On-site method installation by Promega includes training, installation and initial setup 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/

2. Product Requirements

PRODUCT	SIZE	CAT.#
DNA IQ™ System	100 reactions	DC6701
	400 reactions	DC6700

Not for Medical Diagnostic Use.

Refer to the *DNA IQ™ System—Small Sample Casework Protocol Technical Bulletin #TB296* or *DNA IQ™ System—Database Protocol Technical Bulletin #TB297* for information related to product storage and troubleshooting DNA IQ™ chemistry issues. These Technical Bulletins are available at:
www.promega.com/protocols/

Note: The DNA IQ™ System is available in two formats: 100 reactions and 400 reactions. These formats contain sufficient reagents to process 1 × 96-well plate and 4 × 96 well plates of aqueous samples (up to 100µl volume) or lysis samples (samples preprocessed with the DNA IQ™ Lysis Buffer; up to 200µl volume), respectively (for information on aqueous and DNA IQ™ Lysis Buffer sample types, refer to Section 6.B of this protocol). When processing larger sample volumes, you will need to purchase additional Lysis Buffer (Cat.# A8261). When processing samples with greater than 7µl of DNA IQ™ Resin, you may need to purchase additional DNA IQ™ Resin (Cat.# A8251). The automated method will prompt you to confirm that a minimum volume of each reagent is placed on the worktable of the liquid handler.

Items Available Separately

PRODUCT	SIZE	CAT.#
DNA IQ™ Resin	50ml	A8251
Lysis Buffer	150ml	A8261
2X Wash Buffer	70ml	A8271
Elution Buffer	50ml	A8281

Not for Medical Diagnostic Use.

3. Materials to be Supplied by the User

- DNA IQ™ System (If you are processing more than 100µl of each aqueous sample or 200µl of each lysis sample, you will need to purchase additional Lysis Buffer; see Section 2. If you are processing samples with greater than 7µl of resin, you may need to purchase additional DNA IQ™ Resin.)
- Labware for sample preprocessing (see Technical Bulletins listed in Section 4.A)
 - Slicprep™ 96 Device (Cat.# V1391)
 - 1.5ml microcentrifuge tubes (e.g. ClickFit Microtube, 1.5ml Cat.# V4745; Cat.# V4741) and DNA IQ™ Spin Baskets (Cat.# V1221; Cat.# V1225)
 - CW Microfuge Tubes (Cat.# AS8201) and CW Spin Baskets (Cat.# AS8101)
- 99% isopropyl alcohol
- 95–100% ethanol
- DTT, Molecular Grade (Dry Powder; Cat.# V3151, V3155). Prepare a 1M DTT solution.
- Proteinase K (Cat.# V3021 for 100mg) for sample preprocessing as required (see Technical Bulletins in Section 4.A).
- See Sections 5.A and 5.B for instrumentation and labware requirements, respectively.

4. Before You Begin

4.A. Sample Processing Before Automated Processing (As Required)

For samples on solid supports and differential extraction samples, preprocessing must be performed prior to the start of the automated method. For more information about sample preprocessing, consult the *DNA IQ™ System—Small Sample Casework Protocol Technical Bulletin #TB296* or *DNA IQ™ System—Database Protocol Technical Bulletin #TB297*, or contact Promega Technical Services at genetic@promega.com

4.B. Reagent Preparation

Prior to beginning the Identity Automation™ DNA IQ™ System method, prepare the following solutions:

Prepared Lysis Buffer

1. Add 1µl of 1M DTT for every 100µl of Lysis Buffer.
2. Mix by inversion several times.
3. Mark and date label to record addition of DTT.

This solution can be stored at room temperature for up to one month if the bottle is tightly closed.

DNA IQ™ Resin Solution (Prepared Lysis Buffer + Resin)

1. Make the Prepared Lysis Buffer as described above.
2. Thoroughly mix the DNA IQ™ Resin by inversion until the particles are completely resuspended.
3. During the automated method run, combine Prepared Lysis Buffer and DNA IQ™ Resin at the volumes instructed by the graphical user interface of the automated DNA IQ™ method; see Section 6.D, Figure 10.
4. Mix thoroughly by gentle inversion several times. To prevent foaming of the solution, do not mix vigorously.



Note: Prepare the DNA IQ™ Resin Solution fresh for each run. Do **not** store.

1X Wash Buffer

1. Add ethanol and isopropyl alcohol directly to the 2X Wash Buffer bottle.
For DC6700, add 35ml of 95–100% ethanol and 35ml of 99% isopropyl alcohol.
For DC6701, add 15ml of 95–100% ethanol and 15ml of 99% isopropyl alcohol.
2. Replace the cap and mix by inversion several times.
3. Mark label to indicate addition of alcohols.
4. Store at room temperature. Make sure bottle is closed tightly to prevent evaporation.

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™ DNA IQ™ System method 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.



The computer connected to 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™ DNA IQ™ System method on a 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 and RoMa Gripper (Freedom EVOware® Standard software) ¹	1	Contact Tecan
Wash Station, LiHa, with DiTi Waste Chute and Trough Carrier, 100ml, 3-Position ²	1	10650037
Shelf, 16-Position (4 sites with 4 shelves/site)	1	10650627
DiTi Carriers, 3-Position ³	1	10613022
Te-Shake™ Microplate Shaker Unit	1	10760723
Microplate Nest, 1-Position, with Hold Down, Te-Shake™	1	10760724
Mounting Plate, Te-Shake™ 1-Position with 2 additional Microplate Positions	1	30015506
EchoTherm Dry Bath, Torrey Pines Scientific, IC20, Electronic Chilling/Heating ⁴	1	30034627
Microplate Adaptor, Torrey Pines Scientific, IC20/IC22/IC25 ⁴	1	30034628
Mounting Plate, Torrey Pines Scientific EchoTherm Dry Bath, IC20/IC22/IC25 ⁴	1	30034624
Tube Carrier, 1.5ml or 2.0ml Microcentrifuge with Hinged Cap, 16-Position ⁵	6	10613035
Note: If samples are supplied in 96-well format and DNA is eluted into a 96-well format, this carrier is not required.		

¹The method was developed for Freedom EVOware® Standard software, version 2.6, running Windows® 7 using a liquid LiHa configured with eight 500µl syringes. The method volumes are also fully compatible with 1ml 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.

³For Tecan Freedom EVO® 150 or 200 instruments, additional DiTi Carriers, 3-Position may be used. See Section 5.B for information on method requirements for LiHa Disposable Tips (200µl and 1,000µl).

⁴Contact Tecan for information regarding compatibility of the method with other heater options.

⁵The 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; 6 for samples to be supplied in tube format and 6 for DNA to be eluted into tube format. Regardless of the instrument, 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; contact Promega for additional information.

5.B. Labware and Consumables Required

The following items are required for the Identity Automation™ DNA IQ™ System method 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	V8151	MagnaBot® 96 Magnetic Separation Device	1
Promega	Z3301	1/4 inch Foam Spacer	1
Promega	V6741	Deep Well Heat Transfer Block	1
User-selected		Plate stand for 96-well PCR Plate or Strip Tubes ¹	1

¹The plate stand is only required if DNA is eluted into a 96-well format (e.g., 96-well PCR plate or strip tubes). 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 Thermo Fisher Scientific MicroAmp® 96-Well Base (Cat.# N8010531), 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
Tecan	30000629	200µl LiHa Disposable Tips with Filter	<8 racks ¹
Tecan	30000631 or 10612513	1,000µl LiHa Disposable Tips with Filter	<½ rack
Tecan	10613048 or 10613049	Trough, Disposable, 100ml, Polypropylene Natural or Trough, Disposable, 100ml, Polypropylene Gray	3
Promega	V6781 or V1391	2.2ml, Square-Well Deep Well Plate or 96 Deep Well Plate from the Slicprep™ 96 Device (for preprocessed solid samples)	1 ²
Promega	V6771	1.2ml, Round Bottom Deep Well Plate	2
VWR	47743-968	Axygen® Multiple Well Reagent Reservoir with 12-Channel Trough, High Profile, Nonsterile (Corning RES-MW12-HP)	1
User-selected		1.5ml or 2.0ml microcentrifuge tubes containing DNA samples	≤96 ³
User-selected		1.5ml or 2.0ml microcentrifuge tubes for DNA elution	≤96 ⁴
User-selected		96-well PCR plate or strip tubes	1 ⁵

¹The automated DNA IQ™ method requires seven (7) 200µl LiHa Disposable Tips with Filter for each sample processed plus up to 8 additional tips for reagent transfer (e.g., processing 96 samples requires 7 full racks of 200µl tips plus a column of tips from an eighth rack).

²A 2.2ml, Square-Well Deep Well plate is required when samples are supplied in tube format.

³The tubes are not required if samples are in 96-well format.

⁴Tubes are not required if elution of DNA is into 96-well format.

⁵The 96-well PCR plate or strip tubes are not required if DNA is eluted into tubes.

5.C. Tecan Freedom EVO® Initial Worktable Configuration

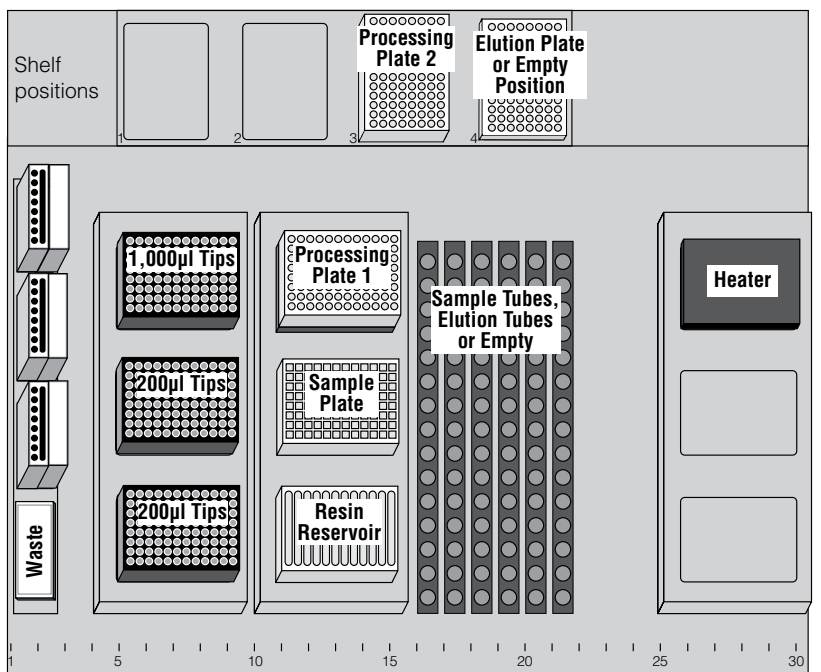


Figure 1. Tecan Freedom EVO® initial worktable configuration. Shown are the minimum requirements for installation of the Identity Automation™ DNA IQ™ System Method on the Tecan Freedom EVO® workstation. The layout shown supports samples supplied in tube or 96-well format as well as elution of the DNA into tube or 96-well format and is an example only; the automated method can be adapted to any worktable layout provided these hardware or equivalent are present. If tubes are not used to supply samples or for DNA elution, the Tube Carriers at Grids 16–21 are not required.

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

- Position 1 to 3 (left) Wash and Waste
- Position 4 (right, rear) Trough, Disposable, 100ml (for Elution Buffer)
- Position 5 Trough, Disposable, 100ml (for 1X Wash Buffer)
- Position 6 (right, front) Trough, Disposable, 100ml (for Prepared Lysis Buffer)
- Position 7 Tip Waste Chute

Grid 4 DiTi Carrier, 3-Position

- Position 1 (rear) 1,000µl LiHa Disposable Tips with Filter
- Position 2 200µl LiHa Disposable Tips with Filter
- Position 3 200µl LiHa Disposable Tips with Filter

Grid 5 Shelf, 16-Position (back of worktable)

Position 1 and 2 (upper left) Empty

Position 3 (upper second position from right) Empty 1.2ml, Round-Bottom Deep Well Plate (Processing Plate 2)

Position 4 (upper right) Empty 96-well PCR Plate or strip tubes on a plate stand (Elution Plate; DNA eluted in 96-well format); Empty (DNA eluted in tube format)

Grid 10 Mounting Plate, Te-Shake™ 1-Position with two additional Microplate Positions and Te-Shake™ Microplate Shaker Unit

Position 1 (rear) Empty 1.2ml, Round-Bottom Deep Well Plate (Processing Plate 1) on a MagnaBot® 96 Magnetic Separation Device, 1/4 inch Foam Spacer

Position 2 (middle) 2.2ml, Square-Well Deep Well Plate or 96 Deep Well Plate from the Slicprep™ 96 Device containing samples (Sample Plate). Place samples in columns 1–12, depending on the number of samples to be processed.

Position 2 (middle) Empty 2.2ml, Square-Well Deep Well Plate (Samples in tube format are transferred to the Sample Plate at the start of the method)

Position 3 (front) Reservoir with 12-Channel Trough (DNA IQ™ Resin Solution prepared in Section 4.B; column 1)

Grids 16–21 Tube Carriers, 1.5ml or 2.0ml Microcentrifuge with Hinged Cap, 16-Position (samples supplied in tube format or DNA elution into tube format)

Positions 1–16 1.5ml or 2.0ml tubes containing samples (samples supplied in tube format)¹

Positions 1–16 empty 1.5ml or 2.0ml tubes (samples supplied in 96-well format and DNA eluted into tubes)^{1,2}

¹Turn all open caps so that the caps do not interfere with pipetting steps.

²The Identity Automation™ DNA IQ™ System method can be installed to allow the samples to be supplied in tube format and the elution of DNA into tube format using only these 6 Tube Carriers positions; see Figure 13 or contact Promega for additional information.

Grid 25 Torrey Pines Heater, Promega Deep-Well Heat Transfer Block

6. Graphical User Interface

The Identity Automation™ DNA IQ™ System method is integrated with an easy-to-use graphical user interface that:

- Allows you to enter sample information (e.g., sample location in tubes or plate, sample identifiers and bar codes, elution tube identifiers and bar codes) manually using a keyboard or hand-held scanner, importing an ordered list or, in the case of samples presented in tubes and DNA eluted into tubes, using the Tecan PosID™ Positive Identification System.
- Allows you to select method options at run time that set the processing requirements (e.g., input labware for samples, sample processing type, sample processing volume, resin volume, elution volume and output labware for eluates).
- Guides you through the physical setup of the Tecan Freedom EVO® workstation (e.g., placement of samples, reagents and labware on the worktable of the liquid handler).

During the on-site method installation, default values for the method options can be set based on your needs. At the start of each run, you can accept the defaults (defaults indicated with either a ✓ or a numerical value) or make new 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 user interface may be customized for preferred language.

6.A. Before You Start the Graphical User Interface

The Identity Automation™ DNA IQ™ System Method uses 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 method. 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 1,000µl 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 setting the tip positions in the EVOware® software, selecting the script and pressing the **Run** button, the following windows will appear:

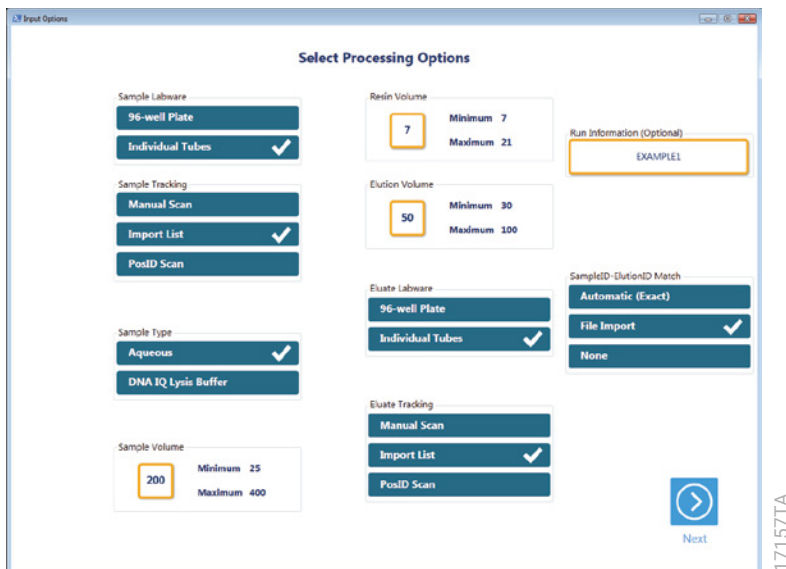


Figure 2. Example ‘Select Processing Options’ window. As appropriate, select button or input numerical value for the Sample Labware type, Sample Tracking type, Sample Type, Sample Volume, Resin Volume, Elution Volume, Eluate Labware type, Eluate Tracking type, and the SampleID-ElutionID Match type for the run. Enter run information in text field (optional).

Sample Labware

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

Sample Tracking

The Sample Tracking option allows you to choose the way identifier and position information is entered for samples. Tracking information may be provided manually (via keyboard or a hand-held scanner), by importing an ordered list containing identifiers and locations, or using the Tecan PosID™ Positive Identification System (for samples presented in tubes only). For more information on how the user interface allows you to enter the sample information, refer to Sections 6.C and 10.

6.B. Example Data Inputs Windows (continued)

Sample Type

The Sample Type option allows you to indicate the way the samples are handled prior to processing using the automated DNA IQ™ System method. **Aqueous** samples refer to any samples that contain no Promega DNA IQ™ Lysis Buffer prior to placing on the liquid handler. **DNA IQ Lysis Buffer** samples refer to any samples that have been preprocessed with DNA IQ™ Lysis Buffer prior to placing on the liquid handler (i.e., samples containing DNA IQ™ Lysis Buffer). Differential extraction samples are treated as either **Aqueous** or **DNA IQ Lysis Buffer** samples, depending on whether they contain DNA IQ™ Lysis Buffer. Within a run, all samples must be of the same type.

Sample Volume


The Sample Volume option allows you to enter the input volume of sample being processed. The volume range allowed is displayed and depends on the Sample Type selected; if **DNA IQ Lysis Buffer** is selected, you can enter 25–800µl; if **Aqueous** is selected, you can enter 25–400µl. Within a run, all samples are processed in the same manner. Therefore, if sample input volume varies, set the value of Sample Volume to the volume of the sample with the largest input volume.

Resin Volume

The Resin Volume option allows you to enter the volume of DNA IQ™ Resin that will be used to process each sample. You can enter a volume of 7–21µl. Within a run, all samples are processed using the same resin volume.

Eluate Labware

The Eluate Labware option allows you to choose the output labware for DNA eluates; 96-well plate or individual tubes (1.5ml or 2.0ml). Within a run, all eluates are eluted into the same labware type.

 If DNA is eluted into 96-well plate format, the sample identifier supplied for the sample is tracked through to the appropriate well of the elution plate (i.e., it is not possible to supply a different per well eluate identifier).

SampleID-ElutionID Match (elution into tube format only)

The SampleID-ElutionID Match option allows you to choose how to associate sample identifiers and eluate identifiers if the DNA is eluted in tube format. For more information on how the method uses the SampleID-ElutionID Match option to direct processing of samples from either a 96-well format or tubes through two intermediate processing plates to the elution tubes, refer to Section 7.B. For more information on the file format required when the **File Import** button is selected, refer to Sections 6.F and 10.

Eluate Tracking (elution into tube format only)

The Eluate Tracking option allows you to choose the method by which identifier and position information are entered for elution tubes. If an eluate tracking option is selected, tracking information may be provided manually using a keyboard or hand-held scanner, by importing an ordered list containing identifiers and locations, or using the Tecan PosID™ Positive Identification System. For more information on how to enter the eluate information, refer to Sections 6.F and 10.

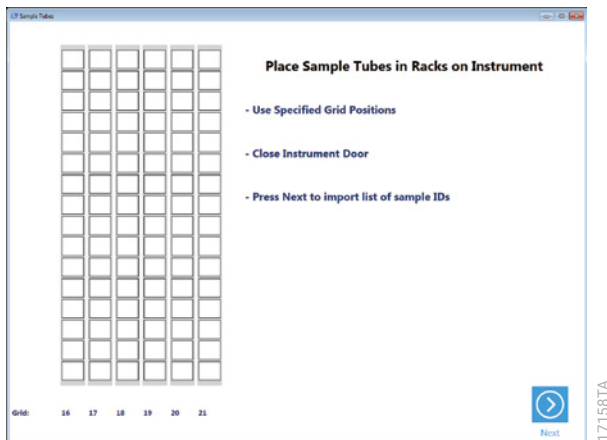


Figure 3. Example ‘Sample Tube’ window. When **Individual Tubes** is selected in the ‘Select Processing Options’ window (Figure 2), a ‘Sample Tube’ window directs you to place the sample tubes in tube racks at specific grid positions on the worktable of the liquid handler. The exact window displayed depends on the sample tracking type selected and the specific configuration of the Tecan Freedom EVO® workstation.

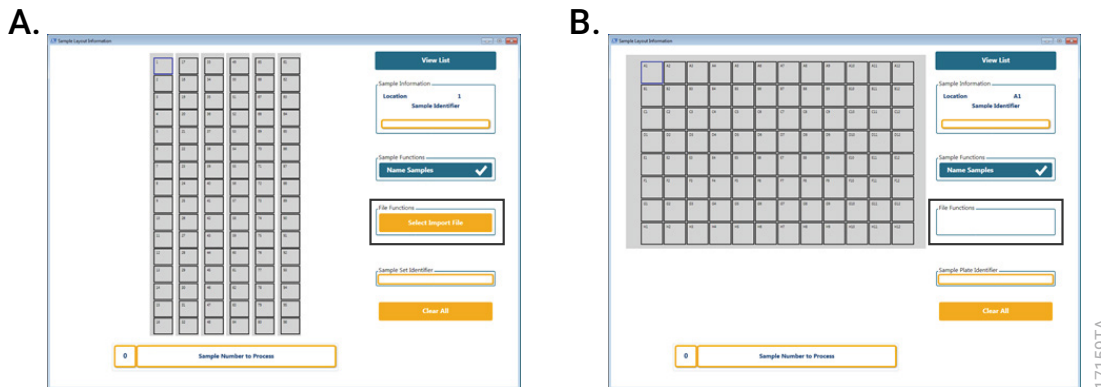



Figure 4. Example ‘Sample Layout Information’ windows. The exact window displayed depends on the Sample Labware and Sample Tracking values selected in the ‘Select Processing Options’ window (Figure 2). The **Select Import File** button is only displayed when sample tracking information is provided via list import (see insert in black box). For more information on the features of the ‘Sample Layout Information’ window, refer to Section 6.C. **Panel A.** The example ‘Sample Layout Information’ window shown supports processing of samples from tubes with sample tracking information entered via list import. Select the **Select Import File** button to import a file containing sample information. **Panel B.** The example ‘Sample Layout Information’ window shown supports processing of samples from a 96-well plate with sample tracking information entered manually. Enter the sample information for the highlighted well in the **Sample Identifier** input box and press the **Tab** key to accept the information and advance to the next well in the plate.

6.C. Example ‘Sample Layout Information’ Windows

The automated Identity Automation™ DNA IQ™ System method allows samples to be presented in a single 96-well plate or up to 96 individual tubes (1.5ml or 2.0ml). You must supply the positions of all samples in the plate or tubes using the ‘Sample Layout Information’ window. Information for the samples may be provided manually using a computer keyboard or hand-held scanner, by importing an ordered list of identifiers and locations or using the Tecan PosID™ Positive Identification System (for samples presented in tubes only).

Features of the ‘Sample Layout Information’ Window.


Pressing the **View List/View Map** toggle button switches the view between a image map of the samples in six 16-position tube racks or in a single 96-well plate (Map View) and a single column list of samples (List View). When samples 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 samples are supplied in a 96-well plate, numbering is based the well position (i.e., A1, B1, C1...F12, G12, H12). To navigate through the sample positions in either the List or Map View, use the mouse or **Tab** key. When manually entering or modifying identifier information, the **Tab** key signals the ‘Sample Layout Information’ window to accept the new or modified identifier information and update the List or Map View.


 The automated Identity Automation™ DNA IQ™ System method processes all wells of a sample plate or all tube positions up to the last well or tube position that contains a sample identifier. All positions must contain a unique sample identifier. The user interface will require that you enter a unique sample identifier for any empty wells or tube positions prior to the final sample well or sample tube (e.g., BLANK1 or BLANKA1).

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

- **White:** Samples that have unique identifiers (or bar codes).
- **Gray:** Empty positions (after the last well or tube position that contains a sample identifier).
- **Salmon:** Samples having either replicate identifiers or sample positions that have no identifier and are located before the last occupied sample position. When multiple samples have the same identifier, the replicate identifier must be changed. Unless all positions up to and including the last filled sample position have a unique sample identifier, you cannot proceed. When using the PosID™ System to scan sample tube barcodes, a tube with a scan error will display be displayed in salmon as “***”; you must manually provide the bar code.

The “Sample Information” box contains the location of the sample (i.e., tubes, 1 to 96; plate, A1 to H12) and the “Sample Identifier” input box. When the cursor is placed in the “Sample Identifier” input box, you may manually supply a unique identifier (or bar code) to an empty location or modify/remove an existing identifier. After adding, modifying or removing the identifier, press **Tab** on the keyboard to accept the change and update the window. To view the ‘Sample Information’ box of a specific sample, select the location of the sample in the Map or View List.

 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 sample using the mouse, the addition, modification or removal of the identifier may be lost.

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

Pressing the **Select Import File** button allows you to select and open an ordered list containing identifiers and locations for samples in a plate (well location) or in tubes (tube rack and tube position information). The file format will be configured based on laboratory specifications during the on-site installation. For information about accepted file formats, refer to Section 10.

Pressing the **Save Sample Set** button sets the sample identifier and sample location and allows the method to proceed. Once the **Save Sample Set** button has been pressed, you are unable to add or remove a sample from the sample set nor can you change a sample identifier or sample location without aborting and restarting the run. The **Save Sample Set** button is only visible when one or more samples are present and any sample status issues (i.e., salmon-colored positions) have been addressed.

The “Sample Set Identifier”/“Sample Plate Identifier” input box contains an optional input field that allows you to enter additional information for the set of samples or sample plate.

Pressing the **Clear All** button removes the identifier information for all samples in the current ‘Sample Layout Information’ window.

In the List View, a “Hide Empty” checkbox allows you to hide any empty sample positions after the last well or tube position that contains a sample identifier.

Example Sample Layout Windows

The ‘Sample Layout Information’ window displayed depends on the Sample Labware and Sample Tracking options selected in the ‘Select Processing Options’ window (Figure 2). The example windows provided illustrate the features of the ‘Sample Layout Information’ window. For example purposes, the windows shown support runs in which the samples are supplied in individual tubes and the sample information (i.e., identifiers and location) are imported via an ordered list.

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



Figure 5. Modifying Information in the ‘Sample Layout Information’ window. Panel A. Map View. Choose the **Select Import File** button (initial window shown in Figure 4, Panel A) navigate to and load an import list; the results of the actions are shown. Salmon-colored positions indicate issues with the imported sample identifier list that must be addressed before the method may continue. Select the **View List/View Map** toggle button to display the sample information in list format. **Panel B. List View.** Modifying Imported Sample Identifier Information. The salmon-colored entries at positions 1 and 2 indicate replicate sample identifiers are detected. Highlight position 1, select the “Sample Identifier” input box, manually provide a unique sample identifier and use the **Tab** key. The salmon-colored entry at position 21 indicates the import file contained no sample identifier information. As there are sample identifiers supplied for positions 22 and 23, manually supply a unique sample identifier for position 21. Highlight position 21, select the “Sample Identifier” input box, provide a unique sample identifier and press the **Tab** key. Finally, to delete sample identifier from a position, highlight the position (e.g., position 23), select the “Sample Identifier” input box, manually delete the sample identifier and press the **Tab** key. **Panel C. List View.** The **Save Sample Set** button is visible when all the sample status issues are resolved.

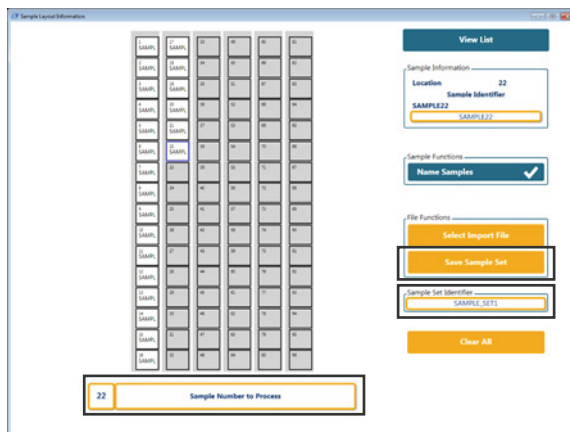


Figure 6. Saving a Sample Set in the ‘Sample Layout Information’ window. The ‘Sample Layout Information’ window provides the number of samples to be processed and the “Sample Set Identifier”/“Sample Plate Identifier” input box to enter additional information for the set of samples (see the black outlined areas at bottom and on lower right side of window, respectively). When the information for all samples has been added, select the **Save Sample Set** button (see black outlined button on upper right side of window).

6.D. Example ‘Worktable Setup’ Windows

The automated Identity Automation™ DNA IQ™ System method 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’ window (Figure 2) as well as the physical configuration of the Tecan Freedom EVO® workstation. The reagent volumes prompted depend on the values of Sample Type, Sample Volume, Resin Volume and Elution Volume selected in the ‘Select Processing Options’ window as well as the number of samples to be processed; the user interface will prompt for the minimum volume required. The example ‘Worktable Setup’ windows shown below support processing of aqueous samples in tubes through to elution of the DNA into 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 move through the various windows.

6.D. Example ‘Worktable Setup’ Windows (continued)

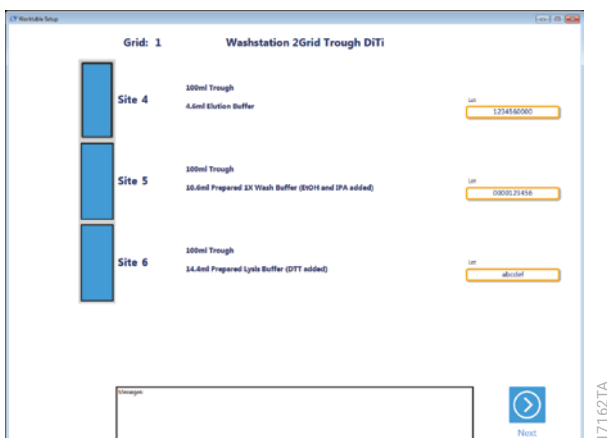


Figure 7. Example ‘Worktable Setup’ Window: Elution Buffer, 1X Wash Buffer and Prepared Lysis Buffer. Reagent Placement on the Worktable. **Optional:** Reagent lot information may be supplied in the boxes on the right side of the window.

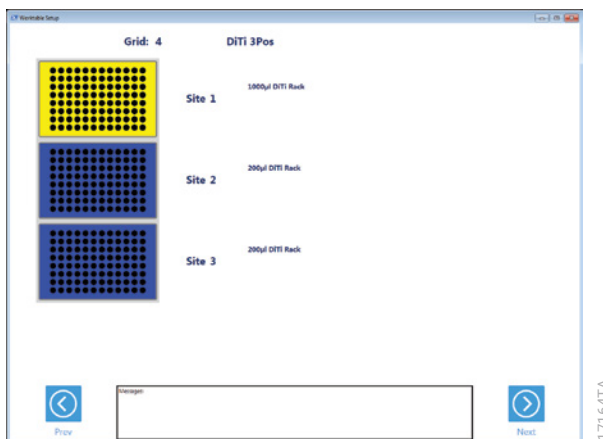
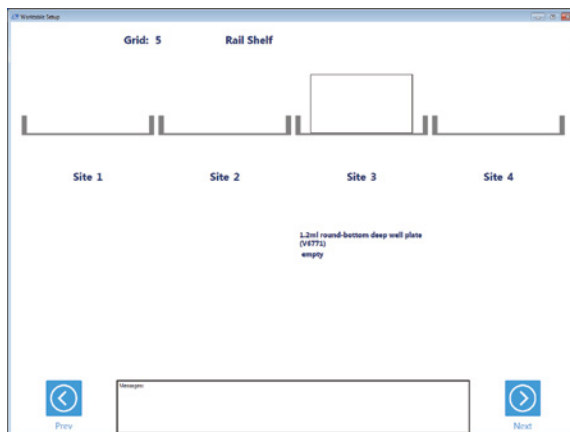
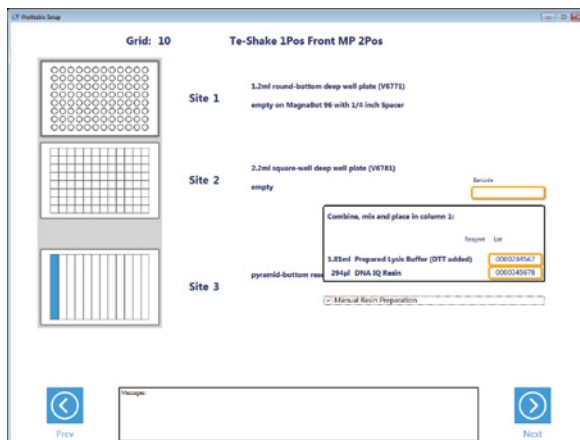


Figure 8. Example ‘Worktable Setup’ Window: Disposable Tips. The Identity Automation™ DNA IQ™ System method relies on Tecan EVOware® software to manage tip handling. As indicated in Section 6.A, you need to set the position of the first 1,000µl tip and the first 200µl tip using the **Set DiTi position** command in the EVOware® software prior to starting the method. During the method run, the EVOware® software will prompt you to place more tips on the worktable when required.



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Figure 9. Example ‘Worktable Setup’ Window: Rail Shelf. Labware placement on the rail shelf depends on the value selected for Eluate Labware in the ‘Select Processing Options’ window (Figure 2). When DNA is eluted into tubes, Site 4 of the rail shelf is empty. When DNA is eluted into a plate, you will be prompted to place an elution plate at Site 4.



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Figure 10. Example ‘Worktable Setup’ window: Empty Processing Plate, Sample Plate and Resin Reservoir. Select the **Manual Resin Preparation** expander to view the volumes of Prepared Lysis Buffer and DNA IQ™ Resin required to prepare the DNA IQ™ Resin Solution (Prepared Lysis Buffer + Resin). Placing the DNA IQ™ Resin Solution (Prepared Lysis Buffer + Resin) in first column of the 12-column reservoir is shown in the expanded view and indicated with blue shading. Regardless of whether samples are supplied in tube or 96-well plate format, a 2.2ml square-well deep-well plate is placed on the robot worktable. If samples are supplied in tube format, the liquid handler transfers samples into the empty 2.2ml square-well deep-well plate. **Optional:** Reagent lot and sample plate bar code information may be supplied in the boxes on the right side of the window.

6.D. Example ‘Worktable Setup’ Windows (continued)

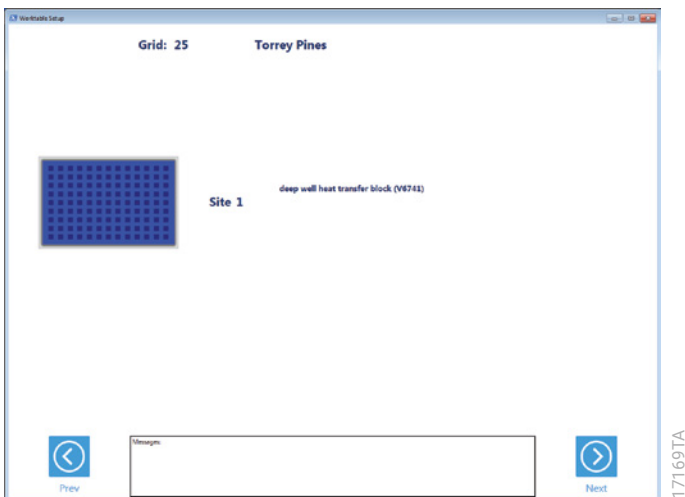


Figure 11. Example ‘Worktable Setup’ Window: Heater with Deep Well Heat Transfer Block. The DNA IQ™ method can support heaters in landscape and portrait orientations. This screen will display the heater option present on your instrument. For manually controlled heaters, you will be prompted to turn on and set the heater to the appropriate temperature at the beginning of the method.



Figure 12. Example ‘Worktable Setup’ window: Worktable Setup Complete. Select the **Prev** button to navigate back through the ‘Worktable Setup’ windows. Click **Next** to continue. Once the **Next** button has been pressed, you are unable to navigate back through the previous ‘Worktable Setup’ windows without aborting and restarting the run.

6.E. Example ‘Elution Tubes’ Window

The automated Identity Automation™ DNA IQ™ System method elutes DNA into a single 96-well plate or up to 96 individual tubes (1.5ml or 2.0ml). When the **Individual Tubes** button is selected in the ‘Select Processing Options’ window (Figure 2), an ‘Elution Tubes’ window directs you to place the appropriate number of empty tubes in tube racks at specific grid positions on the worktable of the liquid handler. The exact window displayed depends on:

- The number of elution tubes required.
- The eluate tracking type selected.
- The sample identifier to elution tube identifier matching option selected.
- The configuration of carriers on the worktable.

In the example provided, the worktable configuration shown in Figure 1 is used and the tube racks on the worktable are used to both supply samples in tubes at the start of the run and to supply empty elution tubes. Because the same tube racks are used for both purposes, the ‘Elution Tubes’ window is displayed after the samples in tubes are transferred to a plate (refer to Section 7.A, Step 3) and the window prompts for the removal of the sample tubes prior to placement of the empty elution tubes (Figure 13; black outline). When different tube racks are used to supply the sample tubes and the elution tubes, the ‘Elution Tubes’ window is displayed prior to the worktable setup windows.

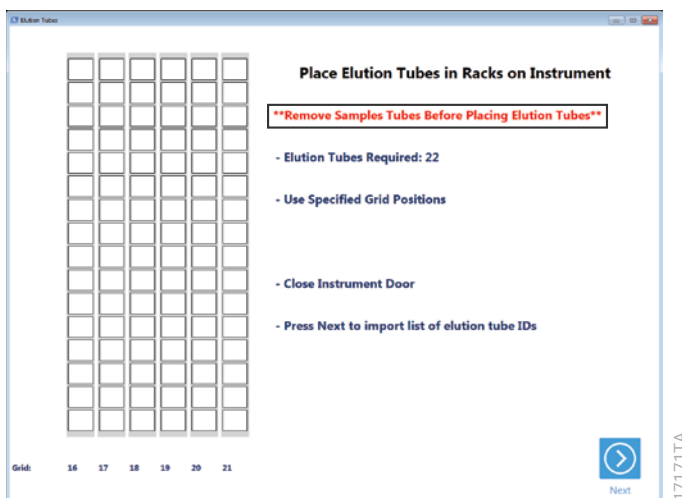




Figure 13. Example ‘Elution Tubes’ window. This screen instructs how to place elution tubes on the system. When the elution tube identifier information is to be supplied either manually or via an import list, the exact placement of the elution tubes at this step must match the location that will be supplied in the subsequent step.

 Elution tube positions shown in white are positions where elution tubes may be placed. Elution tube positions shown in gray (not shown in this example) are not allowed; elution tubes placed in gray positions will be ignored by the method. For more information related to selections in the ‘Select Processing Options’ window (Figure 2) and available eluate transfer locations, refer to Section 7.B.

6.F. Example ‘Elution Layout Information’ Windows (for DNA Eluted into Tube Format Only)

The automated Identity Automation™ DNA IQ™ System method elutes DNA in up to 96 individual tubes (1.5ml or 2.0ml). Through the ‘Elution Layout Information’ window, you can set the position and name (identifier) for the elution tubes. Information for the elution tubes may be provided manually using a keyboard or hand-held scanner, by importing an ordered list of identifiers and locations, or using the Tecan PosID™ Positive Identification System.


 The ‘Elution Layout Information’ window is not displayed when a value of **None** is selected for Eluate Tracking in the ‘Select Processing Options’ window (Figure 2) nor is it displayed when DNA is eluted into 96-well plate format.

Features of the ‘Elution Layout Information’ Window

Pressing the **View List/View Map** toggle button switches the view between a pictorial map of the elution tubes in six 16-position tube racks (Map View) and a single column list of elution tubes (List View). 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). To navigate through the positions in either the List or Map View, use the mouse or **Tab** key. When manually entering or modifying identifier information, the **Tab** key signals the ‘Elution Layout Information’ window to accept the new or modified identifier information and update the List or Map View.


Color coding is used to indicate the status of the elution tube positions. The color coding depends on the selected values for the Eluate Tracking and SampleID-ElutionID Match options made in the ‘Select Processing Options’ window (Figure 2).


- **White:** Elution tubes positions that have unique identifiers based on the options selected.
- **Gray:** Empty positions, positions that contain elution tubes with unexpected identifiers, or positions that are not allowed based on the options selected.
- **Salmon:** Elution tube positions with replicate identifiers. When multiple elution tubes have the same identifier; the replicate identifier must be changed. When using the PosID™ System to scan elution tube bar codes, a tube with a scan error will display be displayed in salmon as “****”; you must manually provide the bar code.

 A salmon-colored position indicates that an elution tube identifier issue must be addressed before the method can continue.

For more information related to selections in the ‘Select Processing Options’ window (Figure 2) and permitted eluate transfer locations, refer to Section 7.B.

The “Eluate Information” box contains the Location of the elution tube (i.e., 1 to 96) and the “Eluate Identifier” input box. When the cursor is placed in the “Eluate Identifier” input box, you may 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 **Tab** on the keyboard to accept the change and update the window. To view the ‘Eluate Information’ box of a specific elution tube, select the location of the elution tube in the Map or View List.

 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 elution tube using the mouse, any changes to the identifier may be lost.

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

Pressing the **Select Mapping File** button allows you to select and open a file containing information that maps sample identifiers with their corresponding elution tube identifiers. The mapping file format will be configured based on laboratory specifications during on-site installation. For information about accepted file formats, refer to Section 10. The **Select Mapping File** button is only displayed when a value of **File Import** is selected for the SampleID-ElutionID Match option. See the ‘Select Processing Options’ window (Figure 2).

When either the **File Import** or **Automatic (Exact)** value is selected for the SampleID-ElutionID Match option in the ‘Select Processing Options’ window (Figure 2), an information box will be displayed that lists all of the elution tube identifiers that are required by the method to proceed.

Pressing the **Clear Mapping Information** button removes the information that maps sample identifiers with elution tube identifiers from the current ‘Elution Layout Information’ window. The **Clear Mapping Information** button is only displayed when a value of **File Import** is selected for the SampleID-ElutionID Match option in the ‘Select Processing Options’ window (Figure 2).

Pressing the **Select Import File** button allows you to select and open an ordered list containing identifiers and locations for elution tubes (tube rack and tube position information). The import file format will be configured based on laboratory specifications during on-site installation. For information about accepted file formats, refer to Section 10. The **Select Import File** button is only displayed when a value of **Import List** is selected for the Eluate Tracking option in the ‘Select Processing Options’ window (Figure 2).

Pressing the **Clear Eluate Information** button removes the identifier information for all elution tube position in the current ‘Elution Layout Information’ window.

Selecting the **Save Eluate Set** button sets the elution tube identifier and elution tube location and allows the method to proceed. Once the **Save Eluate Set** button has been pressed, you are unable to add or remove an elution tube from the eluate set nor can you change an identifier or location without aborting and restarting the run. The **Save Eluate Set** button is only displayed when unique identifiers have been supplied for the required number of tubes in permitted tube positions. When either the **File Import** or **Automatic (Exact)** value is selected for the SampleID-ElutionID Match option in the ‘Select Processing Options’ window (Figure 2), the **Save Eluate Set** button is only displayed when tubes for every expected elution tube identifier have been placed.

The Eluate Set Identifier contains an optional input field that allows you to enter additional information for the set of elution tubes.

In the List View, a “Hide Empty” checkbox allows you to hide any empty positions.

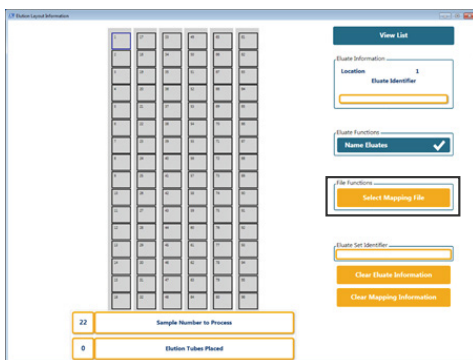
The **Rescan Eluate Tubes** button is only visible when a value of **PosID Scan** is selected for the Eluate Tracking option in the ‘Select Processing Options’ window (Figure 2). Pressing the **Rescan Eluate Tubes** button directs the instrument to rescan the elution tubes using the PosID™ Positive Identification System.

6.F. Example ‘Elution Layout Information’ Windows (for DNA Eluted into Tube Format Only; continued)

Example Elution Layout Windows

The ‘Elution Layout Information’ window displayed depends on the Eluate Tracking and SampleID-ElutionID Match options selected in the ‘Select Processing Options’ window (Figure 2). The example windows provided illustrate the features of the ‘Elution Layout Information’ window. For example purposes, the windows shown support runs in which the elution tube information (i.e., identifiers and location) is imported via an ordered list and the method is expecting you to provide a file that maps each elution tube identifier with a specific sample identifier provided at the start of the run.

A.



B.

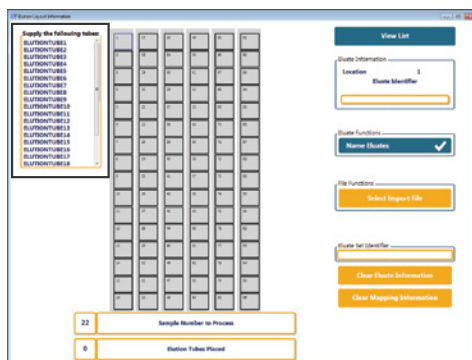


Figure 14. Example ‘Elution Layout Information’ windows. Panel A. The **Select Mapping File** button is visible when you select to provide sample identifier to elution tube identifier information via list import. Choose the **Select Mapping File** button to import a file containing the mapping information for sample identifiers to elution tube identifiers. **Panel B.** After the mapping file has been selected, a scrollable “Supply the following tubes:” information box containing the required elution tube identifiers is displayed (see content outlined in black). The **Select Import File** button is visible when you select to enter eluate tracking information via list import. Choose the **Select Import File** button to import a file containing eluate tube information.

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Figure 15. Modifying Information in the ‘Elution Layout Information’ windows and Save Eluate Set. **Panel A.** Map View. Select the **View List** button to display a list of elution tube identifiers. **Panel B.** List View. The salmon-colored tube positions and the “Supply the following tubes:” box (upper left corner) indicate that the imported list contained two positions with the same elution tube identifier and the list did not contain elution tube identifiers that map to each sample identifier, respectively. Both issues must be resolved before the method can proceed. Position 71 contains an elution tube identifier; however, its gray color indicates the identifier is not mapped to any sample identifier. The tube at position 71 will not be used. Checking the “Hide Empty” box collapses the empty elution tube positions (i.e., positions containing no identifier). **Panel C.** Modifying Imported Elution Tube Identifier Information. The salmon-colored entries at positions 68 and 70 (Panel B) indicate that duplicate identifiers are detected. Highlight position 68, select the **Eluate Identifier** input box, manually provide one of the unique and required identifiers and press the **Tab** key. The listing of identifiers in the “Supply the following tubes:” box (Panel B) indicates additional elution tubes are needed on the worktable. Highlight position 71, choose the “Eluate Identifier” input box, manually correct the incorrect elution tube identifier and use the **Tab** key. To remove all elution tube identifier information or the information that maps the sample identifiers to the elution tube identifiers, select the **Clear Eluate Information** button or the **Clear Mapping Information** button, respectively. When the required information for the elution tubes has been added and no replicate identifiers are detected, the **Save Eluate Set** button will be displayed. Select the **Save Eluate Set** button.

7. Description of the Identity Automation™ DNA IQ™ System Method

7.A. Overview of Liquid Handling Steps

This overview describes the general liquid-handling steps required for the Identity Automation™ DNA IQ™ System method on the Tecan Freedom EVO® workstation.

1. **EVOware® Software:** Within EVOware® set the positions of the first 1,000µl tip and first 200µl tip. Select and start the Identity Automation™ DNA IQ™ System method.
2. **Graphical User Interface:** Select the Sample Labware, Sample Type, Sample Volume, Resin Volume, Elution Volume and Eluate Labware as well as the Sample and Eluate Tracking options. Set up the worktable of the Tecan Freedom EVO® workstation.
3. **Sample Transfer (for samples in tubes only):** The liquid handler transfers samples prepared in 1.5ml or 2.0ml microcentrifuge tubes to an empty 2.2ml Square-Well Deep Well Plate (Sample Plate). If samples are supplied in 96-well format, the method skips this step.
4. **Lysis Buffer Addition:** As required, the liquid handler dispenses Prepared Lysis Buffer to each sample in the Sample Plate.
5. **DNA IQ™ Resin Solution Addition:** The liquid handler adds DNA IQ™ Resin Solution to each sample in the Sample Plate.
6. **DNA Binding:** The Sample Plate is subjected to a series of shaking and incubation steps (10-second shake, 30-second incubation; repeated eight times) to bind the DNA to the DNA IQ™ Resin.
7. **Volume Transfer:** The Sample Plate contents are transferred to the first Processing Plate located on the MagnaBot® 96 Magnetic Separation Device, which collects the resin at the sides of each well.
8. **Sample Lysate Removal:** The supernatant (sample lysate) is removed to the Sample Plate. The Sample Plate now serves as a Lysate Waste Plate.
9. **Lysis Buffer Wash:** The liquid handler adds Prepared Lysis Buffer to each sample well of the first Processing Plate. The plate then is moved to the shaker, and the resin is washed by shaking for 30 seconds.
10. **Lysis Buffer Wash Removal:** The first Processing Plate is moved back onto the MagnaBot® 96 Magnetic Separation Device, and the supernatant is removed to the Lysate Waste Plate.
11. **1X Wash Buffer Addition #1:** The liquid handler adds 1X Wash Buffer to each sample well of the first Processing Plate. The plate is placed on the shaker, and the resin is washed by shaking for 15 seconds.
12. **Clean Plate Transfer:** The liquid handler transfers the 1X Wash Buffer and DNA IQ™ Resin to a second Processing Plate on the MagnaBot® 96 Magnetic Separation Device.
13. **1X Wash Buffer Removal #1:** The supernatant (1X Wash Buffer) is removed from the second Processing Plate and transferred back to the first Processing Plate. The first Processing Plate now serves as a 1X Wash Buffer Waste Plate.
14. **Washes #2 and #3 with 1X Wash Buffer:** The 1X Wash Buffer addition and removal steps are repeated twice for a total of three washes.

15. **Heated Drying:** The second Processing Plate is moved onto the heater (85°C). The system pauses for 5 minutes to evaporate any remaining 1X Wash Buffer in the sample wells.
16. **Elution Buffer Addition:** The liquid handler adds the desired volume (e.g., 30–100µl) of Elution Buffer to each sample in the second Processing Plate. The second Processing Plate is placed on the shaker for 30 seconds to resuspend the resin, then moved to the heater for a 5-minute 85°C incubation to elute DNA from the DNA IQ™ Resin into the Elution Buffer.
17. **Elution:** After a final 30-second shake, the second Processing Plate is moved onto the MagnaBot® 96 Magnetic Separation Device, and the supernatant (Elution Buffer containing purified DNA) is removed to either the Elution Plate or the Elution Tubes.
18. **End of Method:** The Identity Automation™ DNA IQ™ System method is complete. The purified DNA in the Elution Plate or Elution Tubes may be processed immediately or stored at 4°C. The total time for the automated method is dependent on sample labware, sample type, sample volume, sample number and instrument configuration.

Note: The automated method may be configured during on-site method installation to generate one or more report files with run information including sample identifiers, elution tube identifiers (if applicable), user selected options collected at runtime through the graphical user interface, user information and instrument information. Report files can be generated to provide sample layout information compatible with import into the Identity Automation™ methods for DNA Quantitation (PowerQuant® System or Plexor® HY System) and DNA Normalization and PowerPlex® Setup. For more information about the report(s) that the automated DNA IQ™ System method can generate, refer to Section 11.

7.B. Sample Processing Location and Elution Tube Placement

Sample Processing Location

When samples are supplied in 96-well plate format, the well location in the Sample Plate is conserved as the samples are processed through the first and second Processing Plates (Section 7.A, Steps 7 and 12). For example, a sample supplied in well A1 of the Sample Plate will be processed into well A1 of the first Processing Plate and then into well A1 of the second Processing Plate.

When samples are supplied in tube format, the liquid handler transfers samples prepared in 1.5ml or 2.0ml microcentrifuge tubes to an empty 2.2ml Square-Well Deep Well Plate (Sample Plate; Section 7.A, Step 3). Samples in tube positions 1–8 are transferred to column 1 of the Sample Plate, samples in tube positions 9–16 are transferred to column 2 of the Sample Plate, samples in tube positions 17–24 are transferred to column 3 of the Sample Plate and so forth. The well location in the Sample Plate is conserved as the samples are processed through the first and second Processing Plates (Section 7.A, Steps 7 and 12).

Eluate Transfer Location

When DNA is eluted into 96-well format, the well location within the Processing Plates is conserved into the Elution Plate (Section 7.A, Step 17). When DNA is eluted into tube format, the final tube location into which the DNA is eluted depends on the values selected for the Eluate Tracking and SampleID-ElutionID Match options in the ‘Select Processing Options’ window (Figure 2).

7.B Sample Processing Location and Elution Tube Placement (continued)

When the SampleID-ElutionID Match option selected is **None**, eluates are transferred to the elution tubes without regards to either the sample identifier or the elution tube identifier (if provided). The ‘Elution Tubes’ window will prompt you to place the required number of elution tubes at specified positions on the worktable of the liquid handler. The liquid handler transfers the eluates based on their well location within the Processing Plates (i.e., in the same order as samples are placed on the worktable). Eluates in column 1 of the Processing Plates are transferred to tube positions 1–8, eluates in column 2 of the Processing Plates are transferred to tube positions 9–16, eluates in column 3 of the Processing Plate are transferred to tube positions 17–24, and so forth. When the SampleID-ElutionID Match option selected is **None** and Eluate Tracking option selected is **None**, the method will automatically assign eluate identifiers that are the same as the sample identifiers supplied at the start of the method.

When the Eluate Tracking value is set to **Manual Scan**, **Import List** or **PosID Scan**, the ‘Elution Layout Information’ window will prompt you to supply elution tube identifiers and location manually, via an import list or using the Tecan PosID™ Positive Identification System for the expected tube positions on the worktable. The liquid handler will transfer eluates to the elution tubes according to the Sample ID-Elution ID Match option selected.

- **Automatic (Exact):** Eluates are transferred to elution tubes with identifiers that exactly match the sample identifier.
- **File Import:** Import a list containing information that matches sample identifiers to specific elution tube identifiers. Eluates are transferred to elution tubes using the information in this mapping file.

8. Important Considerations

1. Always use aerosol-resistant tips to minimize the risk of cross-contamination.
2. Thoroughly resuspend the DNA IQ™ Resin by shaking vigorously before preparing the DNA IQ™ Resin Solution. Before combining the resin and Prepared Lysis Buffer, invert the resin bottle to ensure that no clumps of resin remain at the bottom of the bottle.
3. If using a manually controlled heater, turn on and set the heater to 85°C when prompted by the automated method.
4. The heater set and display temperatures may differ by ~1°C. This is not uncommon for the heater. A difference of ~1°C at the 85°C set temperature will not affect elution efficiency. During heated elution, samples should reach a temperature of approximately 65°C to achieve complete elution. If necessary, adjust temperature or calibration settings for the heater control unit to ensure that proper temperatures are reached. At the appropriate calibration and temperature settings, the surface of the heat block adaptor should be ~70–72°C.
5. The recovered elution volume in the Elution Plate or Elution Tubes at the end of the method will be less than the volume of Elution Buffer added due to heater evaporation and loss of Elution Buffer on the DNA IQ™ Resin. As much as 5–10µl may be lost when processing using 7µl of DNA IQ™ Resin; higher volumes may be lost when processing with greater than 7µl of DNA IQ™ Resin. If necessary, adjust the amount of Elution Buffer added to recover the desired volume. The method can elute in 30–100µl.
6. Aspirating and dispensing speeds, as well as pipetting heights, are critical to the success of this method.
7. When defining labware, be certain to set Z-max heights carefully at the well bottom of each labware present on the worktable.
8. Sample identifier and eluate 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).
9. The Clean Plate Transfer step of the method (Section 7.A, Step 12) is critical. Without this step, Lysis Buffer may contaminate the eluates.

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® Setup methods on a Tecan Freedom EVO® workstation. A Freedom EVO® 100 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, 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, 100ml, 3-Position ³	1	10650037
DiTi Carrier, 3-Position ⁴	1–3	10613022
Shelf, 16-Position (4 sites with 4 shelves per site)	1	10650627
Microplate Carrier, 3-Position, Landscape (MP 3Pos) or alternative carrier(s) to provide up to 9 microplate positions ⁵	1–2	10612604 or 10612624
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 2 additional Microplate Positions	1	30015506
EchoTherm Dry Bath, Torrey Pines Scientific, IC20, Electronic Chilling/Heating (or equivalent; contact Tecan for additional options on heating units, adaptors and mounting hardware) ⁸	1	30034627
Microplate Adaptor, Torrey Pines Scientific, IC20/IC22/IC25 ⁸	1	30034628

Part Description	Minimum Quantity	Tecan Part#
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 can be used.

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 (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. See Section 5.B for information on method requirements for LiHa Disposable Tips (200µl and 1,000µl). 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 running of 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. 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. Install enough labware carriers to support the maximum number of plates per automated method run. For more information, refer to *Identity Automation™ qPCR Setup Protocol for the PowerQuant® and Plexor® HY Systems on the Tecan Freedom EVO® Workstation #EP047* or *Identity Automation™ DNA Normalization and PowerPlex® Setup Protocol for the Tecan Freedom EVO® Workstation #EP042*.

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 1.3ml–5.0ml. Alternatively, the following reagents 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; contact Promega for additional information. 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 (6 carriers for up to 96 Unknowns and 12 for up to 192; refer to the *Identity Automation™ qPCR Setup Protocol for the PowerQuant® and Plexor® HY Systems on the Tecan Freedom EVO® Workstation #EP047*). 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 setup per automated method run (6 carriers for setup of one amplification plate and 12 for two; refer to *Identity Automation™ DNA Normalization and PowerPlex® Setup Protocol for the Tecan Freedom EVO® Workstation #EP042*).

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

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

Hardware, Labware, and Consumables Required

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

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		1–2 (optional) ³	1–2 (optional) ³

¹Required only for preprocessing 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 can 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 setup 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 setup amplifications from Controls and present manually prepared Standards in tube format (1.5ml or 2ml). Refer to the *Identity Automation™ qPCR Setup Protocol for the PowerQuant® and Plexor® HY Systems on the Tecan Freedom EVO® Workstation #EP047* for more information.

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 the 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 96-well format (e.g., 96-well PCR Plate or strip tubes) and for the amplification plates.

Consumables Required for Full Workflow Automation: 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 rack	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	3055743	Disposable Trough, 25ml, Low Dead Volume (Optional: To prepare PCR or qPCR amplification mix at volumes ≤20ml)		1 ²	1 per run ²
VWR	47743-968	Axygen® Multiple Well Reagent Reservoir with 12-Channel Trough, High Profile, Nonsterile (Corning 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		

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)
Promega	V6821	1.1ml, Square-Well, V-Bottom Deep Well Plate			2
User-selected		96-well PCR plate(s) or 8-well strip tubes for purified DNA samples	1 plate ⁵	1–3 plates ⁵	1 ⁵
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	

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)		2 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.

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

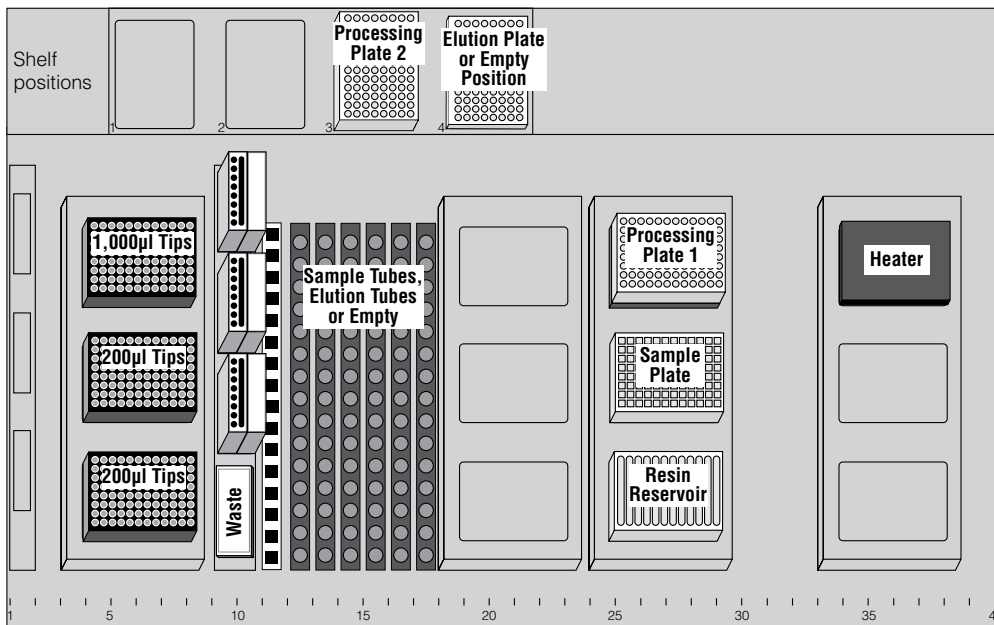


Figure 16. Tecan Freedom EVO® initial worktable configuration. This worktable supports the full suite of Promega automated methods for the Tecan Freedom EVO® workstation, including the DNA IQ™ System, DNA Quantitation (PowerQuant® System or Plexor® HY System) and DNA Normalization and PowerPlex® Setup. The labware shown accommodates samples supplied in tube or 96-well format as well as DNA elution into tube or 96-well format and is an example only; the automated method can be adapted to any worktable layout provided these hardware or equivalent are present. Sample input labware (tube or 96-well format) and elution labware (tube or 96-well format) are selected on a per-run basis; all samples within a run are supplied in a single format and all DNA are eluted in a single format.

Grid 1 Trough Carrier, 100ml, 3-Position (used with DNA Quantitation (PowerQuant® System or Plexor® HY System) and DNA Normalization and PowerPlex® Setup)

Grid 3 DiTi Carrier, 3-Position

Position 1 (rear) 1,000µl LiHa Disposable Tips with Filter

Position 2 200µl LiHa Disposable Tips with Filter

Position 3 200µl LiHa Disposable Tips with Filter

Grid 5 Shelf, 16-Position (back of worktable)

Position 1 and 2 (upper left) Empty

Position 3 (upper second position from right) Empty 1.2ml, Round-Bottom Deep Well Plate (Processing Plate 2)

Position 4 (upper right) Empty 96-well PCR Plate or strip tubes on a plate stand (Elution Plate; only if DNA is eluted in 96-well format)

Position 4 (upper right) Empty (if DNA is eluted in tube format)

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

Position 1 to 3 (left) Wash and Waste

Position 4 (right, rear) Trough, Disposable, 100ml (for Elution Buffer)

Position 5 Trough, Disposable, 100ml (for 1X Wash Buffer)

Position 6 (right, front) Trough, Disposable, 100ml (for Prepared Lysis Buffer)

Position 7 Tip Waste Chute

Grid 11 Tube Carrier, 16mm, 16-Position [Optional; used with DNA Quantitation (PowerQuant® System or Plexor® HY System) and DNA Normalization and PowerPlex® Setup]**Grid 12–17 Tube Carriers, 1.5ml or 2.0ml Microcentrifuge with Hinged Cap, 16-Position** (if samples are supplied in tube format or DNA is eluted into tube format)

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

Positions 1–16 Empty 1.5ml or 2.0ml tubes (if samples are supplied in 96-well format and DNA is eluted into tubes)^{1,2}

¹Turn all open caps so that the caps do not interfere with pipetting steps.

²The Identity Automation™ DNA IQ™ System method can be installed to allow the samples to be supplied in tube format and the elution of DNA into tube format using only these 6 Tube Carriers positions.

Grid 18 Microplate Carrier, 3-Position (used with DNA Quantitation (PowerQuant® System or Plexor® HY System) and DNA Normalization and PowerPlex® Setup)**Grid 24 Mounting Plate, Te-Shake™ 1-Position with two additional Microplate Positions and Te-Shake™ Microplate Shaker Unit**

Position 1 (rear) Empty 1.2ml, Round-Bottom Deep Well Plate (Processing Plate 1) on a MagnaBot® 96 Magnetic Separation Device, 1/4 inch Foam Spacer

Position 2 (middle) 2.2ml, Square-Well Deep Well Plate or 96 Deep Well Plate from the Slicprep™ 96 Device containing samples (Sample Plate). Place samples in columns 1–12, depending on the number of samples to be processed.

Position 2 (middle) Empty 2.2ml, Square-Well Deep Well Plate (Samples in tube format are transferred to the Sample Plate at the start of the method.)

Position 3 (front) Reservoir with 12-Channel Trough (DNA IQ™ Resin Solution prepared in Section 4.B; column 1)

Grid 33 Torrey Pines Heater, Promega Deep-Well Heat Transfer Block

10. Import File Format: Minimum File Requirements

The Identity Automation™ DNA IQ™ System method on the Tecan Freedom EVO® automated liquid-handling workstation allows for the import of files that containing ordered lists of sample identifiers and sample location, eluate identifiers and eluate location (elution into tube format only) and sample identifier-eluate identifier mapping 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); the format will be configured based on laboratory specifications during the on-site installation.

Sample Information 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

Elution Tube Information Import File: Minimum File Requirements

Elution Tube Identifier	Elution Tube Location
eluateID, eluate name or elution tube bar code	tube rack name and tube position in tube rack

Sample Identifier to Eluate Identifier Mapping File: Minimum File Requirements

Sample Identifier	Elution Tube Identifier
sampleID, sample name or sample tube bar code	eluateID, eluate name or elution tube bar code

The file must contain elution tube information for every sample identifier.

Figure 17 shows warning messages that may occur if the Mapping File selected is not in the appropriate file format or does not contain mapping information for all samples placed on the worktable.

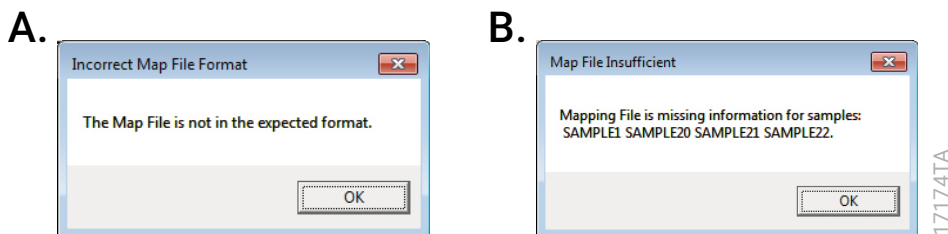


Figure 17. Warning Messages for Import of the Sample Identifier to Eluate Identifier Matching File.

Panel A. Warning message that occurs when the file selected is not in the appropriate file format. **Panel B.** Warning message that occurs when the file selected does not contain mapping information for all samples placed on the worktable. If either type of message occurs, create a new Mapping File using the appropriate format with information that maps every sample to an elution tube identifier.

11. Method Generated Report Files

The Identity Automation™ DNA IQ™ System method on the Tecan Freedom EVO® automated liquid-handling workstation can be configured to generate one or more customizable report files. The file format and information included in each report is configured based on laboratory specifications during the on-site installation. The default report file location is **C:\ProgramData\Tecan\EVOware\database\PromegaReports\DNAIQ_ReportFiles**

Report Title	DNA IQ Setup						
RunDate	201909240906						
User	jsmith						
Instrument Serial Number	123456						
Script	Promega_DNAIQ_V3_0_0						
Run Options							
Sample Batch Identifier	SAMPLE_SET1						
Sample Type	AqueousSamples						
Sample Number	22						
Sample Volume	200						
Sample Labware	Tubes						
SampleID Input Type	Import						
Elution Labware	Tubes						
Elution Volume	50						
ElutionID Input Type	Import						
SampleID - ElutionID Match Option	True						
SampleID - ElutionID Match Style	File						
Resin Volume	7						
Reagent Lots/Barcodes							
Elution Buffer	1234560000						
1X WashBuffer	0000123456						
Lysis Buffer with DTT (trough)	abcdcf						
Lysis Buffer with DTT (resin)	0000234567						
Resin	0000345678						
Run Parameters							
Add Lysis Buffer	True						
Volume of Added Lysis Buffer	350						
Volume of Elution Buffer	50						
Sample Number	Sample Rack	Sample Tube	SampleID	Eluate Number	Eluate Rack	Eluate Tube	ElutionID
1	1	1	SAMPLE1	1	1	1	ELUTIONTUBE1
2	1	2	SAMPLE2	82	6	2	ELUTIONTUBE2
3	1	3	SAMPLE3	83	6	3	ELUTIONTUBE3
4	1	4	SAMPLE4	84	6	4	ELUTIONTUBE4
5	1	5	SAMPLE5	85	6	5	ELUTIONTUBE5
6	1	6	SAMPLE6	86	6	6	ELUTIONTUBE6
7	1	7	SAMPLE7	87	6	7	ELUTIONTUBE7
8	1	8	SAMPLE8	88	6	8	ELUTIONTUBE8
9	1	9	SAMPLE9	89	6	9	ELUTIONTUBE9
10	1	10	SAMPLE10	90	6	10	ELUTIONTUBE10
11	1	11	SAMPLE11	91	6	11	ELUTIONTUBE11
12	1	12	SAMPLE12	92	6	12	ELUTIONTUBE12
13	1	13	SAMPLE13	93	6	13	ELUTIONTUBE13
14	1	14	SAMPLE14	94	6	14	ELUTIONTUBE14
15	1	15	SAMPLE15	95	6	15	ELUTIONTUBE15
16	1	16	SAMPLE16	96	6	16	ELUTIONTUBE16
17	2	1	SAMPLE17	65	5	1	ELUTIONTUBE17
18	2	2	SAMPLE18	66	5	2	ELUTIONTUBE18
19	2	3	SAMPLE19	67	5	3	ELUTIONTUBE19
20	2	4	SAMPLE20	68	5	4	ELUTIONTUBE20
21	2	5	SAMPLE21	71	5	7	ELUTIONTUBE21
22	2	6	SAMPLE22	70	5	6	ELUTIONTUBE22

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Figure 18. Example Report File. The example text file shown is a time-stamped record of run parameters.

11. Method Generated Report Files (continued)

A.

```
S1,1,ELUTIONTUBE1
S5,1,ELUTIONTUBE17
S5,2,ELUTIONTUBE18
S5,3,ELUTIONTUBE19
S5,4,ELUTIONTUBE20
S5,6,ELUTIONTUBE22
S5,7,ELUTIONTUBE21
S6,2,ELUTIONTUBE2
S6,3,ELUTIONTUBE3
S6,4,ELUTIONTUBE4
S6,5,ELUTIONTUBE5
S6,6,ELUTIONTUBE6
S6,7,ELUTIONTUBE7
S6,8,ELUTIONTUBE8
S6,9,ELUTIONTUBE9
S6,10,ELUTIONTUBE10
S6,11,ELUTIONTUBE11
S6,12,ELUTIONTUBE12
S6,13,ELUTIONTUBE13
S6,14,ELUTIONTUBE14
S6,15,ELUTIONTUBE15
S6,16,ELUTIONTUBE16
```

B.

```
WellNum;SampleID
A1;SAMPLE1
B1;SAMPLE2
C1;SAMPLE3
D1;SAMPLE4
E1;SAMPLE5
F1;SAMPLE6
G1;SAMPLE7
H1;SAMPLE8
A2;SAMPLE9
B2;SAMPLE10
C2;SAMPLE11
D2;SAMPLE12
E2;SAMPLE13
F2;SAMPLE14
G2;SAMPLE15
H2;SAMPLE16
A3;SAMPLE17
B3;SAMPLE18
C3;SAMPLE19
D3;SAMPLE20
E3;SAMPLE21
F3;SAMPLE22
```

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Figure 19. Example Report File. Sample Layout File can be imported into Identity Automation™ methods for DNA Quantitation (PowerQuant® System or Plexor® HY System) and DNA Normalization and PowerPlex® Setup. The example report files shown are compatible with import into the graphical user interfaces for the Identity Automation™ qPCR setup method for the PowerQuant® System and Plexor® HY System and the Identity Automation™ DNA Normalization and PowerPlex® Setup method. **Panel A.** The comma delimited list of tube rack (S1, S2...S6), tube position (1–16) and elution tube information (ELUTIONTUBE1, ELUTIONTUBE2, etc.) corresponding to the example shown in Figures 5 and 14 for DNA eluted into tubes. This list contains no header line. **Panel B.** A semicolon delimited list of well location (A1, B1, etc.) and sample identifier information (SAMPLE1, SAMPLE2, etc.) for DNA eluted into a 96-well plate. This list contains a header line.

12. Summary of Changes

The following changes were made to the 12/20 revision of this document:

1. Updated method to include processing samples supplied in 1.5ml or 2.0ml tubes.
2. Updated to include eluting DNA into 1.5ml or 2.0ml tubes.
3. Added new Sections 6, 10 and 11.
4. Placed in new template.

^(a) U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, European Pat. No. 1 204 741 and Japanese Pat. No. 4425513.

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