Agilent

ZAG DNA Analyzer

System Manual

Notices

Document Information

Document No: D0002113 Rev. B
EDITION 01/2022

Copyright

© Agilent Technologies, Inc. 2020-2022

No part of this manual may be reproduced in any form or by any means (including electronic storage and retrieval or translation into a foreign language) without prior agreement and written consent from Agilent Technologies, Inc. as governed by United States and international copyright laws.

Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051

Trademark acknowledgements

ZAG DNA Analyzer system is a trademark of Agilent Technologies, Inc.

ProSize data analysis software is a registered trademark of Agilent Technologies, Inc.

Windows is a registered trademark of Microsoft Corporation

Revisions and Updates

This manual is subject to change without notice. This manual may be revised and updated periodically as components and/or maintenance procedures are modified. These updates will be provided to all manual holders on record. Contact Agilent if you have questions regarding availability of updates. Please take care to keep your contact information current with Agilent so that revisions and updates can be provided in a timely manner.

For Research Use Only

Not for use in Diagnostic Procedures.

Warranty

The material contained in this document is provided "as is," and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

U.S. Government Restricted Rights. Software and technical data rights granted to the federal government include only those rights customarily provided to end user customers. Agilent provides this customary commercial license in Software and technical data pursuant to FAR 12.211 (Technical Data) and 12.212 (Computer Software) and, for the Department of Defense, DFARS 252.227-7015 (Technical Data - Commercial Items) and DFARS 227.7202-3 (Rights in Commercial Computer Software or Computer Software Documentation).

Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In This Guide

Agilent has prepared this manual as a technical reference for the ZAG DNA Analyzer system.

This document includes system overviews, analytical methods, maintenance procedures, software operation, troubleshooting guide, and instrument shutdown procedures. Additional information includes literature references, instrument specification and utility requirements, parts and supply lists, product specification sheets, and system warranty information.

This document is intended for use by technical personnel that are proficient with analytical instrumentation operation and upkeep. A certain level of training and expertise is assumed and fundamentals are not addressed herein. Procedures are presented in a step-by-step format using photos and screen captures. If questions remain after reviewing a given procedure, contact your Agilent Support Representative.

1 System Overview

This chapter gives an instrument overview.

2 ZAG DNA Analyzer Software – File Menu

This chapter describes the ZAG DNA Analyzer software in more detail on the commands of the File menu.

3 ZAG DNA Analyzer Software – Admin Menu

This chapter describes the ZAG DNA Analyzer software in more detail on the commands of the Admin menu.

4 ZAG DNA Analyzer Software – Utilities Menu

This chapter describes the ZAG DNA Analyzer software in more detail on the commands of the Utilities menu.

5 ZAG DNA Analyzer Software – Help Menu

This chapter describes the ZAG DNA Analyzer software in more detail on the commands of the Help menu.

6 ZAG DNA Analyzer Software – Operation Tab

This chapter describes the ZAG DNA Analyzer software in more detail on the Operation tab.

7 ZAG DNA Analyzer Software – Run Status Tab

This chapter describes the ZAG DNA Analyzer software in more detail on the Run Status tab.

8 ZAG DNA Analyzer Capillary Array

This chapter explains the essential operational parameters of the capillary array.

9 ZAG DNA Analyzer Software – Sample Name Entry

This chapter provides information on how to enter the sample names in the ZAG DNA Analyzer software.

10 ZAG DNA Analyzer Software – Using the Bar-Code Reader

This chapter explains how to add and remove solutions to the gel and conditioning solution bottles using the bar-code reader.

11 ZAG DNA Analyzer Software – Automated Analysis

This chapter explains the procedure for automated analysis using the ZAG DNA Analyzer system.

12 Appendix

This chapter provides additional information on part numbers, maintenance procedures, and system settings.

Content

1 System Overview 7

About the System 8

Configured ZAG DNA Analyzer System Dimensions 9

ZAG DNA Analyzer System Connections 10

ZAG DNA Analyzer External Cabinet 12

Top Compartment 13

Side Compartment 14

Drawers 16

ZAG DNA Analyzer Loading and Orientation of 96-Well Plates 18

ZAG DNA Analyzer Loading Samples 19

2 ZAG DNA Analyzer Software – File Menu 20

About this Software 21

System Requirements 22

System Installation 23

Opening the ZAG DNA Analyzer Software 24

Main Screen Toolbar 26

File Menu 27

3 ZAG DNA Analyzer Software – Admin Menu 31

Admin Menu 32

4 ZAG DNA Analyzer Software – Utilities Menu 45

Utilities Menu 46

5 ZAG DNA Analyzer Software – Help Menu 58

Help Menu 59

6 ZAG DNA Analyzer Software – Operation Tab 61

Operation Tab Overview 62

7 ZAG DNA Analyzer Software – Run Status Tab 77

Run Status Tab Overview 78

8 ZAG DNA Analyzer Capillary Array 85

Capillary Array Parts 86

Removal of the Capillary Array 87

Unpacking a new Capillary Array 98

Capillary Array Installation 101

Using the External Array Docking Station for Storage 111

9 ZAG DNA Analyzer Software – Sample Name Entry 116

Sample Name Entry 117

10 ZAG DNA Analyzer Software – Using the Bar-Code Reader 123

Adding/Removing Solutions to the gel and conditioning solution bottles 124

Changing Levels of Water or 1 N NaOH 127

Changing Levels of Conditioning Solution or Waste 128

11 ZAG DNA Analyzer Software – Automated Analysis 129

Automated Analysis 130

12 Appendix 138

Permissible Characters 139

Compatible Plates and Tubes 140

Preventative Maintenance Schedule 142

Capillary Array Cleaning 144

Reservoir Vent Valve Cleaning 150

Capillary Array Window Cleaning 151

1 System Overview

About the System 8

Configured ZAG DNA Analyzer System Dimensions 9

ZAG DNA Analyzer System Connections 10

ZAG DNA Analyzer External Cabinet 12

Top Compartment 13

Side Compartment 14

Drawers 16

Drawer Status 17

ZAG DNA Analyzer Loading and Orientation of 96-Well Plates 18

ZAG DNA Analyzer Loading Samples 19

This chapter gives an instrument overview.

About the System

The ZAG DNA Analyzer system is a multiplexed capillary electrophoresis (CE) instrument for performing automated, high throughput separation and relative quantification of double stranded nucleic acids (DNA). Separation is achieved by applying an electric field through a narrow bore (50 m i.d.) fused silica capillary array filled with various conductive gel matrices designed to sieve DNA molecules of a specific size range. When a high voltage is applied to the capillary array, injected DNA migrates differentially through the gel matrix as a function of length or size, with smaller sized fragments eluting faster than larger sized fragments.

At a point toward the far end of the capillary array, detection of the separated DNA is achieved by fluorescence of a sensitive intercalating dye present in separation gel matrix, which fluoresces when bound to double stranded DNA molecules. The ZAG DNA Analyzer utilizes a high intensity light emitting diode (LED) excitation light source that is focused across the capillary array detection window and imaged onto a sensitive, two-dimensional charge-coupled device (CCD) detector. By monitoring the relative fluorescence unit (RFU) intensity as a function of time during the CE separation, digital electropherogram traces representative of the DNA content of an entire plate of 96 samples can be collected in a single experimental run.

Configured ZAG DNA Analyzer System Dimensions

This chapter provides a basic overview of the ZAG DNA Analyzer hardware and operation. **Figure 1** shows an external view of a fully configured ZAG DNA Analyzer system, which has a compact footprint of 55” on the bench top with a weight of 147.5 lbs (67 kg).

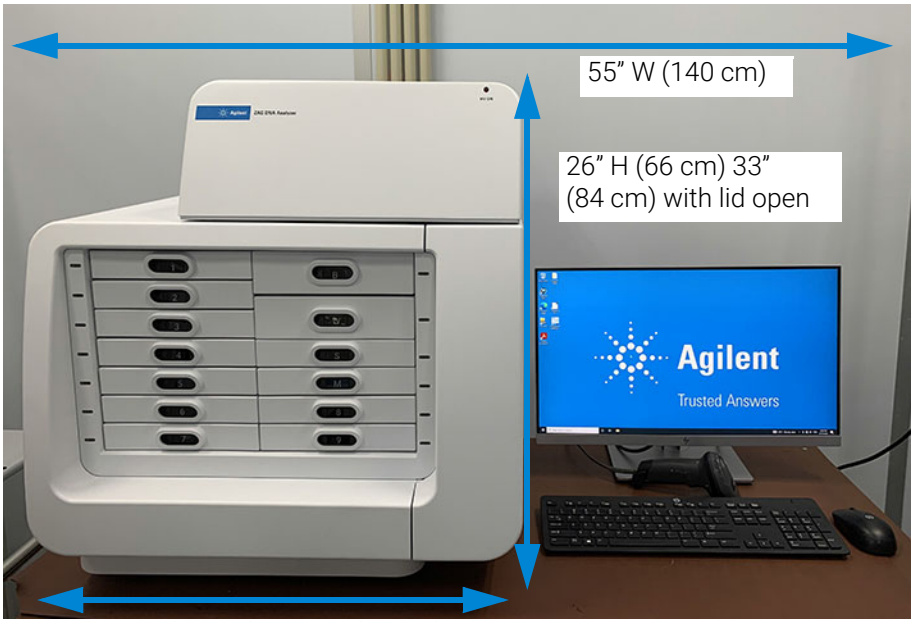


Figure 1 Configured ZAG DNA Analyzer system with computer workstation

ZAG DNA Analyzer System Connections

The back of the ZAG DNA Analyzer contains the communications panel where necessary connections are made to the instrument computer (PC) and electrical outlet for operation (**Figure 2** and **Figure 3**).

The instrument utilizes a standard 120V, 20 Amp, 3-prong outlet.

The use of a double-conversion surge protection or uninterrupted power supply (UPS) device is highly recommended. Contact the corresponding Agilent Sales/Service Representative for specific recommended models.

A minimum of three standard electrical wall outlets should be available to connect the instrument, computer and accessories, although a power strip can be used in place of separate wall outlets if needed.

Each connection is labeled on the PC. The various connections between the system and the ZAG DNA Analyzer are summarized below:

- From the ZAG DNA Analyzer System:
  - Two USB connections to PC USB

    The order/location of connections is critical and the locations have been identified on both the computer and the ZAG DNA Analyzer.
  - Power cord to grounded electrical outlet
- From the PC:
  - Two USB connections to the ZAG DNA Analyzer

    The order/location of connections is critical and the locations have been identified on both the computer and the ZAG DNA Analyzer.
  - Power cord to grounded electrical outlet
  - Connection to monitor, keyboard, mouse etc.

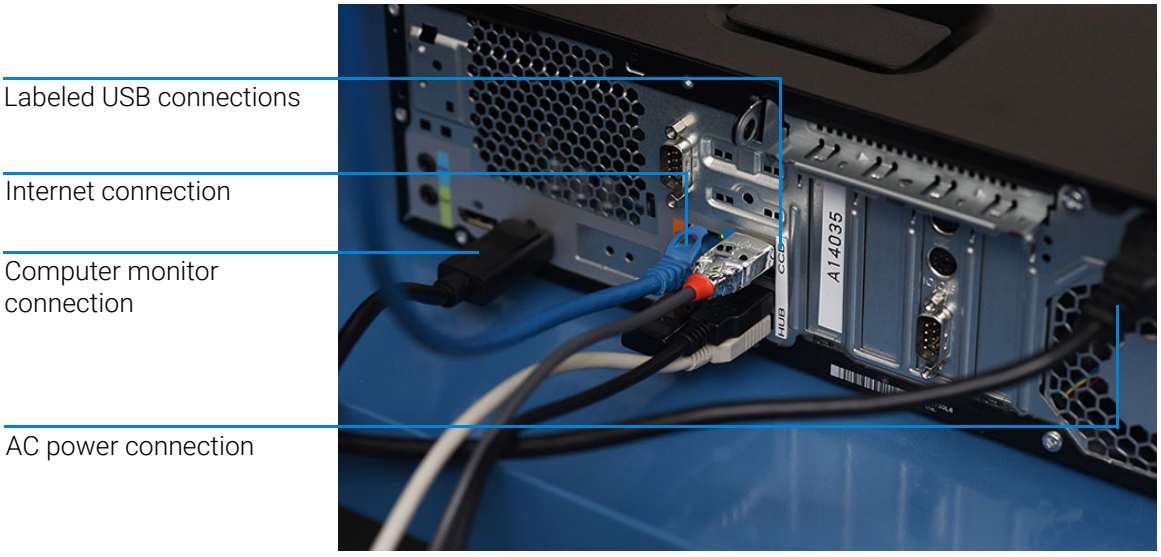


Figure 2 Back of panel of the ZAG DNA Analyzer computer connections

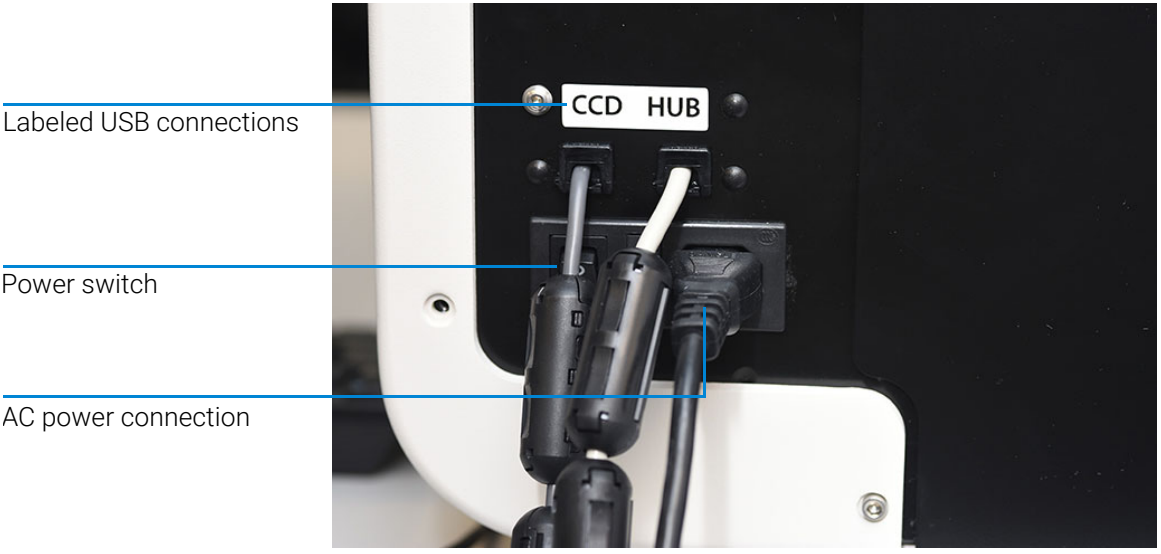


Figure 3 Back of panel of the ZAG DNA Analyzer connections

ZAG DNA Analyzer External Cabinet

There are three primary points of access to the inside of the ZAG DNA Analyzer: the top compartment, the side compartment access door, and the drawers (13 total) (**Figure 4**).

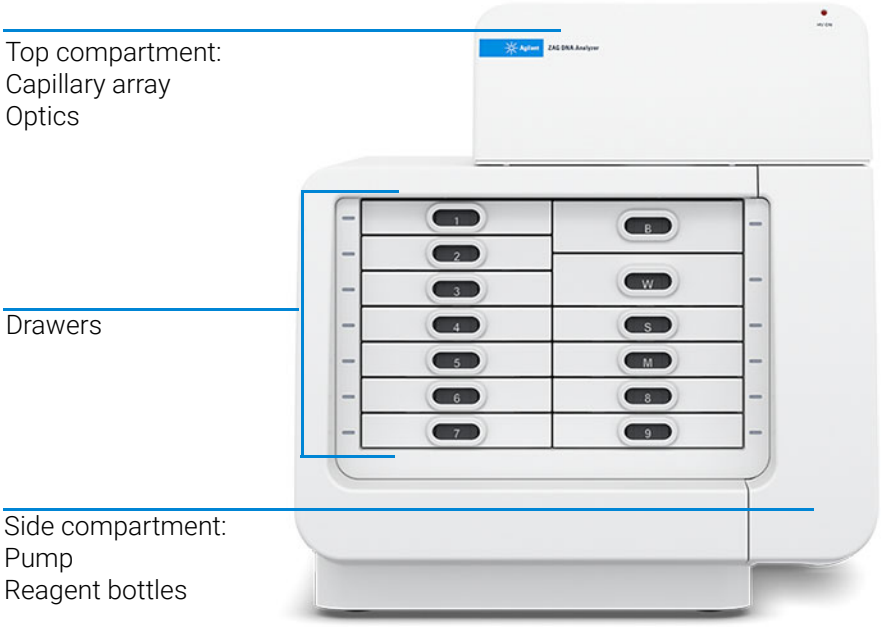


Figure 4 Entry points of the ZAG DNA Analyzer system

Top Compartment

The top compartment provides access to the optical detection platform and 96-capillary array cartridge. A non-accessible compartment on the back of the instrument contains the high voltage power supply, electronics, and the safety interlock system. The safety interlock system shuts off the high voltage in case this door is opened while the instrument is running.

The 96-capillary array cartridge is a replaceable, modular component of the ZAG DNA Analyzer. The user can easily exchange the capillary array cartridge. This process is explained in **Chapter 4**, “ZAG DNA Analyzer Software – Utilities Menu”.

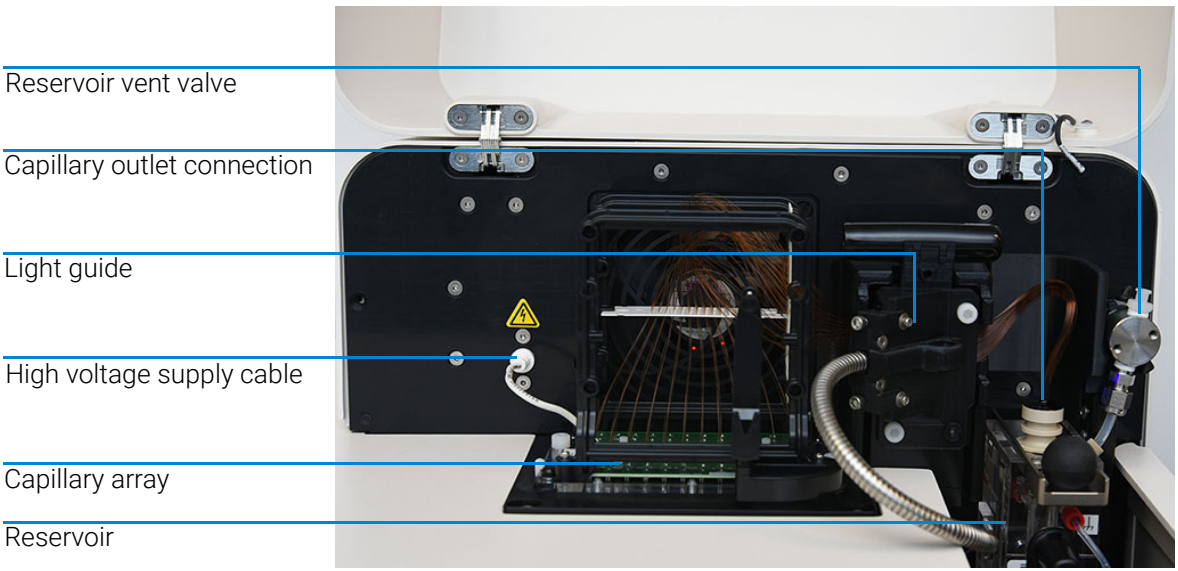


Figure 5 ZAG DNA Analyzer main unit top compartment open

Side Compartment

The side compartment allows access to the high pressure pump, syringe, waste bottle, conditioning solution and gel solutions (gel 1 and gel 2).

The High-Pressure Syringe Pumping system provides automated flushing and filling of the capillary array with conditioning solution and separation gel between experimental CE runs, providing pressurization of the capillaries up to 280 psi.

The system design enables vacuum injection (hydrodynamic injection) of samples in addition to traditional electrokinetic (voltage) sample injection - a feature unique to the ZAG DNA Analyzer and advantageous when working with samples containing high salt matrices.

Two different solutions are fed to and pumped through the capillary array during routine operation:

- **Capillary Conditioning Solution**
- **dsDNA Separation Gel** (gel 1 or gel 2)

The appropriate solution is selected for pumping by way of a 6-way distribution valve.

The system also contains a waste bottle, which collects separation gel and capillary conditioning solution pumped via the waste line from the capillary array reservoir during the filling process.

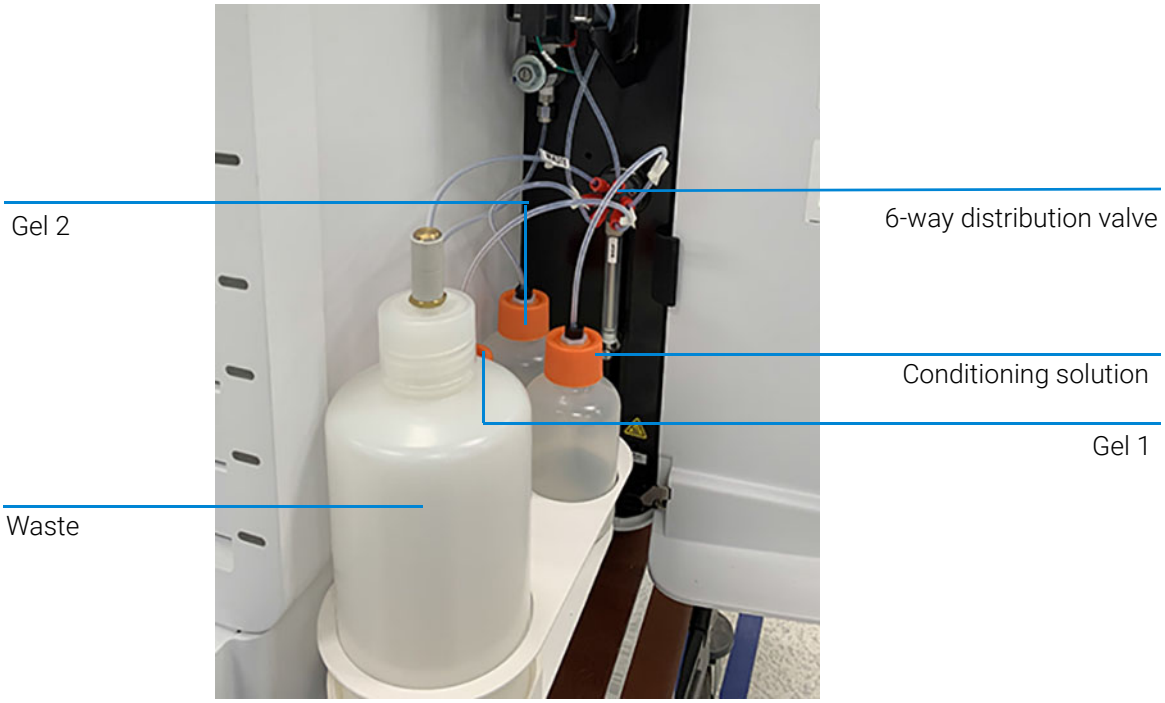


Figure 6 Side door compartment

The three fluid line connections inside the ZAG DNA Analyzer are:

- *Gel line* (gel 1 or gel 2) from syringe pump to gel bottle (gel 1 or gel 2)
- *Conditioning fluid* from syringe pump to conditioning fluid
- *Overflow waste line* from syringe pump to waste bottle

Drawers

The ZAG DNA Analyzer front-panel drawers provide an external interface for loading *Buffer*, *Waste*, *Storage*, *Marker* and *Sample 96-Well Plates* into the system. These can be broken into two types of drawers, system drawers and sample drawers.

System Drawers:

- *Drawer B* (top right drawer): This location is used for the *Inlet Buffer Tray* used during the CE separation.
- *Drawer W* (second drawer from top on right): This location is utilized for a *Waste Tray* when performing a capillary conditioning method where the capillary array is flushed with *Capillary Conditioning Solution* or *Gel*. This tray is also used for an array conditioning between runs.
- *Drawer S* (third drawer from top on right): This location is utilized for the *Storage Solution Tray* when the system in not in use.
- *Drawer M* (third drawer from top): This location is used for loading the *Lower/Upper DNA Marker Tray*.

Sample Drawers:

- *Drawer 1 -9*: These locations are utilized for 96-*Sample Plates 1* through *Sample Plate 9*.

Drawer Status

Status	Description
Drawers B and W are Interlocked	When any of the top two drawers are open, the high-voltage will automatically shut off.
Drawers S, M, 1 through 9 are not interlocked	Sample trays can be exchanged while the instrument is in operation.

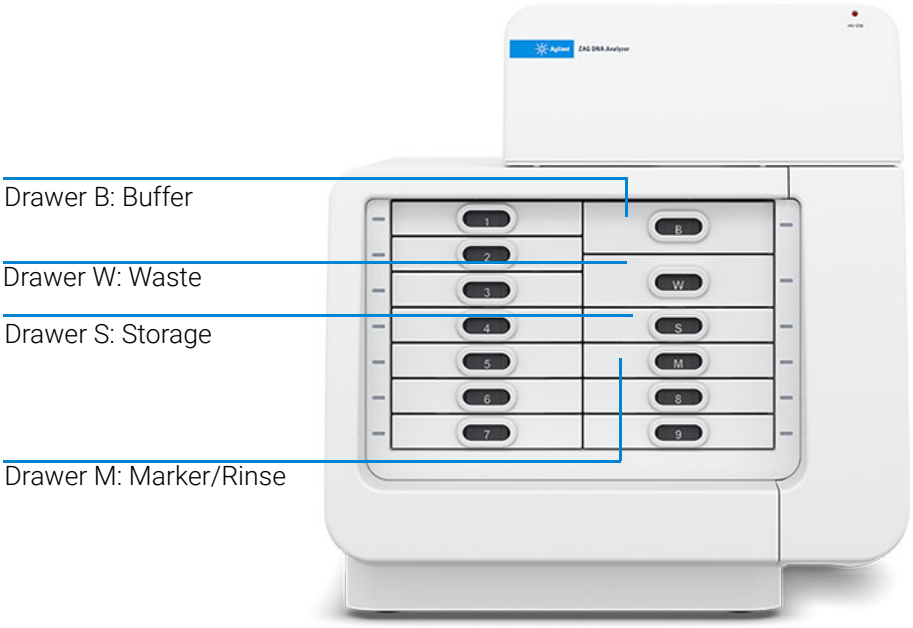


Figure 7 Instrument drawer positions

ZAG DNA Analyzer Loading and Orientation of 96-Well Plates

The ZAG DNA Analyzer is a multiplexed CE system containing a 96-capillary array, which is designed to interface directly with an entire plate of a standard 96-well plate footprint.

Each capillary of the array corresponds to a specific well for a given row in the 96-well sample plate. The capillary array orientation is indexed such that capillary #1 corresponds to well A1, and capillary #96 to well H12.

Well A1 of the 96-well plate should always be oriented to the back left location of the instrument drawer to ensure that the sample well location is correctly assigned and reported in the software.

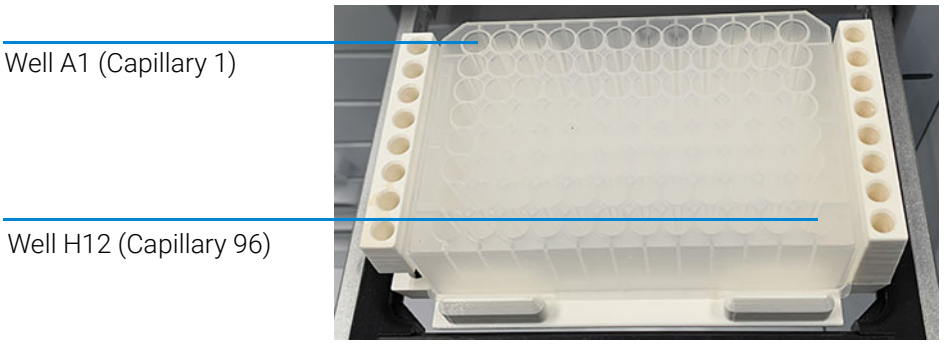


Figure 8 Proper orientation when loading 96-well marker and sample plates

Each drawer location houses a tray carrier containing alignment pins for ensuring proper alignment of the 96-well plate when placed against the capillary array.

The ZAG DNA Analyzer has been designed to operate using specific dimensions and styles of plates.

Plates with similar dimensions may be used, but capillary damage may occur with the use of poor-quality PCR plates.

For a list of compatible PCR plates, refer to **“Compatible Plates and Tubes”** on page 140.

ZAG DNA Analyzer Loading Samples

The ZAG DNA Analyzer requires a minimum volume of 20 µL/well in the sample plate for proper injection.

When preparing lower/upper DNA marker plates for repeated use, a volume of 30 µL/well with a 20 µL mineral oil overlay is recommended.

Ensure the sample has been adequately mixed with the dilution buffer before loading on the instrument.

Vortexing sample is the best way to ensure adequate mixing before analysis.

Check the wells of the sample plate/s after pipetting to ensure that there are no air bubbles trapped in the bottom of the wells. The presence of trapped air bubbles can lead to injection failures.

Air bubbles can be removed from the plates by introducing a brief centrifugation step prior to placing the plates into the tray carrier.

Individual tips are given in each Kit Guide for reference.

2 ZAG DNA Analyzer Software – File Menu

About this Software 21

System Requirements 22

System Installation 23

Opening the ZAG DNA Analyzer Software 24

Main Screen Toolbar 26

File Menu 27

File Manager 27
ZAG DNA Analyzer software 30
Logout 30
Exit 30

This chapter describes the ZAG DNA Analyzer software in more detail on the commands of the File menu.

About this Software

The ZAG DNA Analyzer system employs proprietary software for operation and data analysis.

This software is preloaded on the instrument and checked prior to shipment as part of the instrument qualification.

System Requirements

The software is run under a Windows 10 PC with the following requirements:

Table 1 Minimum computer requirements

Type	Specification
Processor	Intel Core i5 – 8500
SVGA Video	Minimum Resolution 1024 X 768
Memory	4 Gigabytes (1 x 4 GB) DDR4-2666
Available Hard Disk Space	500 Gigabytes
USB Serial Ports	6 ports (2 instrument, keyboard, mouse)
Network	If not using a local database, a network connection to the database server host is desired.

System Installation

To install the ZAG DNA Analyzer software:

1. Place the USB in the USB drive. Navigate to **ZAG DNA Analyzer Installer > setup.exe**, and double-click **setup.exe**.
2. Follow the setup instructions provided by the installation wizard. The default installation directory is C:\Agilent\ZAG DNA Analyzer.

Opening the ZAG DNA Analyzer Software

1 To log into the software, select the ZAG DNA Analyzer software icon (**Figure 9**).

Figure 9 ZAG DNA Analyzer software icon

There are two levels of users available:

- **Administrator**: The administrator login has enhanced access to functions such as allowing the user to edit separation methods.
- **User**: The user login has restricted access that allows only routine operation of the instrument.

2 To login to the ZAG DNA Analyzer software, type **Administrator** or **User** into the **User ID** field of the login window (**Figure 10**).

3 Enter your password.

The first time you login, the password information is left blank.

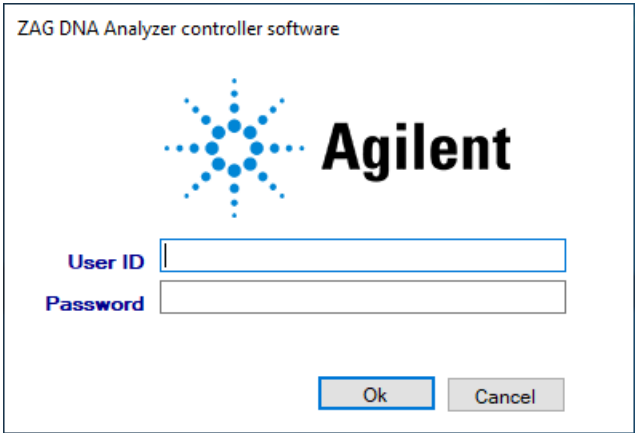


Figure 10 Login menu

4 Click **OK**.

The main screen opens.

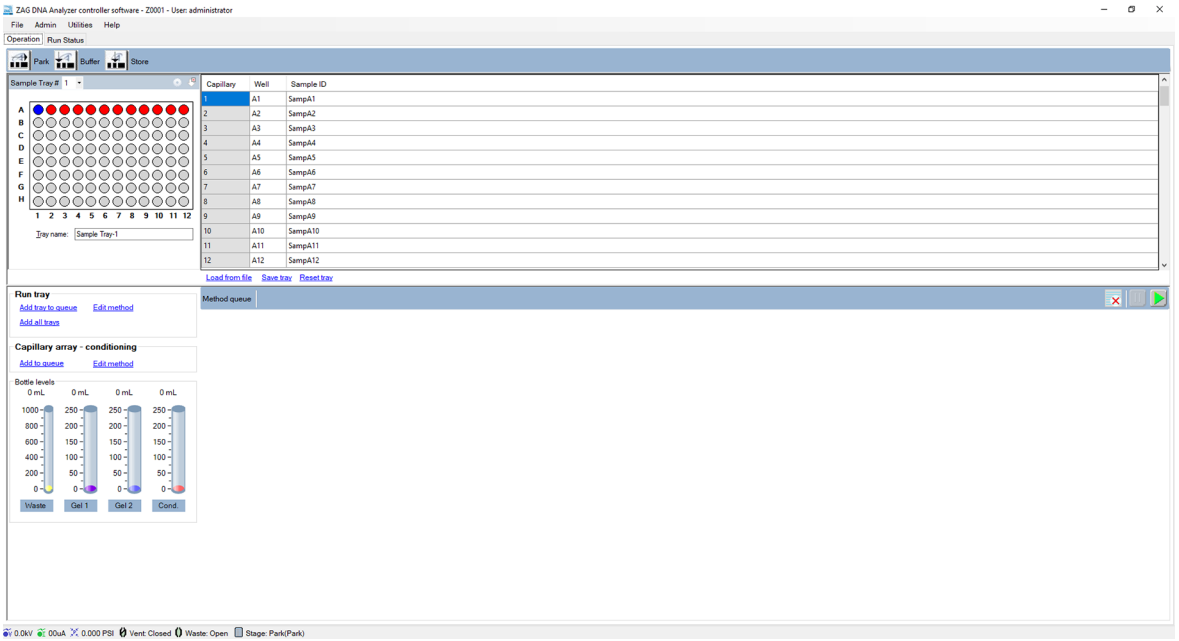


Figure 11. ZAG DNA Analyzer software main screen

More information about the **User** and **Administrator** functions within the software will be discussed in a later chapter.

A password can be set for the system during the Agilent training and installation period at your facility or by using the **Change Password** command of the **Admin** menu described in section **"Change Password"** on page 33.

The login information is used in event and error logging to aid in controlling access to the system, tracking usage and monitoring changes to the system.

Every time you log into the ZAG DNA Analyzer software, you begin on the main screen (**Figure 11**).

Main Screen Toolbar

The main screen toolbar is located at the top of the ZAG DNA Analyzer software main screen as seen in **Figure 11**.

File Menu

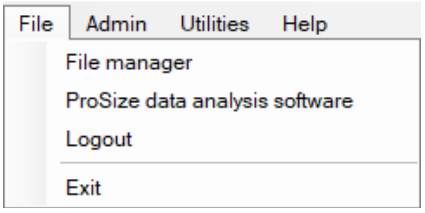


Figure 12 File menu functions

File Manager

The **File manager** command allows electropherogram data to be examined within the ZAG DNA Analyzer software environment.

Files are normally analyzed using the ProSize Data Analysis software, which is covered in the *ProSize Data Analysis Software User Manual*.

The file manager also enables you to correct the capillary alignment for an individual data file.

Selecting the **File manager** function opens a window allowing the user to navigate to a data file. Once a file is selected, the file manager screen opens (**Figure 13**).

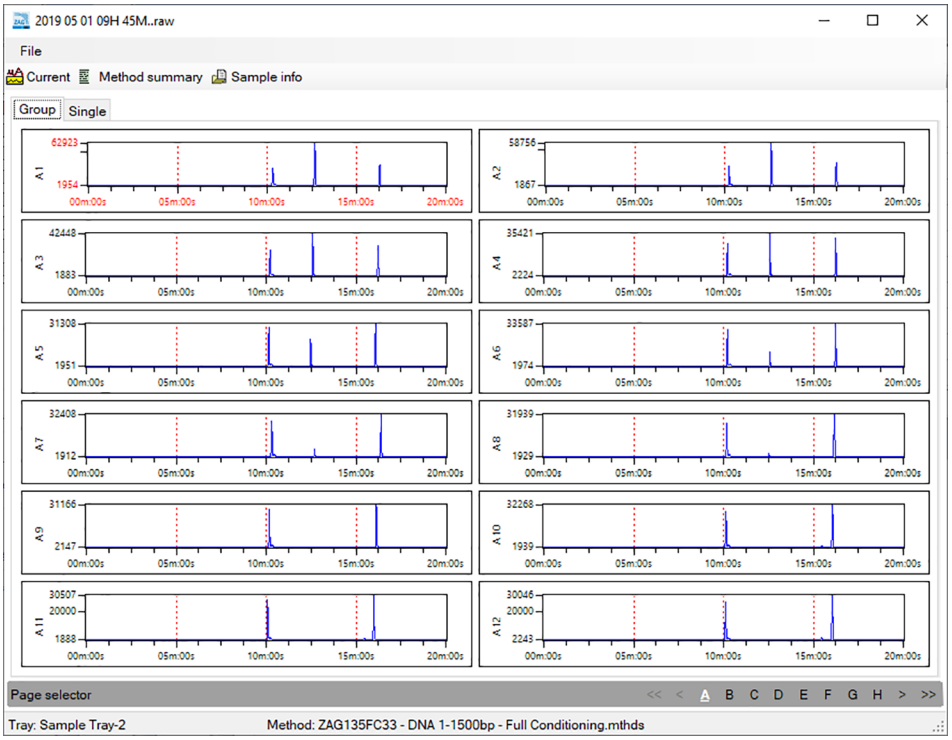


Figure 13 File manager window

The functions of the file manger screen are reviewed in **Table 2**.

Table 2 File manager – file functions

Field	Description
Open	Opens a Windows dialog to navigate to desired data file.
Cap. Alignment	Allows the user to view and manipulate the capillary alignment for the data file opened only. Capillary alignment from a file is discussed in section **"Capillary Alignment"** on page 47.
Print	Allows the user to print the twelve electropherograms to a page.
Exit	Closes the File Manager window.

The **Current**, **Method summary**, and **Sample info** toolbar functions are discussed in **Table 3**.

Table 3 File manager toolbar options

Field	Description
Current	Selecting **Current** allows user to view the current of the separation during the analysis.
Method summary	Selecting **Method summary** shows a summary of the method that was used for the separation.
Sample info	Selecting **Sample info** option shows the user the sample names input for the separation file.

Once the data file is opened in the file manager, the data can be viewed in groups of 12 by row when the **Group** tab is selected. A page selection is located at the bottom of the screen allowing for navigation of all rows in a plate (assuming 96-capillary array data is chosen).

To view a single electropherogram at a time either double left-click on the desired well or select the **Single** tab. A page and well selection is located at the bottom of the screen allowing for navigation of all rows and wells in the plate.

Electropherogram data can be panned, zoomed in, or zoomed out by right-clicking on the chart and selecting the function of interest.

ZAG DNA Analyzer software

Selecting this command opens the ZAG DNA Analyzer software.

Logout

The **Logout** command allows the user to log out the ZAG DNA Analyzer software and to log in as a different user.

After logout the login menu opens (**Figure 10**).

Exit

The **Exit** command closes the ZAG DNA Analyzer software. Alternatively, the user can exit the program by selecting the red **X** at the top right corner of the main screen.

3 ZAG DNA Analyzer Software – Admin Menu

Admin Menu 32
Change Password 33
User Maintenance 34
Archive and Purge Database 35
Configuration 36
Results Report Setup 40
Event report 41
Error Report 43

This chapter describes the ZAG DNA Analyzer software in more detail on the commands of the Admin menu.

Admin Menu

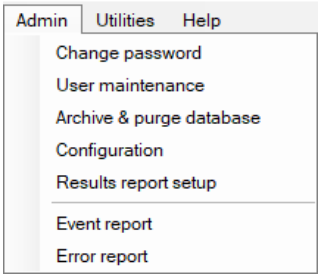


Figure 14 File menu functions

Change Password

The command **Change password** opens the window shown in **Figure 15**.

Changing the password is accessible to all users.

Password requirements:

- Maximum password length is 40.
- Password can contain letters or numbers.
- Passwords are case insensitive.

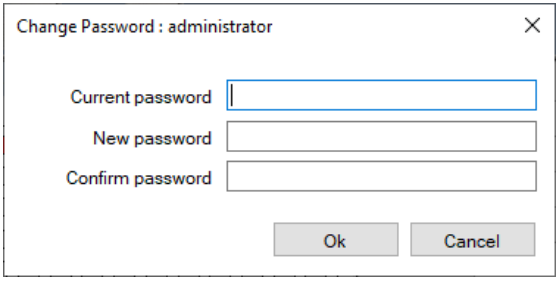


Figure 15 Change password menu

User Maintenance

The command **User maintenance** opens the **User Maintenance** window (**Figure 16**).

In this window, the administrator can add, delete, and modify all users that can access the ZAG DNA Analyzer software.

1 To edit the settings, select the pencil icon .

2 After editing, and if all entries are acceptable to the user, select the check mark .

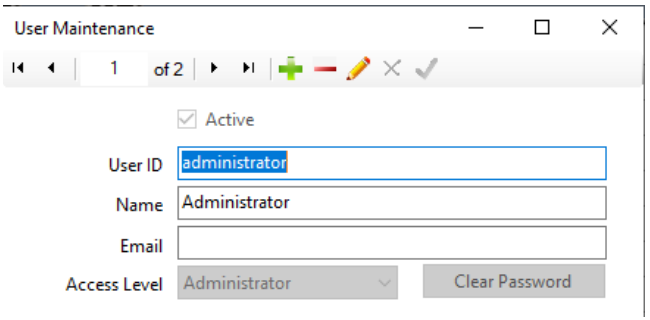


Figure 16 User maintenance window

A summary of the parameters in the **User maintenance** window is illustrated in **Table 4**.

Table 4 User maintenance window parameters

Field	Description
User ID	User ID for login or signature. This ID must be unique for the system.
Access Level	Set the user access level to user or administrator.
User Name	The full name of the user.
Email	Users email address (optional).
Active	Select the check box to activate the user and its user ID. If cleared: The user ID cannot be used.
Clear Password	Sets the Users login password to blank. If a minimum password length has been set, the user will need to change their password on login.

Archive and Purge Database

The **Archive & purge database** command is used to maintain the event and error log database.

Event and error logs are saved in the database and can be retrieved for advanced troubleshooting.

This function allows the user with administrative rights to back-up the data for future use in a different location or on an external storage device.

Configuration

Selecting the **Configuration** command opens the **Configuration settings** window where the administrator can modify **Security Settings**, **Device Settings**, and **Bottle Volumes** for the system.

The **Security Settings** tab allows the administrator to modify the login requirements for all users (**Figure 17**).

A summary of the configurations in the **Security Settings** tab is provided in **Table 5**.

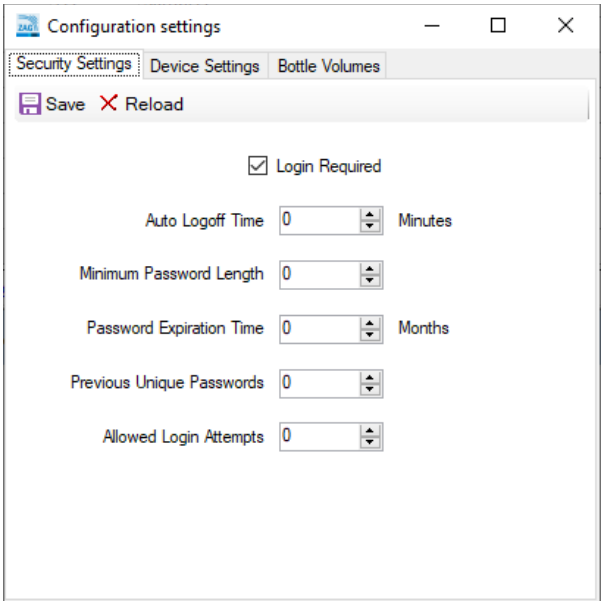


Figure 17 Configuration – Security Settings tab

Table 5 Configuration – Security Settings tab functions

Configuration Option	Range	Description
Login Required	True or False	If True: User must log to the software. If False: No login is required for user level access.
Minimum Password Length	0 to 12	The password must exceed this number of characters.
Maximum Number of Login Attempts	0 to 12	If a user attempts to login with an invalid password, after this many attempts: • That user ID will be made inactive and the error logged. • The failed login attempt is recorded in the event log. • The software is shut down.
Time to Change Passwords	0 to 36 Months	A password (login ID and signature) will expire after the set number of months. If set to zero, there is no password expiration.
Auto Logoff Time	0 to 30 Minutes	If the software is left unattended for length of time, the current user will be logged off. If set to zero, there is no automatic logoff.
Number of Previous Passwords	0 to 4	When a user changes their password, they may not select from this number of previously used passwords. If set to zero, there is no previous used password restriction.

The **Device Settings** tab allows modification of the device settings (**Figure 18**).

The settings should be updated whenever a new capillary array cartridge is installed.

A summary of the configuration options in the **Device Settings** tab is provided in **Table 6**.

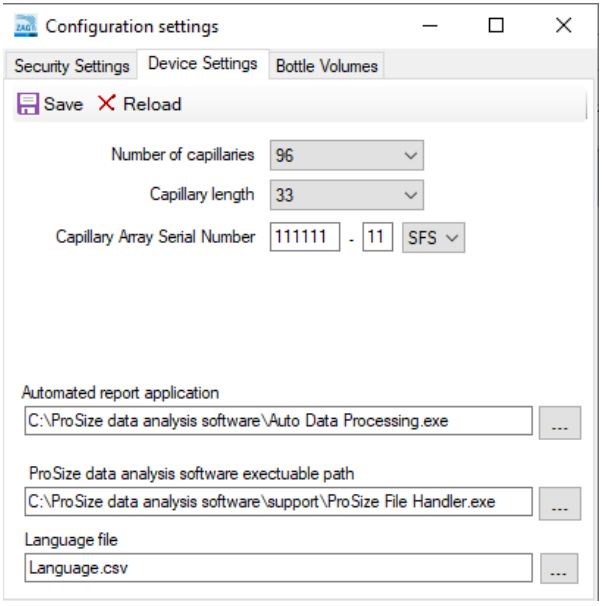


Figure 18 Configuration – Device Settings tab

Table 6 **Configuration – Device Settings tab functions**

Parameter	Access Level	Description
Number of capillaries	Administrator	Value: 96 Note that only a 96 capillary well format is allowed.
Capillary length	Administrator	Value: 33, or 55 Note that this length refers to the effective length of the capillaries in use.
Capillary Array Serial Number	Administrator	The format must be alpha-numeric: 6-digit, 2-digit, 3 alpha
Automated report application	Administrator	Allows for changing the file path used for the automated report application.
ProSize data analysis software executable path	Administrator	Allows for changing the file path used to open the ProSize data analysis software.
Language file	Administrator	Allows user to browse and select a language file for the ZAG DNA Analyzer software (default is english).
Save	Administrator	Saves the chosen settings.
Reload	Administrator	Reloads the previously saved settings.

The **Bottle Volumes** tab allows modification of the reagent bottle volumes (**Figure 19**).

The gel 1, gel 2, conditioning, and waste bottles can be set from 50 mL to 5000 mL by entering the appropriate volume of the container used in the system.

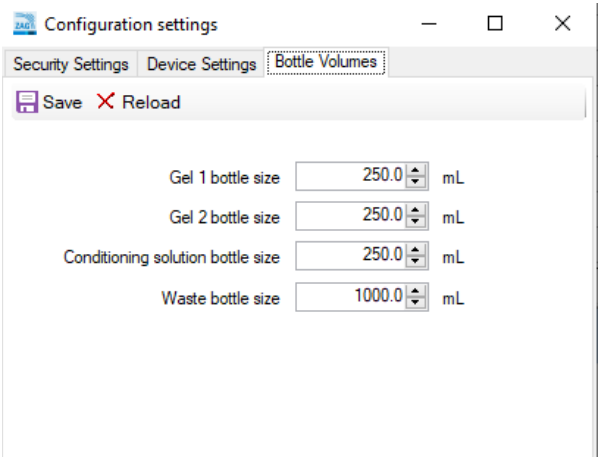


Figure 19 Configuration – Bottle Volumes tab

Results Report Setup

The command **Result report setup** opens the **Automated Report Settings** window (**Figure 20**).

The settings allow the administrator:

- to enable auto-processing, and
- to select the types of reports generated when auto-processing.

For more information about auto-processing, refer to **Chapter** , “Automated Analysis”.

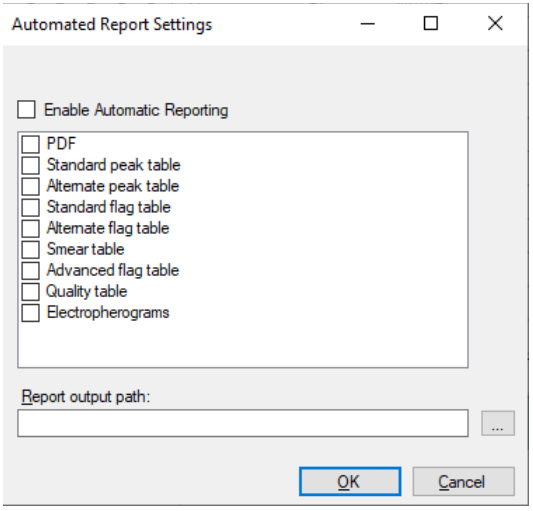


Figure 20 Results report setup window

Selecting **Enable Automatic Reporting** turns the auto-processing function on/off. When the auto-processing function is selected, the program will call a ProSize executable, process the data, and then export the desired results (PDF, standard peak table, etc.). For a complete description of each of these data types, refer to the *ProSize Data Analysis Software User Manual*, or to **Chapter 11**, “ZAG DNA Analyzer Software – Automated Analysis”, which gives a detailed description of auto-processing.

NOTE

In order for auto-processing to work correctly, the name of the ZAG DNA Analyzer software method must exactly match the name of the ProSize software configuration file. For more details, refer to **Chapter 11**, “ZAG DNA Analyzer Software – Automated Analysis”.

Event report

The **Event report** command provides a tabular report of the audit trail of the events that have occurred in the ZAG DNA Analyzer system.

Selecting **Event report** from the **Admin** menu opens the **Select Date Range** window where the user can **Use all dates** or **Use selected date range** (Figure 21).

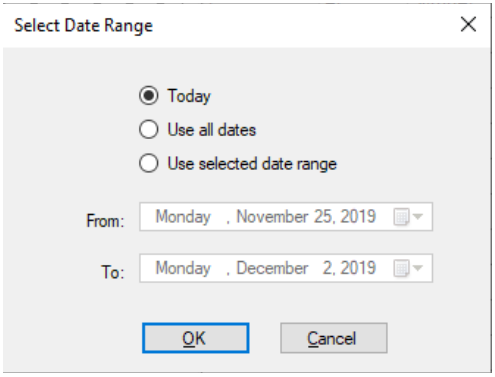


Figure 21 Event report window

Users with both **Administrator** and **User** level access can view the **Event report**.

The **Event report** contains the following information for each event log item:

- User name - user who was logged in
- Computer name - network name of the computer where the event occurred
- Event date
- Event code action
- Description

After selecting the appropriate date range in the **Select Date Range** window and clicking **OK**, an **Event report** is generated (Figure 22).

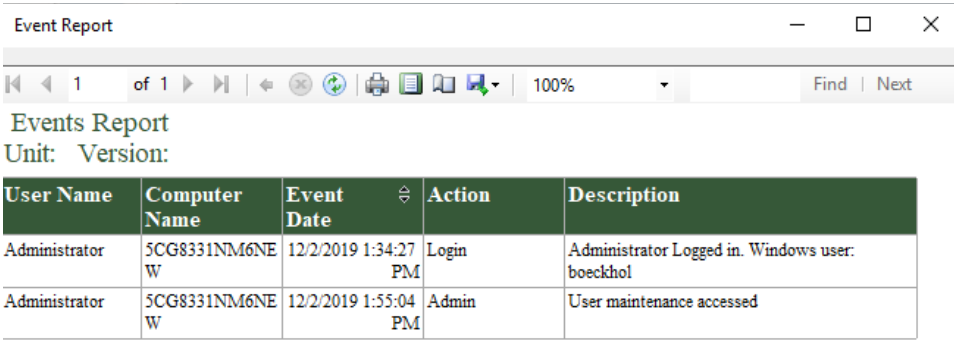


Figure 22 Event report example

The icons along the top of the **Event Report** follow standard Windows function nomenclature and are summarized in Table 7.

Table 7 Event report icons and Descriptions

Icon	Description
1 of 10	Page selection
	Back to parent report
	Stop rendering (i.e. stop report generation)
	Refresh
	Print
	Print layout
	Page setup
	Save
100%	Zoom

Error Report

The **Error report** command is used for advanced troubleshooting.

Selecting the command from the **Admin** menu opens the **Select Date Range** window where the user can **Use all dates** or **Use selected date range** (Figure 23).

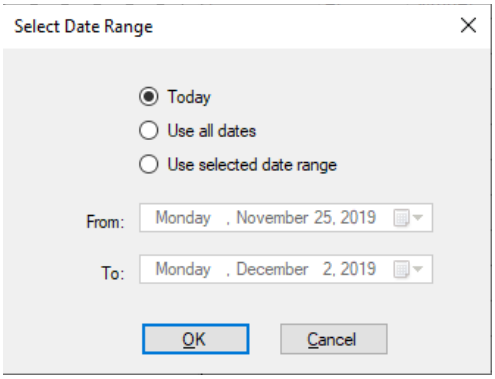


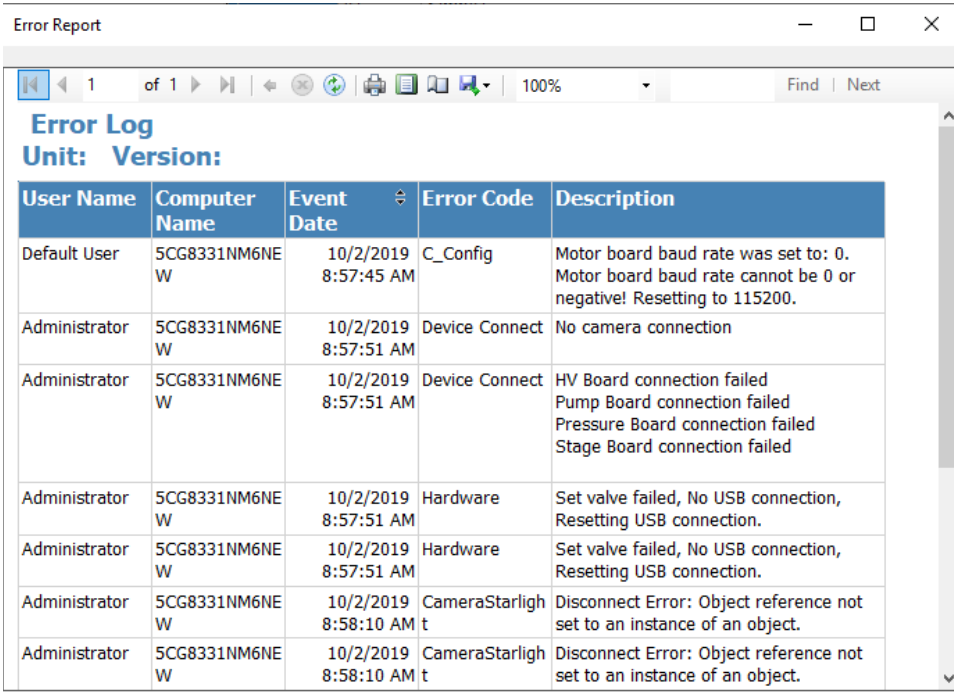
Figure 23 Error report window

The error report captures the following information:

- Software exceptions and Hardware errors detectable by the software
- User Name - The user who was logged in when the error occurred
- Computer Name - Network name of the computer where the error occurred
- Event Date
- Error Code
- Description

After selecting the appropriate date range in the **Select Date Range** window and selecting **OK**, an error report is generated (Figure 24).

The icons along the top of the Error Report follow standard Windows function nomenclature and are summarized in Table 7.



User Name	Computer Name	Event Date	Error Code	Description
Default User	5CG8331NM6NEW	10/2/2019 8:57:45 AM	C_Config	Motor board baud rate was set to: 0. Motor board baud rate cannot be 0 or negative! Resetting to 115200.
Administrator	5CG8331NM6NEW	10/2/2019 8:57:51 AM	Device Connect	No camera connection
Administrator	5CG8331NM6NEW	10/2/2019 8:57:51 AM	Device Connect	HV Board connection failed Pump Board connection failed Pressure Board connection failed Stage Board connection failed
Administrator	5CG8331NM6NEW	10/2/2019 8:57:51 AM	Hardware	Set valve failed, No USB connection, Resetting USB connection.
Administrator	5CG8331NM6NEW	10/2/2019 8:57:51 AM	Hardware	Set valve failed, No USB connection, Resetting USB connection.
Administrator	5CG8331NM6NEW	10/2/2019 8:58:10 AM	CameraStarlight	Disconnect Error: Object reference not set to an instance of an object.
Administrator	5CG8331NM6NEW	10/2/2019 8:58:10 AM	CameraStarlight	Disconnect Error: Object reference not set to an instance of an object.

Figure 24 Error report example

4 ZAG DNA Analyzer Software – Utilities Menu

Utilities Menu 46

Capillary Alignment 47
Hardware I/O 51
Prime 53
Solution Levels 54
Clean Reservoir Vent Valve 56
Results Dashboard 57

This chapter describes the ZAG DNA Analyzer software in more detail on the commands of the Utilities menu.

Utilities Menu

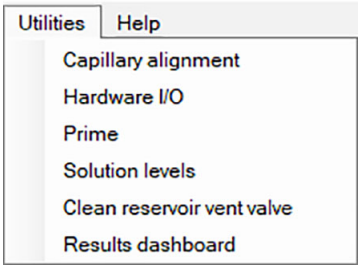


Figure 25 Utilities menu commands

Capillary Alignment

The **Capillary alignment** command is required when a new capillary array is installed. It may also be performed to address issues as part of a troubleshooting exercise.

For the ZAG DNA Analyzer system, the method *Capillary alignment from a file* is the recommended and easiest method of alignment.

When **Capillary alignment** is selected from the **Utilities** menu, the **Capillary Alignment Prep** window opens, prompting the user with the option to fill the capillaries with dye if that is the chosen method (**Figure 26**).

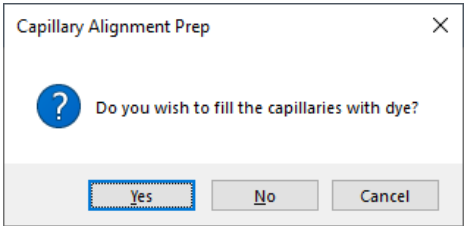


Figure 26 Capillary Alignment Prep window

Method – Capillary Alignment from a File

1 Select **No** when from the **Capillary Alignment Prep** window.

This will open the real time view of the **Capillary Alignment** window (**Figure 27**).

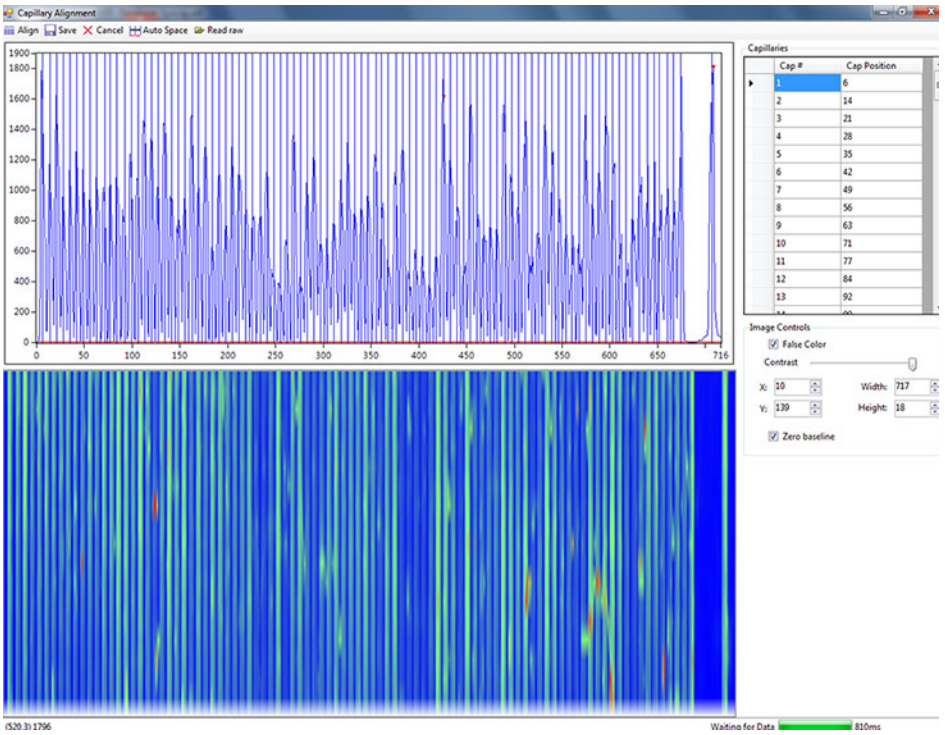


Figure 27 Real Time Capillary Alignment popup window

2 From the top menu of the window, select **Read raw**.

3 Navigate to the location with the raw file using the following Windows prompts.

The default saved location of raw data is:
C:/Agilent/Data/(Date: YYYYMMDD)/(Time: XXHXXM).

4 Select the latest raw file.

5 The *Align from File* window opens (**Figure 28**), allowing the user to align the capillaries from the selected run file. The toolbar of the *Align from File* window is discussed in **Table 8**.

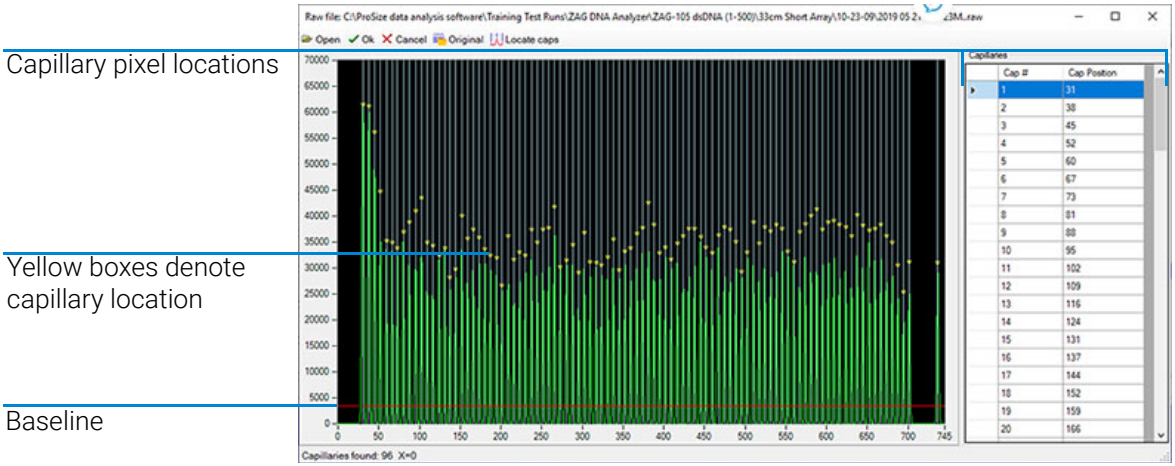


Figure 28 Align from file window for 96-capillary system

Table 8 **Align from file toolbar functions**

Icon	Description
Open	Opens a new file
Ok	Accepts changes to the file (i.e. Capillary locations)
Cancel	Cancels any actions and closes the file
Original	Locates the original capillary positions used when the selected file ran.
Locate caps	Locates the capillaries based on peak positions in the selected open file. Note: Move the red baseline up so that only the peaks of interest are integrated and not noise from the baseline.

6 Left-click on the red baseline and draw it upwards from the bottom of the graph but not above the top of capillary peaks, as shown in **Figure 28**.

7 Select **Locate caps** from the toolbar of the *Align from file* window.

This will locate the capillary peaks and place a yellow box at the apex of the selected capillaries denoting the capillary pixel location.

The bottom left corner of the window states the number of capillaries found. This should be 96 capillaries.

If necessary, adjust the capillary positions:

- To manually adjust a capillary position, left-click on the white line showing the capillary position and drag it left or right to the desired location.
- To zoom-in for desired resolution, right-click and select **Zoom** (and dragging the appropriate area).
- Should the number of capillaries be off due to too many capillary positions chosen or too few capillary positions chosen, redraw the red baseline and repeat this step.
- To insert or delete a capillary position, right-click on the black area of the graph or the capillary pixel location table to the right of the graph.
- Right-clicking on the graph area also allows for zooming in or out on the graph.

8 Once the desired number of capillaries is located, select **OK** from the *Align from file* toolbar. This will save any changes made to the capillary alignment and close the *Align from file* window, returning you to the **Capillary Alignment** window.

9 Select **Save** from the Capillary Alignment window.

From this point forward the instrument will use these saved pixel locations for all future runs.

Hardware I/O

The command **Hardware I/O** is available to users with administrator privileges and is used for troubleshooting the instrument.

Selecting the **Hardware I/O** command from the **Utilities** menu opens the **Hardware testing screen** as seen in **Figure 29**.

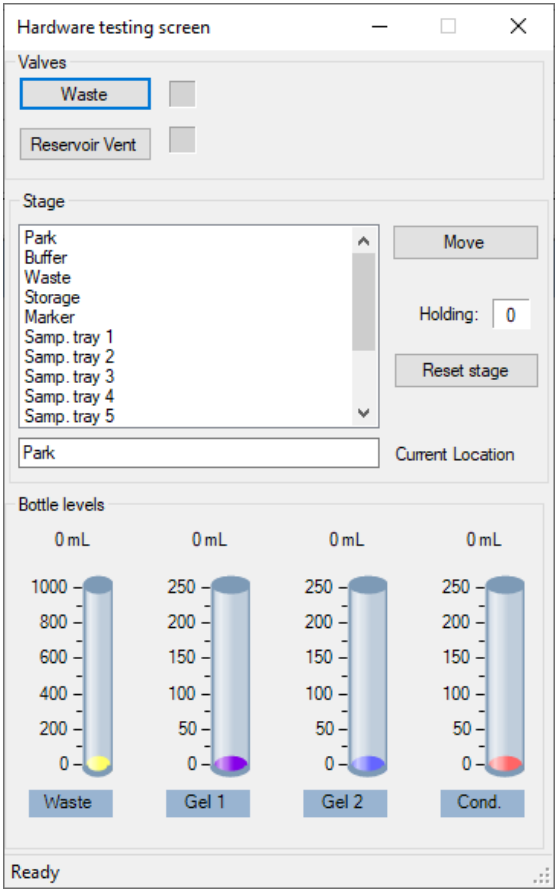


Figure 29 Hardware testing screen

An overview of the functions available in the **Hardware testing screen** is listed in **Table 9**.

Table 9 Functions of the Hardware testing screen

Function	Description
Valve > Waste	Select to open the valve. Clear to close the valve. The status of the valve is indicated by an open/filled circle in the status bar, respectively.
Valve > Reservoir Vent	Select to open the valve. Clear to close the valve. The status of the valve is indicated by an open/filled circle in the status bar, respectively.
Stage > Move	Select to move tray to the selected position.
Stage > Reset Stage	Allows the user to reset the stage position should a drawer be opened before the stage finishes its movement.
Bottle Levels	Gives a visual indication (simulation based on calculated usage) of the amount of reagents available in the system.

Prime

The **Prime** command allows the user to prime any of the three available reagent bottle lines should a new solution be added to the instrument or bubbles be found in the reagent bottle lines.

Selecting the **Prime** command from the **Utilities** menu opens the **Prime** window (**Figure 30**). The **Prime** functions are discussed in **Table 10**.

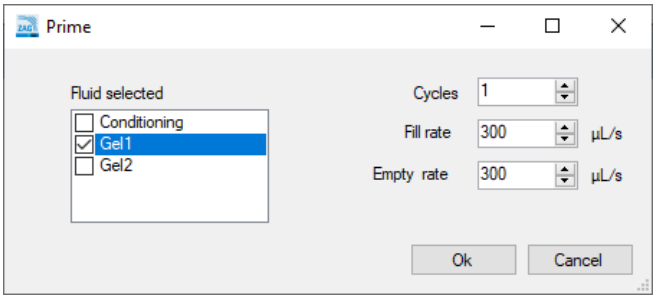


Figure 30 Prime window

Table 10 **Functions of the Prime window**

Function	Description
Fluid Selected	Allows the user to select which reagent line or combination of lines to prime.
Cycles	Refers to number of cycles (1-10) of the syringe to complete. One cycle is generally sufficient.
Fill Rate	Allows the user to adjust the fill rate up and down 0-1000, the default setting is 300 µL/s.
Empty Rate	Allows the user to adjust the fill rate up and down 0-1000, the default setting is 300 µL/s.

Solution Levels

The command **Solution levels** allows the user to adjust the volumes added to the reagent bottles and adjust the waste bottle level when emptied.

The ZAG DNA Analyzer software tracks the solution levels as the instrument is used. This ensures that the instrument has enough fluids for all of the planned runs.

If the solution levels are low, the program will issue a warning and ask the user to adjust the solution levels before it can proceed with a separation.

Selecting the **Solution levels** command from the **Utilities** menu opens the **Check solution volumes** window (Figure 31).

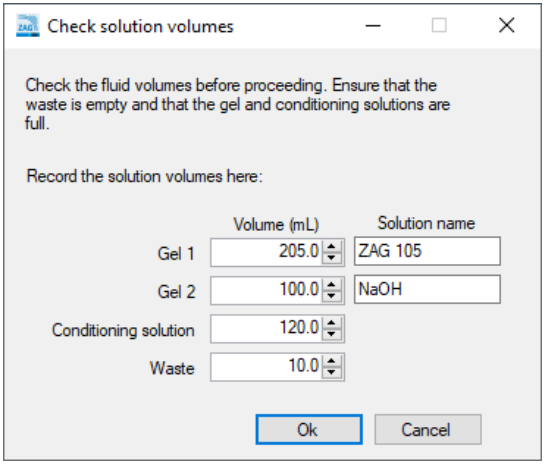


Figure 31 Check solutions volumes window

1 When solutions are re-filled, open this window and enter the correct solution levels (mL) for each container:

   a Use the up and down arrows or type the solution level in each entry field to adjust solution levels.

   b Select **Ok** to save changes to solution levels.

   A prompt for a bar-code opens.

2 Use the bar-code scanner to scan the bar-code on the gel (**Figure 32**).

NOTE

The bar-code is unique to each bottle of gel, and is only valid for the volume of gel purchased. Once the gel runs out, the bar-code is no longer valid, and a new purchased gel must be used.

If conditioning solution levels are changed, no bar-code scanner is needed.

If NaOH or water is being placed into either the gel 1 or gel 2 containers, the bar-codes for these products are located on the inside door of the instrument. Use the bar-code reader to read the bar code of either water or NaOH. There is no limit to the amount of NaOH or water that can be used with these internal bar-codes.

Figure 32 Gel bottle bar code

For the program to run correctly (i.e. to issue the correct warning), it is critical that the solution levels be entered into the program each time that new solutions are placed onto the instrument.

Clean Reservoir Vent Valve

The **Clean reservoir vent valve** command allows the user to clean the reservoir vent valve manually.

Selecting the command from the **Utilities** menu opens the reservoir vent valve, the waste valve, and displays the **Clean Reservoir Vent Valve** window (Figure 33).

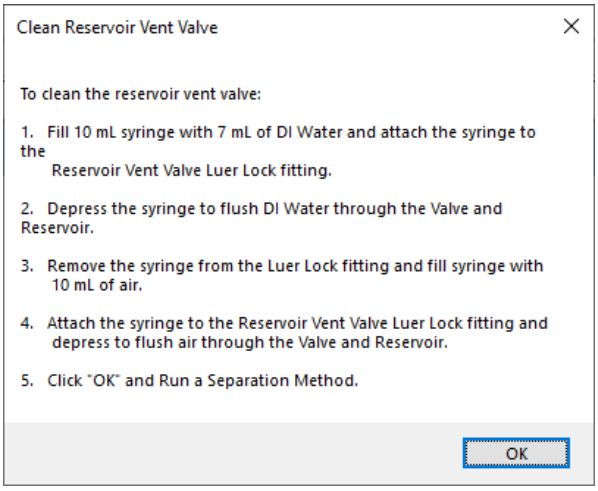


Figure 33 Clean reservoir vent valve window

Follow the steps outlined in Figure 33 to clean the reservoir vent valve. Should you have an older system without the reservoir vent valve luer lock fitting and syringe, contact your Agilent Sales Representative for information on how to acquire them.

Results Dashboard

The command **Results dashboard** allows the user to quickly view the status of auto-processed data.

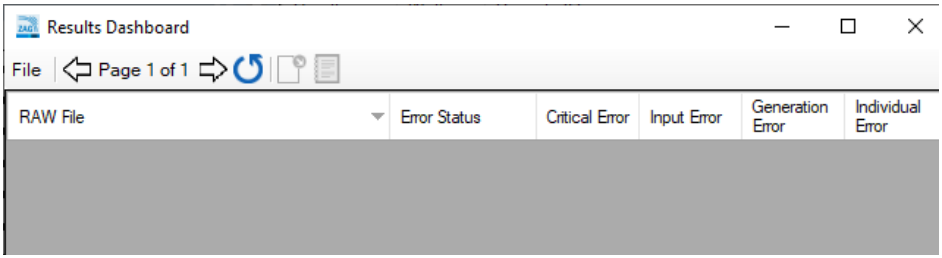


Figure 34 Results Dashboard window

For more information about the Results dashboard and the window toolbar, refer to **Chapter 11**, “ZAG DNA Analyzer Software – Automated Analysis”.

5 ZAG DNA Analyzer Software – Help Menu

Help Menu 59
User Manual 59
Tutorial Videos 59
About 59
About Firmware 60

This chapter describes the ZAG DNA Analyzer software in more detail on the commands of the Help menu.

Help Menu

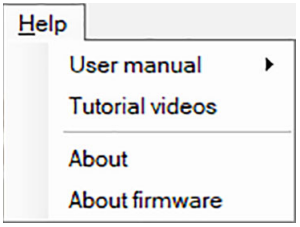


Figure 35 Help menu commands

User Manual

Navigating to the command **User manual** will provide a drop-down list of every chapter of the user manual.

Tutorial Videos

Selecting **Tutorial videos** opens a folder containing videos made to help the user with topics such as *Placing an array on the ZAG DNA Analyzer System* and *Performing a Capillary Alignment*.

About

The **About** command opens an **About ZAG DNA Analyzer** window displaying the version number of software, hardware serial number, and copyright information.

About Firmware

The **About firmware** command opens an **About Firmware** window displaying the version numbers of the voltage board, pump board, and motion control board.

6 ZAG DNA Analyzer Software – Operation Tab

Operation Tab Overview 62

Hotel Position Icons 63
Tray Selection and Sample ID 64
Experimental Run Controls and Adding to Queue 67
Method Queue 74

This chapter describes the ZAG DNA Analyzer software in more detail on the Operation tab.

Operation Tab Overview

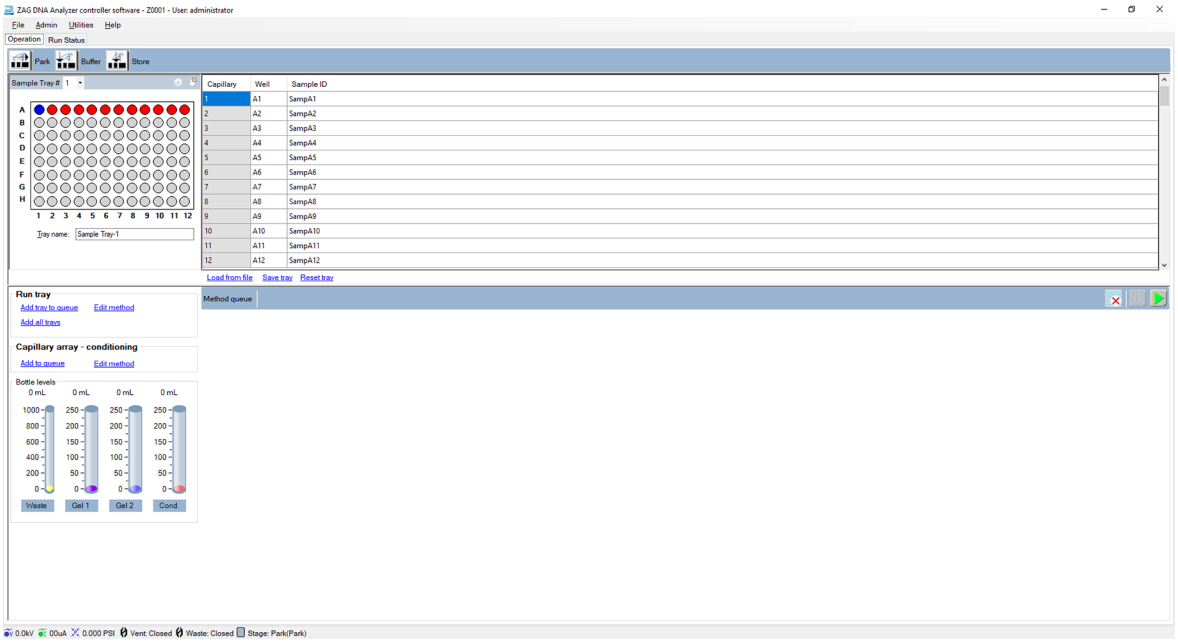
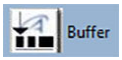
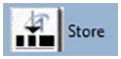


Figure 36 ZAG DNA Analyzer software main screen

Hotel Position Icons

There are three hotel positioning icons located at the top of the **Operation** tab (**Figure 36**). The icons and their functions are discussed in **Table 11**.

Table 11 Hotel position icons

Icon	Description
	This command is used to place the current tray being held back into its respective drawer and move the instrument platform to the bottom of the instrument.
	This command is used to pick up the buffer tray from the buffer drawer and hold it against the capillary array.
	This command is used to place the current tray being held (on the tray carrier) back into its respective drawer and pick up the storage solution tray to hold it against the capillary array.

Tray Selection and Sample ID

Select the sample tray to be used from either the **Sample Tray #** drop-down list or the colored tab tray selection, depending which configuration is set (**Figure 37**).

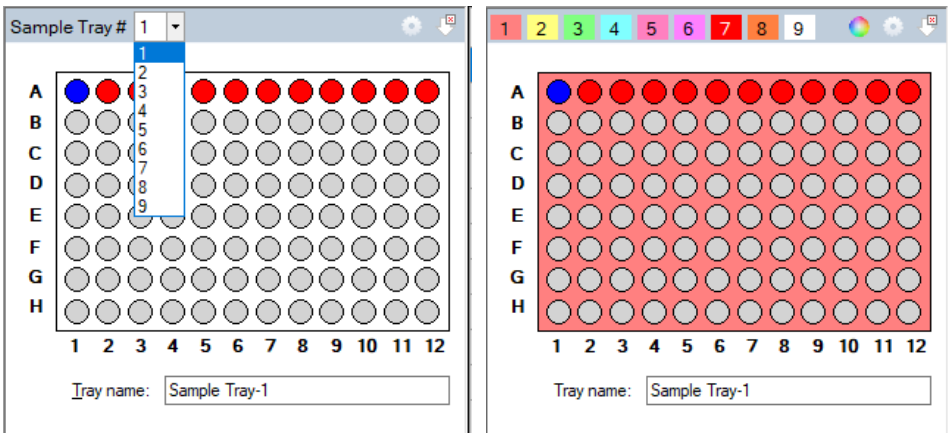


Figure 37 Classic drop-down tray selection (left) or colored tab tray selection (right)

1 In the tray window, select .

The **Select Tray Co...** dialog window opens (**Figure 38**).

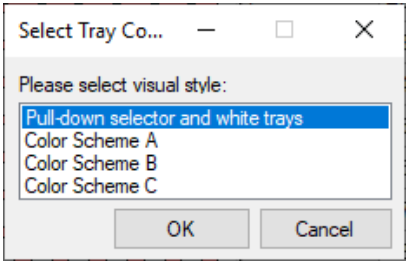


Figure 38 Select tray configuration dialog

2 Choose between the sample tray drop-down list or the colored tab tray selection (**Figure 37**).

3 If you use the tab tray selection window, select to change the color of each sample tray in the **Color selection** window.

4 To select a row from the 96-well plate depicted in the sample and sample tray selection window, left-click once in that row (**Figure 37**). To select a new row, left-click on another row.

5 To clear a row selection, select (**Figure 37**).

The **Tray name** dialog box allows you to input a name for the tray being run (**Figure 37**). Alternatively, select this dialog box and use a bar-code scanner to import sample names for the plate being run. For more information, refer to **Chapter 9**, "ZAG DNA Analyzer Software – Sample Name Entry".

Enter sample information in the **Sample ID** section of the main screen (**Figure 39**). For a 96-cap system, you must select each row individually to manually enter data (Rows A-H).

You can also save or load Sample names and information using .txt or .csv files. These functions are discussed in **Table 12**. For more information, refer to **Chapter 9**, "ZAG DNA Analyzer Software – Sample Name Entry".

Capillary	Well	Sample ID
1	A1	SampA1
2	A2	SampA2
3	A3	SampA3
4	A4	SampA4
5	A5	SampA5
6	A6	SampA6
7	A7	SampA7
8	A8	SampA8
9	A9	SampA9
10	A10	SampA10
11	A11	SampA11
12	A12	SampA12

Load from file Save tray Reset tray

Figure 39 Sample information editor

Table 12 Sample information editor functions

Item	Description
Load from file	Loads sample names from a .txt or .csv based file. See **Chapter 9**, "ZAG DNA Analyzer Software – Sample Name Entry" for more information.
Save tray	Saves the information entered for an entire sample tray.
Save selected row	Saves the information entered for the selected row of a sample tray.
Reset row	Resets the selected row to the default **Sample ID** setting.
Reset tray	Resets the entire sample tray to the default **Sample ID** settings.

Experimental Run Controls and Adding to Queue

The ZAG DNA Analyzer software provides pre-loaded default methods for both capillary array conditioning and experimental methods for each analysis kit offered by Agilent.

The experimental run controls shown in **Figure 40** shows the controls available for **Run tray** and **Capillary array – conditioning**. These options will be discussed below.

Reagent levels of the bottles are also shown.

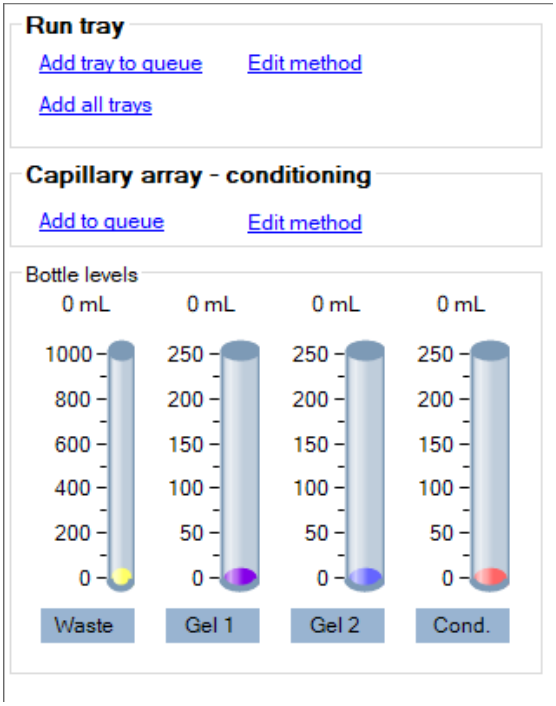


Figure 40 Experimental run controls

Run Tray– Edit Method

Selecting **Edit method** opens the method editor window (**Figure 41**).

Users with user level access does not have the privilege to change method parameters. The option **Edit method** is not available and is replaced with **View Method**.

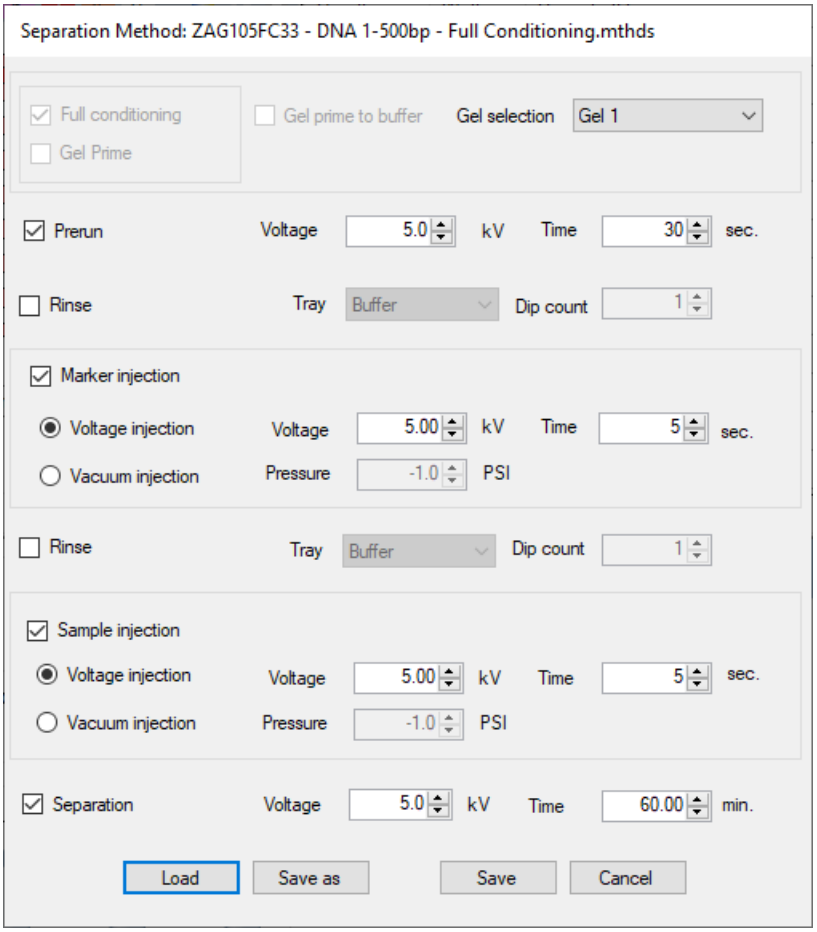


Figure 41 **Add tray to queue** method editor window

The method editor window allows for customization of the run parameters for a CE separation.

Full conditioning, **Gel Prime**, and **Gel prime to buffer** are hard-coded and cannot be disabled.

Selecting the check box next to the individual parameter enables different steps and parameters. The individual parameters are discussed in **Table 13**.

Optimum capillary conditioning values are preloaded and defined for each method. For further definition of these values, refer to each method Kit Guide.

Table 13 Method editor window functions

Item	Description
Gel selection	Using the drop down menu the user can select to use the **Gel 1** or the **Gel 2** reagent bottle position.
Prerun	A short pre-run is recommended to normalize the gel inside the capillaries.
Rinse	The **Rinse** option allows the user to dip into the selected position rinsing the capillary tips and electrodes between the **Prerun** and **Sample injection** or **Marker injection**. The **Tray** position and **Dip count** can be altered as well.
Marker injection	**Marker injection** is enabled when using the qualitative analysis kits. The user has the option of a **Voltage injection** or **Vacuum injection** allowing them to set the **Voltage**, **Pressure**, and **Time**.
Rinse	The **Rinse** option allows the user to dip into the selected position rinsing the capillary tips and electrodes between the **Prerun** and **Sample injection** or **Marker injection**. The **Tray** position and **Dip count** can be altered as well.
Sample injection	The user has the option of a **Voltage injection** or **Vacuum injection** allowing them to set the **Voltage**, **Pressure**, and **Time**.
Separation	The user has the option to alter the **Voltage** and **Time** of the CE separation.

The user can **Load** a new method or **Save as** a new method with a unique name. Select **Save** to accept the changes and close the window, or **Cancel** to close the method editor window without accepting any changes made.

NOTE

When creating a new method with a unique name the user will need to make a corresponding global configuration in the ProSize software with a matching name, refer to the *ProSize Data Analysis Software User Manual* for more complete instructions.

Run tray - Add tray to queue

Selecting the **Add tray to queue** command opens the **Separation setup** window as shown in **Figure 42**.

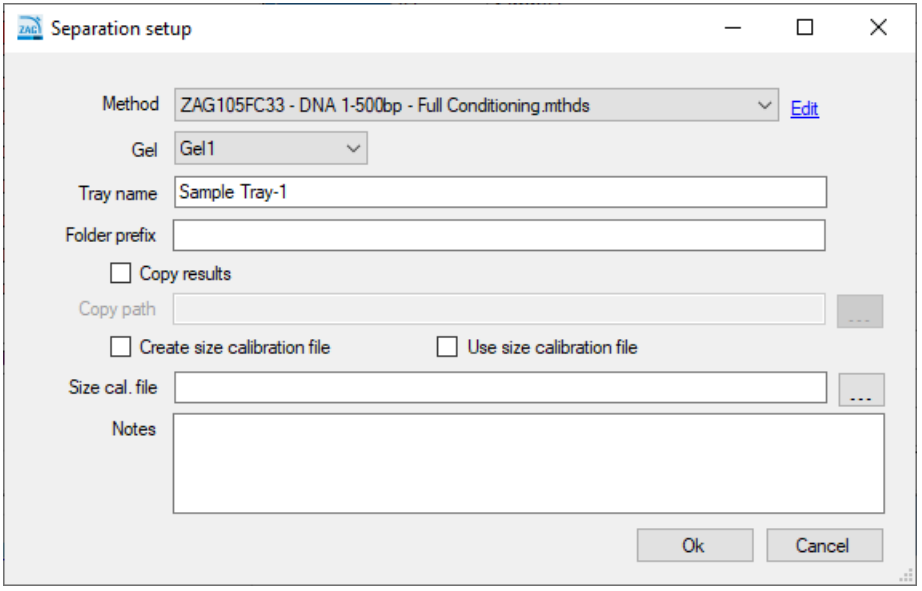


Figure 42 Separation setup window

The settings of the **Separation setup** window are discussed in **Table 14**.

Table 14 Separation setup window functions

Item	Description
Method	Methods can be selected from the drop-down menu. A user with administrator level access can also select **Edit** to change any parameters of the method by opening the method editor window in **Figure 42**. User level access only allows the user to **View** the method file selected.
Gel	The user can toggle the gel bottle location to the desired bottle to use for the separation method without having to alter a predefined method.
Tray name	The **Tray name** appears as input by the user on the main screen or the default name appears. The user may edit this field by typing in the provided box.
Folder prefix	The folder prefix allows the user to add a prefix to the folder name where the results files will be written.
Copy results / Copy path	The default directory location for the data is C:\Agilent\Data. You can select **Copy results** and choose to copy the saved data to a different location by selecting **[...]** option.
Create size calibration file	This is used for automated analysis (**Chapter 11**, "ZAG DNA Analyzer Software – Automated Analysis"). When selected (and auto-processing is enabled), the run is used to create a size calibration file, which is used to calibrate the size of fragments in subsequent files. Upon completion of the run, a size calibration file is named and placed into a file director as defined in the **Size cal. file** section.
Use size calibration file	This is used for automated analysis (**Chapter 11**, "ZAG DNA Analyzer Software – Automated Analysis"). When selected (and auto-processing is enabled) the software uses the size calibration file defined under **Size cal. file** to define the sizes of DNA fragments.
Size cal. file	This is used for automated analysis (**Chapter 11**, "ZAG DNA Analyzer Software – Automated Analysis"). The user defines a name and file location of the size calibration file. When **Create size calibration file** is selected, the software writes a .SCAL file to the defined name and location of the file. When **Use size calibration file** is selected, the software imports and uses the .SCAL file at the defined location.
Notes	Allows to add any additional information the user may require for a set of samples.

After the appropriate method is chosen from the drop-down menu, select **OK** to add the chosen method to the method queue, or **Cancel** to close the screen.

Capillary Array - Conditioning

The ZAG DNA Analyzer software provides a preloaded capillary conditioning method for cleaning the capillary array.

The user can also create a method by selecting **Edit method** shown in Figure 39.

Selecting the check box next to the individual parameter enables different bottles to be used for the conditioning. The individual parameters are discussed in Table 15.

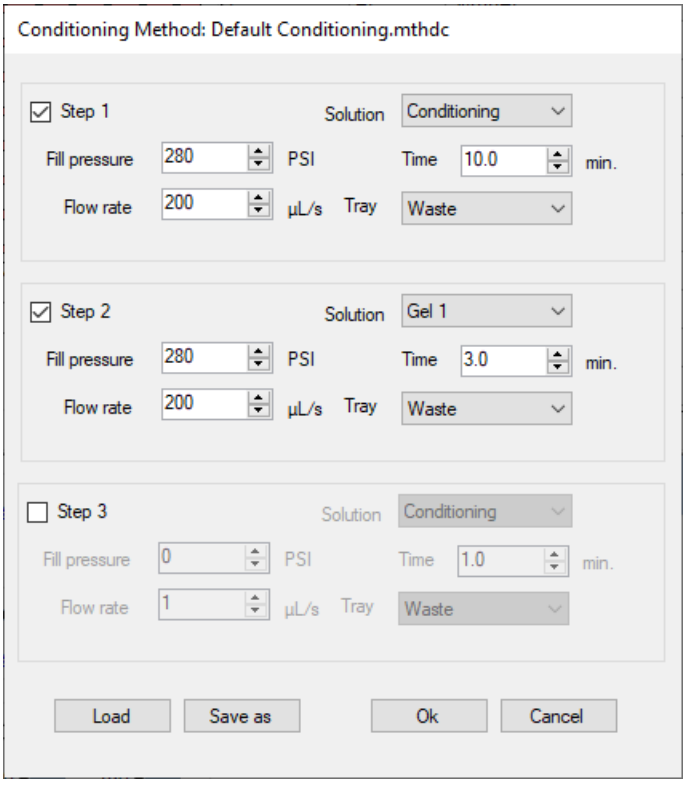


Figure 43 Conditioning method editor

Table 15 Conditioning method editor window setting

Item	Description
Step 1, 2, or 3	Enables/disables the step to be used.
Solution	Allows selection of the conditioning solution, **Gel 1**, or **Gel 2** reagent bottles for use.
Fill pressure	Default value is set to 280 psi. This can be changed from 1 – 300 psi.
Flow rate	Default value is set to 200 µL/s. This can be adjusted from 1 – 1000.
Time	This is set in minutes from 1 – 240.
Tray	Allows the user to select the **Tray** to pump into when conducting the conditioning.

The user can **Load** a new method, **Save as** a new method with a unique name, select **OK** to accept the method and close the window, or **Cancel** to close the method editor window without accepting any changes made.

Select **Add to queue** to open the **Select conditioning method** window shown in **Figure 44**.

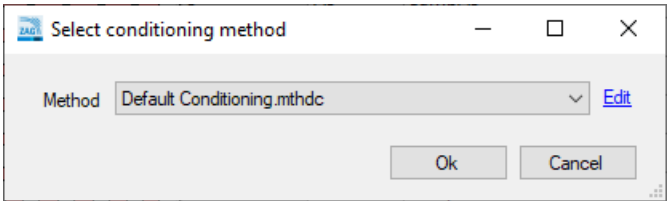


Figure 44 Select conditioning method window

A previously saved method can be chosen from the drop-down menu. A user with administrator privileges can select **Edit** to view the **Conditioning Method Editor** window seen in **Figure 43**. Users with user level access can only select a previously defined method and **View** the method, but not edit a method.

After the appropriate method is chosen from the drop-down menu, select **OK** to add the chosen method to the **Method Queue**, or **Cancel** to close the window.

Method Queue

Once a Sample Tray or Row (12-capillary unit only) has been selected, sample names entered into the **Sample ID** field, and **Add to queue** has been selected the chosen method will be shown in the **Method Queue** (Figure 45).

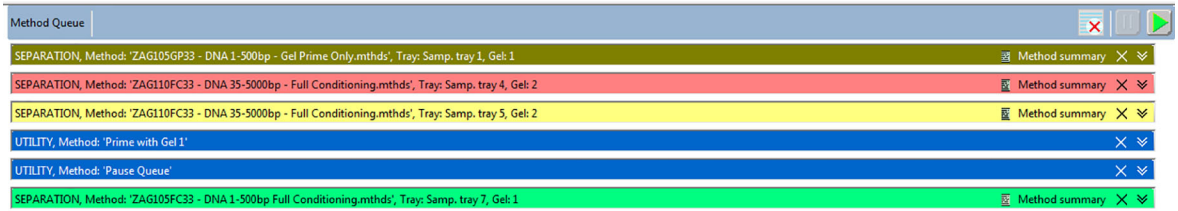


Figure 45 Method queue

Figure 45 shows three sample runs chosen from sample trays 1, 4, and 5, followed by a prime and a pause in the method queue. Finally, a fourth sample run is included (sample tray 7) after the pause.

A **Pause** or **Prime** can be inserted into the method queue by right clicking in the method queue area of the screen. When **Insert Prime** is selected, the **Select solution** window opens, prompting the user to choose the bottle location to prime from a drop-down menu (Figure 46).

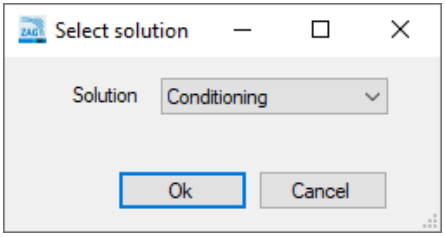


Figure 46 Select solution window

Methods loaded into the method queue can be moved up or down based on the user's needs by left-clicking on the method and dragging it to the desired location in the queue.

To view the parameters for the separation method in the method queue, select **Method summary** next to the separation method.A summary of the method will appear, as shown Figure 47.

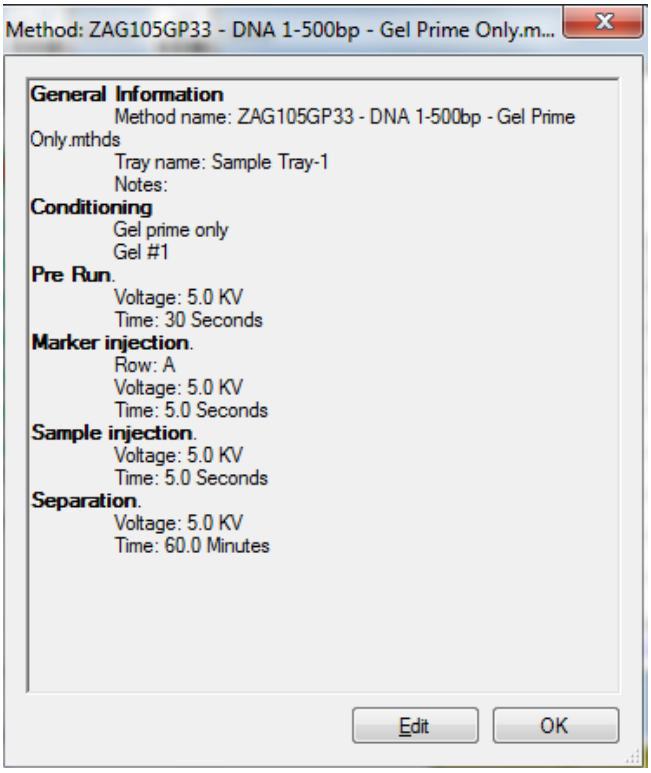


Figure 47 Method summary window

Selection of the **Edit** command from the **Method summary** window allows the user to make final changes to the method if desired.

To delete a specific item on the queue, select **X** next to the separation method. To delete all items in the queue, select the **Clear** icon from the Method Queue menu bar.

To expand the method and show a detailed summary, select the down arrows next to the separation method.

There are three run controls for the method queue, **Clear the Method Queue**, **Pause the Method Queue**, and **Start the Method Queue**. These run controls are described in Table 16.

Table 16 Method queue run controls

Icon	Description
	Clear: Selection of this icon will clear all separation methods, pauses, and primes from the queue.
	Pause: Selection of this icon will pause the method queue. The current method running will still continue. To restart the queue select start icon.
	Start: Selection of this icon will start the method queue. Once started the top method will disappear and the screen will switch to the **Run Status** tab. The next method will move up in the queue.

7 ZAG DNA Analyzer Software – Run Status Tab

Run Status Tab Overview 78
Stage Movement Animation 79
Conditioning Animation 80
Pre-Run / Injection View 81
Real-Time Separation View 82
Status Bar 84

This chapter describes the ZAG DNA Analyzer software in more detail on the Run Status tab.

Run Status Tab Overview

Once Start has been selected (for more information, refer to **Chapter 6**, "ZAG DNA Analyzer Software – Operation Tab"), the display will switch from the **Operation** tab to the **Run Status** tab. The **Run Status** tab has several features, as shown below.

Stage Movement Animation

Whenever the stage moves from one position to another, the animation shows where the ZAG DNA Analyzer plates are moving to/from (**Figure 48**). This provides a real time view of what is happening. An up arrow indicates that a plate is being removed from the drawer position and moved up against the capillary array. A down arrow indicates that a plate is being placed back into the drawer position.

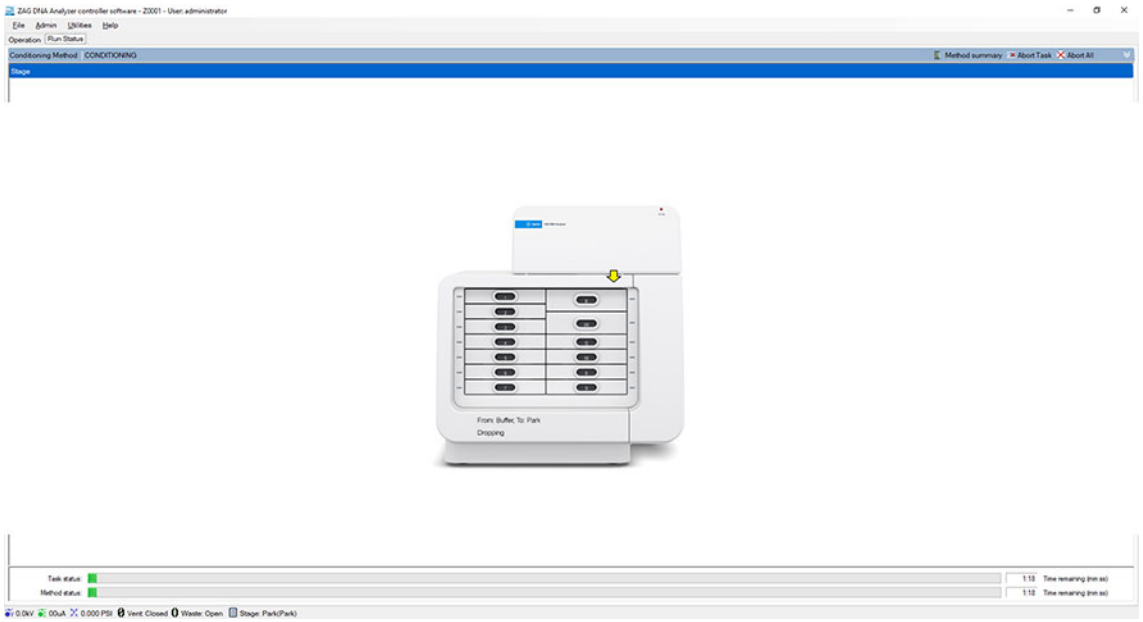


Figure 48 Plate movement animation

Conditioning Animation

When the ZAG DNA Analyzer is pumping conditioning solution or gel, the animation in **Figure 49** is shown. The animation gives a real-time view of exactly what the instrument is doing during a conditioning sequence.

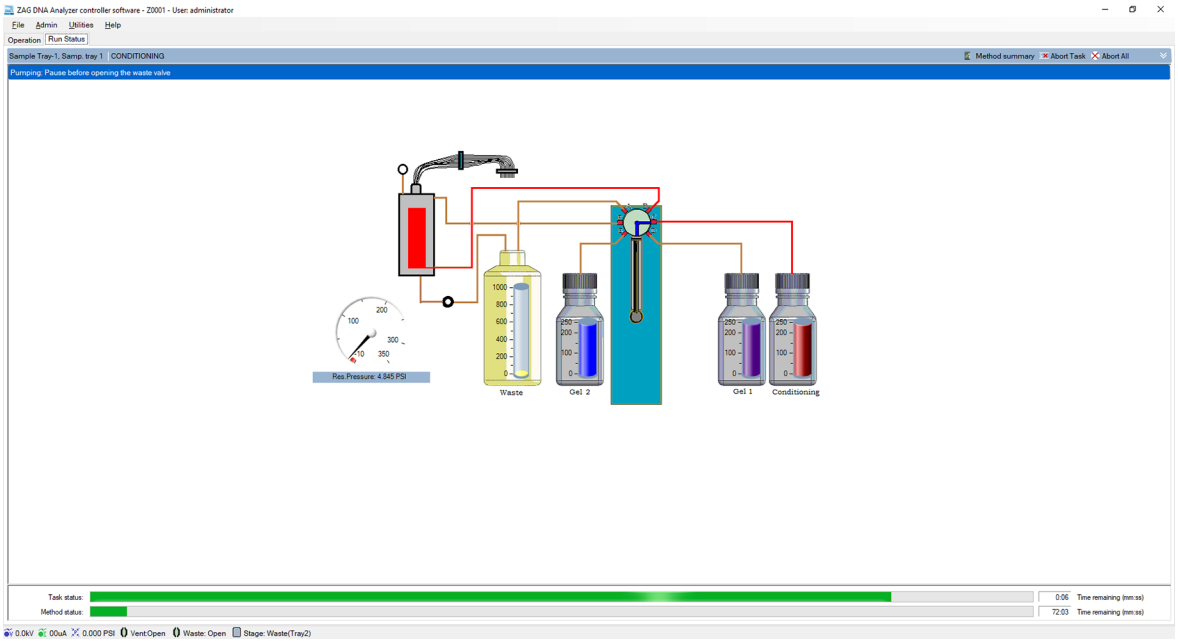


Figure 49 Conditioning animation

Pre-Run / Injection View

When the ZAG DNA Analyzer is completing a pre-run or injection, the screen in **Figure 50** is shown.

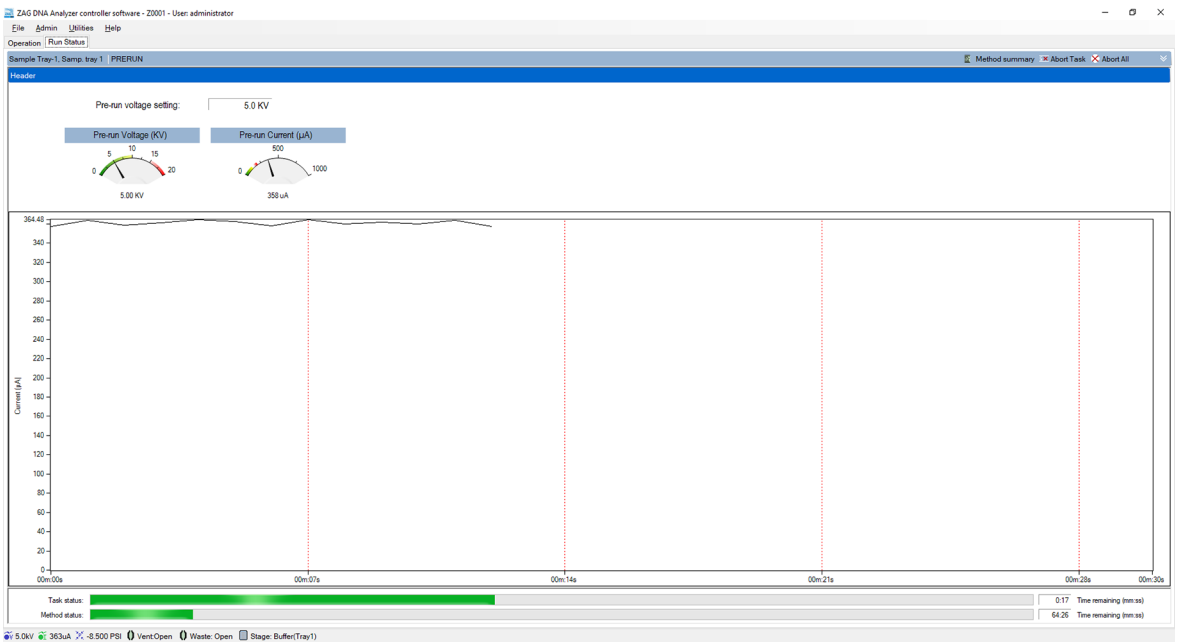


Figure 50 Pre-run/injection screen.

Real-Time Separation View

When the ZAG DNA Analyzer starts the separation, the screen shows the real-time view of the separation (**Figure 51**).

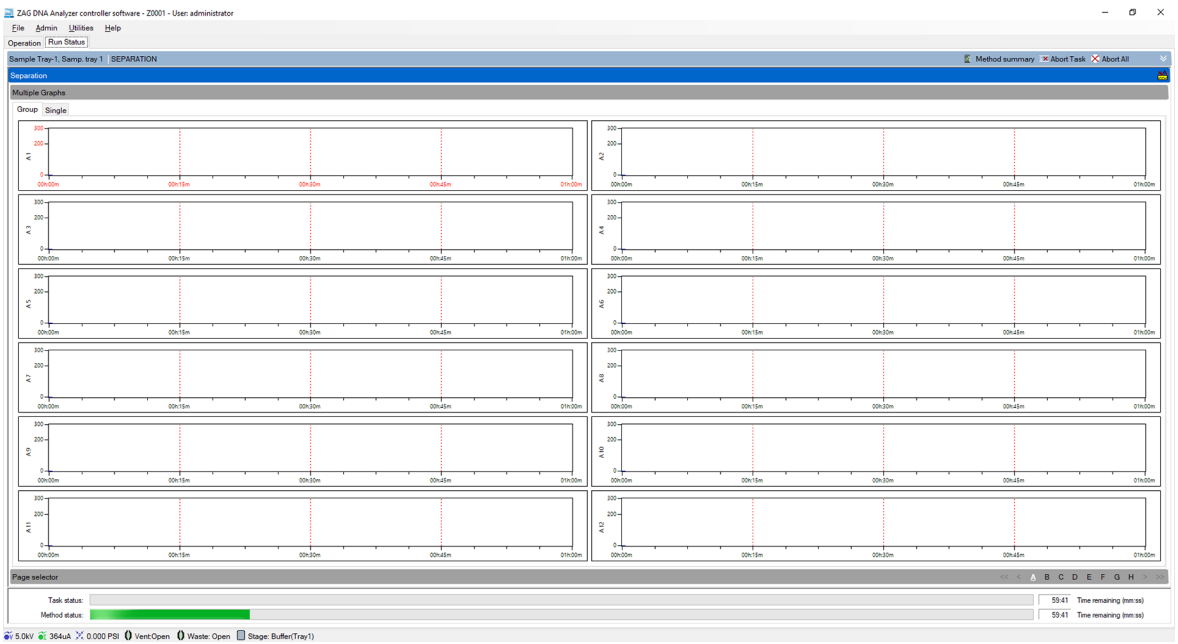


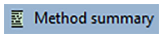
Figure 51 Real-time separation window

The **Group** tab at the top shows the run in a group of 12 electropherograms. The **Single** tab shows individual electropherograms.

When a 96-capillary system is running, the user can select between rows of a group on the bottom right of the electropherograms by selecting the corresponding letter of the row. When viewing the **Single** tab, the user can also select the well number to view.

Other options available from the **Run Status** tab are discussed in **Table 17**.

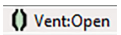
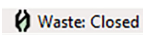
Table 17 Run status tab options

Icon	Description
	Opens a popup window showing the method summary for the current method being run.
	Aborts only the individual task being done, i.e. stage movement, pumping, or injection.
	Aborts the entire method being run and begins the next method in the queue. If no methods are found, the stage returns to the storage position. When selected the user will be presented with a window asking to verify they want to abort.
	Shows the current for the separation being performed.
Task Status	Shows the status bar and time left for each individual task being accomplished, i.e. stage movement, pumping, or injection.
Method Status	Shows the status bar and time left for the entire method to complete.

Status Bar

The bottom bar of the ZAG DNA Analyzer software screen shows a real time status bar containing important information about the instrument status. These functions are discussed in **Table 18**.

Table 18 Instrument Status Information

Icon	Description
	Left-clicking on this icon will show the voltage level for the last 5 minutes.
	Left-clicking on this icon will show the current level for the last 5 minutes.
	Left-clicking on this icon will show the pressure level for the last 5 minutes.
	Denotes if the reservoir vent valve is open or closed.
	Denotes if the waste valve is open or closed.
	Denotes the location of the stage at that point in time.

8 ZAG DNA Analyzer Capillary Array

Capillary Array Parts 86

Removal of the Capillary Array 87

Unpacking a new Capillary Array 98

Capillary Array Installation 101

Using the External Array Docking Station for Storage 111

This chapter explains the essential operational parameters of the capillary array.

Capillary Array Parts

The ZAG DNA Analyzer capillary array allows for direct parallel injection and separation of 96 samples at once.

The capillary array cartridge is located in the upper compartment or hood of the instrument.

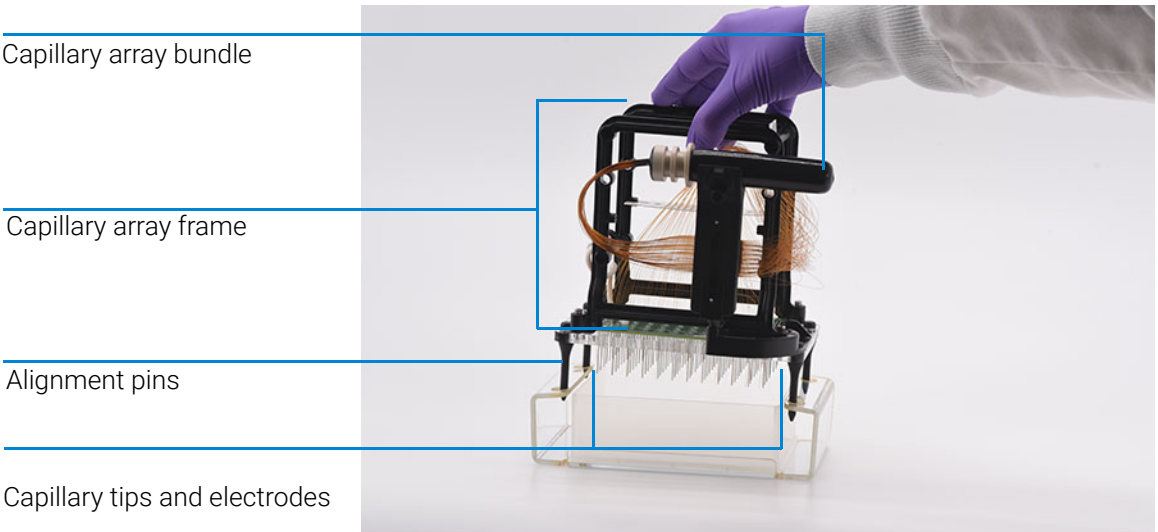


Figure 52 Capillary array parts (96-capillary array shown)

Removal of the Capillary Array

This section provides a guideline to remove the capillary array cartridge from the ZAG DNA Analyzer.

Before proceeding with the capillary array removal, select the **park** icon from the main screen to place the buffer tray back into its drawer and move the stage into resting position.

1 Open the reagent door and top hood of the instrument.

Figure 53 ZAG DNA Analyzer system

2 Unplug the white high voltage supply cable for the top front panel, and place in the holder of the capillary array frame.

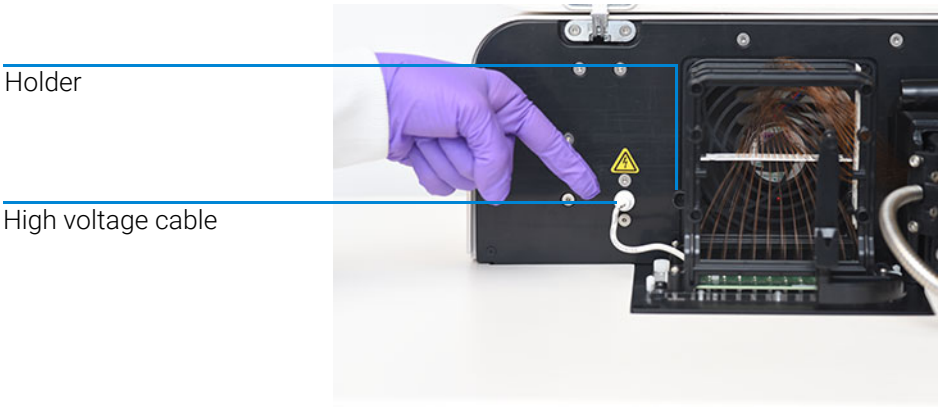


Figure 54 Instrument top compartment – high voltage supply cable

3 Use the provided allen wrench to remove the two white screws that secure the light guide to the array window.

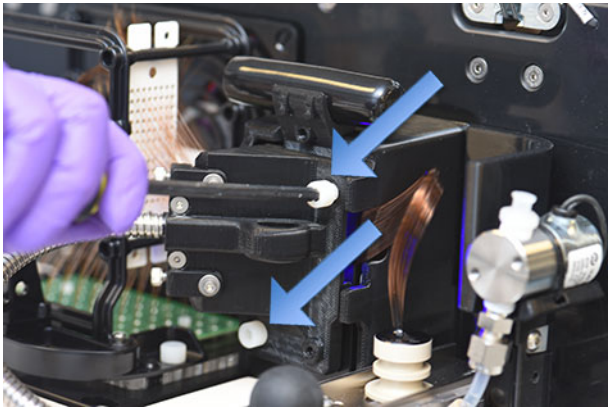


Figure 55 Instrument top compartment – unscrew light guide

4 Remove the light guide.

NOTE

Avoid looking directly at the LED light as the brightness can temporarily affect your vision.

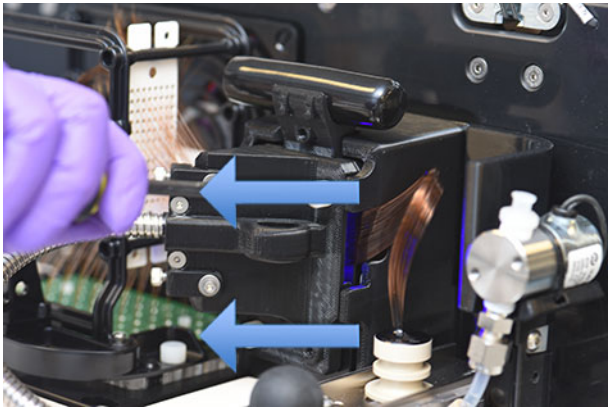


Figure 56 Instrument top compartment – light guide

5 Place the light guide in the reagent door of the instrument.

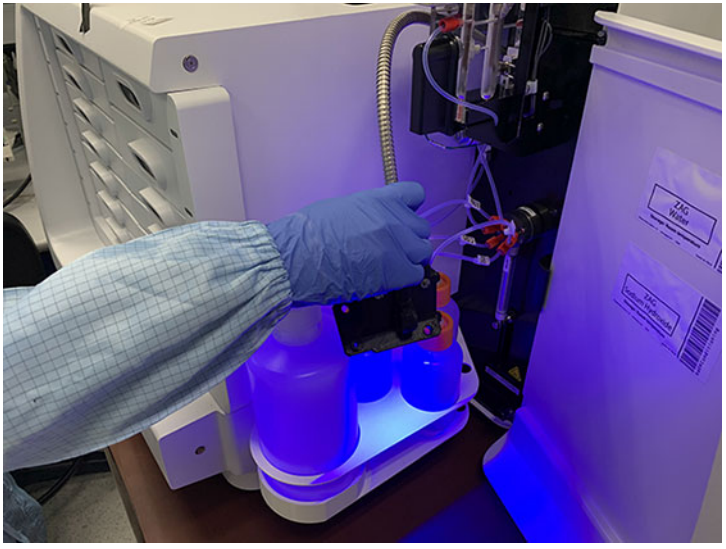


Figure 57 Instrument top compartment – light guide in reagent door

6 Close the reagent door.

Figure 58 Instrument top compartment – reagent door closed

7 Pull back on the capillary reservoir connector slide.

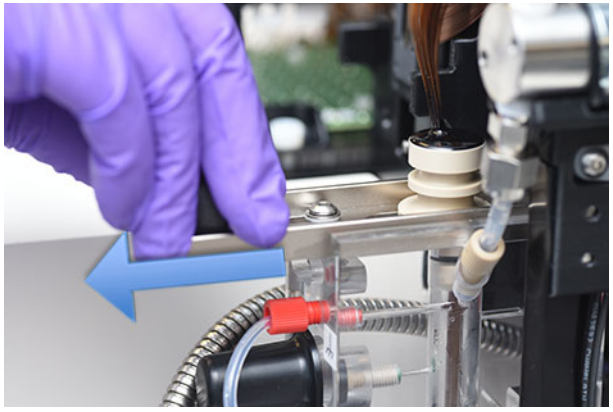


Figure 59 Instrument top compartment – capillary reservoir connector slide

8 Use the capillary reservoir connector tool to loosen the capillary array bundle by prying up on the bundle.

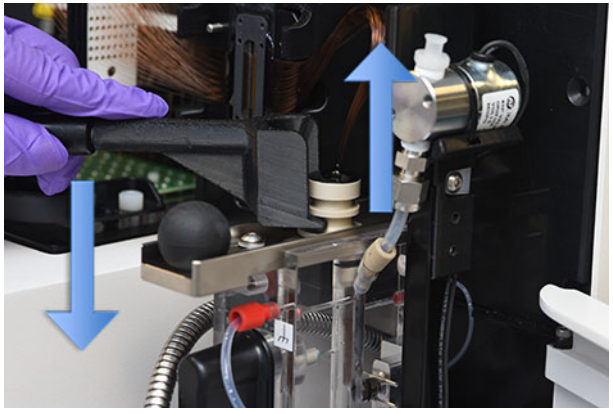


Figure 60 Instrument top compartment – capillary reservoir connector tool

9 Remove the capillary array bundle by pulling up gently.

NOTE

Avoid pulling up hard as to not break any capillaries.

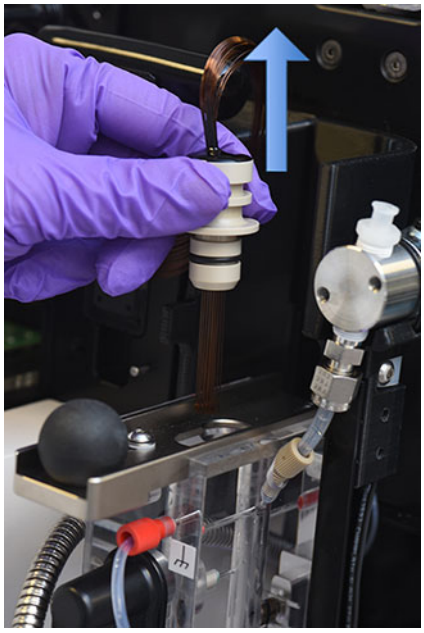


Figure 61 Instrument top compartment – capillary array bundle removal

10 Carefully insert the protective cover over the capillary bundle.

Figure 62 Instrument top compartment – installing protective cover

11 Place the capillary array bundle on the top holder of the capillary array window.

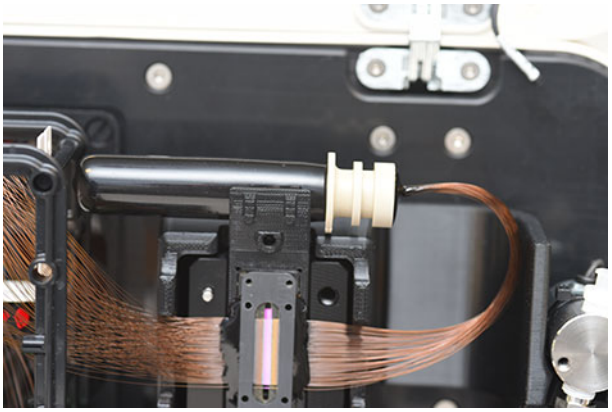


Figure 63 Instrument top compartment – storing covered capillary array bundle

12 Remove the capillary array window from the window holder.

NOTE

Do not press on or touch the capillaries.

13 Flip the array window after removal, so that the capillary array bundle goes from the right to the left side of the array frame.

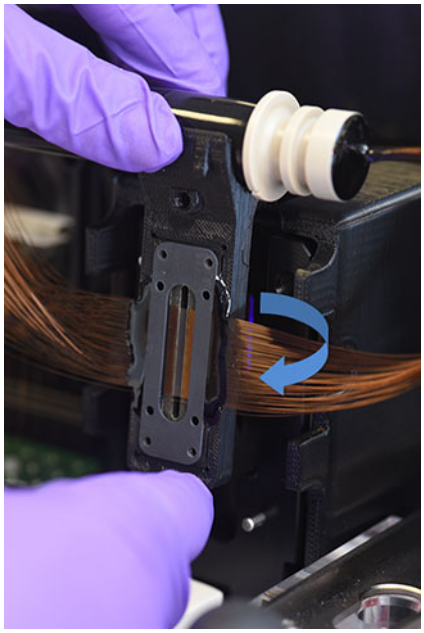


Figure 64 Instrument top compartment – remove capillary array window

14 Attach the array window to the capillary array frame using the attachment screw.

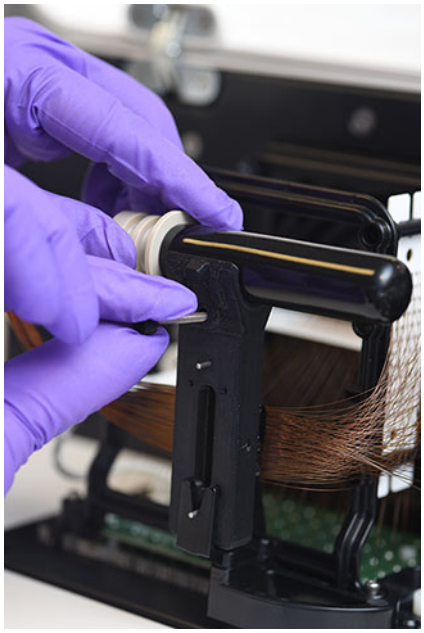


Figure 65 Instrument top compartment – attach array window to capillary array frame

15 Use the provided allen wrench to remove the two white screws holding the capillary array in place.

Figure 66 Instrument top compartment – array attachment screw removal

16 Carefully lift the array straight up to remove it from the ZAG DNA Analyzer.

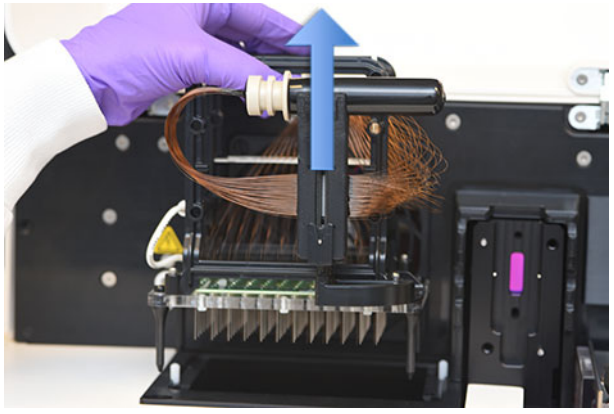


Figure 67 Instrument top compartment – capillary array removal

Once removed from the instrument, the capillary array cartridge is ready for disposal or storage in the ZAG DNA Analyzer external array docking station.

Unpacking a new Capillary Array

This section provides a guideline to unpack a new capillary array from the shipping container and packaging.

NOTE

Save the original packaging when receiving a new capillary array cartridge. This packaging is necessary for proper shipment of a capillary array in the event of a return.

1 Unpack the new capillary array:
   - **a** Open the capillary array shipping box.
   - **b** Remove the foam cover.
   - **c** Remove the packaged capillary array from the shipping box.

Figure 68 Capillary array shipping box

2 Remove plastic shipping cover from the capillary array.

NOTE

Take care not to break capillaries or touch the array window when removing packaging.

Figure 69 Capillary array plastic shipping cover

3 Remove the rubber band securing the capillary array bundle to the capillary array window.

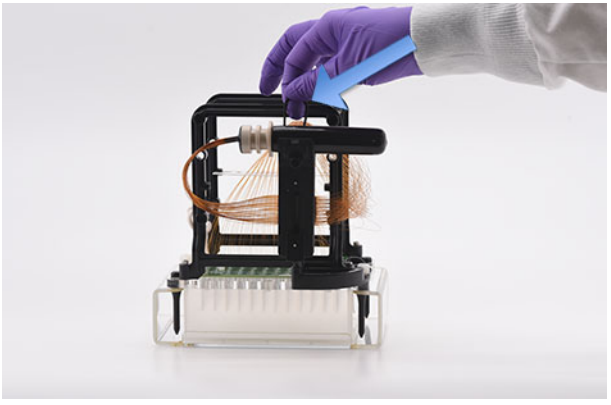


Figure 70 Remove securing rubber band

4 Unscrew the two white nylon screws holding the array to the array docking station.

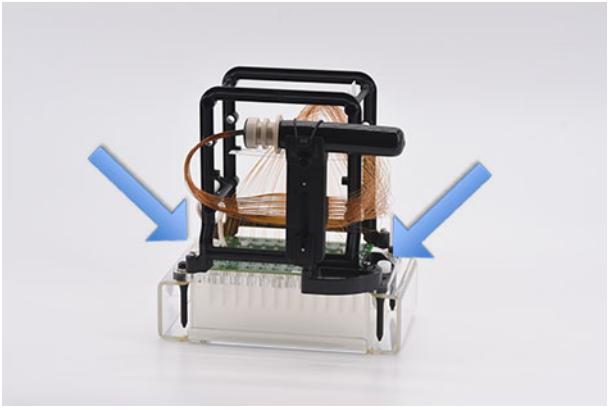


Figure 71 Array docking station screws

5 Carefully lift the array straight up to remove it from the array docking station.

Figure 72 Removal of array from shipment frame

Capillary Array Installation

This section provides a guideline to install a capillary array cartridge into the ZAG DNA Analyzer system.

Before proceeding with installation ensure the instrument is in the **park** position. If it is not in the **park** position, select the **park** icon from the main screen to place the buffer tray back into its drawer and move the stage into the resting position.

1 Open the reagent door and top hood of the instrument.

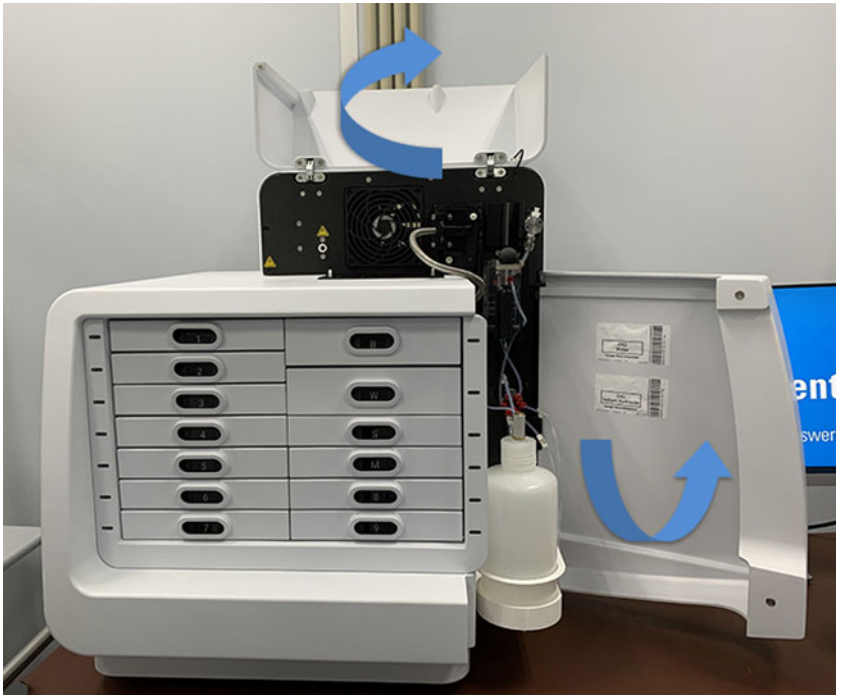


Figure 73 ZAG DNA Analyzer system

2 Carefully place the capillary array into the top compartment of the instrument with the array window facing out.

The four alignment pins should align with the alignment holes in the instrument.

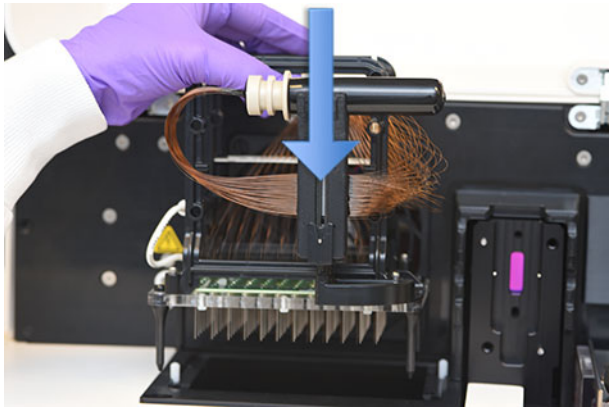


Figure 74 Instrument top compartment – capillary array installation

3 Use the provided allen wrench to install the two white screws holding the capillary array in place.

Figure 75 Instrument top compartment – array attachment screw

4 Remove the array window attachment screw.

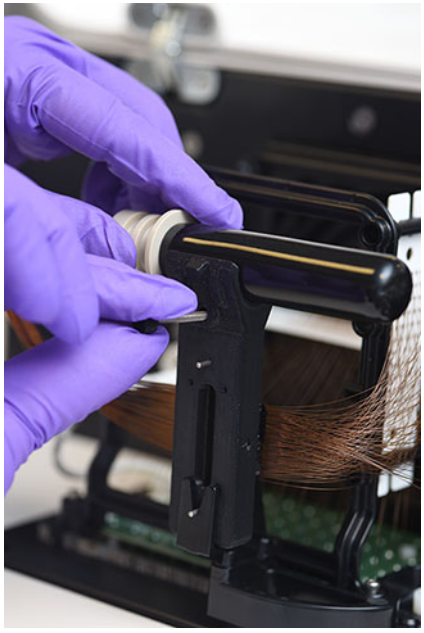


Figure 76 Instrument top compartment – remove array window

5 Carefully flip the array window so that the capillary array bundle goes from the left to the right side of the instrument.

Position the capillary array window into the holder and firmly press it into place.

NOTE

Do not press on or touch the capillaries.

Figure 77 Instrument top compartment – array window placement

6 Remove the capillary array bundle from the top holder of the capillary array window.

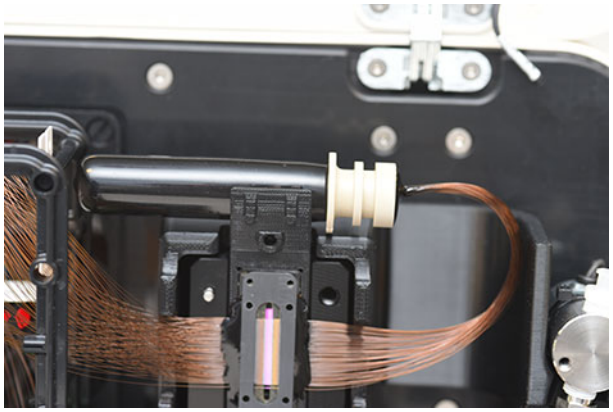


Figure 78 Instrument top compartment – capillary array bundle placement

7 Carefully remove the protective cover from the capillary bundle and place it back on the array window.

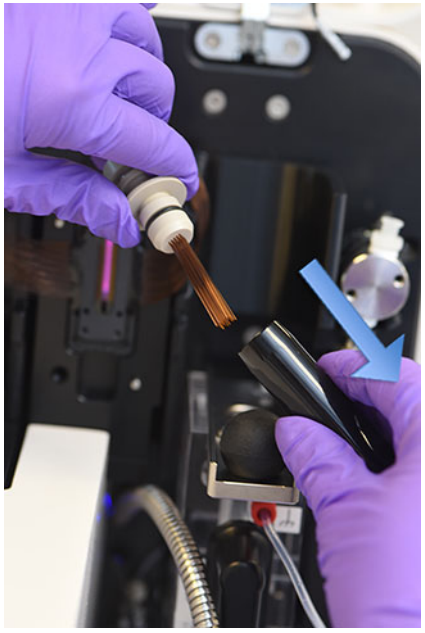


Figure 79 Instrument top compartment – Removal of protective cover

8 Install the capillary array bundle by firmly pushing the capillary array bundle into the reservoir opening until a distinct click is heard.

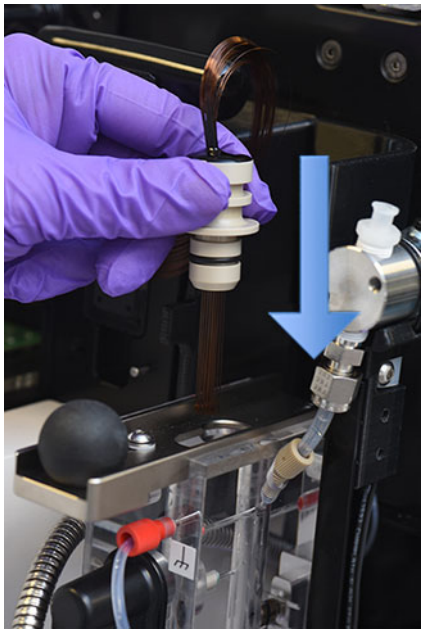


Figure 80 Instrument top compartment – capillary array bundle installation

9 Push in the capillary reservoir connector slide to secure the capillary array bundle.

NOTE

If this step is not followed the capillary array bundle will be damaged upon pressurization.

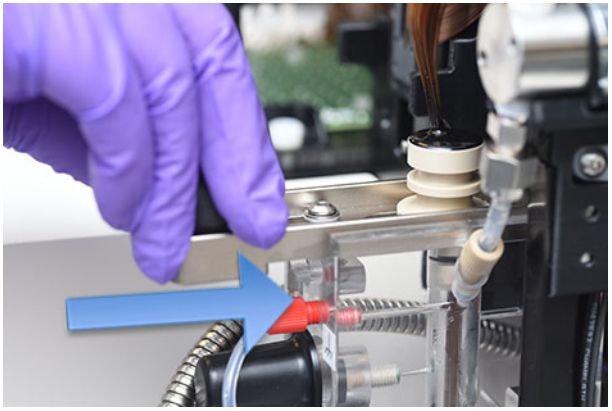


Figure 81 Instrument top compartment – capillary reservoir connector slide

10 Place the light guide over the array window using the two alignment pins.

Ensure that the finger hold faces the right side of the instrument.

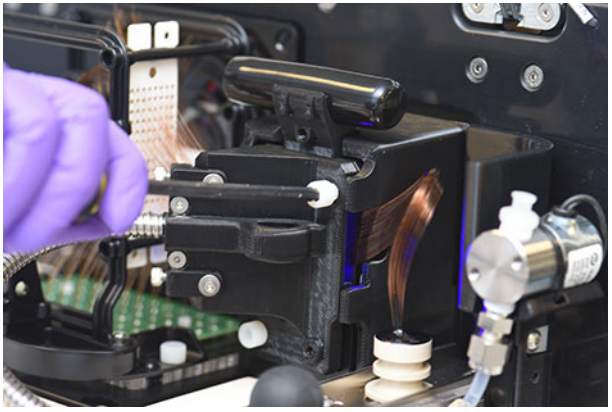


Figure 82 Instrument top compartment – light guide placement

11 Use the provided allen wrench to install the two white screws that secure the light guide to the array window.

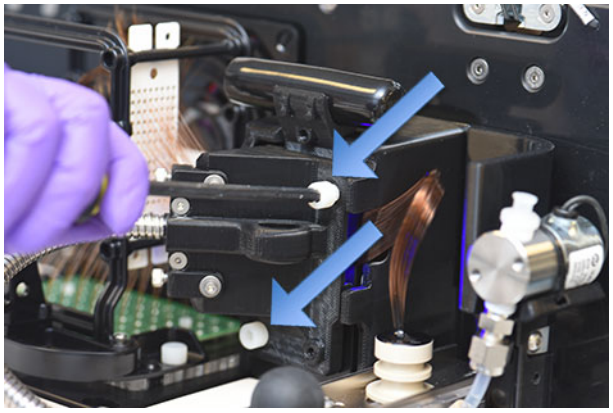


Figure 83 Instrument top compartment – light guide installation

12 Remove the high voltage cable from the array frame holder and firmly push it into the high voltage cable connection.

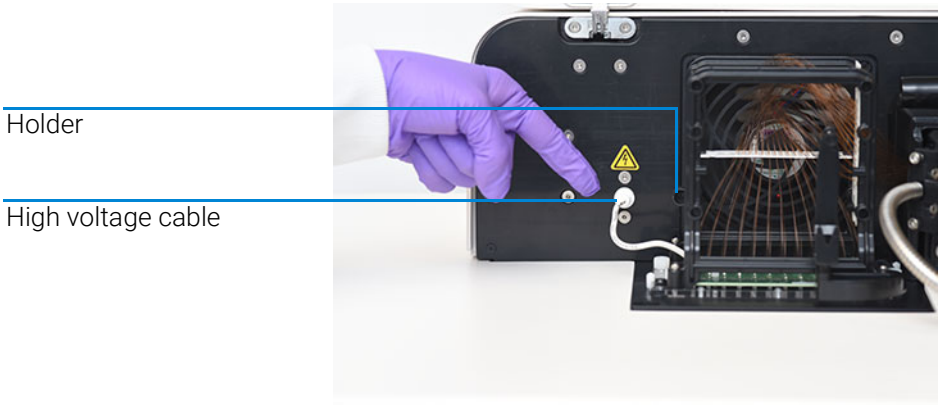


Figure 84 Instrument top compartment – high voltage supply cable

13 Close the reagent door and top hood of the instrument.

Figure 85 ZAG DNA Analyzer system

14 After installation of an array, the ZAG DNA Analyzer will require a capillary alignment as described in **Chapter 4**, “ZAG DNA Analyzer Software – Utilities Menu”.

Using the External Array Docking Station for Storage

This section provides a guideline to store a capillary array cartridge using the external array docking station for long-term storage of the array.

The capillary array can be stored for up to 30 days using the docking station before the storage solution needs to be replaced with new storage solution.

1 Remove the capillary array from the instrument. For detailed instructions, refer to section **“Removal of the Capillary Array”** on page 87.

2 Place the tray base inside the array docking station.

Figure 86 Array docking station with tray base installed

3 Place a 96-deep well tray (Agilent part #P60-20 or Fisher part #12-566-120) into the array docking station with tray base.

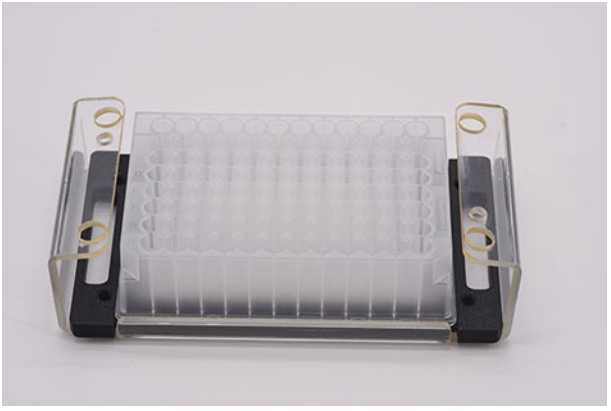


Figure 87 Array Docking Station with 96-deep well plate

4 Fill all wells with 1 mL storage solution

5 Place capillary array into the array docking station using the four leg holes as guides. Ensure that the capillary tips are in the storage solution side of the tray, not left in open air.

6 Insert the two white screws as shown in **Figure 88** to tighten the capillary array into place.

Figure 88 Array docking station with capillary array installed

7 Fill the provided glass vial with 20 mL of capillary storage solution and place into the array spindle storage device.

Figure 89 Array spindle storage device, no storage solution in this example bottle

8 Slide the array spindle storage device onto the capillary array side arm, located to the left of the capillary array window, and screw the locking screw into place (**Figure 90**).

To see a full image of the array with the array spindle storage device installed, refer to **Figure 91**.

Figure 90 Array spindle storage device installation

9 Remove the capillary array outlet spindle from the black storage plug and place it into the array spindle storage device (**Figure 91**).

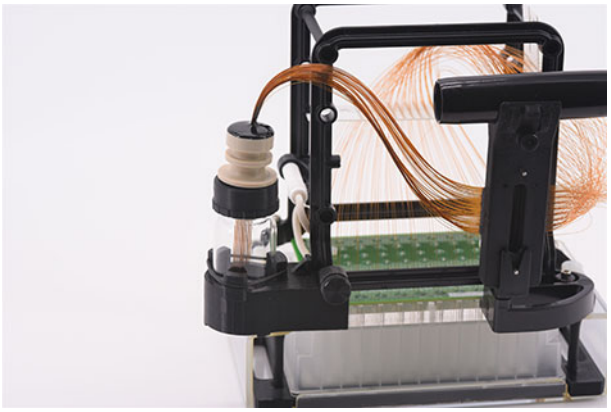


Figure 91 Array docking station with array installed

10 Replace the capillary storage solution monthly; in drier climates it may be required to change the storage solution more frequently, i.e. every 1 to 2 weeks.

9 ZAG DNA Analyzer Software – Sample Name Entry

Sample Name Entry 117

Entering Sample Names Manually 117

Importing Sample Names 118

Importing Sample Names Using a Bar-Code Reader 120

This chapter provides information on how to enter the sample names in the ZAG DNA Analyzer software.

Sample Name Entry

Entering Sample Names Manually

1 From the **Operation** tab, select the tray number, the desired row, and the sample cell.

2 In the field **Sample ID**, enter the desired sample names.

3 Select **Save tray** to save the file as a .txt or .csv (**Figure 92**).

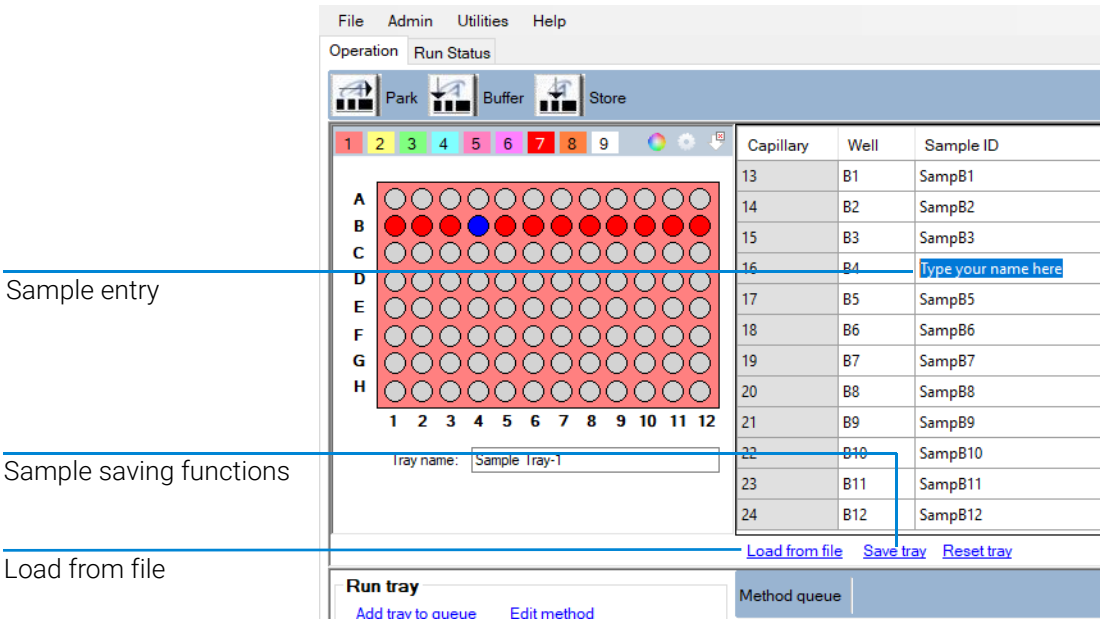


Figure 92 Manual sample entry

Importing Sample Names

✔ The files must be available in .txt or .csv file format.

✔ The data format must comply with the format described below in order for the system to read the files correctly.

1 In the **Operation** tab, select **Load from file** to load a set of saved or previously created sample names.

- For a .txt file, a single column of sample names are used (**Figure 93**).

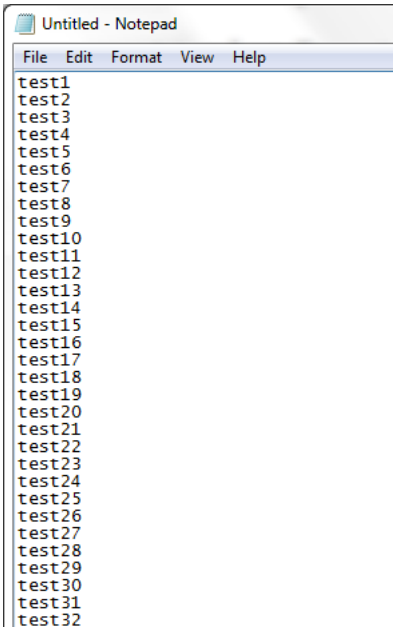


Figure 93 .txt file format (single row of names—no well numbers or row numbers).

- For a .csv file, the format is row number, well number, and sample name (**Figure 94**).

	A	B	C	D	E	F
1	1	A1	SampA1			
2	2	A2	SampA2			
3	3	A3	SampA3			
4	4	A4	SampA4			
5	5	A5	SampA5			
6	6	A6	SampA6			
7	7	A7	SampA7			
8	8	A8	SampA8			
9	9	A9	SampA9			
10	10	A10	SampA10			
11	11	A11	SampA11			
12	12	A12	SampA12			
13	13	B1	test1			
14	14	B2	test2			
15	15	B3	test2			
16	16	B4	test4			
17	17	B5	test5			
18	18	B6	test5			
19	19	B7	test7			
20	20	B8	test8			
21	21	B9	test9			

Figure 94 .csv file format: Row Number, Well number, Sample name

Importing Sample Names Using a Bar-Code Reader

For the purposes of sample name import, a bar-code reader is equivalent to a keyboard. When a bar-code is read the program searches the *Samples* folder for a name that is identical to the bar-code. If a name is found, the file (and the corresponding sample names) is imported.

1 Place the sample name files into the C:/Agilent/Samples folder. If a folder does not exist, create a new *Samples* folder (**Figure 95**). The sample name file can be either a .txt file or .csv file (using the formats described in section **"Importing Sample Names"** on page 118). The sample name files are created by the user or automatically by a LIMS system.

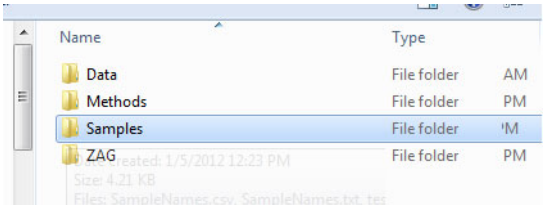


Figure 95 Samples folder

It is critical that the name of the file is identical to what is read by the bar-code reader.

Example:

In **Figure 96**, the name associated with the bar-code is 00060065.

Figure 96 Bar-code 00060065

Thus, the .csv file or .txt file must be given the file name 00060065 and placed in the Samples folder (**Figure 97**).

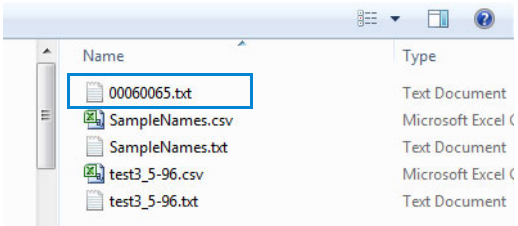


Figure 97 File name 00060065

2 In the field **Tray name** of the **Operation** tab, highlight the tray name with the mouse cursor (**Figure 98**).

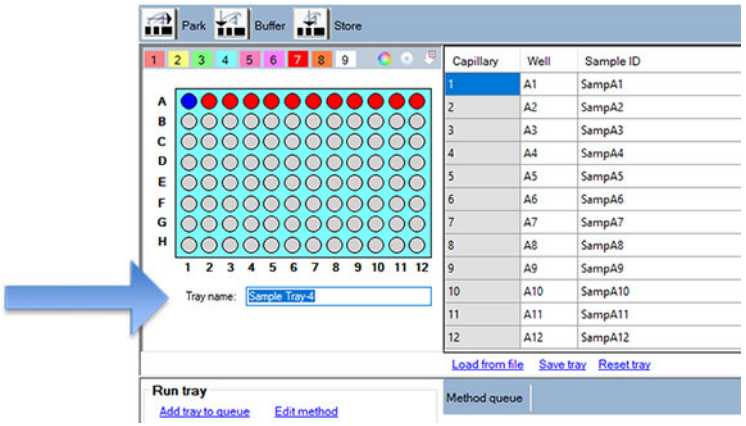


Figure 98 Highlighted of tray name

3 Use the bar-code reader to scan the bar-code on the plate.

The names are automatically imported from the .txt or .csv file in the *Samples* folder (**Figure 99**).

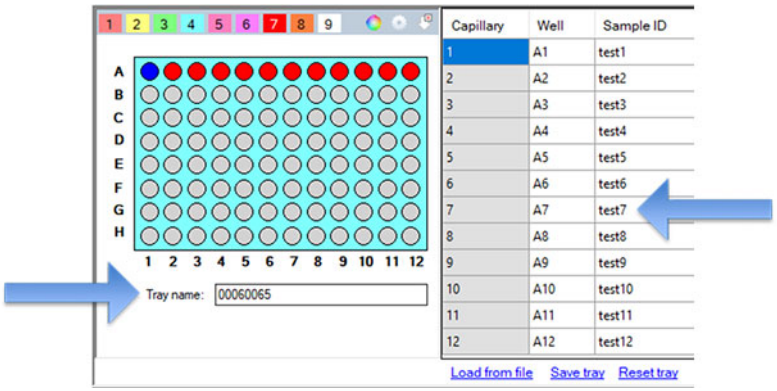


Figure 99 Imported sample names

10 ZAG DNA Analyzer Software – Using the Bar-Code Reader

Adding/Removing Solutions to the gel and conditioning solution bottles 124

Changing Levels of Water or 1 N NaOH 127

Changing Levels of Conditioning Solution or Waste 128

This chapter explains how to add and remove solutions to the gel and conditioning solution bottles using the bar-code reader.

Adding/Removing Solutions to the gel and conditioning solution bottles

There are three general circumstances for which gel levels require adjustment:

- You add gel to the system for the first time.
- The system is running low on gel, and the software prompts you to add more gel.
- You switch a gel (remove one gel and replace it with another).

NOTE

When the system is low on gel, the software automatically opens the dialog **Check solution volumes** shown in **Figure 101**.

1 From the **Utilities** drop-down menu, select **Solution levels** (**Figure 100**).

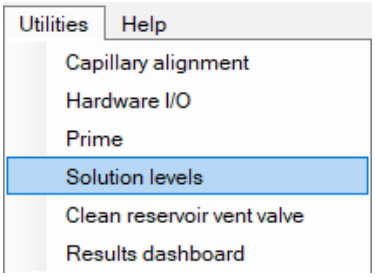


Figure 100 Solution Levels utility

The dialog **Check solution volumes** opens.

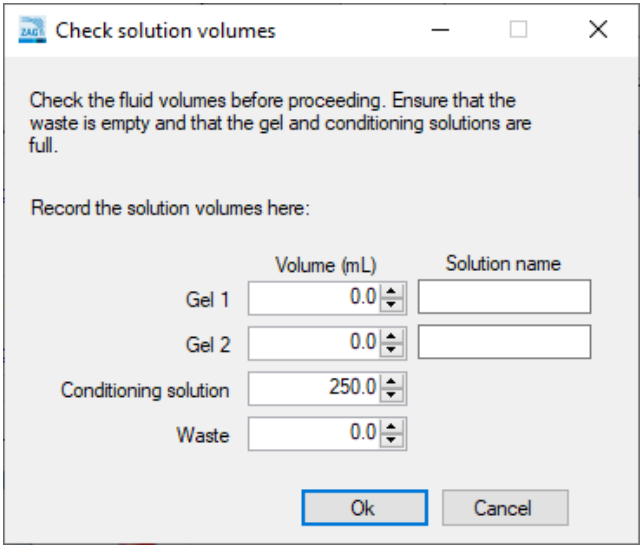


Figure 101 Check solution volumes dialog

2 In the dialog, enter the gel level (based on the volume in the gel bottle).

A prompt opens to enter a bar-code (**Figure 102**).

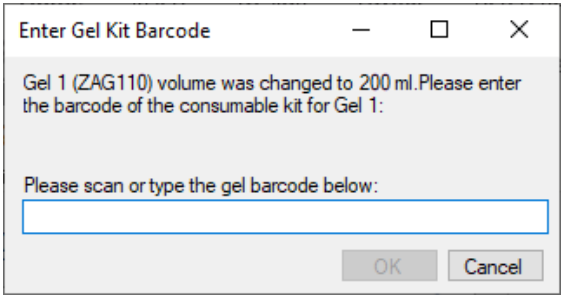


Figure 102 Prompt to enter bar-code

3 Use the bar-code reader to enter the bar-code into the system (**Figure 103**).

Figure 103 Scanning the bar-code of the gel

4 Once the bar-code is accepted, the software responds with an acknowledgment that the bar-code was accepted (or rejected) (**Figure 104**).

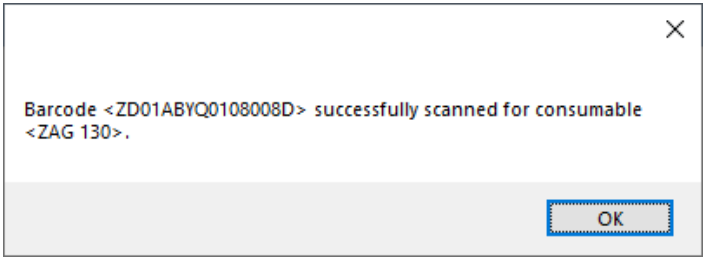


Figure 104 Acknowledgment of bar-code entry

The bar-code is only good for the amount of gel sold in the bottle (500 mL). Once 500 mL of gel is exceeded, the bar-code will no longer be accepted by the system. Thus, a rejection of a bar-code is due to the fact that the code is no longer valid and that the volume allowance for that bar-code has been exceeded.

Changing Levels of Water or 1 N NaOH

When **Water** or **NaOH** are selected as the solution type (**Figure 101**), a bar-code is required to change these levels.

The bar-codes for **Water** and **NaOH** have no limited volume allowance. These bar-codes are located on the inside door of the ZAG DNA Analyzer.

1 When prompted for a bar-code, use the bar-code scanner on the bar-codes attached to the inner door.

Figure 105 Bar-codes for NaOH and Water

Changing Levels of Conditioning Solution or Waste

When changing the level of conditioning solution or waste, simply change the levels in the respective fields (**Conditioning solution**, **Waste**) as indicated in **Figure 101**, and close the window. No bar-code entry is required for these components.

11 ZAG DNA Analyzer Software – Automated Analysis

Automated Analysis 130

Automated (Batch) Analysis Using ProSize 130
Automated Analysis Using the ZAG DNA Analyzer Software 131
Monitoring the Status of the Automated Processed Data 136

This chapter explains the procedure for automated analysis using the ZAG DNA Analyzer system.

Automated Analysis

Automated analysis can be performed using the ProSize software, or the ZAG DNA Analyzer software.

Automated (Batch) Analysis Using ProSize

Once all the analyses are done and saved in a folder, you can batch process an entire set of runs using the batch processing feature of ProSize software.

For more information, refer to Chapter 12 “Batch Processing Using ProSize“ of the *ProSize Data Analysis Software User Manual*, or refer to the video *batch processing*.

Automated Analysis Using the ZAG DNA Analyzer Software

At the end of a run, automated analysis is performed by the ZAG DNA Analzer software (using ProSize), and then the results are automatically exported to the desired folder.

Enabling Automated Analysis

1 From the **Admin** drop-down menu, select **Results report setup** (Figure 106).

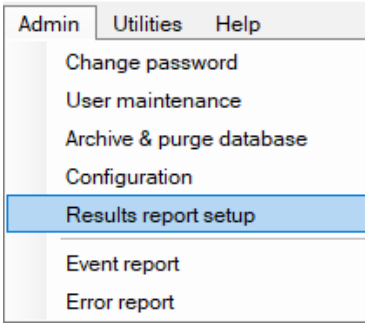


Figure 106 Admin menu

The **Automated Report Settings** dialog opens.

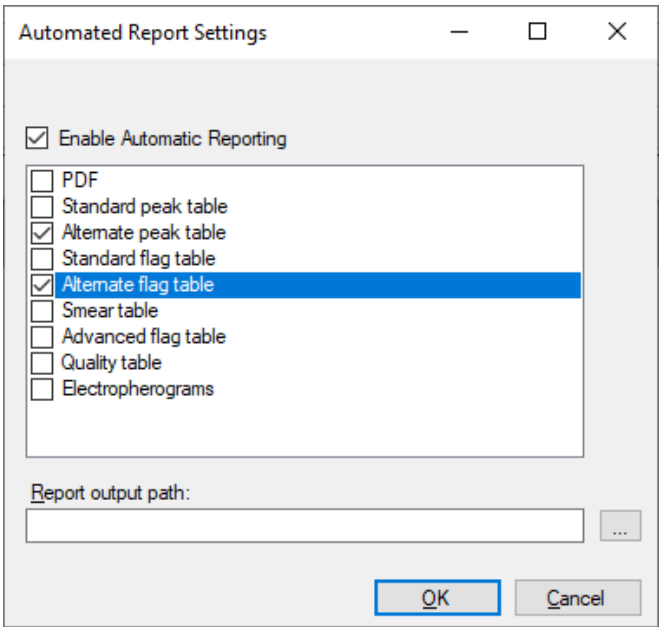


Figure 107 Automated Report Settings dialog

Each of the export options (PDF, Standard peak table, etc.) are described in the Chapter 7, "Exporting Data from ProSize", and Chapter 8, "Generating Reports from ProSize" in the *ProSize Data Analysis Software User Manual*.

2 To enable automated analysis, select the **Enable Automatic Reporting** check box (**Figure 107**).

3 Select the desired export options (PDF, etc.).

The **Report output path** defines where the exported data is placed. If this field is left empty, the exported data will be placed into the original data folder. Create an output folder in a desired location other than the data folder, if desired.

For automated analysis to work correctly, two main criteria that must be met:

- The name of the method in the ZAG DNA Analyzer software (used to acquire the data) must exactly match the name of the configuration file in ProSize software.

  For example, if the method used to run the sample is **ZAG105GP33 - DNA 1-500bp - Gel Prime Only**, then the configuration file in the ProSize software must have the name: **ZAG105GP33 - DNA 1-500bp - Gel Prime Only**.

- The ladder sizing file must be able to be processed by the ProSize software. If the ladder file is not read correctly, then the data will not be processed. This means that the configuration file in the ProSize software must be set correctly, so that the ladder sizing file is correctly read. This also means that ladder well must be of high quality, without anomalous or missing peaks.

  For example, assume a 100 bp ladder is used in well H12, but the configuration file in the ProSize software is set so that the minimum peak height for integration of the ladder is 10000 units. In this case, the ladder is not read correctly by the ProSize software because many ladder elements are missing (**Figure 108**), and the file will not be auto-processed by the ZAG DNA Analyzer software.

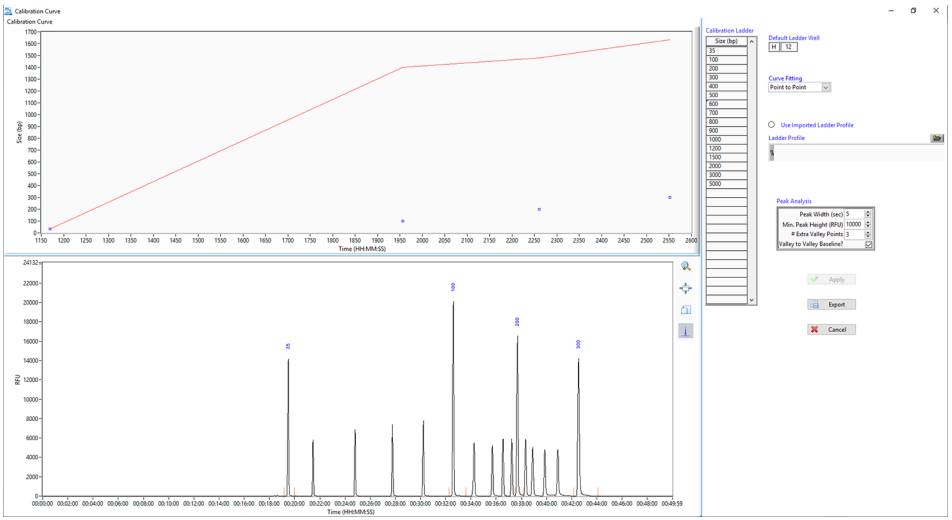


Figure 108 ProSize calibration curve setup

If the configuration file is set with a minimum peak height of 500, then the ladder is processed correctly by the ProSize software, and all the ladder elements are recognized (**Figure 109**).

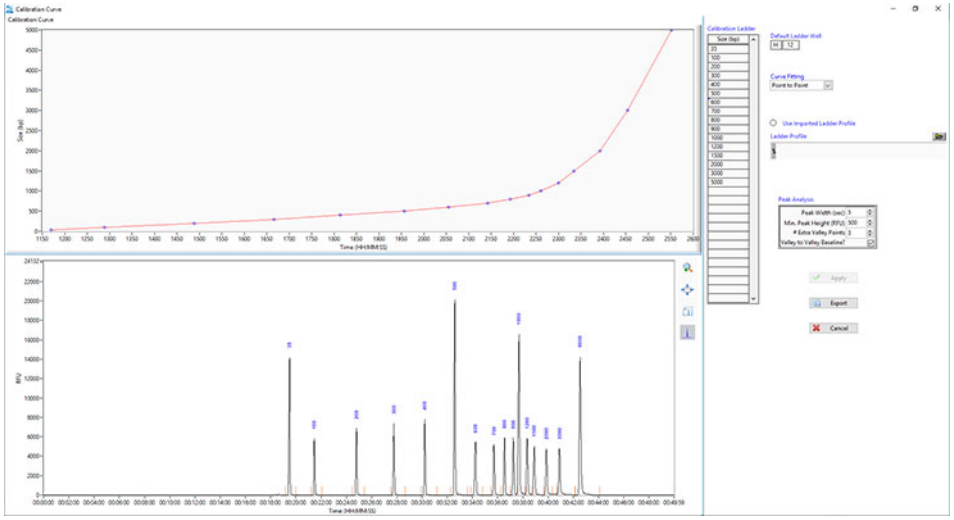


Figure 109 ProSize calibration curve setup

Importing a Ladder File for Automated Analysis

The ZAG DNA Analyzer software uses ProSize software to perform automated post-batch processing. Thus, when changing or preparing certain features of the auto-processing routine, you can utilize the ProSize software to modify configuration files.

Both ProSize data analysis software and ZAG DNA Analyzer software give you the option of using an imported ladder file. For batch or automated processing, the use of imported ladders has several advantages:

- You can use all 96 wells of the sample plate, without having to reserve well H12 for the ladder.
- You can use a high-quality, saved ladder file to process many subsequent files without the need for re-calibration.
- A high-quality ladder file eliminates the chance of a bad auto-processed file due to a poor quality of in-run ladder (i.e. a ladder well that has poor signal, missing, or poorly resolved peaks).

Using the ZAG DNA Analyzer Software to Export a Ladder File and Use an Imported Ladder File

1 Turn on the auto-processing feature (for details, see **"Enabling Automated Analysis"** on page 131).

2 When running a sample, select **Add tray to queue**.

The **Separation setup** dialog opens (**Figure 110**).

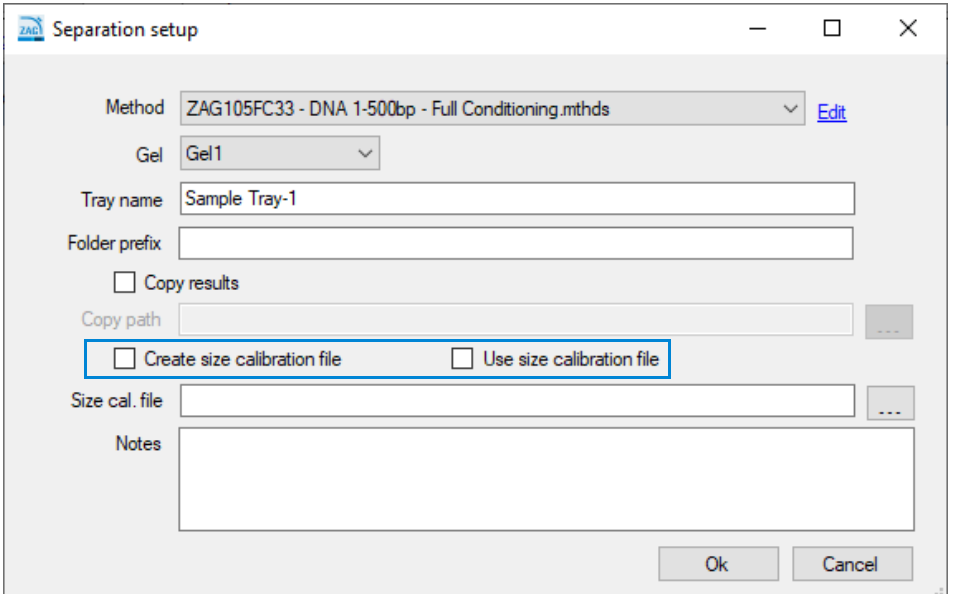


Figure 110 Separation setup dialog

3 Select **Create size calibration file** to create or export a ladder file (.SCAL). At the end of the run, the software automatically uses ProSize software to create the file and place it to the specified location (**Size cal. file**).

Or

Select **Use size calibration file** to import a previously saved ladder file and apply the .SCAL file to the run. This will automatically process and export the desired data (users often run a size calibration ladder once per day, and then use the saved, exported calibration ladder for all subsequent runs of the day).

For more information on size calibration files and how these are created in the ProSize software, refer to Chapter 5 "ProSize Size Calibration Screen" of the *ProSize Data Analysis Software User Manual*.

Monitoring the Status of the Automated Processed Data

The **Results dashboard** command allows you to quickly determine the status of post-processed data.

1 From the Utilities drop-down menu, select **Results dashboard** (Figure 111).

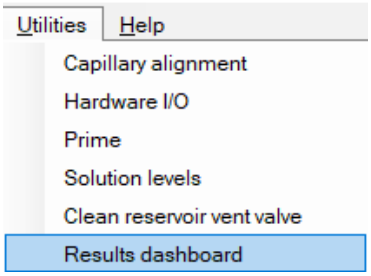


Figure 111 Utilities menu

The **Results Dashboard** window opens. The data files are listed (Figure 112).

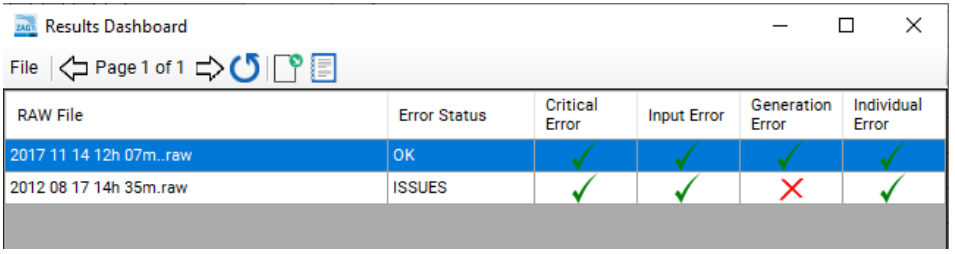


Figure 112 Results dashboard

a Right-click the file, and from the context menu, select **View with Prosize** to open and review the file in ProSize software.

b Select **Error Log** to view the error messages.

A summary of error messages is given in Table 19.

Table 19 Results dashboard error messages

Message	Description
Error Status	Gives a statement of the status of processing. If there is an issue, **ISSUES** will be displayed.
Critical Error	Either a) the method name did not match the configuration file name, or b) the ladder file could not be processed correctly.
Input Error	A user asked for something that could not be generated, such as a flag summary when no flag conditions were set, or a smear table, when the configuration file has no smear conditions.
Generation Error	There was an issue with the generation of a file (.csv, .pdf, or .txt) (usually associated with some operating system error).
Individual Error	There is a problem with an individual capillary, such as a missing upper or lower marker, or unusually broad marker peaks.

The error messages are also recorded in C:\ProSize data analysis software\Error Log. An example error log file is shown in **Figure 113**.

Figure 113 Example Error message

12 Appendix

Permissible Characters 139

Compatible Plates and Tubes 140

Sample/Marker Plates 140
Buffer/Waste Plates 141

Preventative Maintenance Schedule 142

Daily Maintenance 142
Weekly Maintenance 142
Monthly Maintenance 142
Yearly Maintenance 143
As Needed to Restore Separation Performance 143

Capillary Array Cleaning 144

Method A – Flush with CE Grade Water 144
Method B – Submerse Capillary Array Tips/Electrodes in Hot Water 146
Method C – Cleaning the Capillary tips, Electrodes, and Capillary Walls 147

Reservoir Vent Valve Cleaning 150

Capillary Array Window Cleaning 151

This chapter provides additional information on part numbers, maintenance procedures, and system settings.

Permissible Characters

The following tables show which characters are permissible (**Table 20**) and non-permissible (**Table 21**) for a file name.

Table 20 Permissible characters for a file name

Characters	
~	`
!	@
#	$
%	^
&	(
)	_
-	+
=	{
}	[
]	;
’	.

Table 21 Non-permissible characters for a file name

*	:
\	|
”	‘
<	>
?	/

Compatible Plates and Tubes

Sample/Marker Plates

The ZAG DNA Analyzer system has been designed to operate using specific dimensioned semi-skirted and full-skirted PCR plates and deep 96-well plates. A list of approved semi-skirted and full-skirted PCR plates is provided in **Table 22**.

NOTE

Non-skirted PCR plates are not recommended for use with the ZAG DNA Analyzer, as they tend to warp or bow and can therefore interfere with proper sample injection.

Table 22 List of approved PCR plates

Approved Vendor/Part Number	Description
Eppendorf # 951020303 (various colors)	Eppendorf 96-Well twin.tec PCR Plates, Semi- skirted
MidSci Pryme # AVRT1	Pryme PCR Ergonomic Plates, 96x0.2ml, Semi- Skirted, Natural
BioRad Hard-Shell # HSS-9601	Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plates
Greiner Bio-One # 652280	96W PCR Microplate, Polypropylene, Half-Skirt, Natural, No Lid
4titude Framestar # 4ti-0900 or -0770/C	FrameStar 96 semi-skirted
Scientific Specialty # 3450-00	96-Well "Semi Skirt" UltraFlux PCR Plate
Neptune # 3742.X	Semi-Skirted 96-Well PCR Plates
BioRad #HSP9601	BioRad Hard Shell Full Skirt 96-Well PCR Plate
Fisher Scientific #E0030129512	Eppendorf twin.tec 96 Well LoBind PCR Plates, Skirted

NOTE

Contact your corresponding Agilent Sales/Service Representative if a different vendor or style of PCR plate is to be used in order to verify compatibility.The use of PCR plates with different dimensions to the above recommended plates can lead to decreased injection quality and consistency. Damage to the capillary array cartridge tips is also possible.

Buffer/Waste Plates

The ZAG DNA Analyzer uses a specific deep 96-well plate (31 mm height) supplied by Fisher Scientific (Part # 12-566-120) for the buffer and waste plate. This specific plate must be used with the instrument (two plates are supplied upon installation) (shown in **Table 23**).

Standard 1 mL deep well, half height or square well 1 mL 96-well plates should not be used as buffer/waste plates with the ZAG DNA Analyzer as damage to the capillary array will occur.

The same specified buffer/waste plate is also available directly from Agilent in the event that these plates cannot be obtained directly from the manufacturer.

Table 23 List of buffer/waste plates

Item	Vendor / Part #	Description
Buffer/ Waste Deep 96-Well Plates	Fisher Scientific #12-566-120	Fisherbrand 96-Well DeepWell Polypropylene Microplates: Well Capacity 1 mL
Buffer/ Waste Deep 96-Well Plates	Agilent #P60-20	ZAG DNA Analyzer 96-Well Buffer/Waste Tray, case of 50

Preventative Maintenance Schedule

Daily Maintenance

- ✔ Empty the waste bottle and waste tray.
- ✔ Replace the inlet buffer in the buffer tray position.
- ✔ Replace rinse buffer solution when applicable.
- ✔ Ensure there is capillary conditioning solution in the conditioning solution bottle location.
- ✔ Ensure there is gel/dye in the gel bottle location.

Weekly Maintenance

- ✔ Restart the instrument and PC on a weekly basis for optimal running.

Monthly Maintenance

- ✔ Replace the buffer and waste plates with new ones.
- ✔ Replace the capillary storage solution and plate.[1]
- ✔ Replace the gel and conditioning solution bottles with new ones.
- ✔ Inspect the capillary array vent valve for dried gel, clean if necessary.

1 More frequent replacement (i.e. every 1-2 weeks) may be required in low humidity or warmer laboratory environments.

Yearly Maintenance

✔ Replace capillary array after 700 – 1000 injections or one year's time.

As Needed to Restore Separation Performance

✔ Place 0.6 mL of 0.5 N NaOH into the waste tray location and flush the capillary array cartridge with 0.5 N NaOH followed by DI water as described in **"Method C – Cleaning the Capillary tips, Electrodes, and Capillary Walls"** on page 147.

Capillary Array Cleaning

There are three methods to clean/flush a capillary array to remove a clog:

A: Flush with CE grade water

B: Submerse capillary array tips/electrodes in hot water (150 °F – 200 °F)

C: Cleaning the capillary tips, electrodes and capillary walls

A combination of two or more of the methods outlined below may be required in some cases.

Method A – Flush with CE Grade Water

When a capillary array is suspected to have any clogged capillaries, the first step is to flush the array with CE grade water.

1 From the **Operation** tab located on the main screen, select **Add to queue** under the capillary array-conditioning menu.

2 From the drop-down menu of the **Select Conditioning Method** window, select **Method A Flush - Water - 10 min 200 psi.mthdc**.

3 Select **Edit** to ensure that the method matches the parameters seen in **Figure 114**.

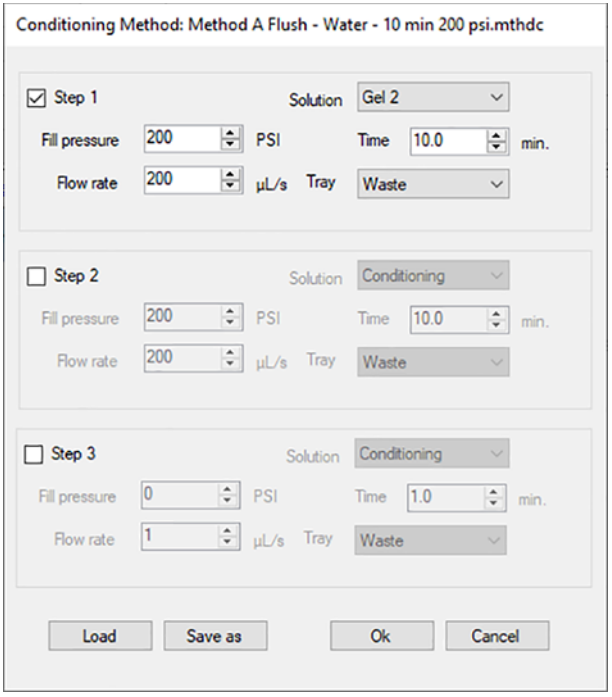


Figure 114 Conditioning parameters **Method A Flush - Water - 10 min 200 psi.mthdc.**

4 If necessary, adjust the method to that shown in **Figure 114**.

5 Click **OK**.

6 Click **OK** again to add the method to the method queue.

7 Open the waste drawer (second drawer from top) and place an empty 96-well deep well plate onto the plate holder.

8 Open the ZAG DNA Analyzer side compartment to replace the gel 2 bottle with a bottle containing CE grade water.

The minimum solution volume required to run Method A Flush for 10 minutes is ≥ 20 mL for a 96-capillary array.

9 Close the door to the instrument side compartment and select **Start** from the method queue to run the capillary conditioning method (for more information, refer to section "**"Method Queue"** on page 74).

10 Once the capillary array conditioning method is complete, open the waste drawer and remove the 96-Well Deep Well Plate.

11 Check the volume of water present in each of the wells used for the flush.

12 For a 10 minute flush there should be ~ 150 µL of CE grade water in each well.

If the waste plate has similar amounts of water present in each well:

1 Empty the 96-Well Deep Well Plate and return it to the waste drawer.

2 Open the side compartment of the ZAG DNA Analyzer and replace the bottle containing CE grade water with an empty bottle and perform a capillary conditioning to remove any residual water from the capillaries.

If a well has significantly less or no water in a well, repeat Method A, or proceed to Method B or C.

Method B – Submerse Capillary Array Tips/Electrodes in Hot Water

1 Select the **park** icon in the main screen to place the plate being held back into its respective drawer and move the stage platform to the bottom of the instrument.

2 Fill a 96-Well Deep Well Plate with 1 mL of hot water (150 ˚F to 200 ˚F) for soaking the tips of the capillary array.

3 For a 96-capillary array, fill each well of the 96-Well Deep Well Plate with 1 mL of hot water.

4 Open the buffer drawer (first drawer from top), and place the hot water filled 96-Well Deep Well Plate onto the plate spacer.

5 Close the buffer drawer securely.

6 From the main screen, locate the hotel positioning icons under the **Operation** tab.

7 Select the **Buffer** icon to position the plate underneath the capillary array.

8 Allow the capillary array to soak for a minimum of 15 minutes to an hour.

9 Select the **Park** icon to return the 96-Well Deep Well Plate to the buffer drawer and place the stage in a resting position at the bottom of the instrument.

10 Perform Method A as described in this Appendix to check the flow of solution through each capillary, or proceed directly to Method C.

Method C – Cleaning the Capillary tips, Electrodes, and Capillary Walls

WARNING

Hazardous solvent

0.5 N NaOH is corrosive and the handling of this solvent can hold health and safety risks. It causes severe eye and skin burns.

- ✔ **Avoid contact with eyes, skin, or clothing.**
- ✔ **Wear eye protection and impervious gloves.**
- ✔ **Refer to the SDS for all warnings and precautions before proceeding.**

The Method C - NaOH flush is the best method for opening clogged capillaries, returning separation to normal as well as ridding the ZAG DNA Analyzer of contamination.

1. Clean the conditioning solution, gel 1, and gel 2 bottle caps with isopropanol or Ethanol.
2. Open the ZAG DNA Analyzer side compartment and replace the gel 2 bottle with a bottle containing 0.5 N NaOH.

   The minimum solution volumes required to run the default capillary conditioning method for 10 minutes is ≥ 20 mL for a 96-capillary array.
3. Place the conditioning, gel 1, and gel 2 lines in the gel 2 bottle containing 0.5 N NaOH.
4. From the **Utilities** menu, select **Prime**.
5. Select the check boxes for **Conditioning**, **Gel 1**, and **Gel 2**.
6. Click **OK** to prime all lines with NaOH.
7. Place a new bottle with new conditioning solution in the conditioning solution position.
8. Place a new bottle with new separation gel/dye mixture in the gel 1 position.

   The minimum solution volumes required to run the default capillary conditioning method for 10 minutes is ≥ 20 mL for a 96-capillary array.
9. From the **Operation** tab located on the main screen, select **Add to Queue** under the capillary array-conditioning menu.
10. From the drop-down menu of the **Select Conditioning Method** window, select the **Method C Flush - 0.5 N NaOH - 10 min 200 psi.mthdc**.

11 Select **Edit** to ensure that the method matches the parameters seen in **Figure 115**.

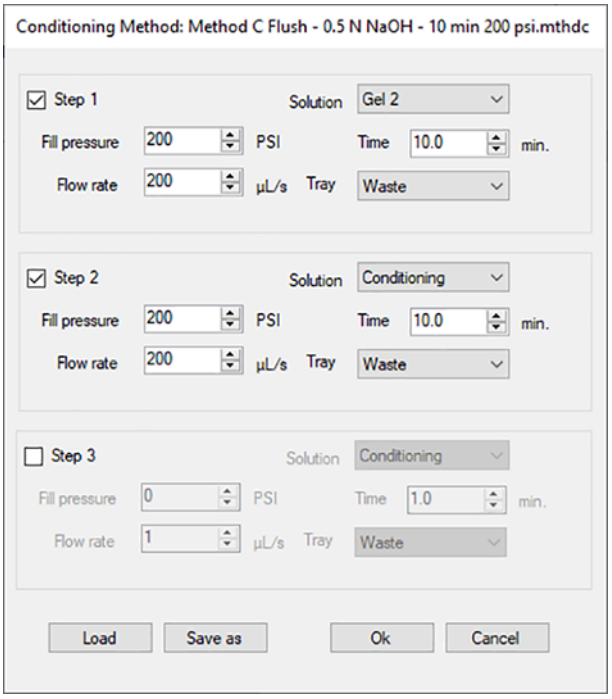


Figure 115 Conditioning parameter **Method C Flush - 0.5 N NaOH - 10 min 200 psi.mthdc**

12 If necessary, adjust the method to that shown in **Figure 115** (**Row** will not be selectable on a 96-capillary instrument).

13 Click **OK**.

14 Click **OK** again to add the method to the method queue.

15 Open the waste drawer (second drawer from top) and place a 96-Well Deep Well Plate filled with 0.6 mL per well of 0.5 N NaOH in all 96 wells for a 96-capillary array onto the plate holder.

16 Close the door to the instrument side compartment and select **Start** from the method queue to run the capillary conditioning method (for more information, refer to section **“Method Queue”** on page 74).

17 Once the capillary array conditioning method is complete, open the waste drawer and remove the 96-Well Deep Well Plate.

18 Check the volume of solution present in each of the wells.

The waste tray wells will be full. Ensure all wells have a similar amount of waste present.

19 Empty the 96-Well Deep Well Plate in the proper aqueous waste disposal area and return it to the waste drawer (second drawer from the top).

CAUTION

0.5 N NaOH is corrosive

0.5 N NaOH can damage the capillary array.

Perform a separation with a Full Conditioning immediately after a 0.5 N NaOH flush or damage will occur to the capillary array.

Reservoir Vent Valve Cleaning

Over time the reservoir vent valve may become clogged requiring cleaning. The ZAG DNA Analyzer has a reservoir vent valve luer lock fitting and syringe allowing you to flush the valve using the **Clean Reservoir Vent Valve** command in the **Utilities** menu.

1 From the **Utilities** menu, select **Clean Reservoir Vent Valve**.

The **Clean Reservoir Vent Valve** window opens (**Figure 116**).

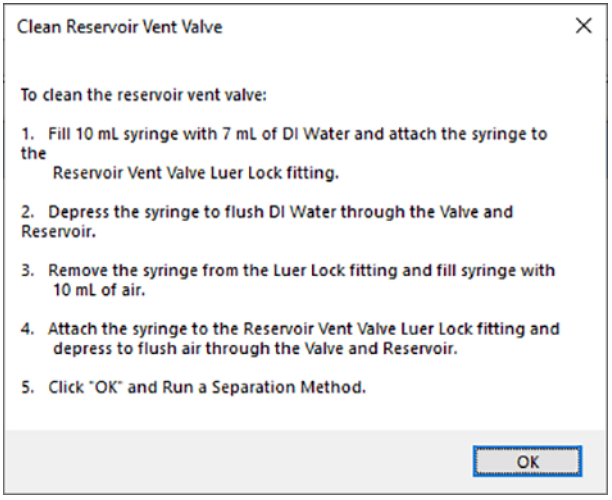


Figure 116 Clean reservoir vent valve window

2 Follow the on-screen instructions to clean the reservoir vent valve.

Capillary Array Window Cleaning

1. Open the side door and hood of the ZAG DNA Analyzer.
2. Remove the light guide from the array window.
3. Remove the bundle end of the capillary array using the capillary array bundle removal tool.
4. Place the bundle in the provided protective cover.
5. Remove the capillary array window from the capillary array window holder. Do not touch the array window.
6. Place a paper tower behind the capillary array window as shown in **Figure 117**.

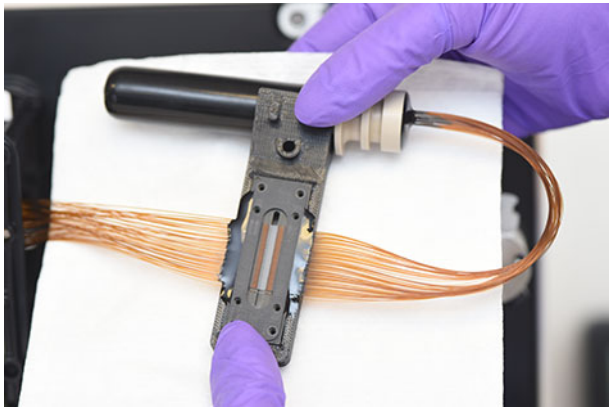


Figure 117 Capillary array window with paper towel behind

7. Using a spray bottle filled with molecular grade ethanol, gently spray the capillary array window.
8. Use a small nylon paintbrush to gently brush the capillaries in one direction while they are still wet. Alternatively a Kim-Wipe can be used to blot the array window dry.
9. Reinstall the capillary array window, bundle, and light guide.
10. Perform a separation on the ZAG DNA Analyzer.
11. Realign the capillaries when finished by navigating to **Utilities > Optical Alignment**.

In This Book

This system manual contains information about the ZAG DNA Analyzer software.

The system manual describes the following:

- system overview,
- software menu commands,
- software tabs,
- capillary array,
- sample name entry,
- automated analysis,
- use of the bar-code reader.

www.agilent.com

© Agilent Technologies, Inc. 2020-2022

Published in Germany
01/2022

Document No: D0002113 Rev. B

Agilent