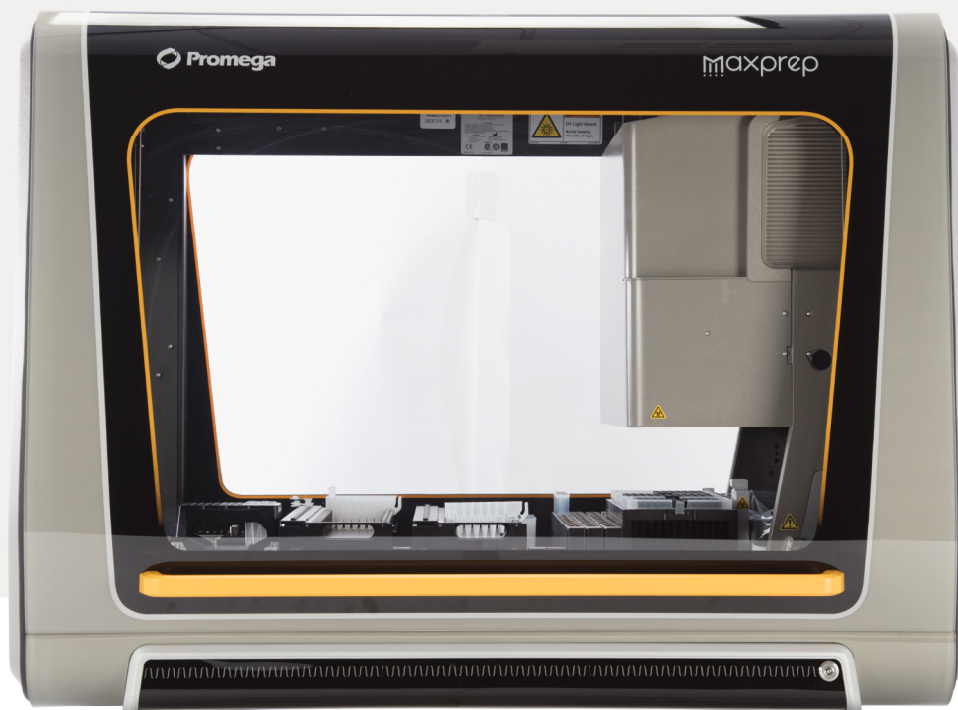# Promega DNA Normalization and STR Setup Method for the Maxprep® Liquid Handler Technical Manual

Instructions for Use of AS9100, AS9101, AS9105, AS9200, AS9201 and AS9205

Table of Contents

All technical literature is available at: **www.promega.com/protocols/**

Visit the website to verify that you are using the most current version of this Technical Manual.

Email Promega Technical Services if you have questions on use of this system: **genetic@promega.com**

1 Description

The Maxprep® Liquid Handler automates normalization and setup of Promega short tandem repeat (STR) amplification reactions including master mix preparation, normalization of samples and placement of controls. Up to a 22,500-fold dilution can be achieved during the normalization of samples. Both full and half volume STR reactions can be prepared by the system based on the STR kit files provided by Promega. Extracted DNA and samples prepared with the Casework Direct Kit are capable of being processed with this method. The Promega DNA Normalization and STR Setup protocol allows the flexibility for administrators to create variant methods in the Maxprep® software that specify normalization and amplification reaction setup options to meet the needs of the laboratory. When starting a method, you can indicate the input and output labware for the method, the Promega STR kit to use, and whether sample concentration data will be imported from the Portal software or a tab-delimited text file.

2   Before You Begin

2.1   Maxprep® Liquid Handler Promega DNA Normalization and STR Setup Method

As new Maxprep® Liquid Handler methods are released, they will be listed on the Promega website and available for download at: **www.promega.com/resources/software-firmware/maxprep-liquid-handler-methods/**

This document presents guidelines for interaction with the Promega DNA Normalization and STR Setup method on the Maxprep® Liquid Handler. Normalization parameters, reaction setup parameters, placement of controls, and behavioral preferences for normalization and Promega STR reaction setup are specified in variant methods and cannot be altered at run time. After selecting a variant method to run, you will enter processing parameters (e.g., Input Labware, Output Labware, Promega STR kit and concentration data location) and be led through a series of user interface screens that detail placement of labware and reagents on the Maxprep® Liquid Handler. The Maxprep® Liquid Handler is provided with a PC and touch-screen monitor. Interactions with the user interface screen can occur using the mouse and keyboard, touch gestures on the monitor or a combination of both approaches.

The Promega DNA Normalization and STR Setup method supports the use of Maxwell® Deck Trays from the following instruments:

Instrument	Cat.#	Technical Manual
Maxwell® FSC Instrument	AS4600	TM462
Maxwell® RSC Instrument	AS4500	TM411
Maxwell® RSC 48 Instrument	AS8500	TM510

Within any individual run you can run either Maxwell® RSC/FSC Deck Trays or Maxwell® RSC 48 Deck Trays as input labware. In this document and in the method user interface, Maxwell® Deck Trays are referred to generically as RSC Deck Trays.

2.2   Materials to be Supplied by the User

- amplification plate (user-specified; e.g., Thermo Fisher MicroAmp™ Optical 96-Well Reaction Plate, Cat.# N8010560)

- 1.5–2.0ml tubes for amplification reaction preparation (e.g., ClickFit Microtube, 1.5ml; Cat.# V4741 and Cat.# V4745)

- 1.1ml, Square-Well V-Bottom Deep Well Plate (Cat.# V6821; one or two as prompted)

- Maxprep® 50µl Conductive Disposable Tips, Filtered (Cat.# AS9301)

- Maxprep® 300µl Conductive Disposable Tips, Filtered (Cat.# AS9302)

- Maxprep® Reagent Reservoir, 50ml (Cat.# AS9304)

- Maxprep® 3-Position Reagent Tube Holder (Cat.# AS9409; up to three additionally required, based on STR kit selected and controls)

- **Optional**: amplification plate base (e.g., Thermo Fisher MicroAmp® 96-well base, Cat.# N8010531 for strip tubes, unskirted or partially skirted amplification plates)

2.3   Selecting a Maxprep® Liquid Handler Promega DNA Normalization and STR Setup Method

To start a method on the Maxprep® Liquid Handler, perform the following steps:

1. Start the Maxprep® software by double-clicking the Maxprep® software icon on the Windows® desktop. Alternatively, select the **Windows Start** button in the lower left corner of the screen, choose **All Programs,** open the Promega folder and select the **Maxprep** software icon.

2. From the Maxprep® software 'Home' screen (Figure 1), press the **Start** button.
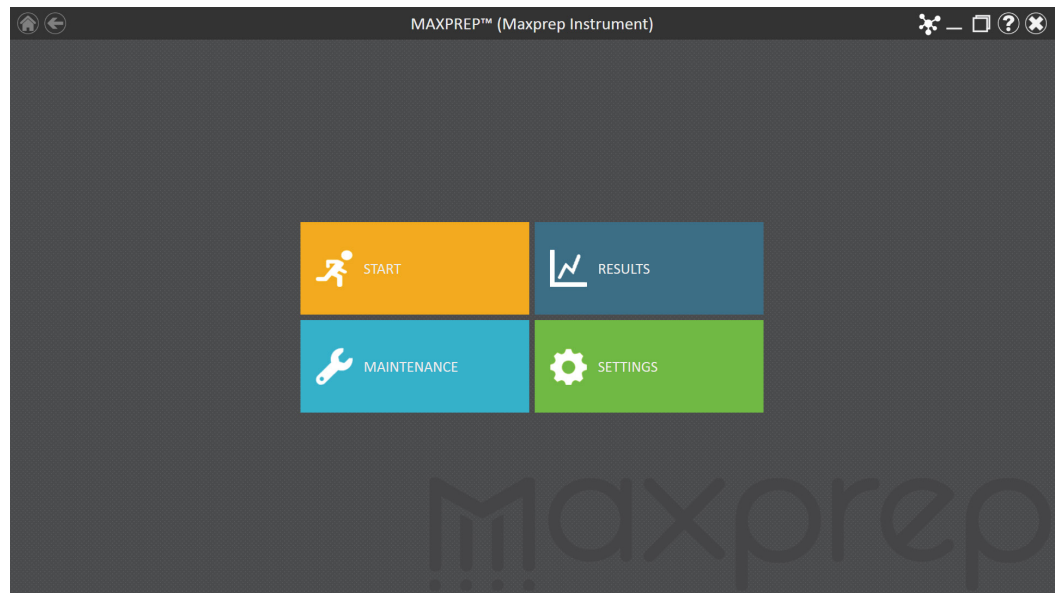
Figure 1. Maxprep® software 'Home' screen. From the Maxprep® software 'Home' screen, select the **Start** button to begin the process of running a method.

3. The 'Methods' screen (Figure 2) displays all of the methods that are installed in the Maxprep® software. Methods are shown either as single line entries or with a folder icon if the administrator has defined lab-specific variant versions of the Promega-supplied methods. Select the folder icon for the Promega DNA Normalization and STR Setup method to expand the list of variant methods that were created.
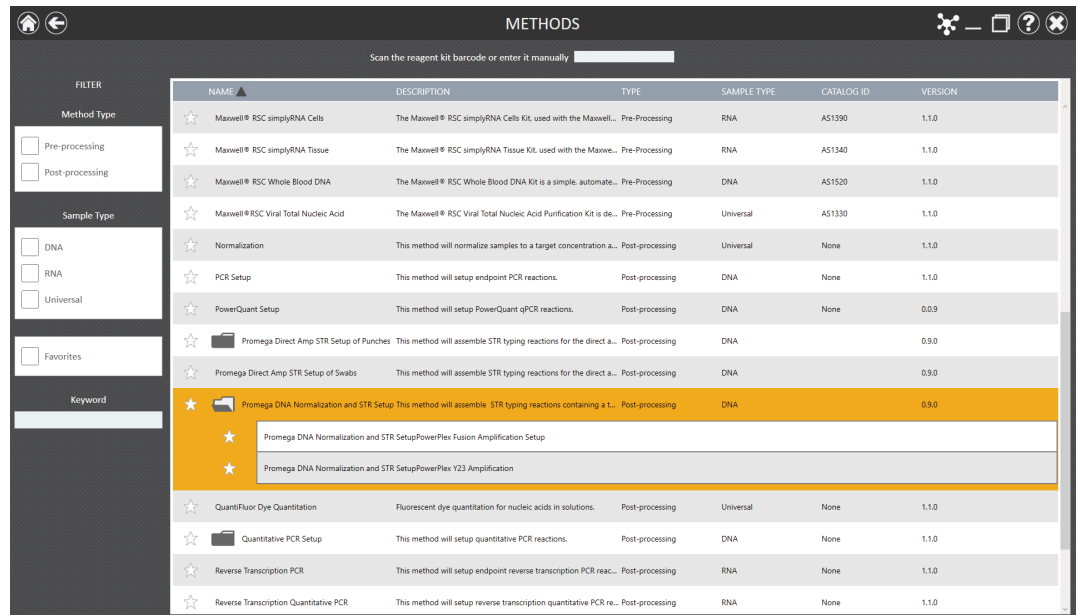
Figure 2. 'Methods' screen. The 'Methods' screen displays the methods installed in the Maxprep® software. Select the method to run, and then press the **Proceed** button.

4. Use one of the following two mechanisms to select the Promega DNA and STR Setup method:

   a. Select the method or variant from the list of installed methods.

   b. Use the Filter check boxes on the left side of the screen to display only post-processing methods, display only methods marked as Favorites or perform a keyword search for a specific word or phrase in any of the descriptive fields for the method. After filtering the list, select the method or variant from the list of displayed methods.

   Regardless of the mechanism used to select a method, the selected method is highlighted and will display a **Proceed** button on the right side of the entry. Press the **Proceed** button to open the Method Run Environment (Figure 3).
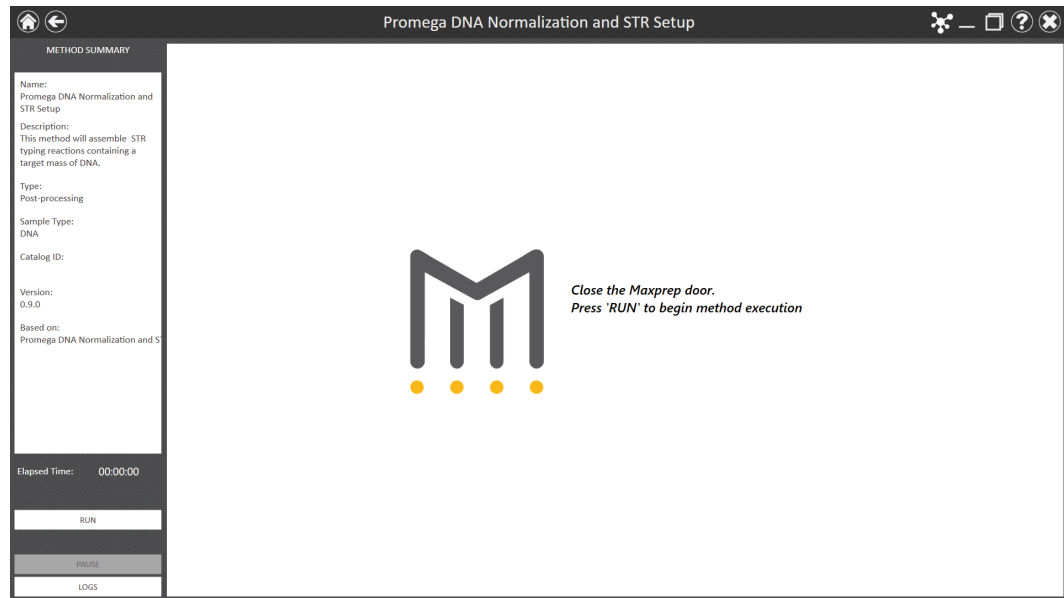
Figure 3. Method Run Environment screen. The Method Run Environment screen displays a summary of the selected method on the left side of the screen. Close the door of the instrument and select the **Run** button to begin method execution.

2.4　Starting a Maxprep® Liquid Handler Method

The Method Run Environment displays the name of the selected method in the title bar and a summary of the information describing the selected method on the left side of the screen. If an incorrect method was chosen, select the **Home** or **Back** button at the top left corner of the screen to return to the 'Home' screen or return to the 'Methods' screen, respectively.

To start method execution, close the door of the instrument and select the **Run** button in the lower left corner of the screen. Once method execution is started, the timer under the *Elapsed Time:* header will start, and the main portion of the screen will indicate that the system is initializing.

2.5   Entering Run-Specific Information

After the system has initialized, a user interface is displayed in the main portion of the Method Run Environment. The user interface requests information necessary to process this method.

Initially you will be prompted to provide information about the Promega STR kit being used for this run (Figure 4). Next, you will be requested to provide information regarding Input Labware, Destination Labware, and whether sample concentration data should be retrieved from the Portal software or imported as a tab-delimited text file (Figure 5).

Note: When creating variant Promega DNA Normalization and STR Setup methods, the administrator defines the parameters specific to the method being performed. These parameters cannot be modified at run time. See Section 4 for information on creating Promega DNA Normalization and STR Setup variant methods.

Figure 4. Providing Promega STR kit information. An initial pop-up screen requests information regarding the lot number for the Promega STR kit being used for this run and a drop-down menu for selection of the specific kit to be used.

Figure 5. General Run Parameters screen. The General Run Parameters screen requests information about input labware, destination labware, Promega STR kit and import of concentration data. Select the **Variant Information** button to view the administrator-defined settings for this method.

1. On the initial pop-up screen, if required by the administrator, enter or scan the kit lot for the Promega STR kit being used for this run to activate the drop-down menu for kit selection. The kit lot information is used to filter the list of available STR kits for this run. Use the drop-down menu to select Promega STR kit settings to use.

   Notes:

   a. When typing in a lot number you must press the enter key after the lot number to accept the entry and activate the drop-down menu for kit selection.

   b. Promega STR kit lot numbers have the structure DC242412345678902020-12-12 where the first 6 characters specify the product catalog number (DC2424), the next 10 characters specify the lot number (1234567890), and the final 10 characters specify the expiration date (2020-12-12; YYYY-MM-DD). Only information entered in this format will be accepted.

   c. If the drop-down menu for kit selection contains no entries based on the kit lot number, it indicates that no STR kit file has been imported into the Maxprep® software for this kit. Contact Promega Techcnical Services (genetic@promega.com) for a kit file specific to the Promega STR kit being used.

2. Use the drop-down menus to specify the Input Labware and Destination Labware to be used for this run. Samples are placed on the system in the Input Labware, and the final Promega STR reactions are assembled in the Destination Labware. Maxwell® Deck Trays (eluates), plates and tubes can all be used as input labware, while only amplification plates can be specified as destination labware options.

3. The 'Import Portal Concentration Data' check box determines how sample concentration data will be imported for this method. If the box is checked, sample concentration data previously imported into the Portal software will be used for all samples identified in this run. If the box is unchecked, you will be prompted to import a tab-delimited text file containing concentration data for the samples to be processed.

4. If you wish to change the STR kit selected for this run, press the Select **STR Kit button.**

5. At the top right corner of the screen is an indication of the Maximum Number of Samples allowable within this method run. The number displayed is based on:

   • The number of sample positions in the input labware

   • The number of controls and ladder wells specified

   • The number of sample replicates

   • The available space in the destination labware

6. To view the administrator-defined settings for this variant, press the **Variant Information** button (Figure 6).

7. After entering values for all items requested on this screen, select the **Next** button.

Run parameters are defined in method variants. Contact your system administrator to edit values.

Description	Value	Min	Max
1.Control 1 Name	None		
2.Control 1 Concentration	0		
3.Control 1 Well Numbers			
4.Control 2 Name	None		
5.Control 2 Concentration	0		
6.Control 2 Well Numbers			
7.Control 3 Name	None		
8.Control 3 Concentration	0		
9.Control 3 Well Numbers			
10.Empty Ladder Wells			
11.Target control mass in reaction	0.5		
1.Quantitation target for normalization	Auto		
2.Target sample mass in reaction	0.5		
3.Min sample mass in reaction	0.2		
4.Max sample mass in reaction	1		
5.Import concentration data from Portal?	True		
1.Allow user to override normalization rules?	False		
2.Allow user to edit quant target for norm?	False		
3.Allow user to edit the target mass?	True		

EXIT

Figure 6. Viewing Promega DNA Normalization and STR Setup information. Press the **Variant Information** button on the interface to view the administrator-defined details for this variant method.

2.5.1 Input Labware Sample Information

After selecting **Next,** the user interface requests information regarding the samples present in the input labware. The nature of the screen will depend on the type of input labware chosen and whether Portal is active.

Maxwell® Deck Trays and Plates as Input Labware

Note: When not connected to the Portal software, the screens shown in Figure 7 are not displayed.
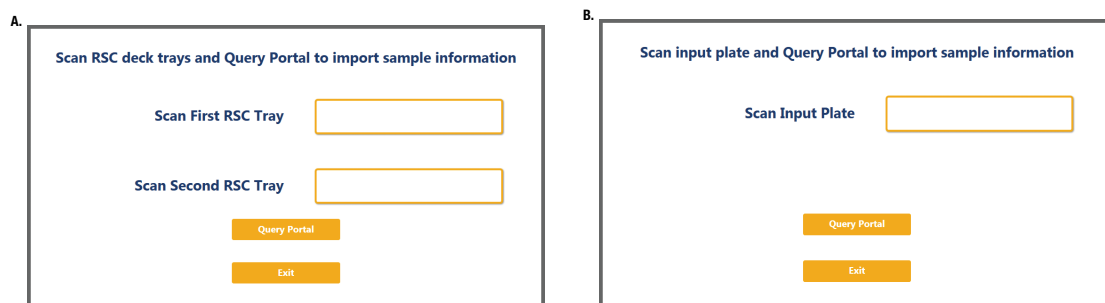
The screens in Figure 7 are only displayed when the Maxprep® software is connected to the Portal software. A bar code is requested for each Maxwell® deck tray or plate to be processed. Select the text box and scan or enter the Maxwell® deck tray or plate bar code(s) for the selected input labware. After entering bar codes for the labware, press the **Query Portal** button to retrieve sample identifiers from the Portal software for the most recent activity associated with the labware. The number of sample identifiers retrieved from the Portal software is displayed on the screen. Press the **Exit** button to display any records retrieved from the Portal software query or to proceed without querying the Portal software and manually enter sample identifiers.

Note: Maxwell® deck trays are generically referred to as RSC deck trays.

A.
Scan RSC deck trays and Query Portal to import sample information

Scan First RSC Tray

Scan Second RSC Tray

Query Portal

Exit

B.
Scan input plate and Query Portal to import sample information

Scan Input Plate

Query Portal

Exit

Figure 7. Querying Portal for input labware bar codes. These screens are displayed only when the software is connected to the Portal software. Press the **Query Portal** button to search the Portal software for sample identifiers associated with the deck tray or plate bar code(s). Press **Exit** to display the sample identifiers retrieved from Portal or to proceed without querying Portal. **Panel A.** Scan or enter bar code information for Maxwell® Deck Tray(s) to be processed. **Panel B.** Scan or enter bar code information for the plate to be processed.

After pressing the **Exit** button, or when not connected to the Portal software, the user interface will display a representation of the labware. If applicable, any sample identifiers returned from the Portal software query are populated on the labware display and shown as black cartridge or well positions. Labware positions with no sample identifiers are displayed as gray cartridge or well positions. To enter or modify sample identifiers, select the desired labware position, then select the text box on the right side of the screen (Figure 8). Scan or enter the sample identifier information for the cartridge position. When entering sample identifiers, press the **Add Sample** button to accept the entry or press **Enter** on the keyboard to accept the information and move to the next position.

If more samples are present on the input labware than can be processed with this variant, a message will be displayed at the top of the interface screen indicating the number of excess samples that will have to be removed by the user before proceeding. To remove samples from processing, select a position, Ctrl-click to select multiple positions or click and drag across several consecutive positions to select them. After selecting positions, press the **Remove Sample** button to remove them from being processed during this run.

Note: When not connected to the Portal software, the **Requery Portal** button is not displayed.

If sample identifiers are removed or edited incorrectly, it is possible to reset the labware to the original information retrieved from Portal. Press the **Requery Portal** button to repeat the process of retrieving sample identifiers from the Portal software for a piece of labware based on the labware bar code.

Once satisfied with the labware positions and sample identifiers to be processed, press the **Next** button to proceed to the next user interface screen.

A.
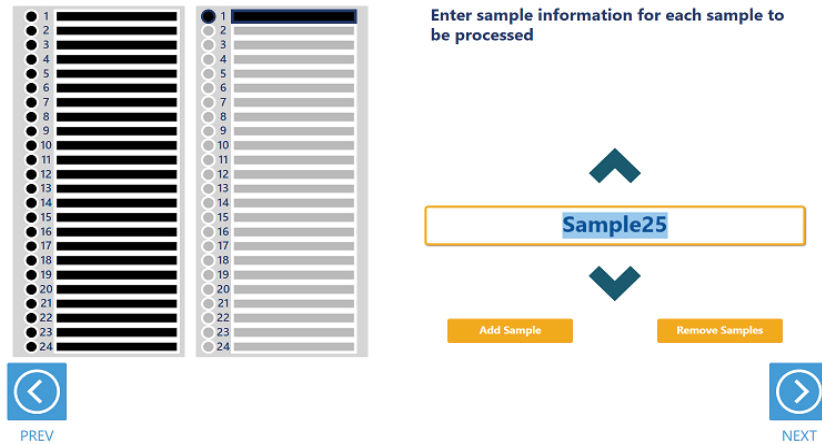
B.
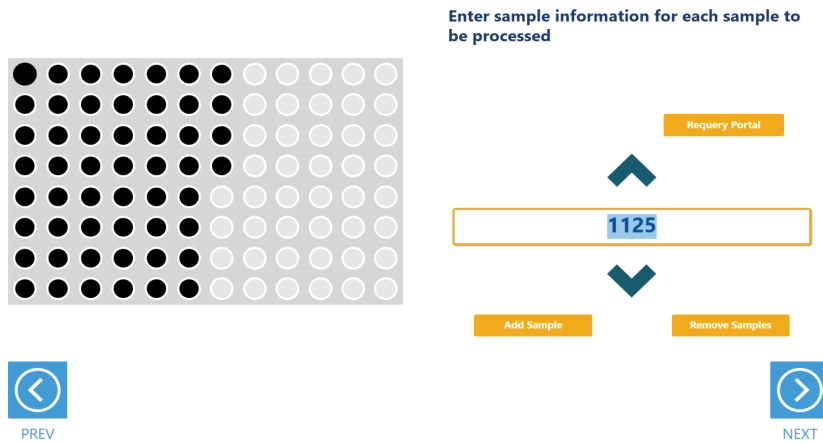
Figure 8. Entering sample identifiers. Input labware is displayed on the interface and allows entry of sample identifier information. Any labware positions that have sample identifier information (either retrieved from Portal software or manually entered) are displayed in black. To enter sample identifiers, select the labware position and then select the text box on the right side of the screen. Scan or enter sample identifiers and press the **Add Sample** button to accept the sample identifier or press **Enter** on the keyboard to accept the sample identifier and move to the next position. **Panel A.** Interface screen for Maxwell® Deck Trays as input labware. **Panel B.** Interface screen for a plate as input labware.

Tubes as Input Labware

When selecting tubes as an input labware type, the interface will prompt you to scan bar codes on three tube racks at Tracks 22–24 (Figure 9). Scanning tube racks will determine the type of tube rack being used, the types of tubes defined for that rack type and the sample identifiers for each tube.
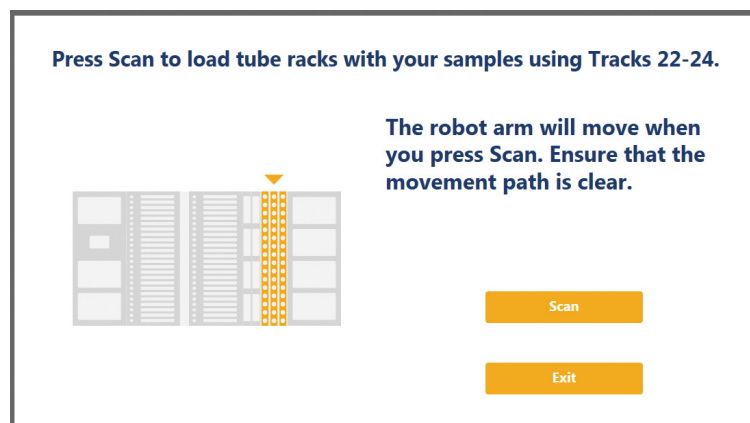
Figure 9. Scanning tube racks. Tube racks at Tracks 22–24 must be scanned when tubes are selected as an input labware type. Scanning tube racks will determine the type of tube rack being used, the types of tubes defined for the rack type and the sample identifiers for each tube.

To scan sample tube racks:

1. Remove tube racks from the system. Load tubes into the tube racks with bar codes visible in the windows on the right side of the carrier.

2. Load the tube racks on the instrument to identify the type of tube rack being used and scan bar codes on the individual tubes.

    a. Press the **Scan** button.

    b. The instrument arm will slowly move to the position for scanning the first primary sample tube rack.

    c. With the tube rack removed from the system, the green loading light where the tube rack should be loaded will flash.

    d. Place the back edge of the tube rack on the loading platform so that the rearmost bar code on the tube rack (rack identifying bar code) is just in front of the bar code scanner.

    e. Slide the tube rack into the system until it fully contacts the back of the instrument. During this time the bar code scanner will flash each time it reads a tube position.

    Note: Do not remove or insert tube racks while the instrument arm is moving. After scanning a tube rack, wait until the instrument arm has moved to the next position before inserting a tube rack.

    f. When the tube rack is fully seated in the instrument, the loading light at the front of the instrument will stop flashing. Approximately 5 seconds after the tube rack is loaded into the instrument, the instrument arm will slowly move to the right for loading of the next position.

g. Repeat steps c–f for all three tube racks. If sample tubes are not present in all three carriers, press **Stop** when the instrument arm moves after scanning the last carrier containing tubes.

3. Once all tube racks are scanned, the interface will display the number of samples that were successfully scanned. Press the **Exit** button to proceed. If multiple tube labware definitions exist for a given rack, a popup screen is displayed to select which tube type is being used on that rack (Figure 10). Select the tube type being used for each rack from the drop-down menus. All tubes on an individual tube rack must be of the same type.

Figure 10. Choosing a tube type. When multiple tube types are defined for a tube rack, you need to select which tube type is being used on any given rack. Each tube rack can use a different tube type, but all tubes within a single rack must be of the same type.

The next user interface screen displays the three tube racks with a green indicator at any position where bar code information was scanned (Figure 11). Select a tube position and confirm the bar codes for each tube using the **Up** and **Down** arrow keys to navigate through tube positions. If no bar codes are present on the tubes, you are required to enter sample identifier information for each position to be processed. Select the tube position and enter sample identifier information. Press **Enter** on the keyboard to accept the sample identifier and advance to the next tube position. A green indicator will be displayed on the tube racks where sample identifiers have been manually entered.

If more samples are present on the input labware than can be processed with this variant, a message will be displayed at the top of the interface screen indicating the number of excess samples that will have to be removed before proceeding. To remove samples from processing, select a tube position and press **Delete** on the keyboard. Use the **Up** and **Down** arrow keys on the keyboard to navigate through tube positions to delete additional sample identifiers. To rescan tube bar codes, change tube racks or specify a different tube type, press the **Rescan Tubes** button. This will reopen the screen used to scan tube racks (Figure 9).

Once satisfied with the labware positions and sample identifiers to be processed, press the **Next** button to proceed to the next user interface screen.
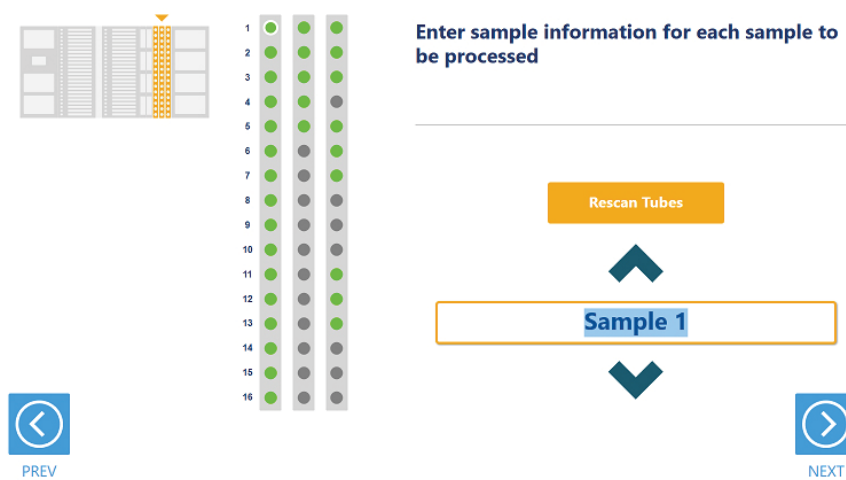
Figure 11. Tube bar code confirmation/entry. Tube positions for which bar code information was scanned are indicated in green. All samples to be processed must have sample identifier information. If no bar codes are being used, manually enter a sample identifier for each position to be processed by selecting the position and entering an identifier.

## 2.5.2   Import of Sample Identifier and Concentration Information

All samples must have concentration information associated with them to be processed with this method. Sample concentration information can be imported either from the Portal software or a tab-delimited text file based on the check box setting on the General Run Parameters screen (Figure 5). Below are instructions for importing concentration data from both sources. Use the instruction set appropriate to the source of concentration data being used in the run.

Importing Concentration Data from Portal

Concentration data must previously have been imported into the Portal software using Portal Access (see the *Portal Access Software Technical Manual* #TM549 for information on creating assays and importing concentration data into the Portal software). This method assumes the use of a human-specific multiplexed quantitative PCR assay to quantitate samples. The assay should have quantitation targets corresponding to Autosomal concentration, Y concentration, Degradation concentration (optional) and IPC.

The screen shown in Figure 12 is used to specify the concentration assay that was used to quantitate the samples in this run.

1. Use the drop-down menu to select a quantitation assay from the Portal software. Quantitation targets used in this assay will automatically be interpreted as containing Autosomal concentration data, Y concentration data, Degradation concentration data, and IPC data based on their names in the assay. We recommend that when setting up an assay in Portal Access, the quantitation target names be specified as Auto, Y, Deg, and IPC to make their automatic identification as seamless as possible. The assay should contain concentration data for Autosomal concentration, Y concentration, Degradation concentration (optional) and IPC.

2. If the automatic designation of target naming does not match the actual targets that correspond to the specified concentration data, press the **Reassign Targets** button to correct the target assignment. Figure 13 displays the screen for reassigning targets. On this screen, use the drop-down menus for each target to indicate which target name corresponds to each of the Autosomal, Y, Degradation, and IPC targets. The check boxes to the left of the different targets can be used to identify which quantitation target is the primary target to be used for normalization of samples. After making selections, press the **Close** button to close this screen.

3. After the assay has been selected and any target reassignment has been performed, the software will automatically attempt to find concentration data specific to the samples being processed in this run. On the lower right side of the screen are text boxes indicating the total number of samples requested to be processed in this run and the number of samples identified with concentrations in the Portal software for this assay. Only samples with concentration data will be processed in this run.

4. Once you are satisfied with the import of concentration data, press the **Next** button to proceed to the next screen. Continue to Section 2.7 Instrument Setup Instructions in this document. Pressing the **Back** button will return you to the General Run Parameters screen.
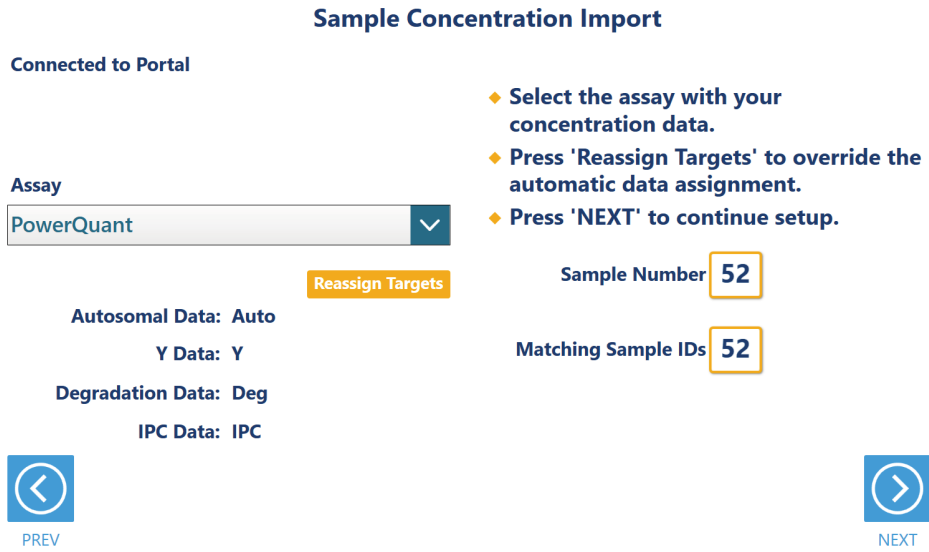
Figure 12. Importing concentration data from Portal. On this screen specify the assay used to quantitate samples to be processed in this run.
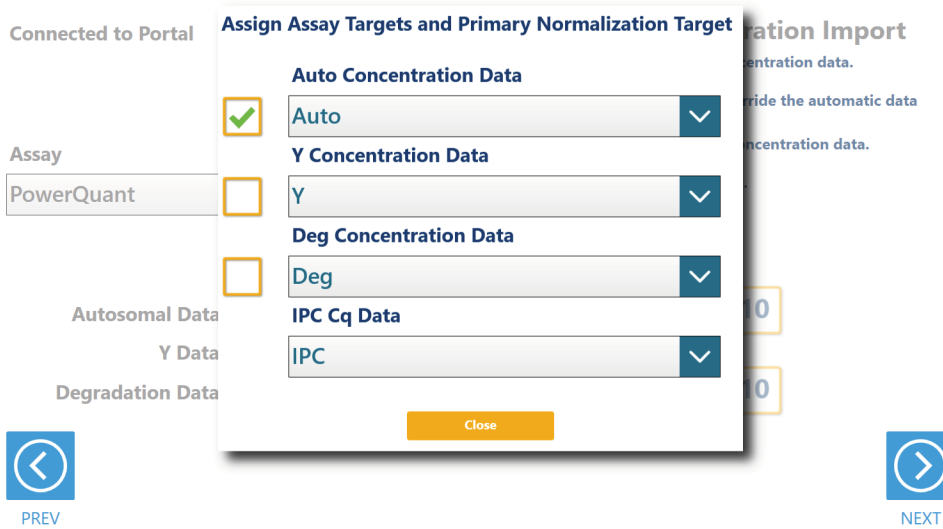
Figure 13. Reassigning quantitation targets. Use the **Reassign Targets** button to change the targets associated with each concentration type and to specify the primary concentration target for sample normalization.
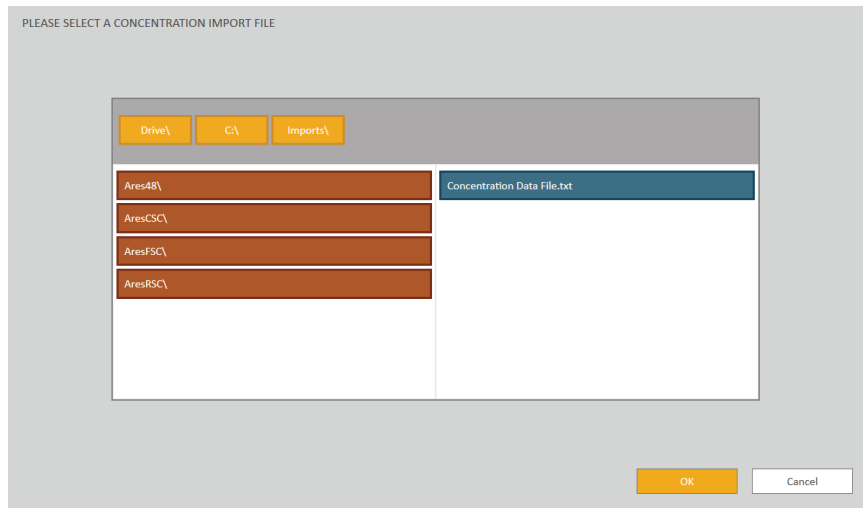
Importing Sample Identifier and Concentration File

An alternative to retrieving concentration data from the Portal software is to import a file specifying the sample identifiers and concentrations of all samples that are to be processed. Use the file browser (Figure 14) to select an import file. The current path will be shown in yellow boxes along the top of the screen. Touch any aspect of the path to navigate to the desired folder. Touch the **Drive** button to select the drive in which to search. Folders within the current path are shown as red rectangles on the left side of the screen. Files will be shown as blue rectangles (yellow when selected) on the right side of the screen. Click the blue rectangle corresponding to the desired import file to highlight it, and then press the **OK** button.

The import file must be a tab-delimited text file containing a table of information with a row of column headers. Our recommended column headers are SampleID, Auto, Y, Deg, and IPC. Setting the column headers in your file to these values will facilitate column identification during import. The table must contain sample identifier information and concentration information for two or three concentration targets (Autosomal concentration, Y concentration, Degradation concentration (optional) and an IPC. Each sample entry in the file must consist of a single row of information. There is no filtering for file extension in the file browser, so files with a .txt, .csv, .tsv, or any other extension will be available for import.

After selecting an import file, the user interface will attempt to identify the header row of the data table and assign column information to SampleID and quantitation target categories (Figure 15). The selected data file is displayed at the top of the screen. Press the **Select File** button to choose a different tab-delimited text file for import if necessary. The user interface assumes three quantitation targets and an IPC are specified in the concentration file. If only two quantitation targets and an IPC are present in the file, use the Quantitation Target Number drop-down menu to adjust the number of quantitation targets. If the user interface has incorrectly identified the header row for the data table, use the Header Row drop-down menu to indicate which row of the file contains header information. On the bottom left side of the screen, the column headers associated with each quantitation target category are listed. If the user interface has incorrectly interpreted which column header specifies which quantitation target, press the **Reassign Targets** button to open a pop-up screen on which you can specify column headers for quantitation targets and the primary target for normalization (Figure 16). Use the drop-down menus on this screen to identify the column headers that correspond to each quantitation target and the IPC. Use the checkboxes on the left side of the screen to specify the primary quantitation target that should be used for normalization of samples.

Once all information pertaining to the structure of the import file is set, select the **Import** button to match the file contents to the sample identifier information associated with the input labware. On the right side of the screen, the number of samples specified on the input labware and the number of matching sample identifiers that were identified in the import file are indicated. Once satisfied with the import file settings, press **Next** to proceed to the next user interface screen.

Figure 14. Import File Browser. Use the import file browser to identify the tab-delimited text file containing sample identifier and concentration information. Yellow boxes at the top of the screen indicate the current drive and path. Use the red boxes to select folders within the current path. Import files are displayed as blue boxes on the right side of the screen. Select a valid import file, and press **OK** to proceed.
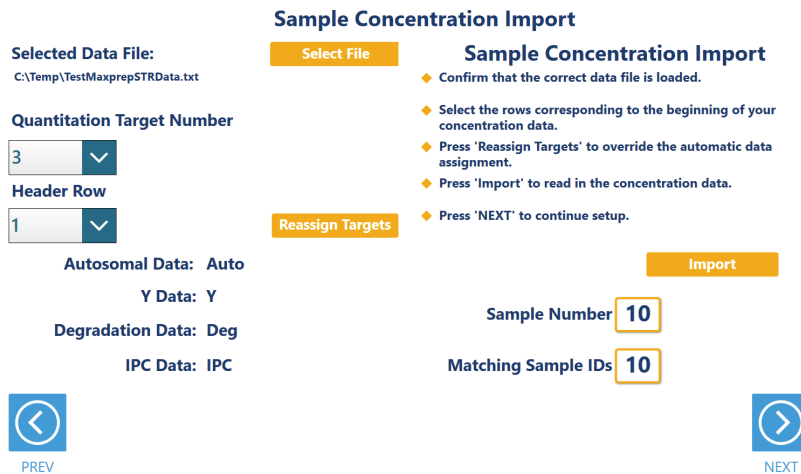
Figure 15. Specifying Import File Structure. Use the drop-down menus on this screen to specify the number of quantitation targets and position of the Header row in the import file. Press **Reassign Targets** to correct the columns of information associated with each quantitation target and the IPC as well as indicating the primary quantitation target for normalization. Press **Import** to assess the number of sample records and the number of matching sample identifiers present in the import file. To select a new import file, press the **Select File** button.

Figure 16. Reassigning columns to quantitation targets. On this screen use the drop-down menus to correct any misidentified columns associated with quantitation targets or the IPC. Use the check boxes on the left side of the screen to specify the primary quantitation target that should be used for normalization of samples.
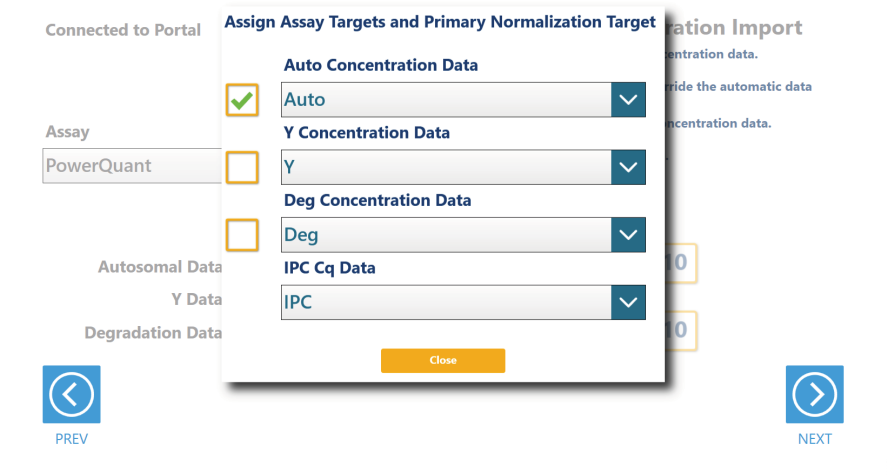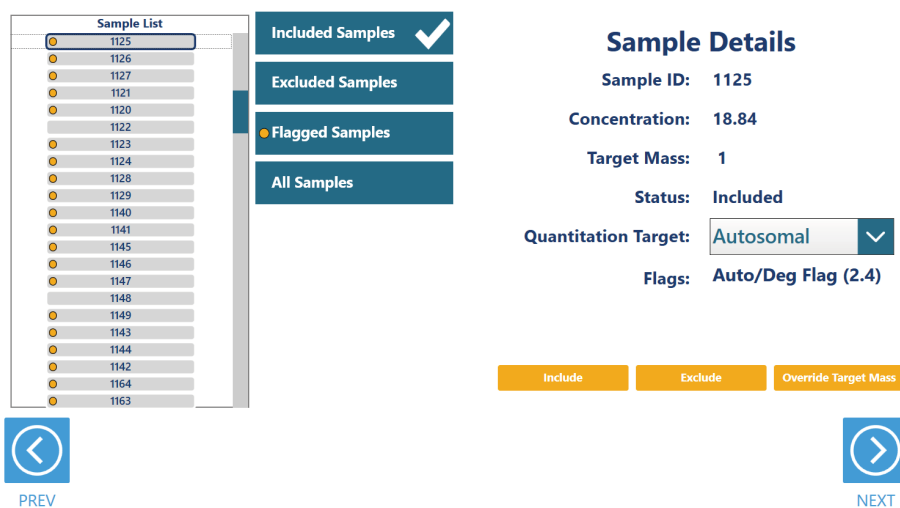
Sample Review and Modification

Once concentration values have been assigned to samples, the user is presented with a screen to review and modify settings for samples to be included in this normalization and amplification setup (Figure 17). A list of samples that matched the imported concentration data set is displayed on the left side of the screen. This list can be filtered using the **Included Samples**, **Excluded Samples**, **Flagged Samples,** and **All Samples** buttons to display samples according to the table below.

Filter Button	Description
Included Samples	Samples that will be included in the normalization and amplification setup based on the variant method normalization rules specified by the administrator.
Excluded Samples	Samples that will be excluded from further processing based on the variant method normalization rules specified by the administrator.
Flagged Samples	Samples that have quality flags based on the variant method rules specified by the administrator. Samples can be flagged based on [Autosomal]/[Y] ratio, [Autosomal]/[Degradation] ratio, and deviation from the average IPC Cq value.
All Samples	All samples with matching concentration values in the imported concentration data set.

Figure 17. Sample Review and Modification. A list of samples is displayed on the left side of the screen. Filter the list using the **Included Samples**, **Excluded Samples**, **Flagged Samples**, and **All Samples** buttons. Sample details for the selected sample are displayed on the right side of the screen. Based on administrator settings, use the Quantitation Target drop-down menu to change the quantitation target used to identify the concentration for the sample, and use the **Include**, **Exclude**, and **Override Target Mass** buttons to modify individual sample settings.

On the right side of the screen the details associated with a selected sample are displayed. Select a single sample to view and modify the details associated with that sample. Sample details include the Sample ID, concentration value for the specified quantitation target, target mass that will be included in the reaction, status indicating whether the sample will be included or excluded in the normalization and amplification setup, specified quantitation target, and any quality flags associated with the sample. Use this information to review the samples and see whether the sample will be processed based on the administrator normalization rules.

Based on administrator settings, it may be possible to modify values for samples. For any individual sample you can use the Quantitation Target drop-down menu to choose a different quantitation target concentration value for normalization and amplification setup. Individual or multiple samples can be modified using the buttons at the bottom of the Sample Details. To modify multiple samples, select a position, Ctrl-click to select multiple positions or click and drag across several consecutive positions to select them. Use the **Include** and **Exclude** buttons to alter the processing status for all selected samples. Use the **Override Target Mass** button to change the target mass to add to the amplification reaction for selected samples.

Once you are satisfied with the processing status for all samples, press the **Next** button to continue to the amplification plate map or press the **Prev** button to return to sample concentration import.

Amplification Plate Map

After any modifications to the samples that will be used for normalization and amplification setup, the user interface presents a screen that displays the amplification plate map (Figure 18). This is an informational screen only and cannot be modified. The locations of samples, controls and ladder wells (as appropriate) are indicated on the plate with samples displayed in black, controls in yellow and empty ladder wells in gray. Select any well position on the amplification plate map to view the identifying information for that well. After reviewing the amplification plate map, press the **Next** button to begin instrument setup or press the **Prev** button to return to the sample review and modification screen.
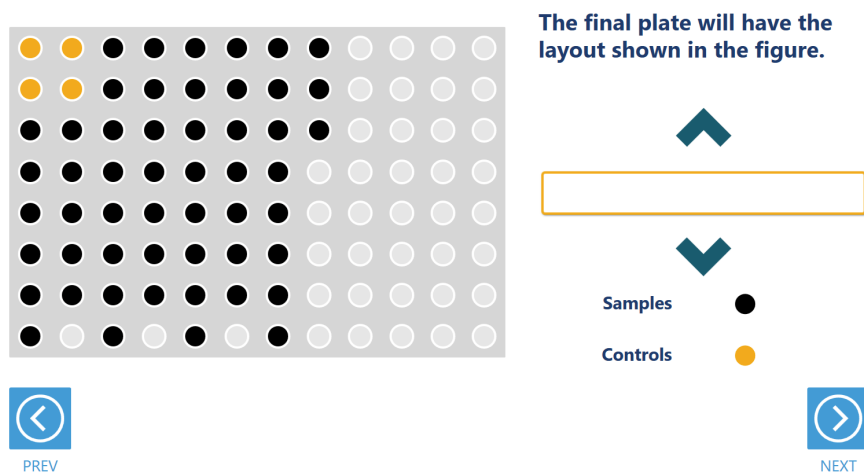
Figure 18. Amplification Plate Map. An Amplification Plate Map is displayed to show the positions of samples, controls and ladder wells (as appropriate). Samples are shown as black wells, controls as yellow wells and empty ladder wells as gray wells. Select any well position to view identifying information for that well.

2.6   Instrument Setup Instructions

Instrument setup proceeds from left to right on the Maxprep® Liquid Handler. The loading platform of the Maxprep® Liquid Handler uses a track system to indicate where carriers are loaded on the system. In these instructions each carrier referenced will list the starting and ending track numbers that are spanned by the carrier. Make sure that carriers are placed correctly on the instrument at each step of the user interface. Screens are presented to indicate the types of labware that should be placed on the instrument, volumes of reagents that should be provided, and to allow entry of reagent bar code information.

All deck setup screens display a miniature view of the entire instrument in the upper left corner of the screen with the current position highlighted. Below this image is a summary of the selected run parameters. On the right side of the screen is a depiction of the carrier and instructions for what should be present at each position on the carrier. For each position where labware or reagents must be placed there is a check box. As labware is loaded at each position, check the box to indicate that the instruction was performed. It is not possible to proceed to the next instrument setup screen until a check is present in all check boxes on the screen.

2.6.1   Utility Carrier (Tracks 1–6)

The Utility Carrier is a fixed position and cannot be removed from the instrument. It contains a Heater/Shaker unit at Site 1 (back of the instrument), instrument tools (gripper paddles and plunger tools) at Site 2 and two labware positions at Site 3 and Site 4 (front of the instrument; Figure 19).

Follow the information on the right side of the screen regarding placement of labware. For instance, on the screen in Figure 19, there should be no labware present on the Heater Shaker device at Site 1, the Gripper Paddles and Plunger Tools are present at Site 2, a 1.1ml Square-Well Plate (Cat.# V6821) is present at Site 3, and an Amplification Plate is present at Site 4.

For the Amplification Plate at Site 4, selecting the **Enter Plate Details** button or the check box at Site 4 displays details and allows entry of bar code information for the Amplification Plate (Figure 20). Place the indicated Amplification Labware on Site 4. By default the bar code for the Amplification Plate is set to the date and time the method was started. Select the **Labware Barcode** text box to scan or enter a different bar code for the Amplification Plate.

Note: Strip tubes, unskirted and partially skirted amplification plates should be seated in an Amplification Plate Base before being placed on the system. Fully skirted amplification plates can be placed directly on the system.

After placing labware, check the boxes next to each position to activate the **Next** button at the bottom right corner of the screen. Press the **Next** button to continue with instrument setup.
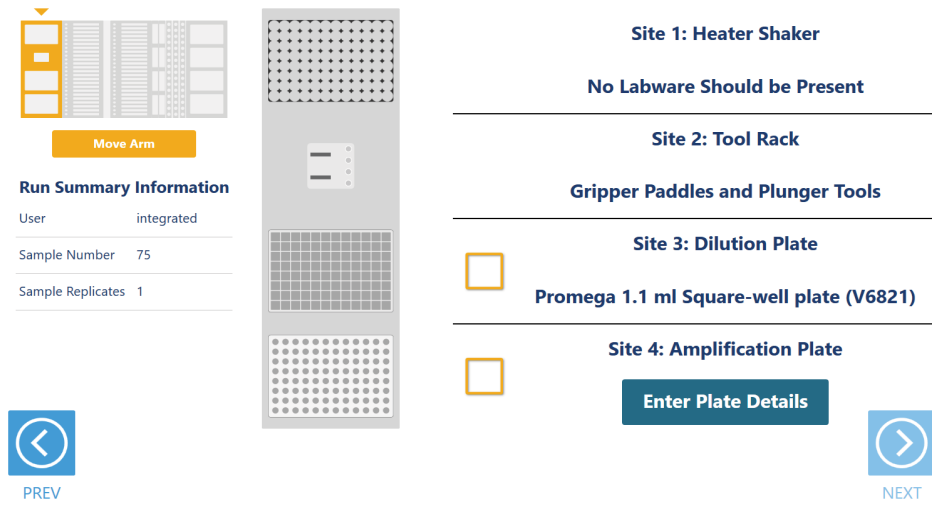
Figure 19. Deck Setup screen for the Utility Carrier. This carrier cannot be removed from the system. It contains a Heater/Shaker for incubation of samples, tools and two labware positions. Place the indicated labware on the carrier, and check the boxes to activate the **Next** button. Press **Next** to continue instrument setup.
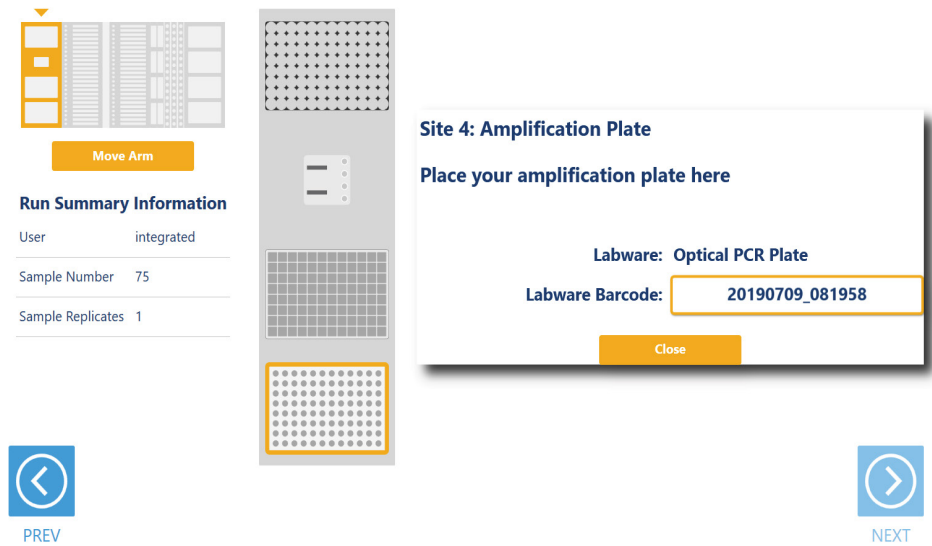
Figure 20. Amplification Plate Details screen. For the Amplification Plate at Site 4, press the **Enter Plate Details** button to display information about the plate. Select the Labware Barcode text box to scan or enter a bar code for the Amplification Plate. The default bar code for the plate is the date and time the method was started.

2.6.2   Maxwell® Deck Tray Carriers (Tracks 7–12, 14–19)

The Maxwell® Deck Tray Carriers are used to place Maxwell® Deck Trays or a plate on the instrument as input labware. Instructions for both input labware types are shown in the following section.

Notes:

a.   Refer to the miniature instrument image on the left side of the screen to see which position the displayed Maxwell® Deck Tray Carriers should occupy on the instrument.

b.   The Maxwell® Deck Tray Carriers are not intended to be directly next to each other on the instrument. A Reagent Carrier or an empty track should be placed at Track 13 between the Maxwell® Deck Tray Carriers to make sure they are loaded correctly.

Maxwell® Deck Trays as Input Labware

There are two types of Maxwell® Deck Tray Carriers, one for 16-position Maxwell® Deck Trays and one for 24-position Maxwell® Front and Back Deck Trays. The choice of which deck tray type will be used for the run is made when the input labware is selected for the run. These carriers can be removed from the system and replaced depending on the type of deck trays that are being used for this run. The Promega DNA Normalization and STR Setup methods use eluates in the Maxwell® elution tube position as samples for the run. In the center of the screen (Figure 21) is an image of the deck trays where the positions to be processed are indicated as black cartridges. If only one deck tray is to be processed, the other deck tray will be shown as a gray rectangle with no cartridges present. Elution tubes should be securely loaded with the caps open on the deck trays at the indicated positions. Place Maxwell® Deck Trays, minimally containing the specified number of elution tubes placed at the elution positions, on the Maxwell® Deck Tray Carriers. Shown on the right side of the screen are the bar codes for each Maxwell® Deck Tray. The first tray is placed on the left carrier at Tracks 7–12, and the second tray is placed on the right carrier at Tracks 14–19.

Check the boxes next to the deck tray bar codes (if scanned on the Query Portal screen) as you load the Maxwell® Deck Trays on the Maxprep® Liquid Handler. Once the boxes are checked, the **Next** button in the bottom right corner of the screen will become active. Press the **Next** button to continue with instrument setup.
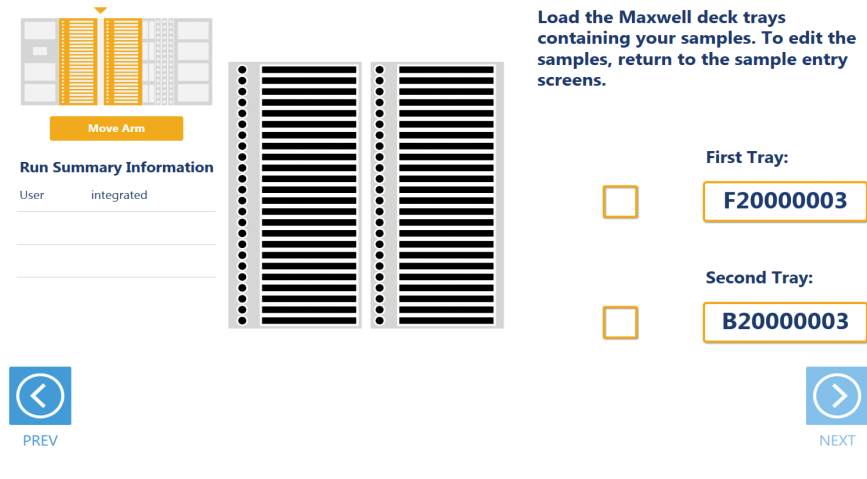
Figure 21. Maxwell® Deck Tray Carriers. These carriers are used to hold 16-position Maxwell® Deck Trays or 24-position Maxwell® Front and Back Deck Trays. Place Maxwell® Deck Trays with elution tubes at the indicated positions on the deck tray carriers as instructed.

Plate as Input Labware

When a plate is selected as an input labware type, the front-most plate position on the left Maxwell® Deck Tray Carrier is used (Figure 22). Either Maxwell® (16-position) Deck Tray Carriers or Maxwell® RSC 48 Deck Tray Carriers can be used for positioning the sample plate. Remove the Maxwell® Deck Tray (if present) and the elution magnet from the left Maxwell® Deck Tray Carrier. Place the sample plate on the left Maxwell® Deck Tray Carrier as indicated. The bar code of the sample plate (if scanned on the Query Portal screen) is shown on the right side of the screen. After placing the sample plate, check the box to activate the **Next** button. Press the **Next** button to move to the next instrument setup screen.
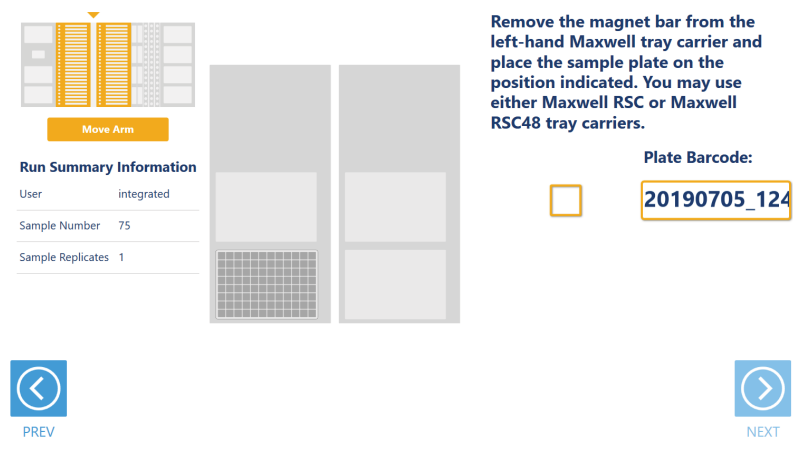

Figure 22. Plate Input Location. When a plate is used as input labware, it will be placed at the front plate position on the left Maxwell® Deck Tray Carrier. Make sure to remove the elution magnet from the carrier..

2.6.3   Reagent Carriers (Tracks 20, 21)

The Reagent Carrier screens display the reagents that should be placed at each site on a reagent carrier (Figure 23). Sample diluent is placed in a Maxprep® Reagent Reservoir, 50ml.

Note: Refer to the miniature instrument deck layout on the left side of the screen to see which position the displayed reagent carrier should occupy on the instrument.
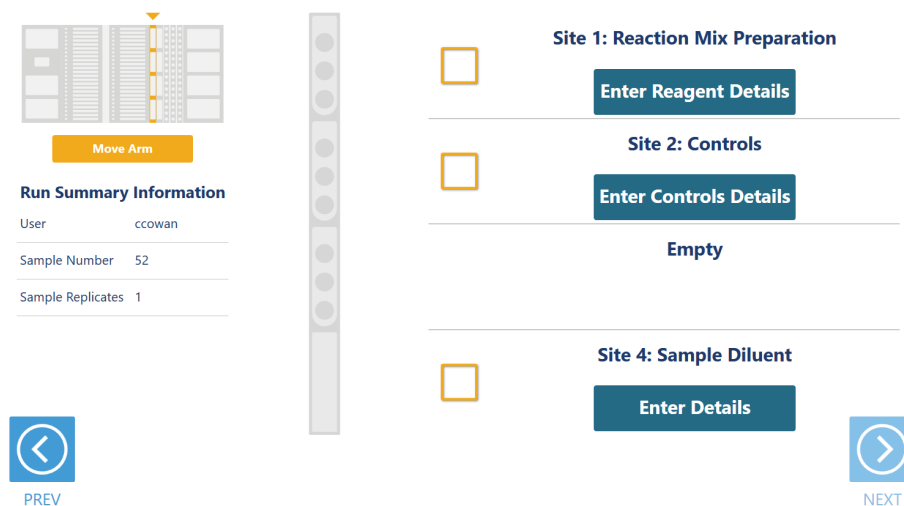
Figure 23. Reagent Carrier screens. The name of the reagent to be placed at each carrier site is indicated. Press the **Enter Reagent Details** button for any individual site to see details and enter reagent lot information.

Press the **Enter Reagent Details** button to see details specific to the reagent and site on the reagent carrier. When finished viewing instructions for a reagent site press **Close** to leave the reagent details and return to the main reagent carrier screen. The act of viewing reagent details will check the reagent check box. All check boxes must be checked to activate the **Next** button on the main reagent carrier screen. Press **Next** to move to the next instrument setup screen.

For tube-based reagents on the first reagent carrier (Table 1), place a Maxprep® 3-Position Reagent Tube Holder (Cat.# AS9409) at the site and add individual tubes containing the reagent specified at the indicated positions in the tube holder. If there is not sufficient reagent present in a tube to complete the run, a warning prompt will be shown to place a new tube of reagent.

Note: If 5X AmpSolution™ Reagent is required for the reaction setup, place a Maxprep® 3-Position Reagent Tube Holder (Cat.# AS9409) at Site 3 as prompted, and add up to three tubes containing the total volume of 5X AmpSolution™ Reagent specified.

When reagents are to be placed at a reagent site in a trough (Table 1), transfer at least the indicated volume of reagent into a Maxprep® Reagent Reservoir, 50ml, and place it at the designated site on the carrier. If there is not sufficient reagent present in the trough to complete the run, a warning prompt will be shown to add more of the specified reagent to the trough position for this reagent.

Table 1. First Reagent Carrier[1]

Site	Reagent Type	Labware	Details
1	Reaction mix preparation	Up to three 1.5ml tubes, empty (as specified by the user interface)	This is where the amplification reaction mix (PowerPlex® Master Mix, Primer Pair Mix, [optional] 5X AmpSolution™ Reagent) is prepared by the instrument
2	Controls (optional)	1.5ml or 2.0ml tubes containing controls	The names of the controls are shown. Place controls in open tubes from back to front in the Maxprep® 3-Position Tube Holder as indicated. Enter bar codes for each control as desired.
3	5X AmpSolution™ Reagent (optional)	Up to three tubes of 5X AmpSolution™ Reagent	When required for reaction setup, this site holds 5X AmpSolution™ Reagent. Load up to three tubes of 5X AmpSolution™ Reagent to satisfy the minimum volume displayed for this run. Enter a bar code for the 5X AmpSolution™ Reagent as desired.
4	Sample diluent	Maxprep® Reagent Reservoir, 50ml, containing normalization diluent	Add at least the minimum volume of sample diluent indicated to the Maxprep® Reagent Reservoir, 50ml, at this site. Enter a bar code for the sample diluent as desired.

[1]Shown in this table are all possible reagents that could be present on Sites 1−4 of the First Reagent Carrier. Depending on the Promega STR Kit being used and the variant method settings, the method will prompt the user to place the required reagents and labware at each site.

The second reagent carrier is used to hold amplification components (Table 2). Touch the **Enter Reagent Details** button to see details specific to the reagent(s) that should be placed at each site on the reagent carrier Figure 23). The details are specific to the type of reagent placed at a given site. When finished viewing instructions for a reagent site, press **Next Site** to move to the next occupied site on the reagent carrier, or press **Close** to leave the reagent details and return to the main reagent carrier screen. The act of viewing reagent details will cause the check box for the reagent to be checked. All check boxes must be checked to activate the **Next** button on the main reagent carrier screen. Press **Next** to move to the next instrument setup screen.

Table 2. Second Reagent Carrier[1]

Site	Reagent Type	Labware	Details
1	PowerPlex® Master Mix	Up to three tubes of PowerPlex® Master Mix	This site holds the PowerPlex® Master Mix. Load up to three tubes of PowerPlex® Master Mix to satisfy the minimum volume displayed for this run. Enter a bar code for the PowerPlex® Master Mix as desired.
2	PowerPlex® Primer Pair Mix	Up to three tubes of PowerPlex® Primer Pair Mix	This site holds the PowerPlex® Primer Pair Mix. Load up to three tubes of PowerPlex® Primer Pair Mix to satisfy the minimum volume displayed for this run. Enter a bar code for the PowerPlex® Primer Pair Mix as desired.
3	Enzyme (optional)	Up to three tubes of enzyme.	When required for reaction setup, this site holds amplification enzyme. Load up to three tubes of enzyme to satisfy the minimum volume displayed for this run. Enter a bar code for the enzyme as desired.
4	Amplification-Grade Water (optional)	Up to three tubes of Water, Amplification Grade	When required for reaction setup, this site holds Water, Amplification Grade. Load up to three tubes of Water, Amplification Grade to satisfy the minimum volume displayed for this run. Enter a bar code for the Water, Amplification Grade, as desired.

[1]Shown in this table are all possible reagents that could be present on Sites 1–4 of the Second Reagent Carrier. Depending on the Promega STR Kit being used, the method will prompt the user to place the required reagents at each site.

For reagents on the second reagent carrier, place a Maxprep® 3-Position Reagent Tube Holder (Cat.# AS9409) at each site as prompted, and add up to three tubes containing the total volume of reagent specified. The instrument will use the reagent present in the first reagent tube until it is consumed, then it will move to the second and third tube in the Reagent Tube Holder. If there is not sufficient reagent present in all three tubes to complete the run, a warning prompt will be shown to place a new tube of reagent at the first position for this reagent.

2.6.4   Sample Tube Carriers (Tracks 22, 23, 24)

When tubes are selected as the input labware, the tube carriers at Tracks 22–24 will be displayed (Figure 24). Scanning or entry of sample tube bar codes occurred earlier in the method, and the tube racks should already be present at these three track positions. Confirm that tubes are present at the positions indicated in green on each of the tube racks displayed. All tubes within each tube carrier should be of the same type. Check the box to activate the **Next** button. Press the **Next** button to proceed to the next instrument setup screen.
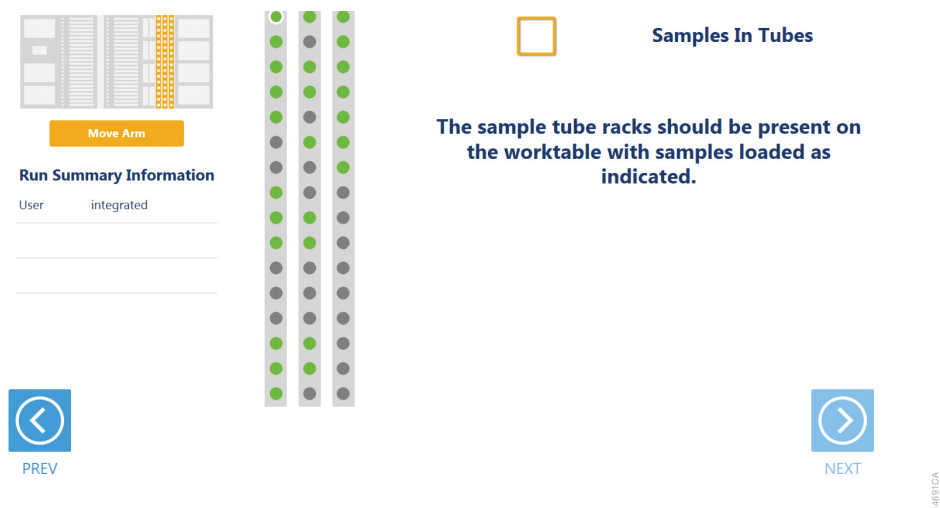

Figure 24. Sample Tube Rack screen. When tubes are the input labware, the three sample tube racks are displayed. Confirm that tubes are present at each position indicated in green and that all tubes within a rack are of the same type.

2.6.5 Tip and Plate Carrier (Tracks 25–30)

Tips and a normalization plate (if prompted) are placed on the tip and plate carrier (Figure 25). Site 1 on this carrier is a plate position that is used to hold a 1.1ml Square-Well V-Bottom Deep Well Plate (Cat.# V6821) for normalization (if prompted).
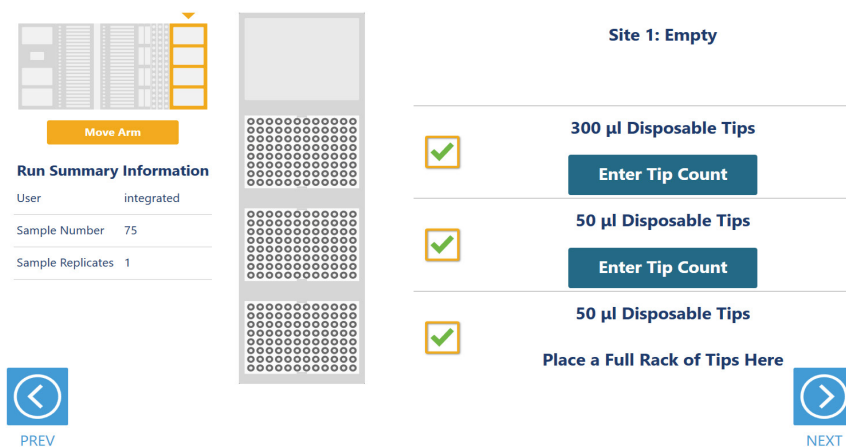
Figure 25. Tip and Plate Carrier screen. Place the specified labware at each site. When required, Site 1 contains a 1.1ml Square-Well Deep Well Plate (Cat.# V6821) for normalization of samples. Sites containing a tip rack indicate the type of tips that should be placed there and whether a partial or full rack of tips needs to be placed. For partial tip racks, press the **Enter Tip Count** button to specify the first available tip in the partial tip rack.

Sites 2–4 on this carrier are used to hold tip racks. Place tips of the specified type at each site on the carrier. Sites at which either a full or partially filled tip rack can be placed will have an **Enter Tip Count** button. Press the **Enter Tip Count** button and select the first available tip present in the tip rack (Figure 26). Entering a tip count and pressing the **Close** button will act to check the check box for the specified site. If a tip count is not entered, the software will assume a full rack of tips is located at the specified position. For all other sites, once you have added the requested labware, check the check box next to the site. Only after all check boxes on the screen are checked will the **Next** button become active. Press the **Next** button to move to the next screen.

Note: Confirm that the appropriate tips are placed at each position before starting the method. Tip types used at each position differ between preprocessing and post-processing methods.
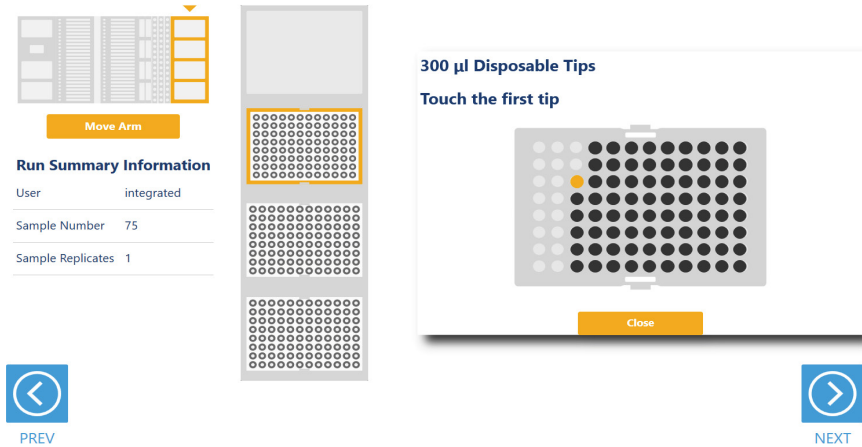
Figure 26. Selecting the first tip in a partial tip rack. For sites at which a partial or full tip rack can be placed, press **Enter Tip Count** to specify the first tip present (in gold) in the partial tip rack. Press **Close** to return to the display of all sites on the carrier. Check all check boxes after loading labware to proceed.

2.6.6   Final Screen

The final screen of the instrument setup presents a list of items that should be checked before starting the run. Confirm that:

- All carriers are completely seated in the instrument

- All labware is loaded at the correct locations

- Tip waste bin is empty if necessary

- The door of the instrument is closed

Once the items in the list are checked and the door of the instrument is closed, press **Next** to start automated sample processing (Figure 27). This is the last chance to return to any of the instrument setup screens and confirm instrument setup before sample processing begins.

During the run, if you need to pause instrument operation to resolve any unforeseen issues, press the **Pause** button in the lower left corner of the screen. If you need to abort the run for any reason after you have paused the instrument, press the **Abort** button in the lower left corner of the screen.

Setup Complete

Run Summary Information

User	integrated
Sample Number	75
Sample Replicates	1
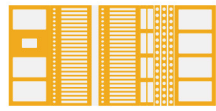
- ◆ **Ensure that all carriers are completely pushed back.**
- ◆ **Verify that all reagents and labware are loaded at the correct locations.**
- ◆ **Empty the waste bin, if necessary.**
- ◆ **Close the door.**
- ◆ **Press 'NEXT' to begin processing.**

PREV

NEXT

Figure 27. Final instrument setup screen. This screen presents a list of items that should be checked before starting automated processing. It also provides a final chance to return to any of the previous instrument setup screens before automated processing begins. To start processing, close the instrument door and press the **Next** button.

2.7 Maxprep® Liquid Handler Promega DNA Normalization and STR Setup Protocol

The Maxprep® Liquid Handler will normalize samples and prepare Promega STR reactions as indicated by the settings in the variant and Promega STR kit selected for this run. The following steps are performed by the Maxprep® Liquid Handler:

1. Based on the total number of samples and controls, Promega STR reaction mix consisting of the following components is prepared in a 1.5ml tube:

   a. Primer Pair Mix specified by the Promega STR kit selected

   b. Amplification Enzyme specified by the Promega STR kit selected

   c. Master Mix specified by the Promega STR kit selected

   d. 5X AmpSolution™ Reagent or Enhancer Volume specified by the Promega STR kit selected

   e. Water, Amplification Grade, Volume specified by the Promega STR kit selected

2. **Optional:** The system checks that there is sufficient volume in the sample diluent trough to dilute samples and controls if dilution is necessary.

3. **Optional:** Transfer of sample diluent from the sample diluent trough to the dilution plates if dilution is necessary.

4. **Optional:** Transfer of Samples to first dilution well and mixing in dilution labware.

5. **Optional:** Transfer of Controls to first dilution well and mixing in dilution labware

6. **Optional:** Transfer of Samples from first dilution well to second dilution well and mixing in dilution labware.

7. **Optional:** Transfer of Controls from first dilution well to second dilution well and mixing in dilution labware.

8. Transfer of amplification reaction mix to amplification plate wells.

9. Transfer of normalized samples to amplification plate wells.

10. Transfer of normalized controls to amplification plate wells.

Method is complete. Open the instrument door, remove the amplification plate, seal or cap the plate, and then briefly centrifuge the plate to bring contents to the bottom of the wells and remove any air bubbles. Prepare the plate as per your amplification protocol. Remove primary samples, plates and used tips from the waste bin and discard as hazardous waste following your institution's recommended guidelines. Either discard or tightly cap and store remaining reagents.

Consumables for Maxprep® methods are designed to be used with potentially infectious substances. Use appropriate protective equipment (e.g., gloves and goggles) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

3   Potential Errors and Warnings

The Maxprep® Promega DNA Normalization and STR Setup method is designed to provide notification and resolution options for several potential error and warning states. Below are listed several possible error and warning states that may be observed during a method. Always carefully read any warning or error messages to find out how to proceed. If in doubt about how to proceed with any error state, the safest option is to choose the **Abort** button to end the run.
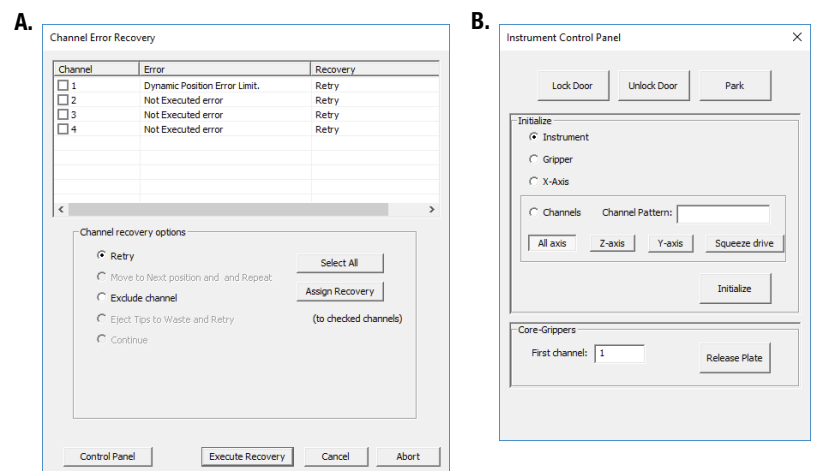
3.1   Intrusion Sensor

The intrusion sensor on the Maxprep® Liquid Handler checks for any potential physical obstructions that could prevent gantry movement on the instrument. Prior to any instrument movement (e.g., initialization, bar code scanning, arm movement, resuming a method after a pause, performing a maintenance method), always be sure to check that all carriers on the system are fully pushed to the back of the instrument and that nothing could impede the progress of gantry movements. An LED on the left front side of the deck provides a visual indicator of the intrusion sensor state. When no physical obstruction is detected, the LED is orange, but when the LED is green, a carrier or other physical obstruction has been detected by the intrusion sensor. Make sure that all carriers are fully seated on the system and that the intrusion sensor LED is orange before proceeding with any instrument operation that will result in movement of the gantry.

If the intrusion sensor does detect a physical obstruction to the gantry movement the system will display an error message (Figure 25) when the gantry attempts to move. This error does not result in an aborted run and the run can continue if you follow the steps below.

Note: During automated bar code scanning, if the intrusion sensor detects an obstruction, the error below will not be displayed and recovery is not possible. In this case, it will be necessary to abort the run, power cycle the Maxprep® Liquid Handler, and start the method run again.

1. On the 'Channel Error Recovery' screen (Figure 28, Panel A), press the **Control Panel** button in the lower left corner to open the "Instrument Control Panel" screen (Figure 28, Panel B).

2. On the 'Instrument Control Panel' screen, press the **Unlock Door** button. Open the door of the instrument and push all carriers back. Confirm that the intrusion sensor does not detect an obstruction by checking that the intrusion sensor LED on the left side of the instrument is orange.

3. Close the instrument door and then press the **Lock Door** button on the 'Instrument Control Panel' screen.

4. In the Initialize area of the 'Instrument Control Panel' screen, make sure the radio button for Instrument is selected and then press the **Initialize** button. The gantry of the Maxprep® Liquid Handler will initialize.

5. Close the 'Instrument Control Panel' screen using the **X** button in the upper right corner of the screen.

6. On the 'Channel Error Recovery' screen, in the Channel Recovery Options area, press the **Select All** button. This will check the boxes for each channel at the top of the screen.

7. Press the **Execute Recovery** button at the bottom of the 'Channel Error Recovery' screen to retry the gantry movement and continue the method run.

Figure 28. Error caused by a physical obstruction detected by the intrusion sensor. Panel A. When the intrusion sensor on the Maxprep® Liquid Handler detects a physical obstruction that would impede gantry movement prior to gantry motion, the error above is displayed. **Panel B.** Follow the instructions in the text above to reinitialize the instrument and continue processing.

3.2   Unable to Load Tips

As part of a normal method run it is necessary for the instrument to load tips on the pipetting channels. The instrument will attempt to load tips at a position twice and will automatically move to the next tip position. If the instrument runs out of tips of a given type, a warning will be displayed to replace that tip type with a full tip rack.

3.3   Insufficient Tips

While the PowerPlex® Reaction Setup with Normalization method was developed to minimize the chance of consuming all available tips during a run, there is still a possibility that a warning message will be displayed indicating that there are no longer tips available on the tip and plate carrier (Figure 29). If this warning occurs, the instrument will pause operation and unlock the door. Open the door of the instrument, add the appropriate tip type to the first site for that tip type, close the door and press **OK** to resume processing.
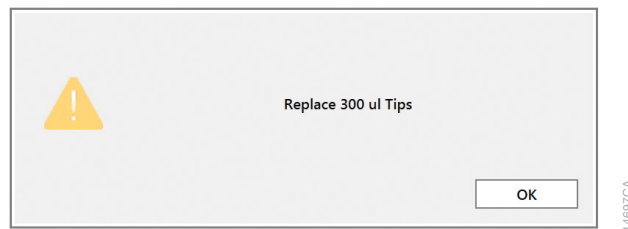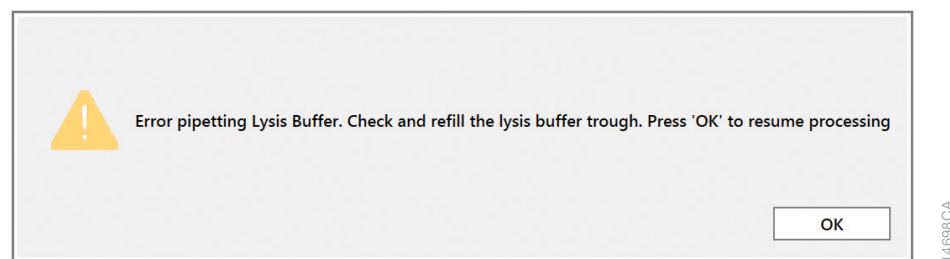

Figure 29. Insufficient tips on the system to complete a method. In the event that all tips of a given tip type are consumed during a run, the instrument will pause and unlock the door. Open the door, load more tips of the indicated type on the tip and plate carrier, close the door and press **OK** to resume processing.

3.4   Insufficient Sample

The system does not check that sufficient sample is present in the input labware. Make sure to provide sufficient volume of samples based on the sample volume indicated in the method or method variant.

3.5   Insufficient Liquid

If the system detects an insufficient volume of reagent in the reagent tubes or trough to transfer the volume of reagent specified for the run, a warning is displayed (Figure 30), the system will pause and the doors will unlock. Open the instrument door and ensure that sufficient reagent is present at the position in question by adding more reagent to the sample diluent trough. Make sure the door of the instrument is closed before pressing the **OK** button.

Figure 30. Insufficient reagent on the system to complete a method. In the event that insufficient reagent is detected in reservoir, a warning will be displayed; the instrument will pause and unlock the door. Open the door, replenish the indicated reagent, close the door and press **OK** to resume processing.

3.6   Hardware Crash

In the course of a method run, if the instrument experiences a physical crash of the pipetting channels into an object, an error message is displayed (Figure 31). This error message will indicate that there was a Hardware Error and the instrument has lost steps. For any situation where a physical crash of the instrument has occurred, press the **Abort** button to end the method run. Follow the instructions below for an aborted run.
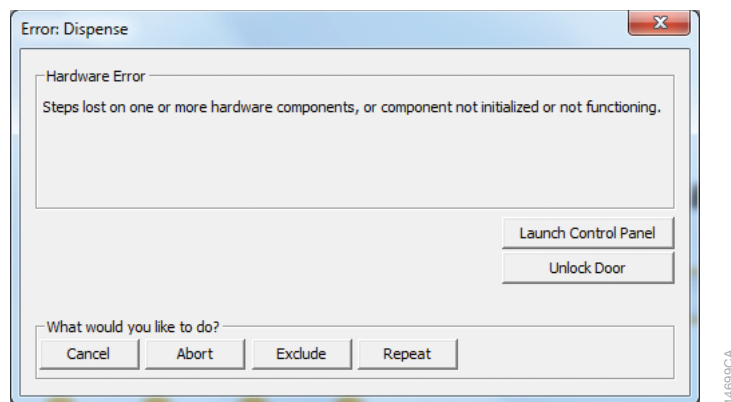

Figure 31. Hardware Crash. In the event that there is a physical crash of hardware components that results in an error message, press the **Abort** button on the error screen to end the method run.

3.7   Aborted Run

Once the PowerPlex® Reaction Setup with Normalization method has started, aborting a run should be considered a last resort. However, there are situations where manually aborting a run (by pressing the **Abort** button in the lower left corner of the screen) may be necessary. Alternatively, there may be conditions where an instrument error could cause a run to be aborted. In the case of an aborted run you will see a warning message (Figure 32) indicating that the instrument should be powered off. If the instrument has experienced a physical crash or there are plungers, tools or tips loaded on the pipetting channels, after powering the instrument off, resolve the crash state by following the instructions regarding recovering from an instrument crash found in the *Maxprep® Liquid Handler Technical Manual* #TM509. Power the instrument back on and restart the Maxprep® software. After any instrument crash, if there is not visible damage to the pipetting channels, it is recommended to run the Weekly Maintenance method that will check instrument calibration. If the Weekly Maintenance method produces failing results, contact Promega Technical Services to schedule a service visit prior to further use of the instrument.

Note: Wait 1 minute after power cycling the Maxprep® Liquid Handler before starting the software to allow for communication to be re-established.
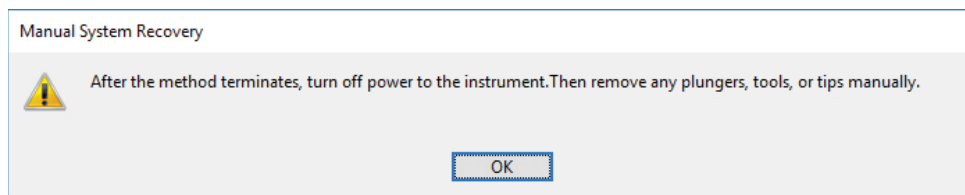
Figure 32. Aborted Method. A message will be displayed after aborting a run indicating that the instrument should be powered off. After turning the instrument off, open the door and manually resolve the crash state as instructed in the *Maxprep® Liquid Handler Technical Manual* #TM509. After any instrument crash, contact Promega Technical Services to determine whether the instrument may require a service visit.

3.8   Instrument Connection Errors

After turning the instrument on, wait at least 1 minute after the green light on the automated bar code scanner turns on before starting a method run. If the instrument has not performed its internal initialization before starting a run, one or more warning messages may be displayed (Figure 33). These warning messages will indicate an error in instrument connection. A message indicating "Unable to ping instrument" is displayed if the instrument is not powered on. Power the instrument on and wait at least 1 minute after the green light on the bar code scanner turns on before attempting to restart the method. Press the **OK** button, wait several seconds and attempt to start the method again.
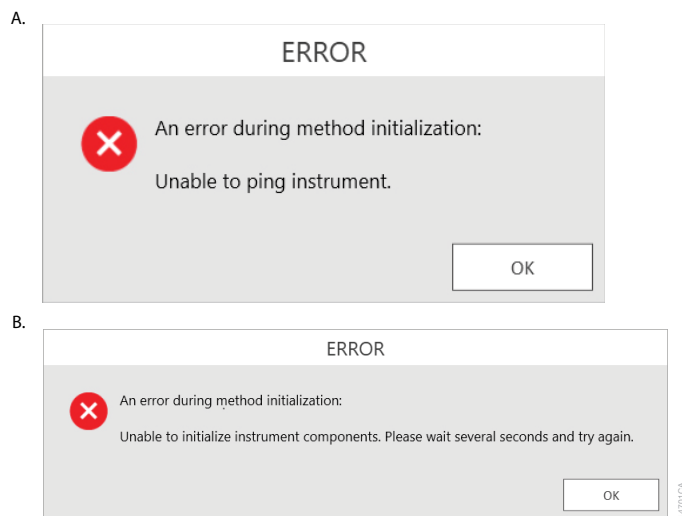
Figure 33. Instrument Connection Errors. Connection errors will be displayed during a run if the instrument has not completed its full internal initialization before a method run is started. The errors shown in **Panels A** and **B** may be displayed. Confirm that the instrument is powered on and has fully initialized, then press the **OK** button, wait several seconds and attempt to start the method again.

4 Variant Method Variables

Administrators can create laboratory-specific variants of the Promega DNA Normalization and STR Setup method. The general process of creating variant methods is discussed in the *Maxprep® Liquid Handler Technical Manual* #TM509 (Section 4.3, Methods). This chapter describes the variant variables that can be adjusted by administrators for the Promega DNA Normalization and STR Setup method. For all variables listed, the administrator can define the default value of the variable within the displayed minimum and maximum allowable values. Not all variables set within the variant methods are user-adjustable at run time.

4.1 Promega DNA Normalization and STR Setup Variant Variables

The Promega DNA Normalization and STR Setup method automates the normalization of samples and amplification reaction setup for Promega STR kits. Variant variables allow the administrator to create independent versions of the method to specify a multitude of preferences and adapt the method to laboratory needs.

4.1.1   Controls

These variables define the layout of controls on the amplification plate. Up to three different control types and ladder wells can be used within a run, and replicate well locations can be defined for each control type or ladder wells.

Note: See Figure 34 in the Appendix, Section 6 for a visual representation of well numbering in the plate.

Setting	Details
1. Control 1 Name	Name of the first control. Used to identify the control during instrument setup.
2. Control 1 Concentration	Concentration of the first control to be used. This value will be used to normalize the control to the specified target mass in the reaction. Enter a concentration of 0 to specify a negative control where only sample diluent will be added to the wells.
3. Control 1 Well Numbers[1]	Well locations where the first control should be placed in the amplification plate. For replicate control wells, specify the well locations as a comma-delimited list (e.g., 1, 17).
4. Control 2 Name	Name of the second control. Used to identify the control during instrument setup.
5. Control 2 Concentration	Concentration of the second control to be used. This value will be used to normalize the control to the specified target mass in the reaction. Enter a concentration of 0 to specify a negative control where only sample diluent will be added to the wells.
6. Control 2 Well Numbers[1]	Well locations where the second control should be placed in the amplification plate. For replicate control wells, specify the well locations as a comma-delimited list (e.g., 2, 18).
7. Control 3 Name	Name of the third control. Used to identify the control during instrument setup.
8. Control 3 Concentration	Concentration of the third control to be used. This value will be used to normalize the control to the specified target mass in the reaction. Enter a concentration of 0 to specify a negative control where only sample diluent will be added to the wells.
9. Control 3 Well Numbers[1]	Well locations where the third control should be placed in the amplification plate. For multiple control wells, specify the well locations as a comma-delimited list (e.g., 3, 19).
10. Empty Ladder Wells[1]	Well locations that should be reserved on the amplification for the placement of ladders during CE setup. These well locations will be empty on the amplification plate.
11. Target Control Mass in Reaction	The target mass that will be used for normalization of controls during the reaction setup.

[1]Well numbering proceeds down columns and across the plate. See Figure 34 in the Appendix, Section 6 for a visual representation of well numbering in the plate.

Note: Placement of empty ladder wells and controls on the plate occurs sequentially. If the same well location is specified for an empty ladder well and controls 1–3, only the empty ladder well will be at that location. If multiple controls are specified for the same location, the highest numbered control will be the only one placed at that location.

4.1.2   Normalization

These variables specify preferences related to the location of concentration data and normalization of samples.

1. Quantitation Target for Normalization
    Based on this setting, the concentration of the sample determined for the specified quantitation target will be used to normalize samples into the Promega STR reaction. Acceptable values for this field are Autosomal, Y or Degradation. Unchecking the "User Modifiable" check box will prevent users from being able to reassign quantitation targets at runtime.

2. Target Sample Mass in Reaction
    This variable determines the desired sample mass to which samples will be normalized in the final Promega STR reaction. Consult the technical manual for the Promega STR reaction being prepared to identify the mass of DNA for which the reaction was optimized.

3. Min Sample Mass in Reaction
    This variable determines the minimum sample mass that is acceptable to use in the final Promega STR reaction. For samples whose concentration is too low to achieve the target sample mass in the reaction, they will be directly transferred to the reaction to achieve a mass equal to or greater than the minimum sample mass in the reaction. Samples that cannot achieve the minimum sample mass in the reaction will be excluded from processing. The value for this setting should correspond to the minimum sample mass validated by the laboratory for the corresponding Promega STR kit. To amplify all samples regardless of target mass in the reaction, set the Min Sample Mass in Reaction to zero.

4. Max Sample Mass in Reaction
    This variable determines the maximum sample mass that is acceptable to use in the final Promega STR reaction. For samples whose concentration is too high to be normalized and achieve the target sample mass in the reaction, they will be normalized to achieve a mass equal to or less than the maximum sample mass in the reaction. Samples that cannot be normalized to achieve the maximum sample mass in the reaction will be excluded from processing. The value for this setting should correspond to the maximum sample mass validated by the laboratory for the corresponding Promega STR kit.

5. Import Concentration Data from Portal
    When checked, concentration data for samples will be imported from the Portal software. This requires that sample concentration data is being uploaded into the Portal software using the Portal Access software (see the *Portal Access Software Technical Manual* #TM549). If unchecked, users must import a tab-delimited text file containing sample identifiers and concentrations to use for normalization.

4.1.3   Permissions

1. Allow user to override normalization rules?
    The administrator defines the rules for normalization of samples in the variant method. Checking this box allows the user to override these rules at run time. If this box is checked, then during the run the user has the ability to: override the target mass for any sample, override the quantitation target to use for normalization of any sample, include any samples that were automatically excluded from processing, and exclude any samples that were automatically included from processing.

4.1.4   Quality Markers

1. Flag high Auto/Y ratios?
    Check this box to flag samples if they have an Autosomal concentration to Y concentration ratio higher than the Auto/Y ratio flag threshold. Uncheck this box if you do not wish to flag samples regardless of their Autosomal concentration to Y concentration ratio.

2. Auto/Y ratio flag threshold
    Use this setting to specify a minimum Autosomal concentration to Y concentration ratio for which samples will either be flagged or excluded from processing. Whether samples are flagged or excluded based on this setting is determined by additional settings in the Quality Markers group.

3. Exclude Auto/Y Flagged Samples?
    Check this box to automatically exclude samples from processing if they have an Autosomal concentration to Y concentration ratio higher than the Auto/Y ratio flag threshold. Uncheck this box to include samples for processing regardless of their Autosomal concentration to Y concentration ratio.

4. Flag high Auto/Deg ratios?
    Check this box to flag samples if they have an Autosomal concentration to Degradation concentration ratio higher than the Auto/Deg ratio flag threshold. Uncheck this box if you do not wish to flag samples regardless of their Autosomal concentration to Degradation concentration ratio. This setting is only applied if sample concentration data sets contain Degradation concentration data.

5. Auto/Deg ratio flag threshold
    Use this setting to specify a minimum Autosomal concentration to Degradation concentration ratio for which samples will either be flagged or excluded from processing. Whether samples are flagged or excluded based on this setting is determined by additional settings in the Quality Markers group.

6. Exclude Auto/Deg Flagged Samples?
   Check this box to automatically exclude samples from processing if they have an Autosomal concentration to Degradation concentration ratio higher than the Auto/Deg ratio flag threshold. Uncheck this box to include samples for processing regardless of their Autosomal concentration to Degradation concentration ratio. This setting is only applied if sample concentration data sets contain Degradation concentration data.

7. Flag high IPC Value?
   Check this box to flag samples based on their IPC Cq value. Individual sample IPC Cq values are compared to the average IPC Cq value for all samples within the run. The sample will be flagged if the deviation of the sample IPC Cq value from the average IPC Cq value for all samples in this run is equal to or greater than the setting for maximum deviation from the average IPC Cq. Uncheck this box if you do not wish to flag samples regardless of their IPC Cq value.

8. Maximum deviation from average IPC
   Use this setting to specify a maximum deviation from the average IPC Cq value for all samples within this run for which samples will be flagged or excluded from processing. Whether samples are flagged or excluded based on this setting is determined by additional settings in the Quality Markers group.

9. Exclude high IPC Samples?
   Check this box to automatically exclude samples from processing if they exceed the maximum deviation from the average IPC Cq. Uncheck this box to include samples for processing regardless of their IPC Cq value.

4.1.5   Samples

1. Maximum sample volume to consume
   Use this variable to specify the maximum volume that may be consumed from the original sample, per replicate amplification setup. This value limits the volume that can be used for both direct transfer and dilution of samples prior to amplification setup. Ideally, this value will be equal to or greater than the maximum sample volume allowed in the amplification reaction.

   Setting a maximum sample volume to consume that is less than the sample volume allowed in the reaction may cause restrictions in sample normalization. One other factor to keep in mind is that the maximum sample volume to consume is per replicate. So if a maximum volume to consume of 15µl and a replicate number of 3 are set, the method could potentially consume 45µl of that sample.

2. Sample Replicates
   Specify the number of replicate Promega STR reactions that should be prepared for each sample. Values of 1 to 3 replicates are possible. The entire set of samples is placed on the amplification plate as a group, and then the entire group is replicated (Figure 18). As the number of sample replicates increases, the total number of reactions that can be processed on a given amplification plate decreases.

3. Are samples in Casework Direct Buffer?
   Check this box if samples are provided in Casework Direct Buffer for this run. These samples will use liquid handling specific to the Casework Direct Buffer. If samples are purified nucleic acid, leave this box unchecked.

4. Matching text to identify reagent blanks.
   Enter a text string that will be used to identify reagent blanks in input sample identifiers. If this string is contained with any sample identifier, the sample will be identified as a reagent blank sample that should not be processed for amplification reaction setup.

5. Quantitation target count
   Specify the number of quantitation targets included in the concentration data set. This variable can have a value of 3 (Autosomal, Y and IPC targets) or 4 (Autosomal, Y, Degradation and IPC targets).

4.1.6   STR

1. Is STR kit lot required?
   Check this box if you want to require that users scan or enter kit lot information for the Promega STR kit being used for this run. Uncheck this box if you do not want to require users to enter kit lot information for the Promega STR kit being used for this run.

2. Must STR kit be within expiration?
   Check this box if you want to require that the STR kit lot scanned at the beginning of the method be within the expiration date. If checked, the user will not be able to proceed throughout method setup unless the STR kit is within its expiration date.

5   Troubleshooting

For questions not addressed here, please contact your local Promega Branch, Office or Distributor. Contact information available at: **www.promega.com**. Email: **techserv@promega.com**

Symptoms	Causes and Comments
Cannot modify method run parameters	The most likely cause of this is that the administrator has disabled the ability of a user to modify this option in the variant method being run. If the administrator has specified that users cannot change the value of a specific variable during a run, it will not be possible to alter the value at run time. Check the settings specified for the variant method that is being run.
The desired labware type is not an available input or output labware option	Many common labware types are available; however, if a specific labware used in the laboratory is not present, contact Promega Technical Services to help resolve this issue.
Cannot advance to the next instrument setup screen	Confirm that all check boxes present on the screen are checked. If at a sample entry screen, confirm that the number of sample positions indicated in the input sample labware exactly matches the number of samples that was set for the run. It is not possible to proceed from a labware setup screen if there is a mismatch between the number of samples that can be processed and the sample number specified.
Instrument error or crash during method run	Double-check that the labware specified during instrument setup exactly matches the labware being used for the method run. Plate movement and pipetting are dependent on precise labware definitions. Do not substitute "similar" labware for the labware specified in the method.
Inaccurate pipetting	Make sure that Daily and Weekly Maintenance methods are being run on a regular basis. They will check that the pipetting functions of the Maxprep® Liquid Handler are working within expected parameters.

6   Appendix

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

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Figure 34. Plate map well numbering conversion. On the side and top of the plate image are the alphanumeric row and column labels. Within the wells are the corresponding numeric designations for each well.

7 Related Products

PRODUCT	SIZE	CAT.#
Nunc 2.0ml Deep Well Plates (Sterile)	60/pack	AS9307
2.0ml Deep Well Plates (Non-Sterile)	60/pack	AS9309
Maxwell® RSC Plunger Pack	1 each	AS1670
Maxprep® 1000µl Conductive Disposable Tips, Filtered	40/pack (3,840 Tips)	AS9303
Maxprep® 300µl Conductive Disposable Tips, Filtered	60/pack (5,760 Tips)	AS9302
Maxprep® 50µl Conductive Disposable Tips, Filtered	60/pack (5,760 Tips)	AS9301
Maxprep® 3-Position Reagent Tube Holder	1 each	AS9409
Maxprep® Plunger Holder	1 each	AS9408
Maxprep® Reagent Reservoir, 50ml	28/pack	AS9304
Maxprep® Waste Bags, Clear	100/pack	AS9305
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® FSC Deck Tray	1 each	AS4016
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® FSC Instrument	1 each	AS4600
Maxwell® CSC Instrument	1 each	AS6000
Maxwell® CSC 48 Instrument	1 each	AS8000
1.1ml, Square-Well V-Bottom Deep Well Plate	25/pack	V6821

8 Summary of Changes

The following changes were made to the 12/24 revision of this document:

1. Added catalog # AS9105 and AS9205.

2. Made general text edits throughout document.

3. Updated document font.

It is the manufacturer's responsibility to provide equipment electromagnetic compatibility information to the customer or user.

It is the user's responsibility to ensure that a compatible electromagnetic environment for the equipment can be maintained in order that the device will perform as intended.