BiOptic Inc.

Qsep₁ & *Qsep₁*-Plus Bio-Fragment Analyzer User Manual

Innovation **Revolution**

Contents

Safety Information

To ensure the integrity of the equipment, reliability of results, and safe operation, please carefully read and comply with the following instructions:

1. Keep the $Qsep_1$ Bio-Fragment Analyzer away from other electronic devices and voltage sources to prevent interference and potential damage.
2. For safe operation, review the operation manual and laboratory safety guidelines provided with the system.
3. If you have any questions or concerns regarding the operation or safety of the system, please contact BiOptic Inc. through the official website or reach out to your local BiOptic representatives.

Warnings

1. Ensure that both the instrument and the $Qair_{box}$ are connected to an appropriate power source.
2. Use a MAINS power cord within an adequate rating.
3. Do not operate the system if any of the power cords are damaged.
4. Do not remove the covers of the $Qsep_1$ Bio-Fragment Analyzer to perform maintenance on the electrical components, as it is based on capillary gel electrophoresis separation driven by high voltage.

Cautions

1. Use only the components and consumables provided by BiOptic Inc.
2. **DO NOT** perform the following actions:
   - Disassemble the $Qsep_1$ Bio-Fragment Analyzer and its associated parts.
   - Bump or jolt the $Qsep_1$ Bio-Fragment Analyzer.
   - Move or disconnect the $Qsep_1$ Bio-Fragment Analyzer from the external computer or air pump when in action.
   - Open the sample door or cartridge door while the $Qsep_1$ Bio-Fragment Analyzer is in operation.
   - Remove the cartridge while the $Qsep_1$ Bio-Fragment Analyzer is in operation.
   - Power off in action $Qsep_1$ Bio-Fragment Analyzer.
3. Before shutting down or disconnecting the $Qsep_1$ Bio-Fragment Analyzer, unlatch and remove cartridge from the instrument, storing it in the clam-shell container. Failure to do so may cause the gel at the cartridge tip to dry out and damage the cartridge.
4. N_2 gas could also be used as an air source to ensure the air quality and prevent dirty

air from damaging the device.

5. Make sure to press the plastic connection ring before pulling the air tube out from the back of the instrument.

6. Empty the condensate collected in the condensation trap of air pump periodically to avoid the mist from damaging $Qsep_1$ Bio-Fragment Analyzer.

Introduction

We appreciate your purchase of the $Qsep_1$ Bio-Fragment Analyzer. In order to guarantee a fast and convenient experience with this advanced, fully automated, and user-friendly system, we kindly request that you carefully review these instructions before using it. For simplicity, please note that throughout the user manual, the term "$Qsep_1$ Bio-Fragment Analyzer" will be used collectively to refer to both the "$Qsep_1$ Bio-Fragment Analyzer" and "$Qsep_1$-Plus Bio-Fragment Analyzer".

Notices

©BiOptic Inc. 2024

Unauthorized reproduction of this manual in any form or language, including translation into another foreign language, is strictly prohibited without the prior agreement and written consent from BiOptic Inc., in accordance with international copyright laws.

The trademarks "$Qsep$" and "$Q\text{-}Analyzer$" are registered trademarks of BiOptic Inc. The trademark "Microsoft Windows" is a registered trademark of Microsoft Corporation in the United States and/or other countries. All product and company names mentioned are trademarks or trade names of their respective companies.

Symbols

Symbol	Description
$C\epsilon$	CE mark for European Conformity
FC	FCC mark of the United States Federal Communications Commission

*FCC ID for RFID Module: 2AUQB

Warranty Statement

Limitation of Liability

The $Qsep_1$ Bio-Fragment Analyzer, along with its software *Q-Analyzer* and associated reagents, is specifically intended for electrophoresis analysis in general biochemistry laboratories and is intended for Research Use Only. It is important to note that BiOptic Inc. does not assume responsibility for the accuracy and reliability of the results obtained, as well as the safety of the operation, for any profit-driven purposes, diagnostic applications, or non-experimental research.

PRIOR TO OPERATING THE INSTRUMENT, IT IS CRUCIAL TO CAREFULLY READ ALL PRODUCT MANUALS AND STRICTLY ADHERE TO THE PROVIDED INSTRUCTIONS.

BiOptic Inc. holds no responsibility or liability for any personal injury, property damage, or other losses that may occur as a result of failure to comply with or properly familiarize oneself with the manuals, or due to improper operation of the devices.

Warranty Statement

The $Qsep_1$ Bio-Fragment Analyzer comes with a one-year warranty, effective from the date of shipment. The warranty covers all repairs due to damage, defects in materials or mechanical breakdown. In the event that a purchased product is defined as defective due to materials, or mechanical breakdown during the warranty period, please contact us at service@bioptic.com.tw for assistance.

System Overview

The $Qsep_1$ Bio-Fragment Analyzer , developed by BiOptic Inc., is an automated CE (capillary electrophoresis) system that offers enhanced efficiency. It utilizes pen-shaped disposable gel-cartridges, eliminating the need for time-consuming procedures such as gel preparation, sample loading, and capillary changing. The automation provided by the $Qsep_1$ Bio-Fragment Analyzer streamlines the process and facilitates easy result retrieval.

While the $Qsep_1$ Bio-Fragment Analyzer is designed to accommodate 8-well and 12-well trays, its counterpart, the $Qsep_1$-Plus Bio-Fragment Analyzer, offers expanded versatility by supporting 8-well, 12-well, and 16-strip or 18-strip trays (for instruments equipped with firmware version 12 or above). This addition of an extra tray option in the $Qsep_1$-Plus Bio-Fragment Analyzer enhances its flexibility for various applications. The integrated pre-programmed test methods eliminate the complexity associated with capillary electrophoresis experiments, making them more accessible even for operators without extensive training.

With the $Qsep_1$ Bio-Fragment Analyzer, concerns regarding variations caused by different operators and their impact on result accuracy and reproducibility are minimized. The following sections will provide an overview of the product and describe the functions of $Qsep_1$ Bio-Fragment Analyzer and the gel-cartridge. It is important to thoroughly review this section to fully appreciate the capabilities of the $Qsep_1$ Bio-Fragment Analyzer.

⚠️ **Caution:** It is advised that the operator strictly adhere to the instructions provided in this manual. Operating the equipment in a manner not specified may compromise the protection offered by the equipment.

Installation

System Components

Each $Qsep_1$ Bio-Fragment Analyzer package comes with the followings:

$Qsep_1$ Bio-Fragment Analyzer (C100001) or $Qsep_1$-Plus Bio-Fragment Analyzer (C100001-P)

Software Key (C101010)*

$Qair_{box}$ (C104301-00)

Standard and High Resolution Cartridge Kit (C105200)

Alignment Markers and Size Markers

Note: The software key works as a USB drive and contains setup application of $Q\text{-}Analyzer$ and $Qsep_1$ Bio-Fragment Analyzer User Manual.

External Features of *Qsep₁*

Figure 1   Front View of *Qsep₁*

Function	Description
A: Indicator Lamp	Blink blue light when powering on *Qsep₁*; flash green light when it is ready to be connected with Wi-Fi and flash red light when HV is on.
B: Cartridge Door	Open the cartridge door to insert/remove the cartridge and close it after finishing the action.
C: Sample Door	Open the sample door to insert/remove the sample tray before/after use by clicking "Open Sample Door".

Figure 2   Back View of *Qsep₁*

Function	Description
A: Power Connector	Provide power to *Qsep₁* using an adapter and a power cord.
B: Main Power Switch	Switch to turn *Qsep₁* on/off before/after use.
C: Air Flow Inlet Port	Connect to an external pump using an 1/8-inch tube.
D: Cable Port	Connect *Qsep₁* to the operating computer with an Ethernet cable.

Unpacking

Before initiating the unpacking process, thoroughly inspect the shipping boxes for any visible damage. If you notice any signs of apparent damage, contact the package transporter to address the issue.

Upon confirming the integrity of the packaging, proceed to unpack the $Qsep_1$ Bio-Fragment Analyzer system components. Take the time to review all accompanying documents, including the packing list, quick start guide, factory report, and performance qualification documentation. Refer to the packing list to ensure all expected items are present.

Conduct a careful examination of the $Qsep_1$ Bio-Fragment Analyzer to confirm that it is free from damage and that there all components are securely in place. Should any damage or discrepancies be identified, contact BiOptic Inc. or your local distributor for assistance.

It is advisable to retain the original packaging and protective foam for potential transportation or shipment needs. Detailed instructions for packing the $Qsep_1$ Bio-Fragment Analyzer can be found in section Packing. Carefully place the $Qsep_1$ Bio-Fragment Analyzer onto the bench in its intended location. For guidance on system location, please refer to section System Location.

System Location

To ensure the best performance of the $Qsep_1$ Bio-Fragment Analyzer, it is essential to provide a suitable environment with the following recommended placement conditions:

- Use a flat and stable laboratory bench that is free from devices generating vibrations.
- Ensure there is a clear space above the instrument, approximately 711 mm (28 inches), to allow for easy insertion of gel cartridge.
- Ensure there is a clear space behind the instrument, approximately 76 mm (3 inches), to facilitate proper air ventilation and accommodate electrical and air tubing connections.
- Maintain the ambient temperature within a range of 18°C to 26°C (65°F to 78°F).
- Maintain the optimal operating humidity within 40 to 95% RH range, non-condensing.
- Keep in mind that the optimal altitude for the $Qsep_1$ Bio-Fragment Analyzer is -20 m to 2,000 m (-65 ft to 6,500 ft).

⚠️ **Warning**: Avoid storing, transporting, or using the $Qsep_1$ Bio-Fragment Analyzer in conditions where the temperature could fluctuate significantly, leading to water

condensation in the instrument. Condensation may damage the electronic components of the instrument. If the instrument is transported in cold weather, refrain from removing it from its original box immediately, as it may cause condensation. Make sure to warm up the $Qsep_1$ Bio-Fragment Analyzer inside its original box before unpacking it to prevent any potential damage.

Power Requirements

The input voltage of $Qsep_1$ Bio-Fragment Analyzer is 12 V DC, and the maximum power is 80 W. A suitable power adapter with cord, MEAN WELL GSM90B12-P1M, is included in the package (100-240 V AC, 50-60 Hz). The dimensions are 24 cm(L) x 21 cm(W) x 30 cm(H) and the weight of the instrument is 5.5 kg. Direct control the instrument with Ethernet cable or remote control with Wi-Fi. Connect the air source within the range of 50 psi-65 psi.

Note: Use a surge protector outlet for $Qsep_1$ Bio-Fragment Analyzer and the computer to avoid electromagnetic interferences and damages to the inner electrical devices.

Computer Requirements

	Minimum	Recommended
Operating System	Windows 10 64 bit	Windows 11 64 bit
Processor (CPU)	Intel Core i5	Intel Core i7
Memory (RAM)	8G RAM	16G RAM
Hard Disk Drive	500 GB	1 TB
Monitor	1920 x 1080	1920 x 1080
Port	RJ45 x 1 USB 3.0 x 1	RJ45 x 2 USB 3.0 x 2

System Setup

To setup the system, please follow these step-by-step instructions:

1. Begin by unpacking the $Qair_{box}$ and securely attach the air tube with the connector. Plug the power cord into the $Qair_{box}$.

2. Connect the power cord and, if necessary, the Ethernet cable to the $Qsep_1$ Bio-Fragment Analyzer. Verify that the power cord is labeled $Qsep_1$. On the other end of the Ethernet cable, connect it to the operating computer. Finish by plugging the other end of the air tube into the $Qsep_1$ Bio-Fragment Analyzer.
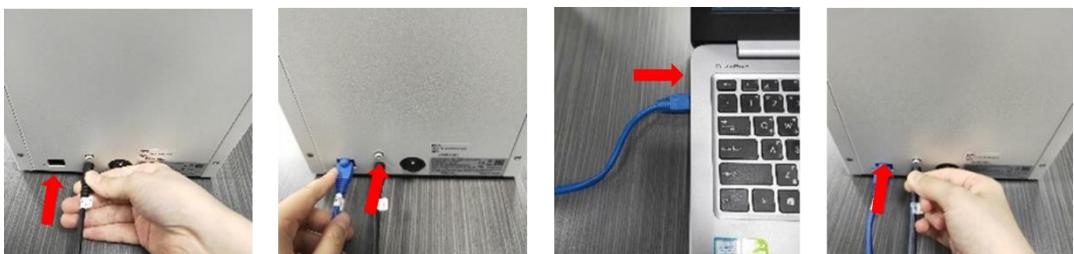
3. Before powering on the $Qsep_1$ Bio-Fragment Analyzer, ensure the protective foam has been completely removed. You can scan the QR code on the sticker for an instructional video.

4. Power on both the $Qair_{box}$ and the $Qsep_1$ Bio-Fragment Analyzer to initiate your analysis.

By carefully following these steps, you will successfully set up the system for optimal performance during your analysis.

Software Installation

Q-Analyzer is a software specially designed to operate *Qsep₁* Bio-Fragment Analyzer. The software key required for installation can be found in the package. To initiate the installation process, click on "**Q-Analyzer-Setup.exe**". The installation steps are outlined below:

1. Start the Setup Wizard by clicking on "Q-Analyzer-Setup.exe".


2. Specify the default data folder.
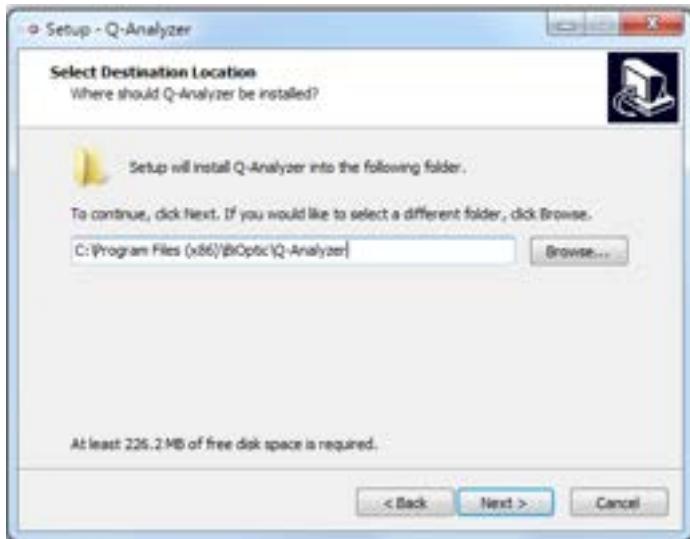
3. Choose the program destination.
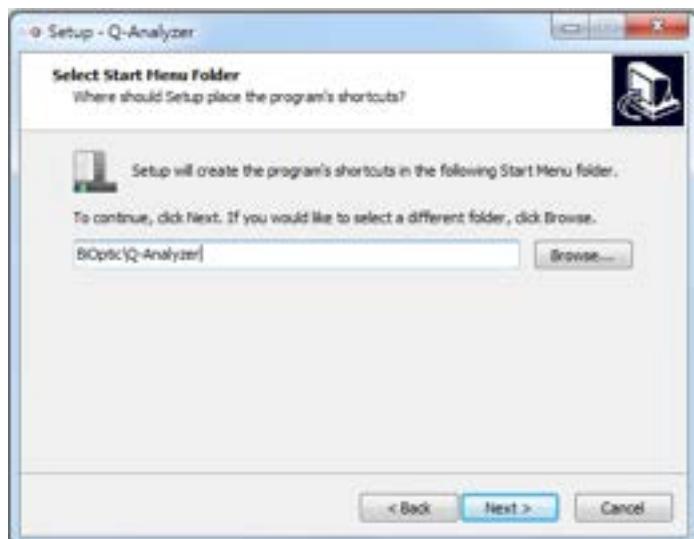
4. Create a desktop shortcut and select the drivers for installation.
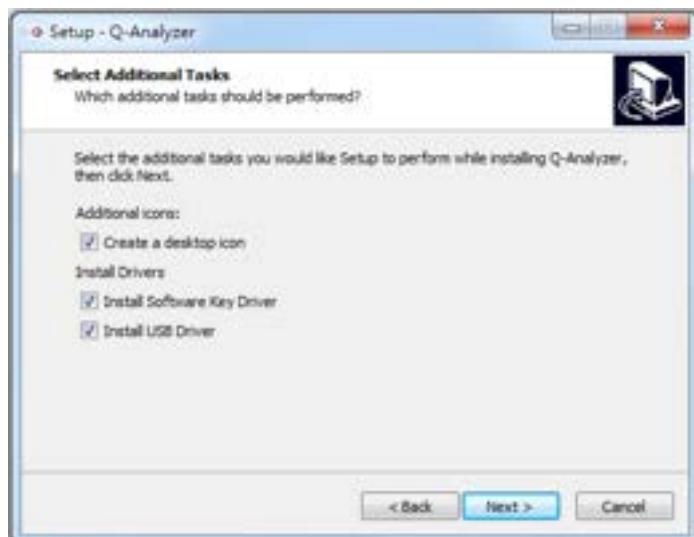
5. Review installation settings and click on "Install" to begin the process.
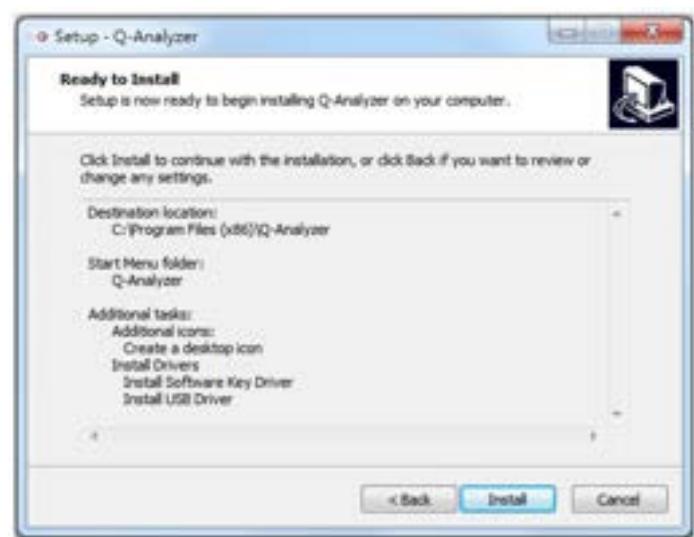
6. The installation of *Q-Analyzer* will be in progress.
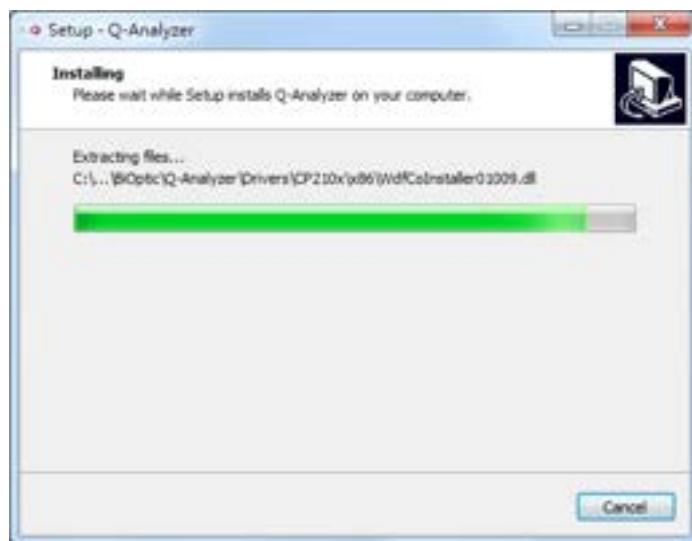
7. Once the installation is complete, *Q-Analyzer* will be successfully installed.

Once the *Qsep₁* Bio-Fragment Analyzer is connected, launching *Q-Analyzer* will bring the **Main Window** on the screen. In the Main Window, you can access the instrument page, which is used to establish communication with the device (Figure 3). When connected to the *Qsep₁* Bio-Fragment Analyzer, the image of the instrument will be displayed in color.
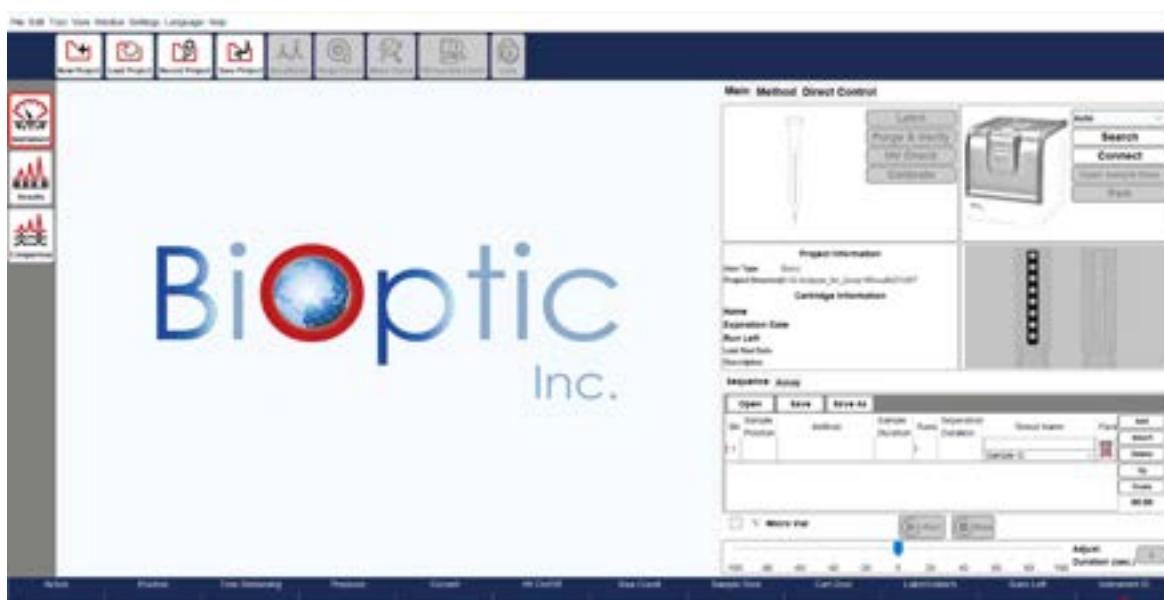
Figure 3   Software Overview

Software Mode

Q-Analyzer, *Q-Editor*, and *Q-Viewer* are integrated into the installed software.

Q-Analyzer setup includes all three programs, eliminating the need to install them separately. To install the software, please double-click the icon and follow the instructions provided on-the screen. When the *Q-Analyzer* icon is double-clicked, the software will automatically detect the status and launch the corresponding program based on the following conditions: *Q-Analyzer*, *Q-Editor,* or *Q-Viewer*.

Table 1. Software Authentication Table

Condition Mode	Advanced Software Key	Basic Software Key	Instrument Connected	Q-Editor License	None
Password	✓	✓			
Q-Analyzer (Advanced)	✓				
Q-Analyzer (Basic)		✓	✓		
Q-Editor				✓	
Q-Viewer					✓

Table 2. Software Function Table

Condition Mode	Instrument Control (Advanced)	Instrument Control	Post Data Analysis	Read	Note
Q-Analyzer (Advanced)	✓	✓	✓	✓	Create Method
Q-Analyzer (Basic)		✓	✓	✓	
Q-Editor			✓	✓	License bound to the PC
Q-Viewer				✓	

Even without connecting to the *Qsep₁* Bio-Fragment Analyzer or inserting the software key, you can click on "**To View**" to launch the *Q-Viewer* after launching *Q-Analyzer*. The *Q-Viewer* is specifically designed to display result files and serves this program exclusively.

Figure 4   Input your license

Table 3. Software Interface Table

Mode	Description
Q-Viewer	
Q-Editor	
Q-Analyzer	

Software Key

Software Key Activation

To ensure the initial use of the software key, it is necessary to activate it beforehand. Insert the software key into a computer that has an available USB port prior to launching *Q-Analyzer*.

Basic Software Key (Blue String)

Advanced Software Key (Green String)
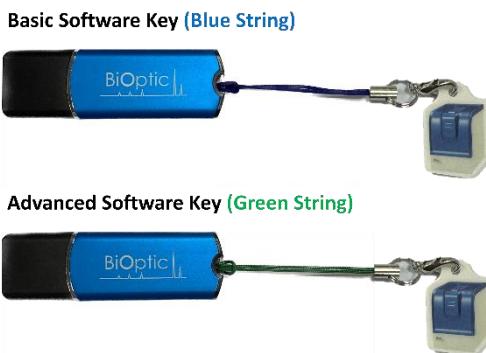
Figure 5   Software Keys

Upon starting *Q-Analyzer*, the system will automatically lead you to the activation page (Figure 5). On this page, you need to enter the Company Name, User ID, and Password. After confirming the password, you will be directed to the login page (Figure 6).
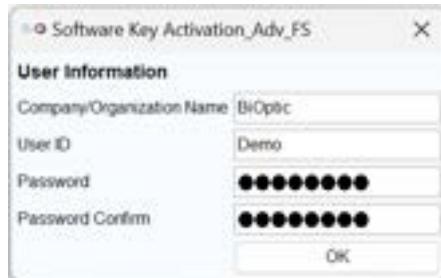
Figure 6   Software Key Activation

*Note: The password should be between 8 and 20 characters in length and can consist of a combination of characters and/or numbers. The password is case-sensitive. If you wish to change the password of the software key, you can navigate to the **Change Password** option in the **Menu → Setting** after launching *Q-Analyzer*.

Figure 7   Login Page

Table 4. Software Key Function Table

| Tab	Basic Software Key	Advanced Software Key
Method		
Direct Control		
Board Setting	N/A	

Forget Password

In case you forget the password, the following steps can be followed to reset it:

1. Right-click on the *Q-Analyzer* desktop icon to find and open the **Toolbox**.
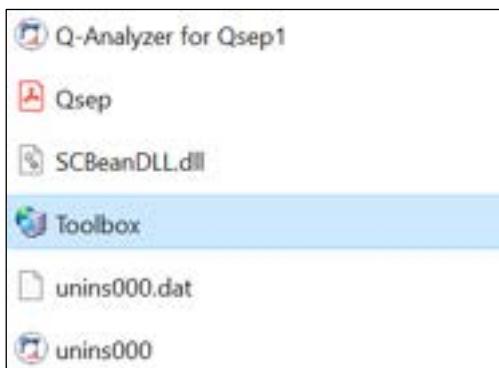
→ Insert the software key and click on "Toolbox".

→ If the software key is detected, the **Next** button will become active.

→ Click on "Next" to access the Toolbox window.

2. Click **Unblock Software Key**

The action will reset the password "00000000".
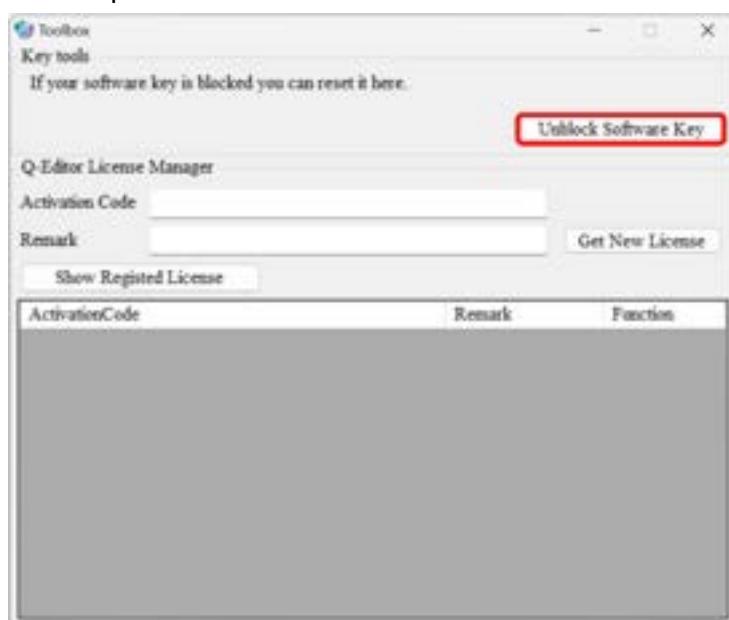
Q-Editor License

Q-Editor mode is designed for offline post data analysis, requiring a registered license that is bound to the computer. Each software key can provide up to five *Q-Editor* licenses for different computers.

To import the license, follow the steps outlined below:

1. Launch *Q-Analyzer* without connecting the instrument or inserting the software key. The message box prompting you to **Input your license** will appear on the screen. Click on **Copy to clipboard** in the message box.

2. Right-click on the *Q-Analyzer* desktop icon to find and open the **Toolbox**. If the software key is detected, click on **Next** to access the Toolbox window.
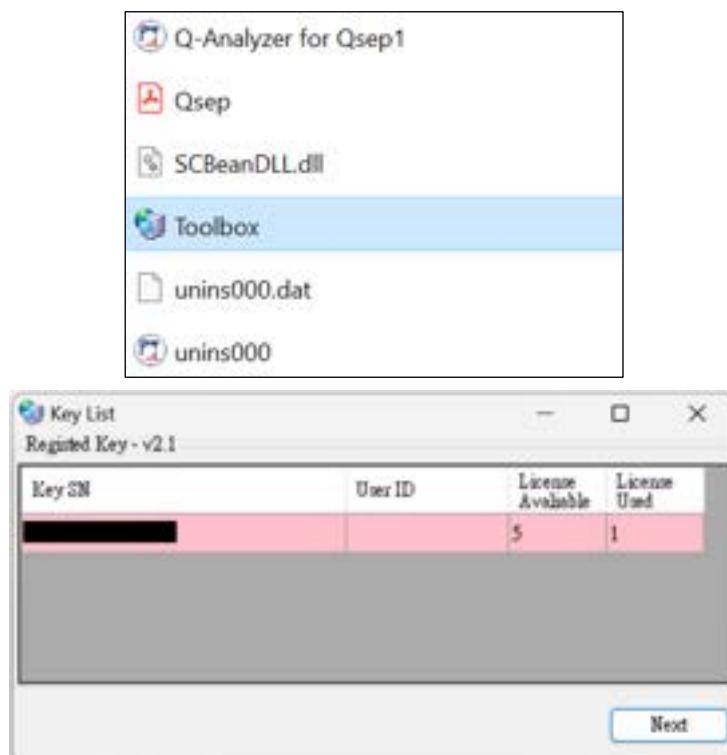
*Note: The available and used number of *Q-Editor* license will be displayed in the table.

3. Get *Q-Editor* license file.

   A. Paste the activation code copied from **Input your License Toolbox**.

   B. Enter information in the **Remark** field before obtaining the new license.

   C. Click on **Get New License** to generate a new license and then select **Download** to save the license. This license will be used to launch the *Q-Editor* when there is no instrument connected or software key inserted.
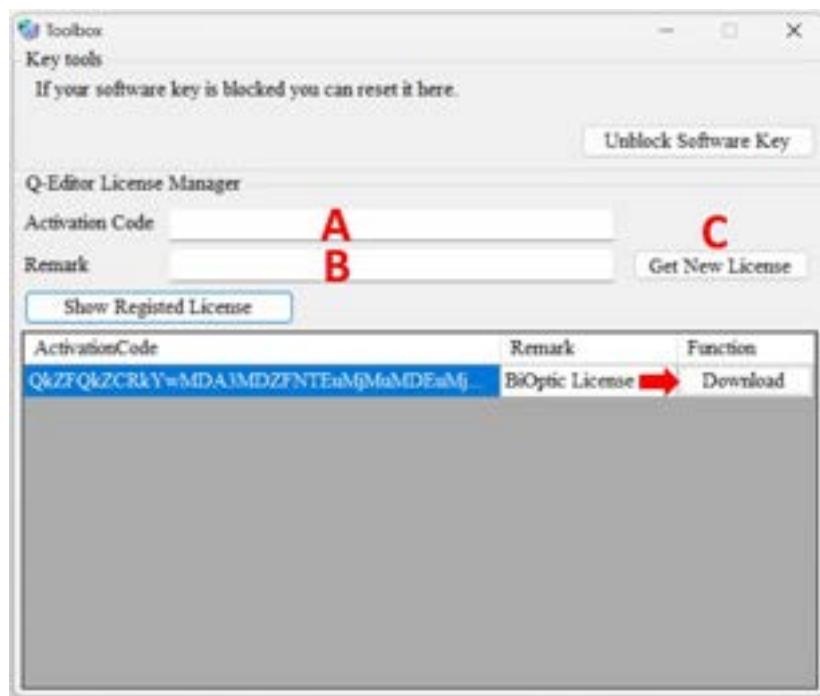

*Note:** If the computer has already been registered with the software key, you can retrieve the license by clicking on **Show Registered License** to download the license file again.

4. Return to the **Input your license** window and click on **Import License** to import the downloaded file. This will enable access to *Q-Editor* on the computer.

Packing

Instructions for Packing and Transporting the *Qsep₁* Bio-Fragment Analyzer:

1. Unlatch the cartridge and open the cartridge door to remove the cartridge from the instrument.
2. Click on "Change Sample" for sample removal and "Change Buffer" to extract the buffer tray from the instrument.
3. Click on "Lock**"** in the Toolbar to position the tray holder in the lock position. If you have the protective foam, insert it to better protect the motor, or the tray holder will go to the lock position directly. Confirm the lock status through the pop-up window. Proceed by closing the *Q-Analyzer* and powering off the instrument.
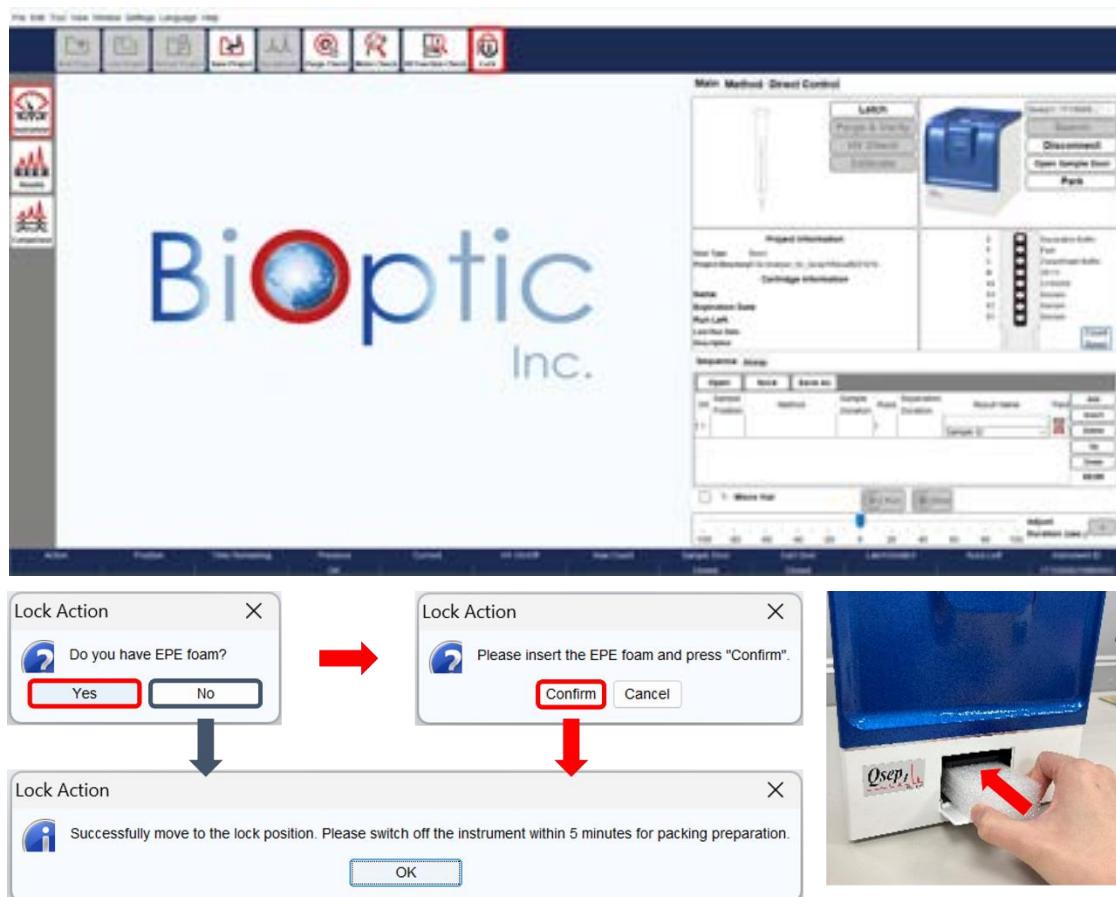
4. Unplug the Ethernet cable, if used, from the back of the instrument.
5. Unplug the power cord from the back of the instrument.
6. Remove the air tube by simultaneously pressing the connector and pulling the air tube.
7. Place the *Qsep₁* Bio-Fragment Analyzer into the original packing it was shipped in.

Operation

Once *Q-Analyzer* is launched, the Main Window will be displayed on the screen. You can access the **Instrument** function within this window to establish communication with the device (Figure 8). In this section, we will focus on introducing the features of the **Main** tab and how to operate *Qsep₁* Bio-Fragment Analyzer (Figure 8 A). The other function tabs will be discussed in section *Q-Analyzer* User Interface.

The software will automatically generate a default project named according to the operation date. Additionally, you have the option to create projects for experiments with different objectives by clicking on **New Project** in the Toolbar (Figure 8 B) before connecting the instrument.
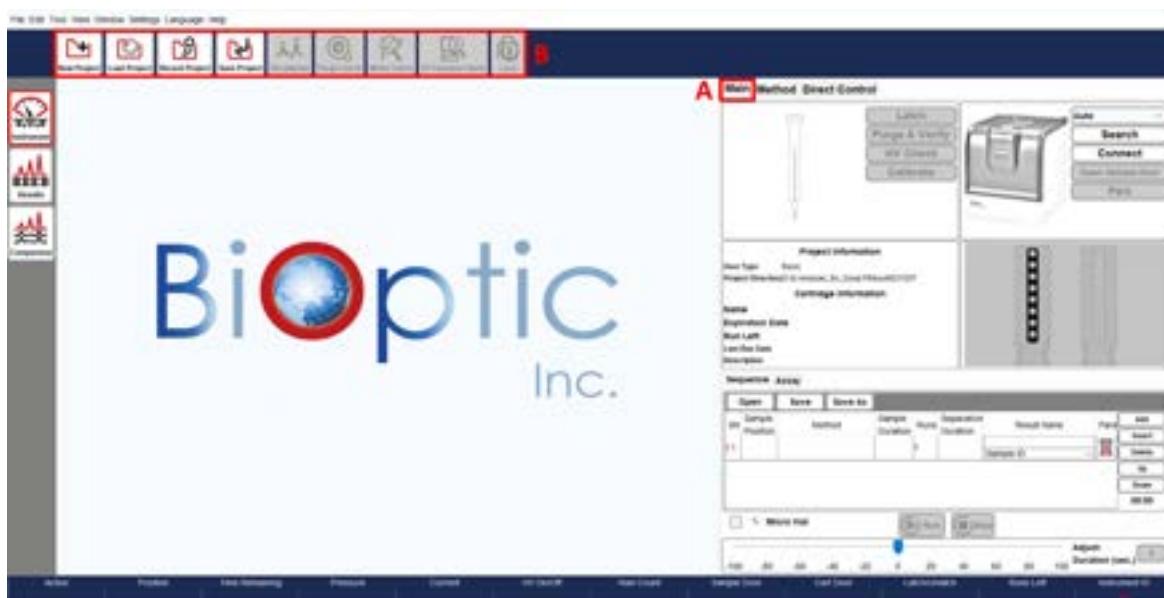
Figure 8   Main Window of the Instrument Mode

Getting Started with *Q-Analyzer*

Q-Analyzer is a software specially designed for operating the *Qsep₁* Bio-Fragment Analyzer. To use the software, establish a connection with Wi-Fi or an Ethernet cable (with power on), or insert the software key into an available USB port on the computer.

*Note: For the *Qsep₁* Bio-Fragment Analyzer, the tray holder is secured using protective foam. Before powering on the device, it is crucial to remove the protective foam. Failure to do so may result in motor damage.

Figure 9   Launch *Q-Analyzer*

There are two versions of the software key: Basic and Advanced, each providing different levels of access to software functions. Each key is associated with its respective user account. Basic users can only utilize the pre-programmed test methods, while Advanced users can create customized test methods and utilize additional functions. The Basic software key is included in the instrument package. For more detailed information, please refer to section Software Key.

Connection Assistance

To ensure a successful connection between the *Qsep₁* Bio-Fragment Analyzer and the computer, please follow these steps:

If using Wi-Fi:

1. Turn on the power of the *Qsep₁* Bio-Fragment Analyzer.
2. Connect with Wi-Fi when the LED flashes green light by checking AP source and finding the SSID of the *Qsep₁* Bio-Fragment Analyzer, which is the combination of Qsep1 and instrument serial number. Enter the password, which is the middle 8 digits of the instrument serial number, to connect.
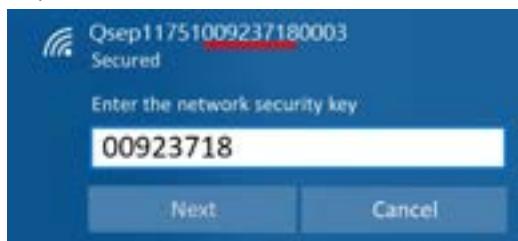
3. Click the software icon to launch the *Q-Analyzer*.

If using an Ethernet cable:

1. Turn on the power of *Qsep₁* Bio-Fragment Analyzer.
2. Connect the *Qsep₁* Bio-Fragment Analyzer to the computer using an Ethernet cable.
3. Click the software icon to launch the *Q-Analyzer*.

Click on the **Connect** button on the Control Panel. Once the button text changes to **Disconnect** and the displayed image of *Qsep₁* Bio-Fragment Analyzer turns into color, the connection is established (Figure 10 A).

Figure 10   Instrument Connection

Upon connection and disconnection, a **Purge Check** message box will appear (Figure 11). Click on **Purge Check** and follow the provided instructions to complete the process. Performing the Purge Check ensures that air tube is not clogged before use and prevents gel from accidentally entered the air tube after use.

*Note: To prevent permanent damage to the system, it is recommended to execute the **Purge Check** whenever prompted by the software.
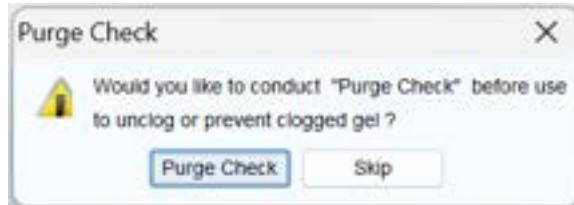
Figure 11   Purge Check Message Box

Buffers, Markers, and Samples Preparation

To operate the *Qsep₁* Bio-Fragment Analyzer, you need to prepare samples, buffers, and markers, and then place them in their designated positions on the sample trays (Figure 12).
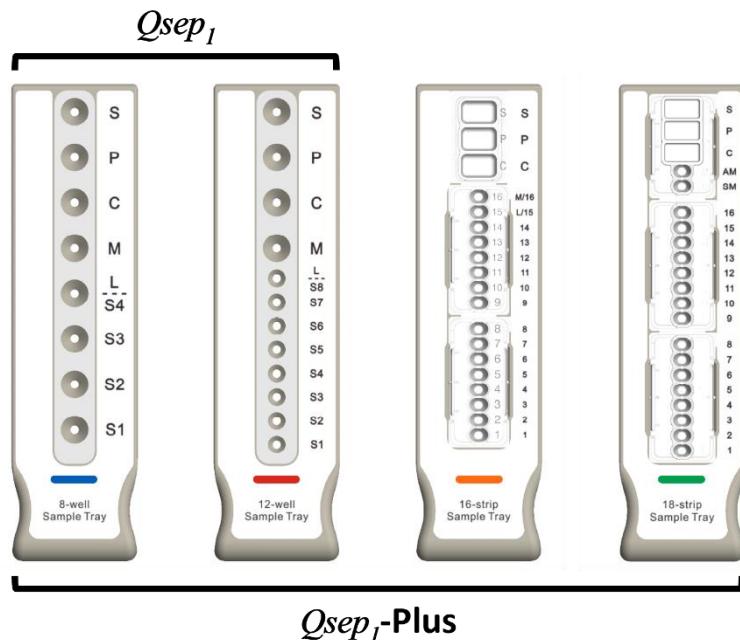
Figure 12　　Sample Tray Types

Compatible Sample Tubes

Refer to the table below to identify the tubes that are compatible with 8-well, 12-well, 16-strip, and 18-strip trays. It is important to note that volume requirements vary between trays, and the micro vial is intended for a single-use injection; disposed of it after use.

Table 5. Compatible Sample Tube Table

Tray Type \ Sample Tube	Name	Catalog No.	Volume Requirement	Image
8-well Sample Tray	Micro Vial	C104250	≥ 2 µL	
	0.1 mL PCR Tube	-	≥ 10 µL	
	0.2 mL PCR Tube	C-STRIP-1	≥ 20 µL	

12-well Sample Tray	0.1 mL Strip Tube	C104252	≥ 10 µL	
16-strip Sample Tray (*Qsep₁*-Plus Only)	16-strip Sample Tube	C104254	≥ 10 µL	
18-strip Sample Tray (*Qsep₁*-Plus Only)	18-strip Sample Tube	C104257	≥ 10 µL	

*Note: Ensure adherence to specified volume requirements for optimal performance of the $Qsep_1$ Bio-Fragment Analyzer. Dispose of micro vials responsibly after a single use.

Buffer & Marker Preparation

Prepare the buffer by placing the Separation Buffer tube in the S well, d_2H_2O tube in the P and C wells, and the Alignment Marker tube in the M well of the buffer tray. For specific volumes, refer to Figure 13 below detailing the required volume for each tube in the buffer tray. Optionally, position the Size Marker tube in the L well, which can be also used for samples.

*Note: A pop-up window will prompt users to consider changing the buffer after completing 20 runs. While this is initially optional, it will transition to a mandatory action after reaching 30 runs.
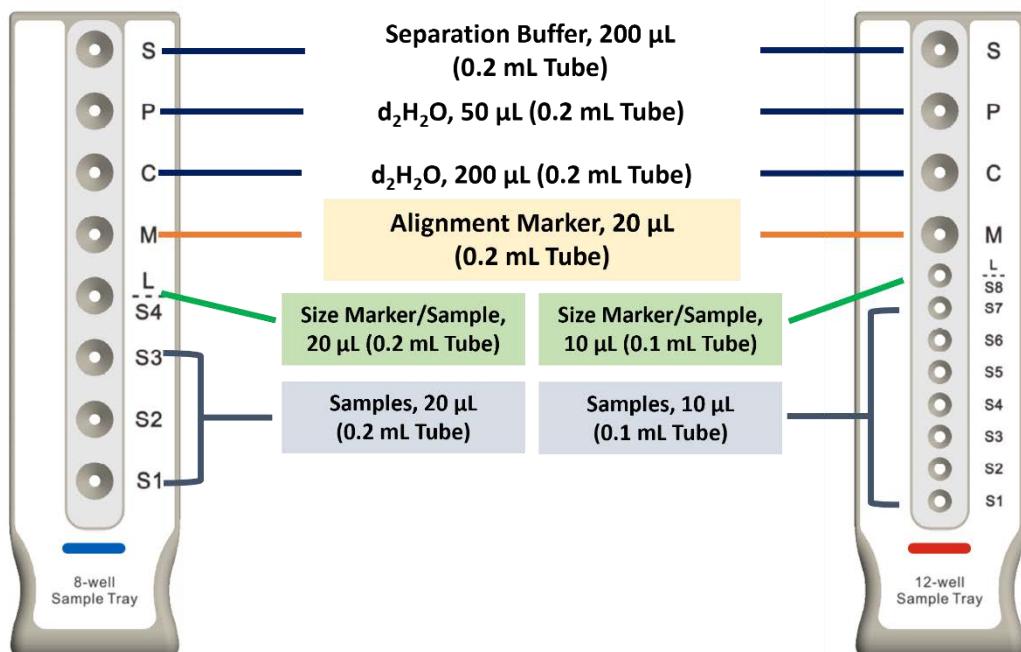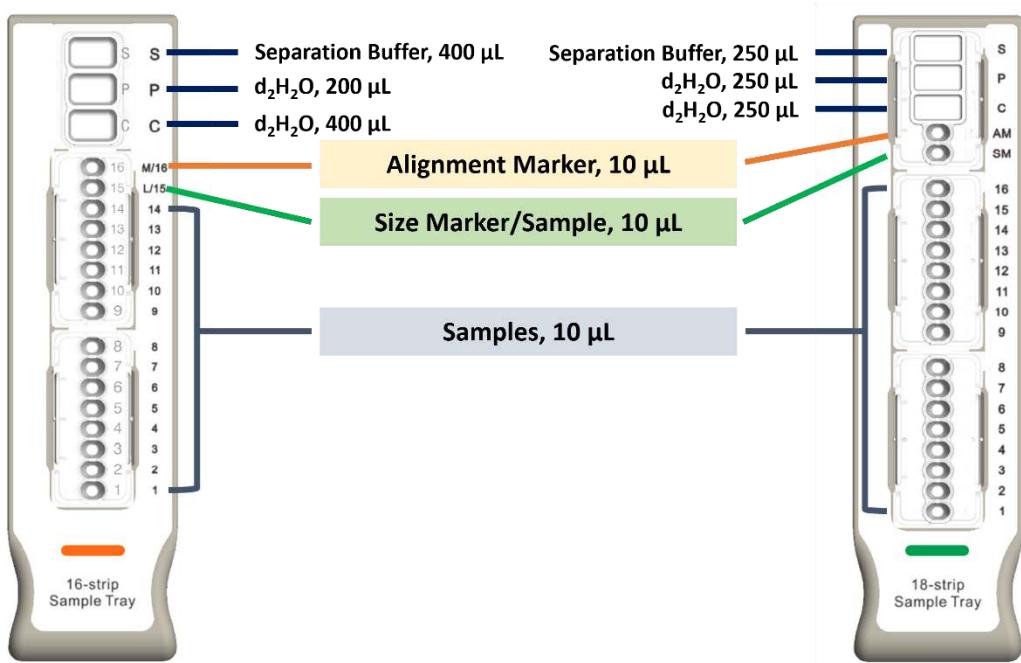
Figure 13   Sample Tray Setup

Sample Preparation

For sample preparation, consult the respective Cartridge User Guides for recommended sample concentration and additional information. Determine the required volume for sample tubes by referencing either the Cartridge User Guide or Figure 13.

After completing the preparation steps as described earlier, follow these instructions to continue:

1. Click on **Open Sample Door** to move the tray holder to the door (Figure 10 C), and slide the sample tray into the instrument. Press the tubes tightly down into the wells.
2. Click on **Park** to move the tray holder to the park position (Figure 10 D).

Cartridge Preparation

Qsep₁ Bio-Fragment Analyzer is specially designed to be compatible with specialized cartridges manufactured by BiOptic Inc. These cartridges have a modular design, eliminating the need for time-consuming gel preparation. It's crucial to ensure that the cartridge is within its limited lifetime, to ensure efficiency and effectiveness. Regularly check the expiration date to maintain reliable and accurate results during analysis.
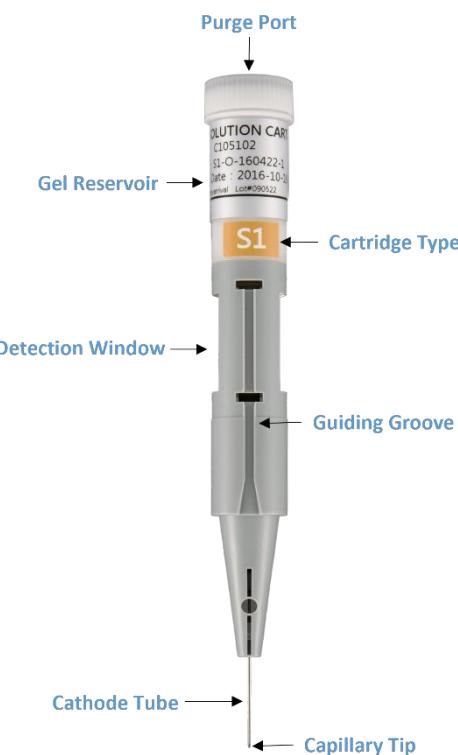
Figure 14   Pen-Shaped Gel Cartridge

Unpacking

Please follow the instructions below for unpacking the cartridge:

1. Open the moisturizing bag and extract the clam-shell container, containing the cartridges.
2. Vertically pull out the cartridge from the clam-shell container.
3. Insert the pin into the small hole on the cartridge cap and press it all the way in until you hear a distinct pop sound. Repeat this step 2-3 times.
4. Proceed to insert the unpacked cartridge into the *Qsep₁* Bio-Fragment Analyzer for calibration.

For more comprehensive instructions, please refer to the unpacking guide included in the cartridge kit.

Insertion

Next steps for cartridge insertion:

1. Open the cartridge door located on the top of the instrument by pressing the white button (Figure 15).
2. Before inserting the cartridge, make sure the tray holder is in the park position by clicking on **Park**.
3. Ensure that the guiding groove of the cartridge is facing the front (Figure 15).
4. Push the cartridge down to the bottom and close the cartridge door to complete the process.

Figure 15   The guiding groove of the cartridge shall face the front

Final steps for cartridge setup:

1. Click on the **Latch** button on the control panel to secure the cartridge in place.
2. The cartridge information section on the left will display details such as Cartridge Number, Expiration Date, and Runs Left. The image of the cartridge will also be

displayed in color (Figure 16).

3. Note that upon the initial use of the cartridge, it must pass the HV check and calibration process. For more detailed information, please refer to section Calibration.
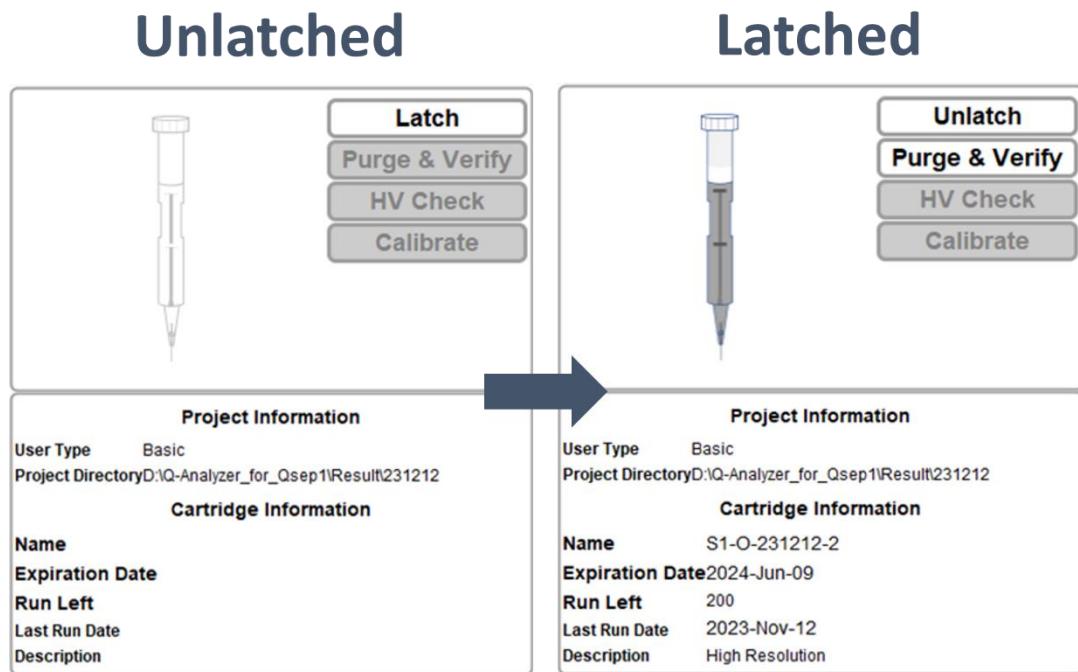
Figure 16   Before and after the cartridge is latched

Calibration

To ensure the quality of a new cartridge after shipment, calibration is necessary before its initial use.

The purpose of the verification is to conduct a **HV Check** to ensure that the current of the cartridge remains stable under HV (high voltage) conditions. If the current is stable, the result will indicate "passed". The **Calibrate** function confirms the quality of the cartridge by performing a test on the alignment marker/quantitative marker. It checks whether the signal of the alignment marker/quantitative marker can be detected when using the cartridge.

To perform the calibration, follow these steps:

1. In addition to buffers, prepare a fresh alignment marker/quantitative marker with an appropriate amount in a specified tube. Position the marker at M well as detailed in section Buffers, Markers, and Samples Preparation for comprehensive instructions.
2. Ensure that the sample tray is correctly positioned on the tray holder.
3. Insert the new cartridge and close the door, following the instructions outlined in section Insertion.
4. Click on **HV Check** to begin the verification process. Confirm that the working current of the cartridge is above 2 µA for the HV Check to pass. If successful, proceed to the next step.
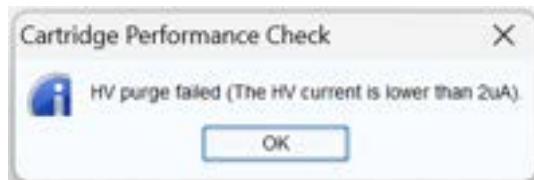
5. Once the HV Check passes, perform the calibration by clicking on **Calibrate**. Some cartridges may present alignment marker options in a pop-up window for you to choose from.
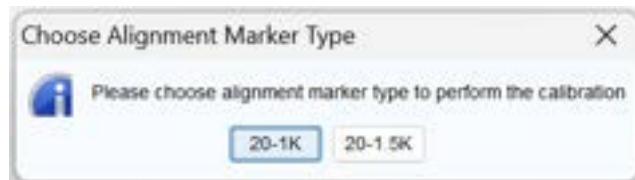
Upon successful calibration, the cartridge is ready to use, and the screen will display a "Passed" message. If a "Failed" message appears, refer to the unpacking guide included in the cartridge kit for troubleshooting instructions. If the calibration fails repeatedly for five times, please contact your local distributor for technical support.

Pre-Programmed Method

Each cartridge, contains various testing protocols, referred to as "Method", which incorporates sequential steps to perform the test. Different cartridges and pre-programmed methods are employed in CE experiment, tailored to the size range and concentration of the sample. Furthermore, you have the flexibility to create their own Methods according to their requirements.

In the Method Selector (Figure 17), you can access the methods that correspond to the appropriate size range, alignment marker, and cartridge type. When the cartridge is latched, *Qsep₁* Bio-Fragment Analyzer automatically detects its type and displays only the recommended methods for that particular cartridge type.
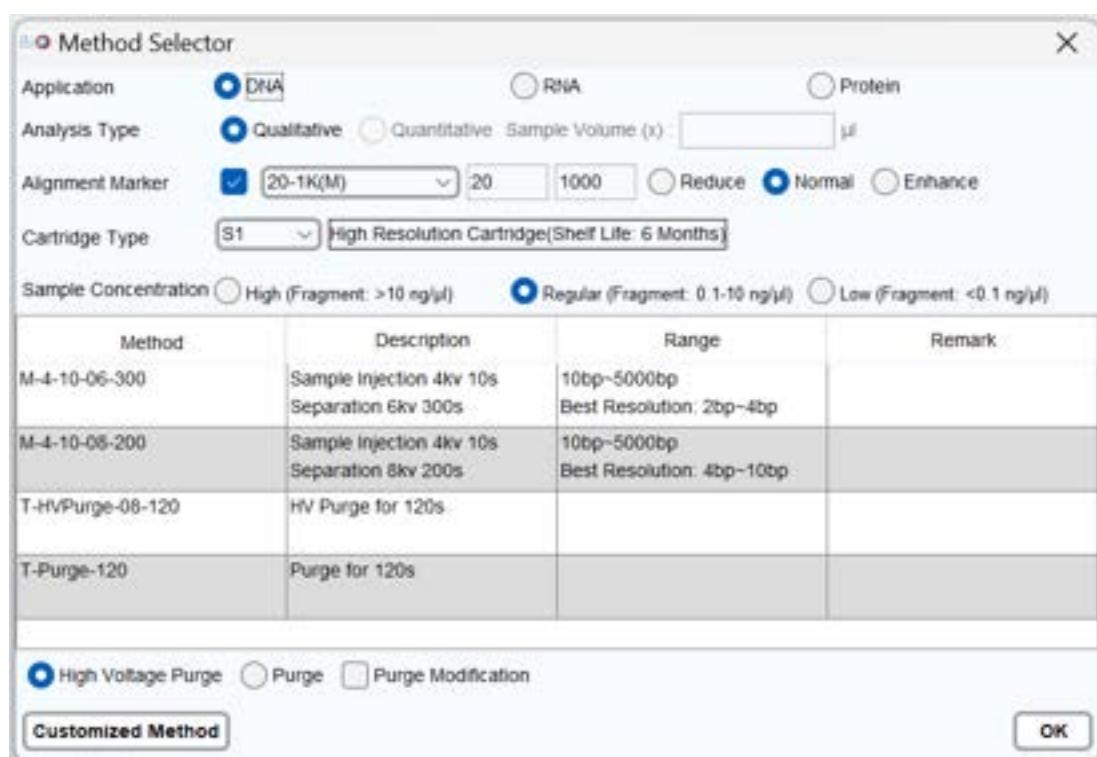
Figure 17   Method Selector

Creating A Sequence

After latching and verifying the cartridge, follow the instructions below to fill in the necessary details in the **Sequence** (Figure 18). The Sequence provides seven setting options to customize the experiment. Edit the sequence by **assigning the sample position**, **selecting the separation method**, **entering the result name**, and **adjusting the parameter**. Once done, click **Add** to add another row for a new sequence and, click **Run** to execute the sequence analysis after confirming.
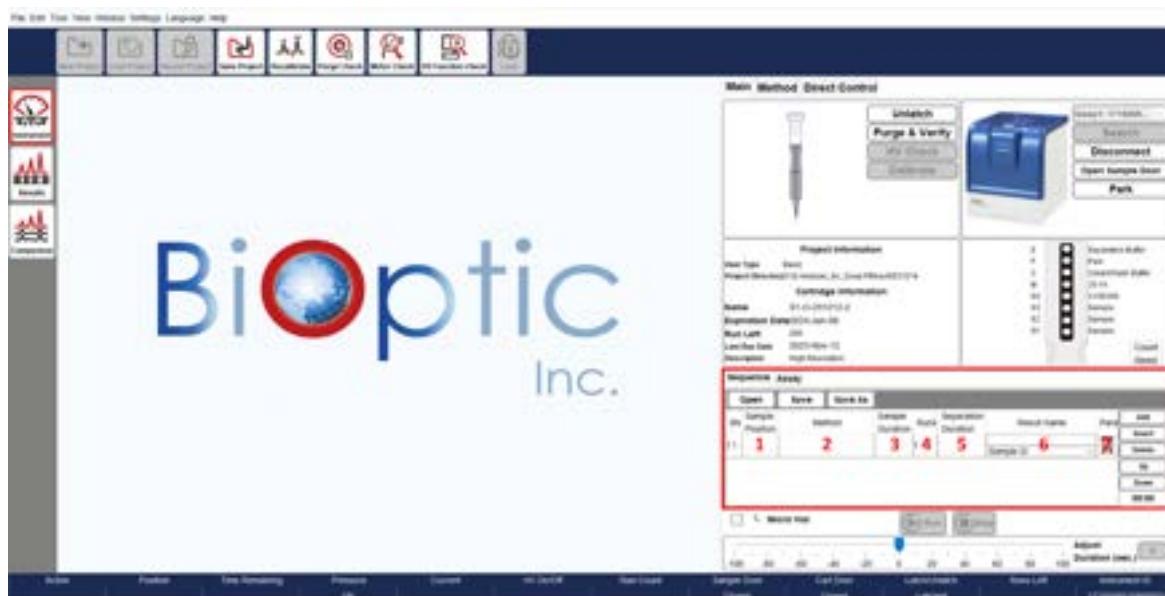
Figure 18   Edit Sequence

1. **Sample Position:** Select your desired sample position from the pop-up window.
The simulation sample tray displayed will depend on the inserted sample tray. The available Alignment Marker will be listed in pull-down menu. Your selection will fix the marker within the method selector. Optionally, you can choose the Size Marker to create a ladder.
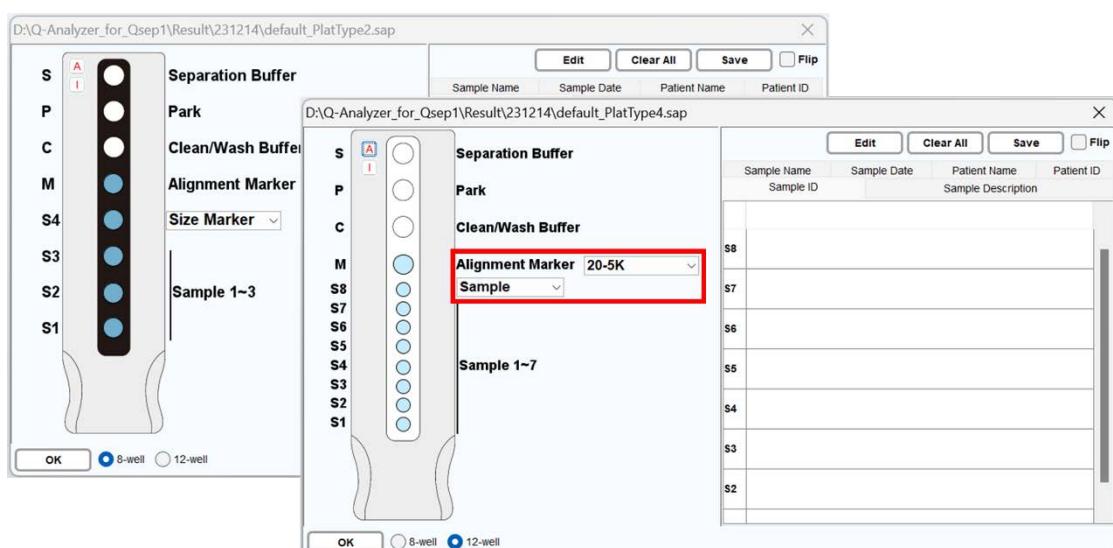
✓ Utilize the table below to choose the Alignment Marker and Size Marker (optional). They should be based on sample size and be placed in appropriate positions.

Sample Size	Alignment Marker	Size Marker	Cartridge Type
20 bp – 1,000 bp	C109100 20 bp & 1,000 bp Position: (M)	C109200 15 bp – 622 bp Position: (L)	S1- High Resolution Cartridge Kit S2- Standard Cartridge Kit N1- High Sensitivity Cartridge Kit
20 bp – 1,500 bp	C109109 20 bp & 1,500 bp Position: (M)	C109200 15 bp – 622 bp Position: (L)	S1- High Resolution Cartridge Kit S2- Standard Cartridge Kit N1- High Sensitivity Cartridge Kit
20 bp – 5,000 bp	C109102 20 bp & 5,000 bp Position: (M)	C109301 50 bp – 3,000 bp Position: (L)	S1- High Resolution Cartridge Kit S2- Standard Cartridge Kit N1- High Sensitivity Cartridge Kit
20 bp – 15,000 bp	C109110 20 bp & 15,000 bp Position: (M)	C109400 100 bp – 10,000 bp Position: (L)	S3- Kilobase Cartridge Kit N3-HS-Kilobase Cartridge Kit
20 bp – 60,000 bp	C109100 20 bp & 1000 bp Position: (M)	C109700 500 bp – 23,000 bp Position: (L)	S3- Kilobase Cartridge Kit N3-HS-Kilobase Cartridge Kit

2. **Method**: Choose the appropriate method for your sample.

The method selector window will appear, suggesting pre-programmed methods based on the cartridge type and the sample conditions. Specify the necessary items:

✓ Select **DNA, RNA** or **Protein** application based on the cartridge.

✓ Choose the desired analysis type, either qualitative or quantitative This option is available exclusively for S1, S2, and N1 cartridges (Cat. C105201-Q, C105202-Q, and C105205-Q). The quantitative function accurately measures sample concentration and is applicable when the quantitative marker (C109109-500Q) is selected during the sample position setup on the simulated sample tray. The quantitative marker serves as an internal marker for adjusting the calculation. Follow these steps for quantification:

A. Enter the sample volume mixed with the quantitative marker.
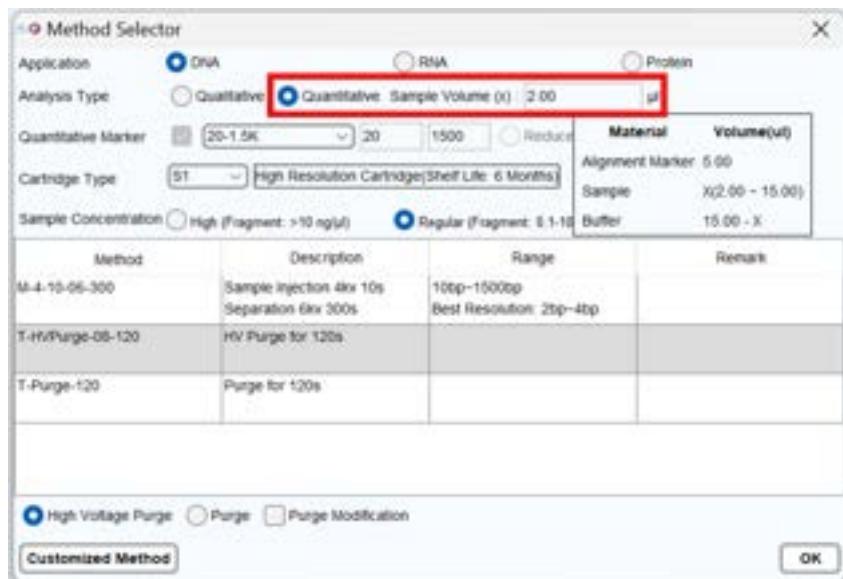
B. Select the recommended method and start the analysis.
C. The concentration will be displayed in the result file, indicating the sample concentration before mixing with the quantitative marker.
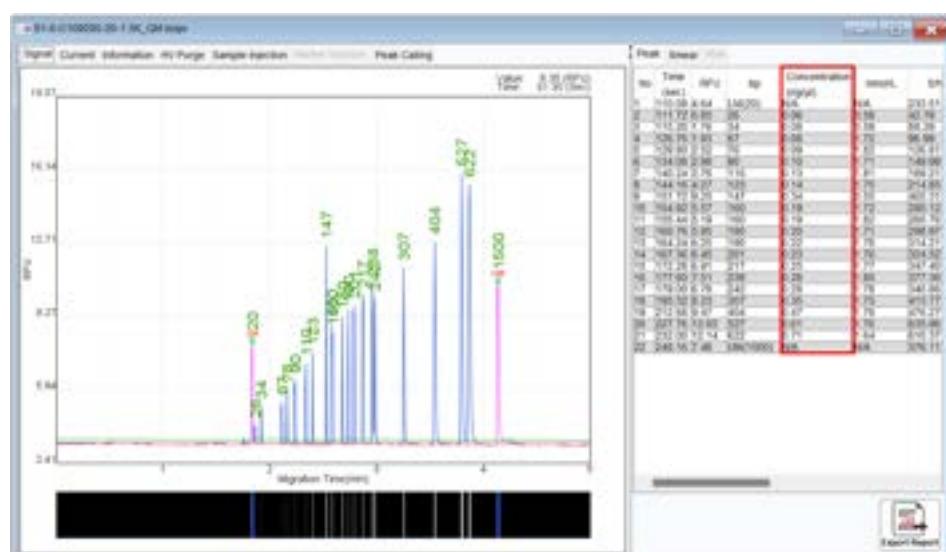
✓ Deselect the **Alignment Marker** checkbox to exclude the alignment marker injection, affecting the subsequent calculation.

✓ Adjust the **Sample Concentration** options based on the sample conditions.

After selection, available pre-programmed methods will be listed.
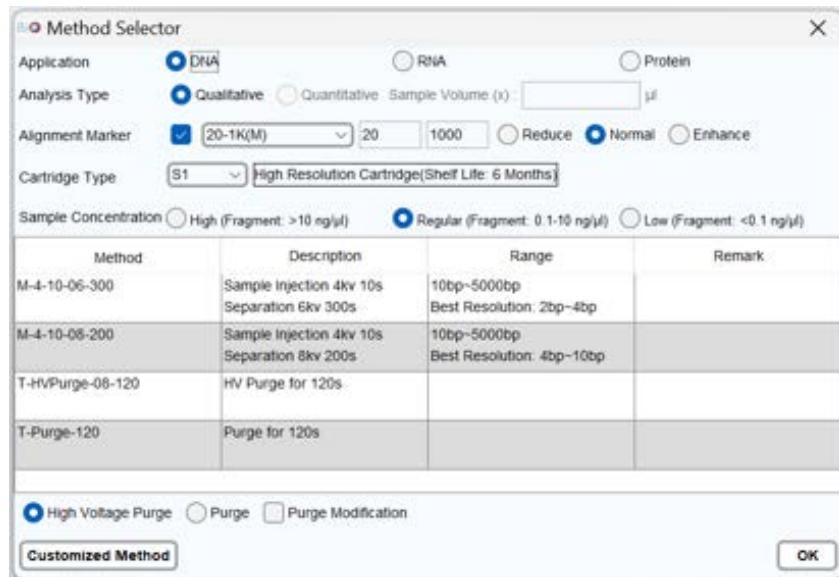
The pre-programmed method is named based on the following rules:
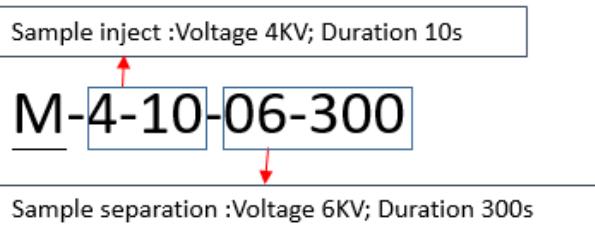
Higher voltage and longer sample injection time enhance the signal. Higher separation voltage shortens the separation time but reduces the resolution. Lower separation voltage increases the resolution but lengthens the separation time. The sample separation corresponds to the size range of base pairs.

3. **Sample Duration**: Set the duration of the sample injection time.
The selected method will display the **Sample Duration** in the sequence table. Modify the duration based on the sample condition and preference. The sample duration can be adjusted within a range of 1 to 300 seconds. However, it is recommended, for optimal performance, to set the duration between 1 to 20 seconds.

4. **Run**: Set the number of runs.
The same sequence will be repeated for the specified number of times. If there is more than one run, the run number will be included at the end of the result name.

5. **Separation Duration**: Set the duration of the separation process.
The separation time can be affected by the experimental condition such as temperature and the concentration of separation buffer. The **Separation Duration** of the selected

method will be displayed in the sequence table. Adjust if necessary, recommended to be 30 seconds longer after the upper marker appears.

6. Result Name: Specify the result file name.

The result file will be saved with information about the sequence, position, and execution. For example, the file name format may be *<Result Name>_<pull-down option>_S1S1_R1.bopx*, where S1, S1 and R1 represent the sequence number, sample position, and the order of execution, respectively. To preview the result name, move the cursor to the Result Name columns.
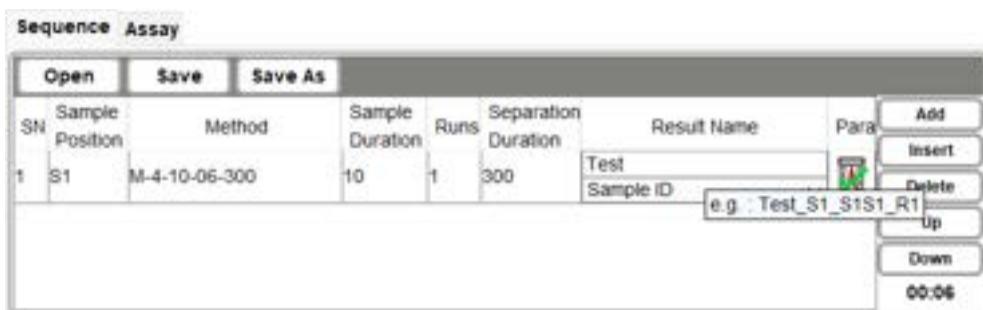
7. Para: Configure the settings for this method.
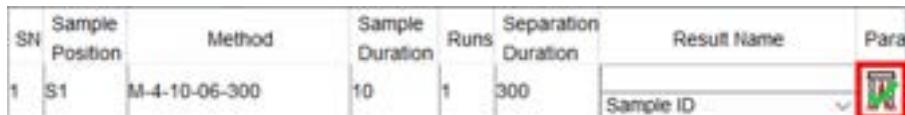
The software will calculate the result using the default reference table. If you wish to use your own created reference table, click on **Browse,** and select the file. Baseline and Peak Threshold can also be adjusted.
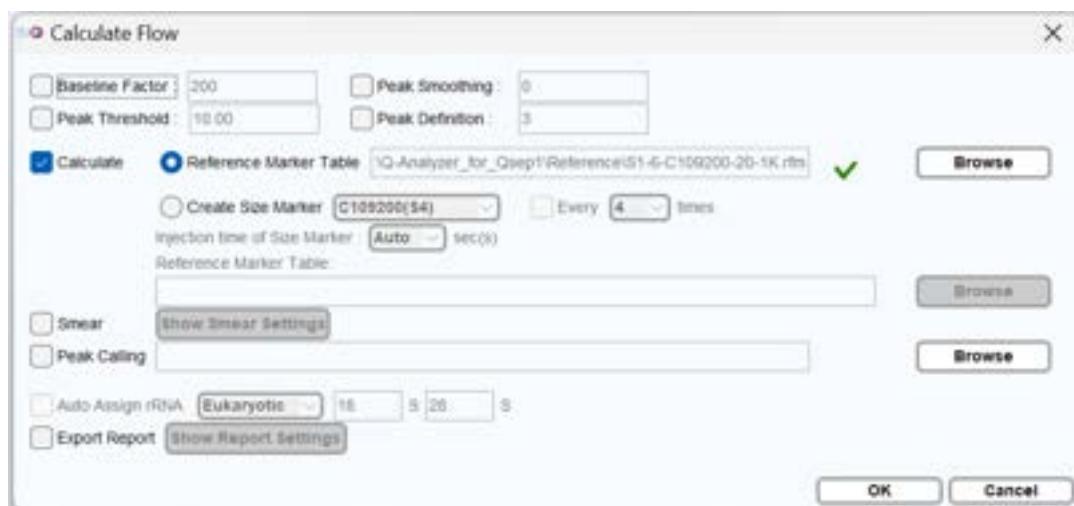
Alternatively, if you need to improve the accuracy of the base pair calculation, use **Create**

Size Marker to periodically update the reference table with the size marker information. Subsequent sample tests will then be calculated based on the updated reference table.

*Note: The size marker used to **Create Size Marker** will correspond to the alignment marker chosen during the sample position setup.

You can assign your own reference marker instead of using built-in reference marker when:

(1) The signal pattern differs from the built-in reference marker, and the software cannot correctly recognize the new pattern.

(2) The size of upper marker is different from what is used. For example, if using 20 bp-1,000 bp, the upper marker must be 1,000 bp.

To execute **Create Size Marker**, follow these steps:

A. Place a fresh Alignment Marker in S well and Size Marker in L.

B. Click on the **Para** icon in the sequence.

C. Check the **Create Size Marker** option.

You can also specify the frequency (**Every __ times**) to execute the size marker if higher accuracy of the sample sizing is needed.
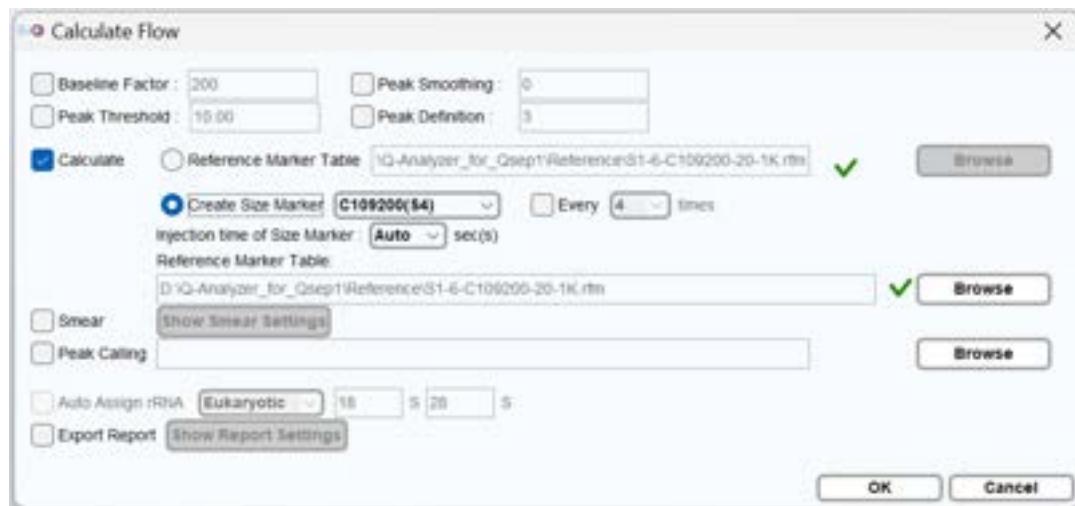
Smear: Selecting **Smear** checkbox applies Smear analysis to the results.

Peak Calling: Selecting the **Peak Calling** checkbox applies Peak Calling analysis to the results.

Auto Assign rRNA: When the RNA application is chosen in the Method Selector, the **Auto Assign rRNA** checkbox will be automatically selected. You have the option to choose

between Eukaryotic and Prokaryotic, and define SSU/LSU based on your needs.
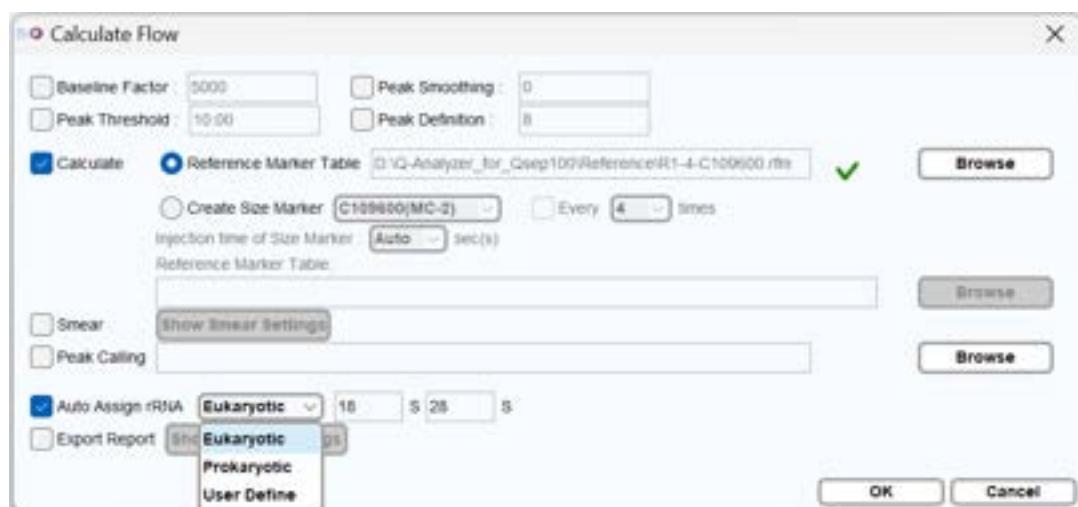
Export Report: If the **Export Report** checkbox is selected, click on **Show Report Settings** and set the **Report Format** according to your preferences.
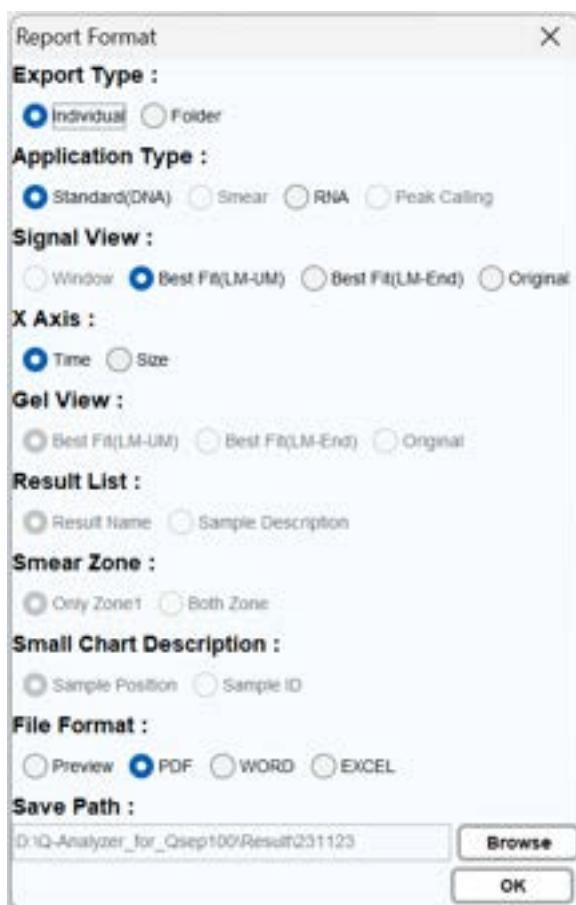
Editing Sample Position

To set the **Sample Position**, you can select the corresponding location on the simulation tray on the pop-up Sample File window (Figure 19). The selected well will be marked in red. To undo the selection, simply click on it again.
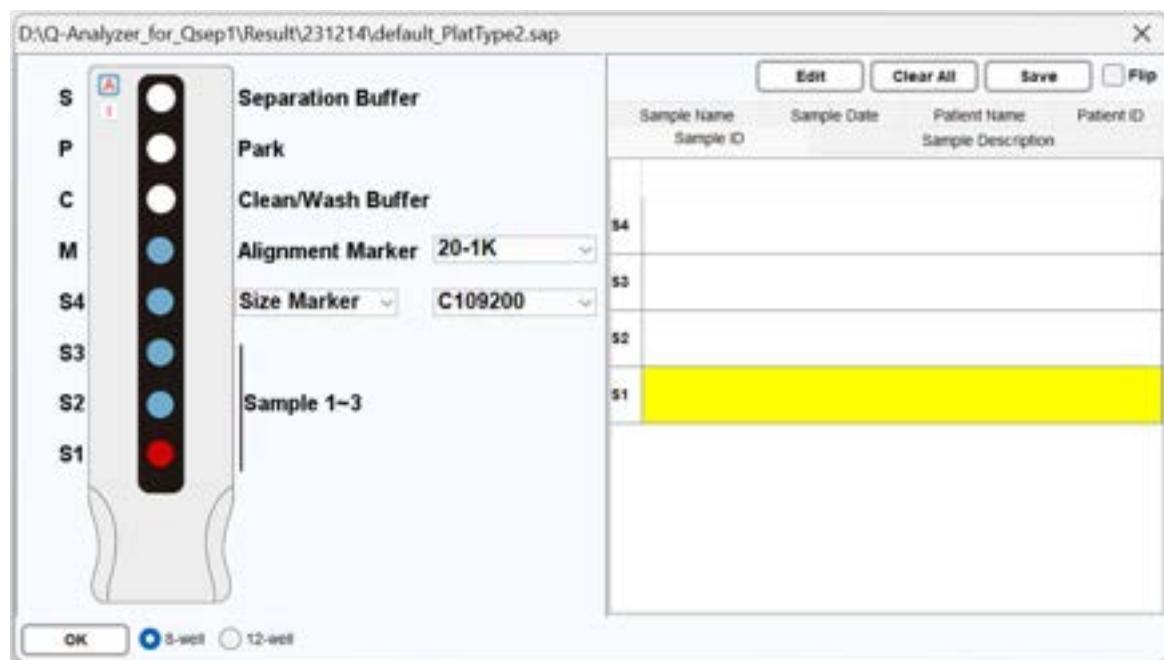

Figure 19   Sample File Window

To select all sample positions by clicking the "A" button and invert the selection by clicking the "I" button in the upper left corner. When multiple samples have been selected to execute the method for multiple times, the system will process the marked sample positions in numerical order. For example, if positions S1, S2, and S3 positions are selected and assigned to execute the method three times, the sequence will be processed in the following order: S1, S2, S3, S1, S2, S3, S1, S2, S3.

Notes for samples can be edited on the Sample Loader window (Figure 20). After clicking on **Edit**, the Sample Loader window will appear on the screen. Use the **Load** button to enter multiple sample descriptions, the **Clear All** button to delete all sample information, and the **Save** button to save the edited sample information. After entering the required information such as time and serial number, click **OK** to start the process.

The information in the excel file (Figure 21) can be loaded into the window and saved by clicking **OK.**
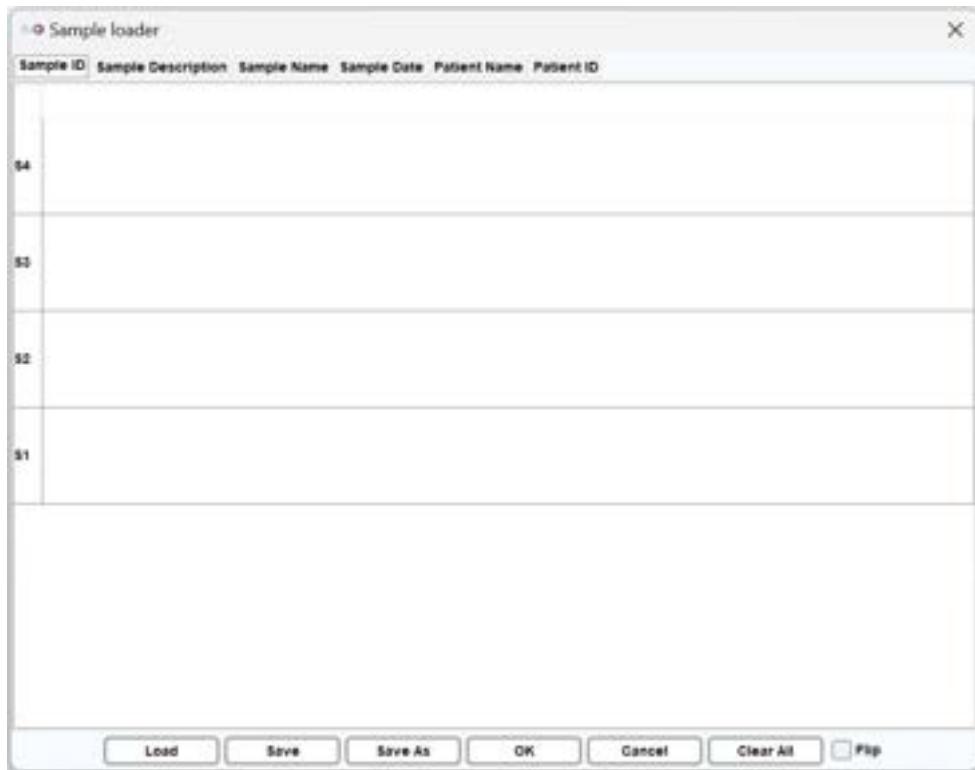
Figure 20　Sample Loader Window

Figure 21　Excel File for Sample Loader

*Note: The information of the samples in the excel sheet must follow the sequence shown in Figure 20. An example file can be found in the installation directory. The default workspace is C:\Users\<username>\Q-Analyzer\Sample.

Each row in the Excel file represents a single sample, and the information for each sample will automatically map to the corresponding tab.

*Note: The "Sample Position" and "Sample Date" must follow specific formats. Sample Position should be in the format of "A-P"-01; and Sample Date should be in the format of yyyy/mm/dd.

Running A Sequence

Once a sequence is created, initiate it by clicking on **Run**. Once the sequence is in progress, the "Run" button will transform into "Pause". If necessary, you can click on "Pause" or "Stop" to temporarily pause or halt the sequence. It's important to note that once the sequence has commenced, none of the applied parameters can be modified.
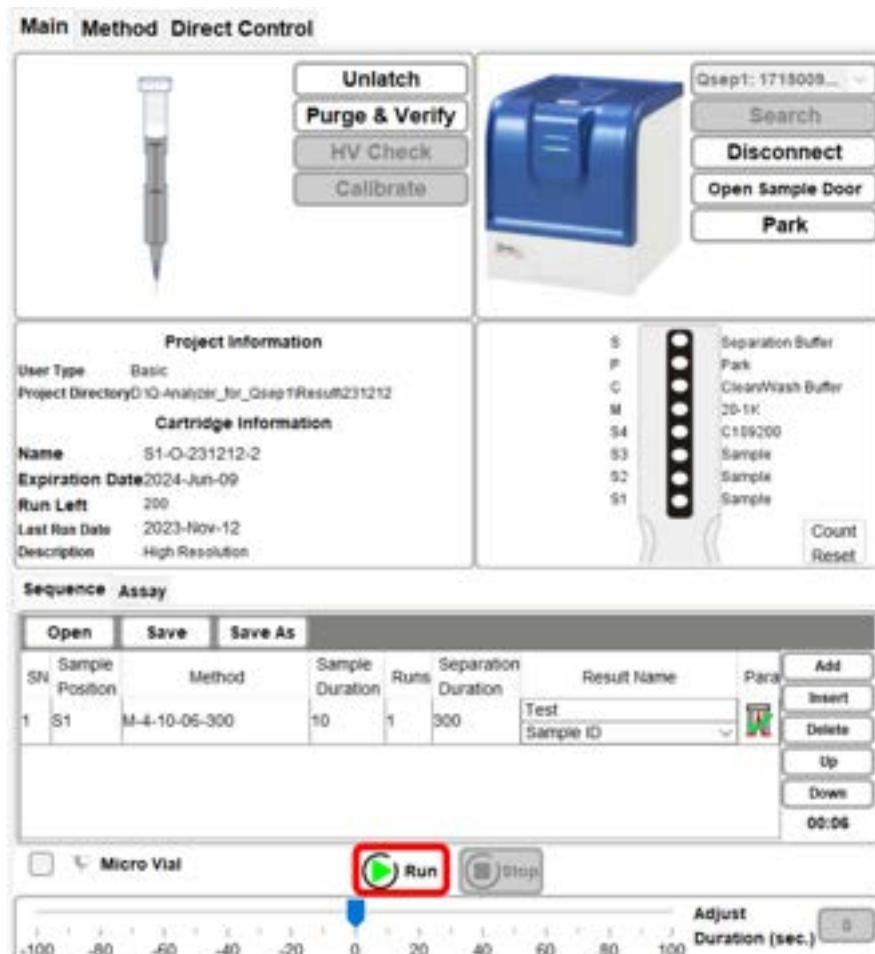
Figure 22   Running a sequence

Assay Preparation

To conduct an assay, please refer to the operation manuals or user guides available on the official website: [BiOptic Support – Reagents](#) for detailed instructions on preparing your samples.

Running An Assay

Once the sample is ready, follow the operation manuals or user guides for detailed instructions on running an assay. Alternatively, you can follow the summarized steps below:

1. Before latching the S1 or S2 cartridge, click on the **Assay** tab to view all available assay options.
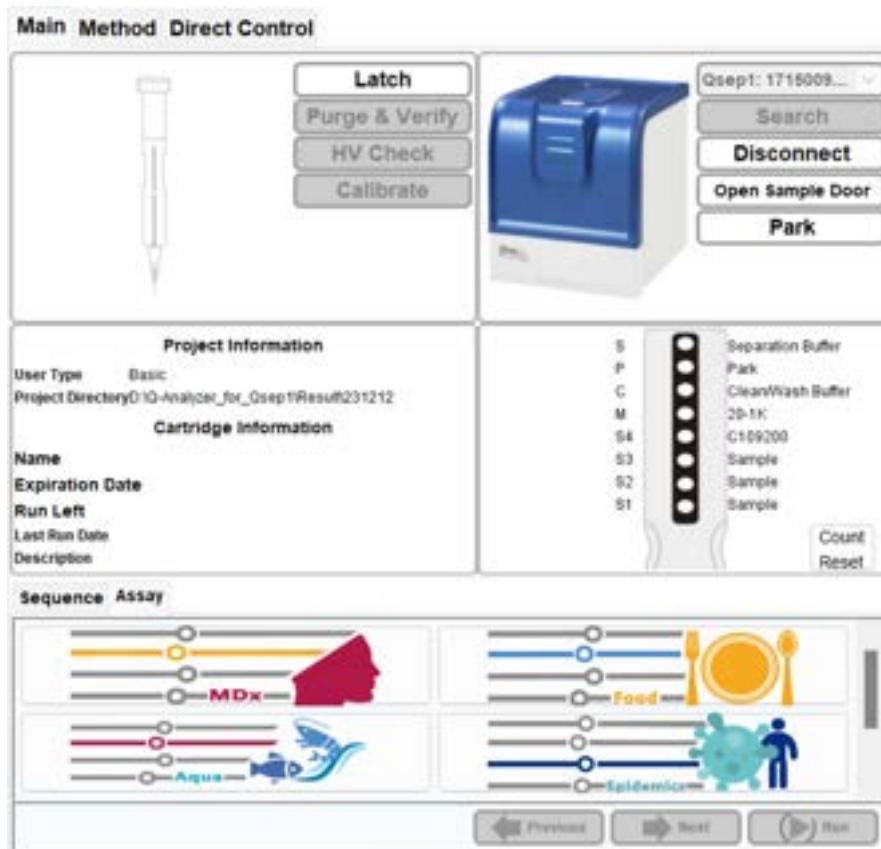
2. Using the High Resolution (S1) Cartridge as an example, after latching the cartridge, available assays will be displayed.
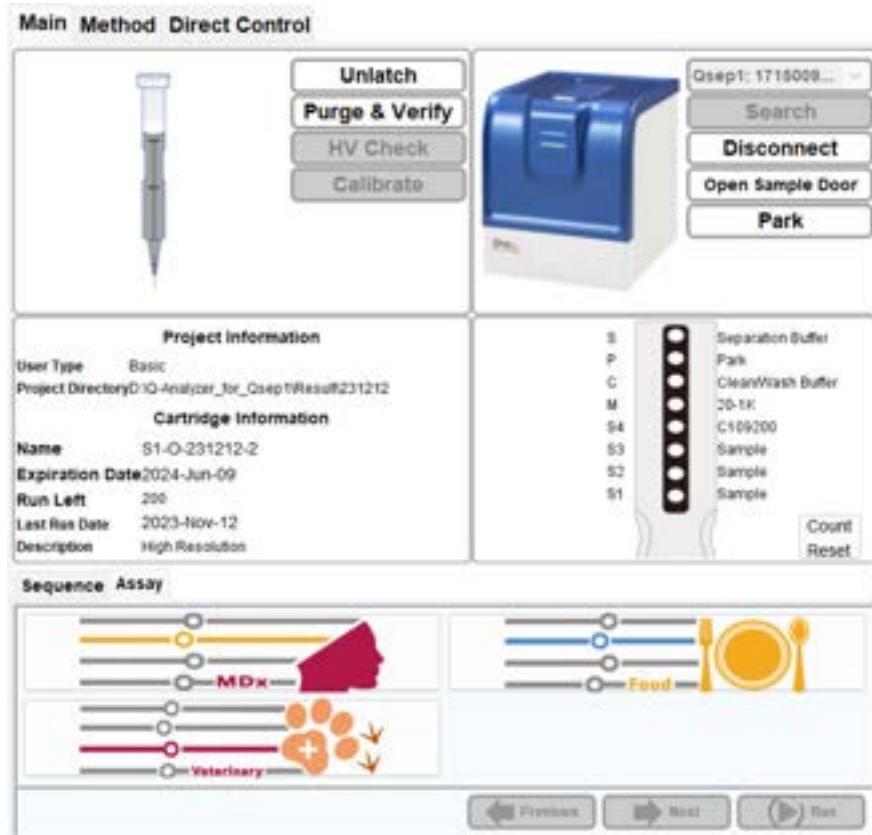
3. Select the desired *Qexp* series to enter the next layers.
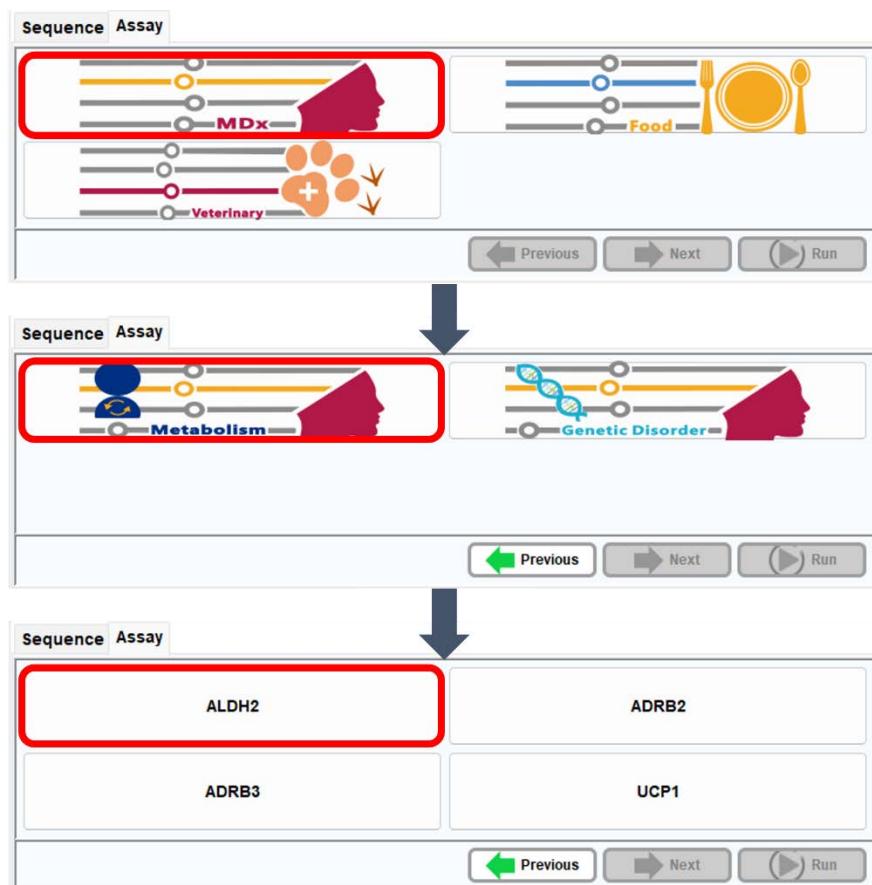
4. Choose the desired assay and follow the instructions on the window to place the buffer, markers, and samples.
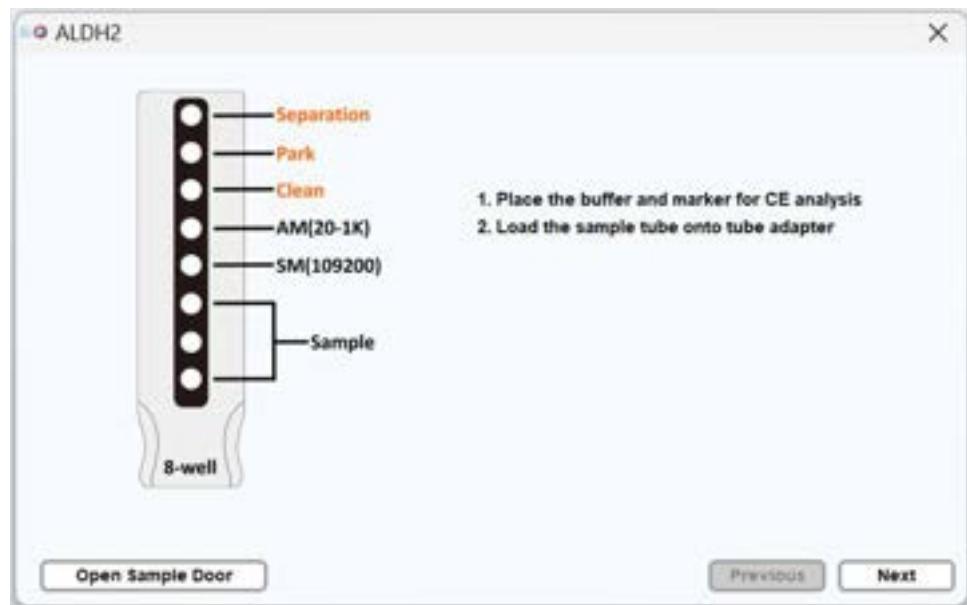
5. Select the sample position and enter result prefix name and information if necessary.
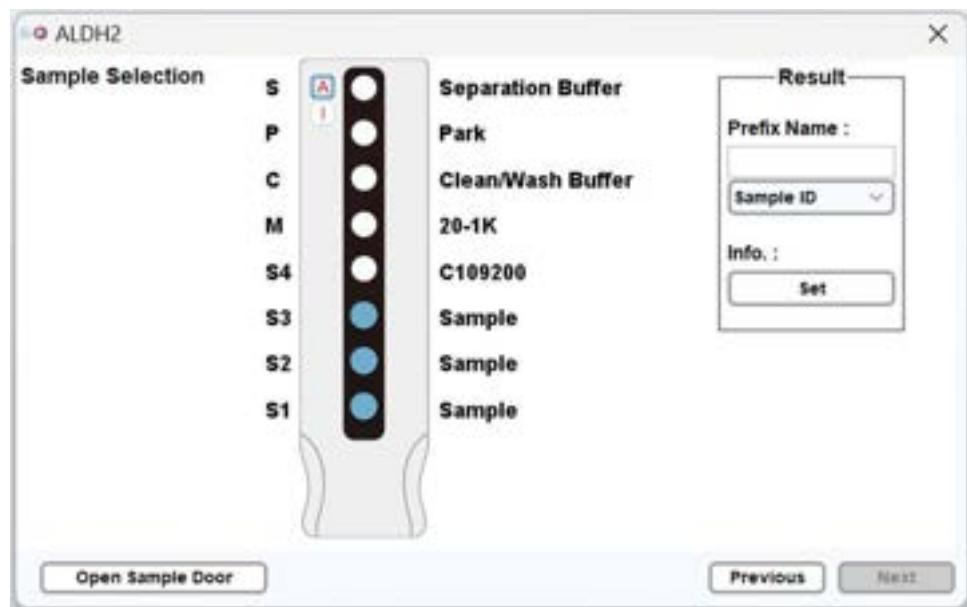
6. Initiate the assay by clicking on **Run**. Once the assay is in progress, the "Run" button will transform into "Pause". If necessary, you can click on "Pause" or "Stop" to temporarily pause or halt the assay. Please note that once the assay has commenced, none of the applied parameters can be modified.

Q-Analyzer User Interface

Main Window
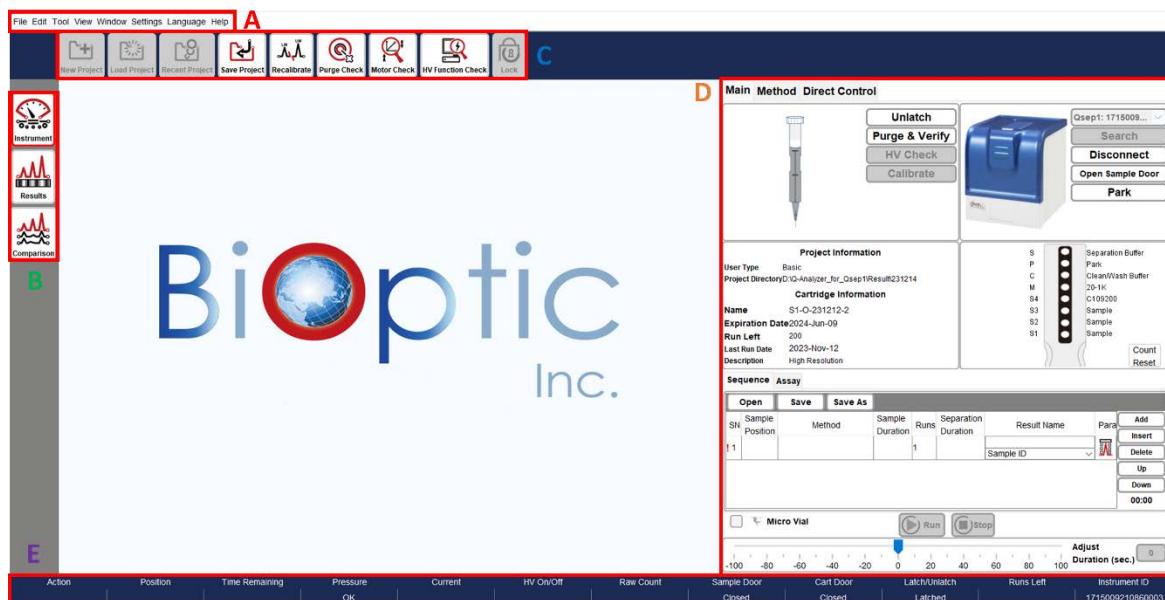
Figure 23   Main window

A: Menu Bar
B: Function bar
C: Toolbar
D: Operation Region
E: Status Bar

System Overview

Upon launching *Q-Analyzer*, the Main Panel (Figure 23) will appear, providing essential information about the *Qsep₁* Bio-Fragment Analyzer, including its status, data/results display, and post-data analysis functions.

Menu Bar

All available functions can be accessed from the Menu bar. For more detailed information, please refer to section <u>Menu Bar</u>.

Function Bar

The Function Bar consists of four icons. Selecting the **Instrument** icon allows you to enter the control panel and operate the instrument (section <u>Control Panel</u>). The **Results** or **Comparison** icons enable you to display and analyze data. For more information, please refer to section <u>Function Bar and Toolbar</u>.

Toolbar

The Toolbar contains various icons that correspond to the selected **Function**. These icons represent the most frequently accessed functions. Detailed information about each icon can be found in section <u>Function Bar and Toolbar</u>.

Operation Region

The Operation Region displays the main window and the corresponding panel after selecting the desired icon from the Function Bar.

Status Column

At the bottom of the main panel, the Status Column provides information about the system's current status. This includes of the pressure levels (okay or low), the status of the cartridge door/sample door (opened or closed), and whether the cartridge is latched or not.

System Status Bar

The columns display in the Status Bar are described as follows (Figure 23 E).

Column	Description
Action	Displays the actions that are being performed.
Position	Indicates the position of the cartridge tip.
Time Remaining	Shows the remaining time for the action to be completed.
Pressure	Indicates the status of the applied pressure. • Fail indication means that the pressure is lower than the system requirement. • If the pressure is decreasing, the description in the column changes from **black to yellow to red**.
Current	Displays the amount of current (in µA) flowing through the capillary.
HV On/Off	Shows the status of the High Voltage power supply.
Raw Count	Displays the original signal.
Sample Door	Indicates whether the sample door is **Opened** or **Closed**.
Cart Door	Indicates the cartridge door is **Opened** or **Closed**.
Latch/Unlatch	Indicates the cartridge is **Latched** or **Unlatched**.
Runs Left	Displays the number of remaining runs in the sequence.
Instrument ID	Shows the instrument ID of the connected $Qsep_1$.

Function Bar and Toolbar

Q-Analyzer is responsible for managing and overseeing *Qsep₁* Bio-Fragment Analyzer, including data collection, processing, and result reporting. To facilitate user navigation, the functions within *Q-Analyzer* are arranged into bars and tabs.

The **Function Bar** consists of three primary icons: **Instrument**, **Results**, and **Comparison**. Upon launching the software, the default function displayed is **Instrument**. Each function corresponds to a distinct **Toolbar**, which contains a list of commonly used functions. More detailed information regarding each function can be found in the following sections.
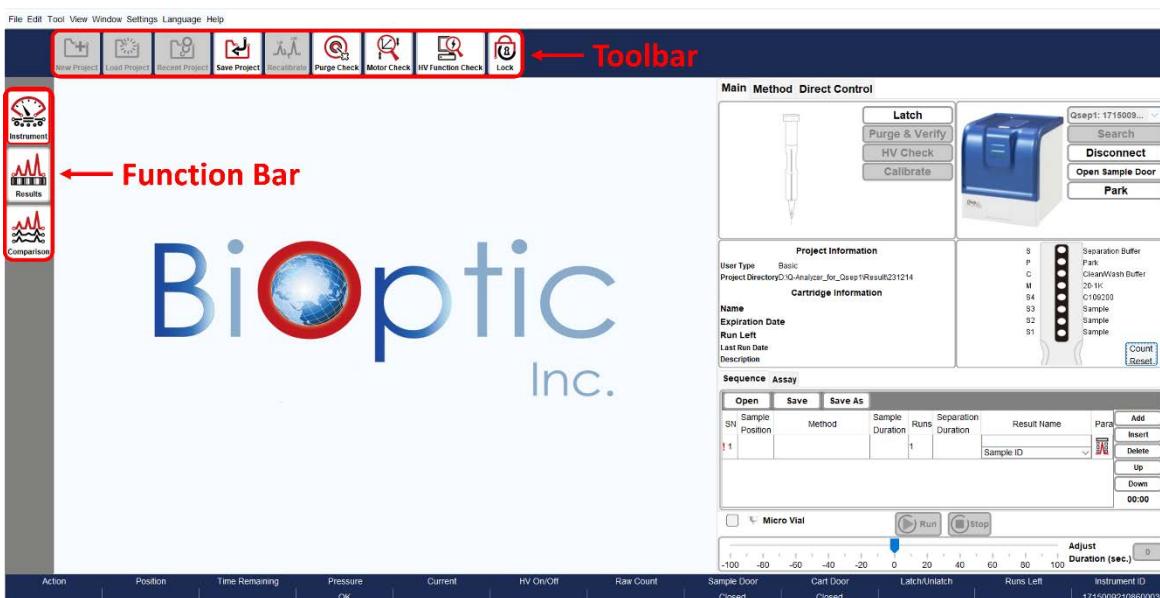
Figure 24   Function Bar and Toolbar

Instrument

Instrument function serves as the primary control for operating $Qsep_1$ Bio-Fragment Analyzer. Instructions for using the control panel can be found in section Control Panel.

Prior to connecting to $Qsep_1$ Bio-Fragment Analyzer, the Toolbar tabs, such as **New Project**, **Load Project**, **Recent Project**, and **Save Project**, are enabled. You have the option to create a new project or load an existing project if needed (Figure 25). Projects can be used to differentiate between different experiments. By naming the **New Project**, the results will be saved in the specified directory. The default workspace is C:\Users\<username>\Q-Analyzer\Result.
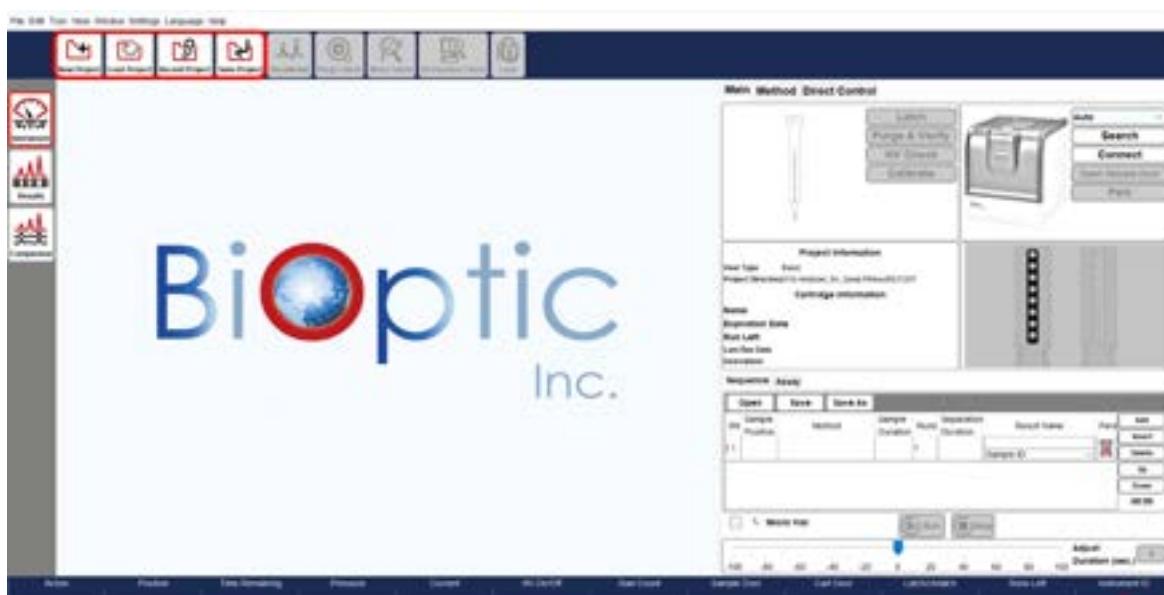
Figure 25   Instrument function before instrument connection

Figure 26   New Project

Once the instrument is connected, the additional icons on the Toolbar become enabled, including **Purge Check**, **Motor Check**, **HV Function Check** and **Lock** (Figure 27). Upon latching the cartridge, the **Recalibrate** icon becomes enabled as well (Figure 28).

These functions allow you to perform basic checks on the instrument and cartridge.
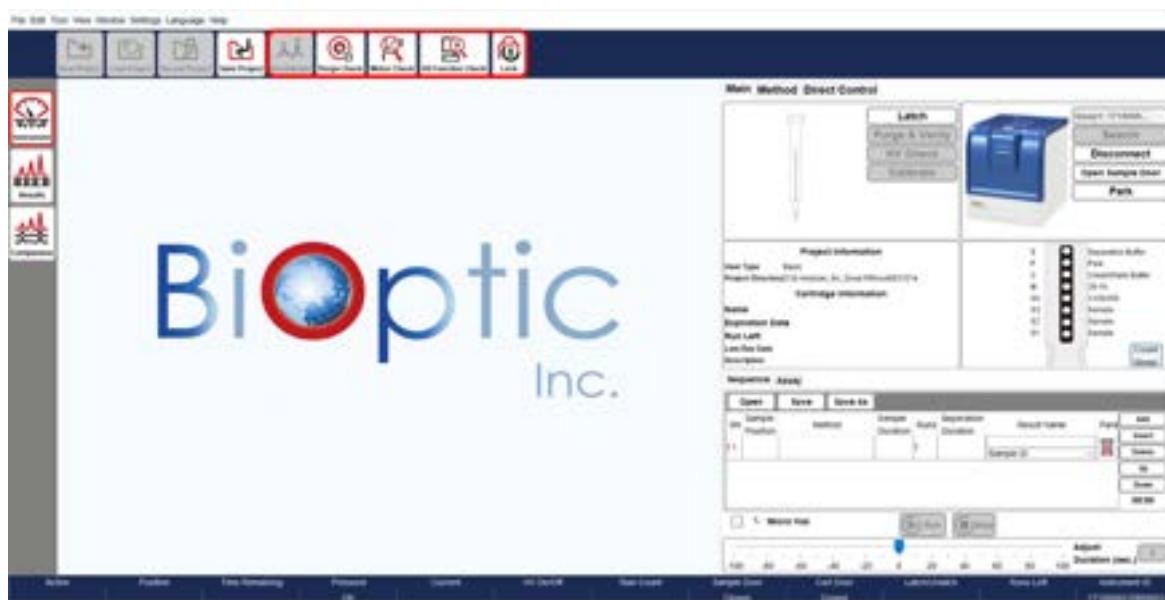
Figure 27   Instrument function before latching the cartridge
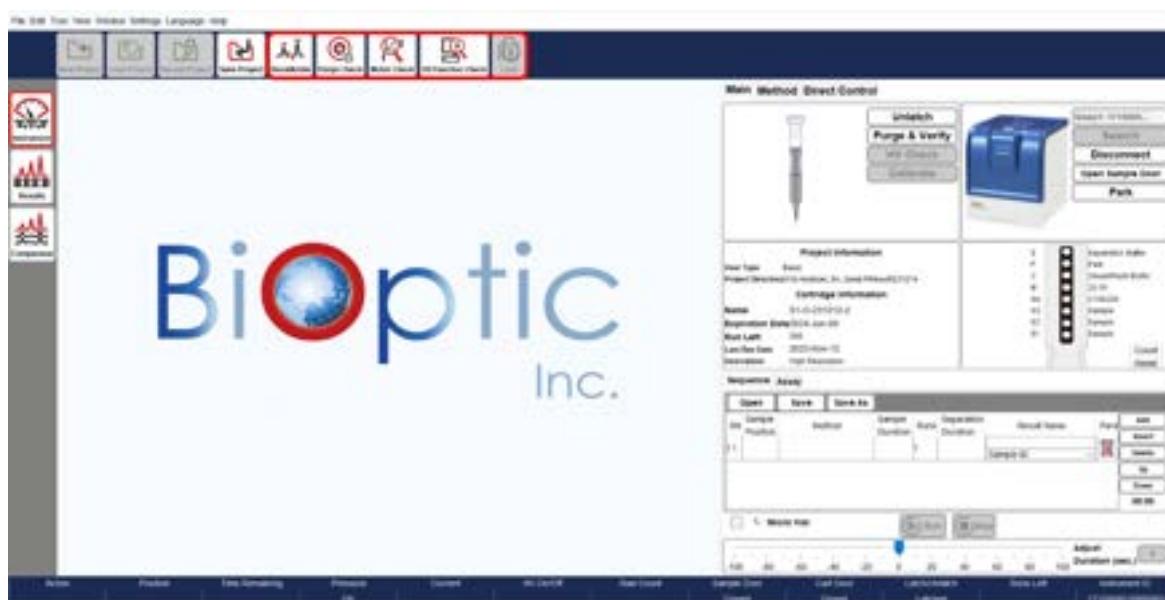
Figure 28   Instrument function after latching the cartridge

Recalibrate

Recalibration assists in correctly identifying the alignment marker (lower and/or upper markers) and updating the cartridge factor. Recalibration is recommended in the following situations:

- The alignment marker has been replaced.
- The cartridge has been stored for more than two weeks since the last execution.
- The software fails to identify the alignment marker correctly.

To **Recalibrate**, follow these steps:

1. Ensure that the fresh Alignment Marker (e.g., 20-1K) is placed correctly (e.g., MA1).
2. Confirm that all buffers are in good condition.
3. Click on **Recalibrate** in the Toolbar and follow the on-screen instructions.

4. Select the appropriate voltage and alignment marker that correspond to the **Method** to be used for the following experiments.

If recalibration fails, it essentially indicates a calibration failure. In the event of recalibration failure, please follow the troubleshooting steps outlined in the unpacking guide included in the cartridge kit or download them from the official website: [BiOptic Support – Consumables](#) for detailed instructions.

Purge Check

Purge Function Check verifies that air is being properly expelled from the JET-NOZZLE during the purging process. It is recommended to perform a purge check before and after using *Qsep₁* Bio-Fragment Analyzer.

To check the air flow, press the JET-NOZZLE down to the end using your thumb. If the JET-NOZZLE can be easily pressed down, the pressure is insufficient. If no air comes out, there may be gel clogging the air tube. Cleaning the air tube at the cartridge door is recommended. For maintenance and repair services, please contact BiOptic Inc. or your local distributor.

Figure 29   Purge Function Check

Motor Check

Motor Function Check verifies motor position and settings.

Follow the instructions provided on the window to open the sample door and cartridge door, and remove the cartridge from the instrument to proceed. You have the option to save the test result after clicking on Start.
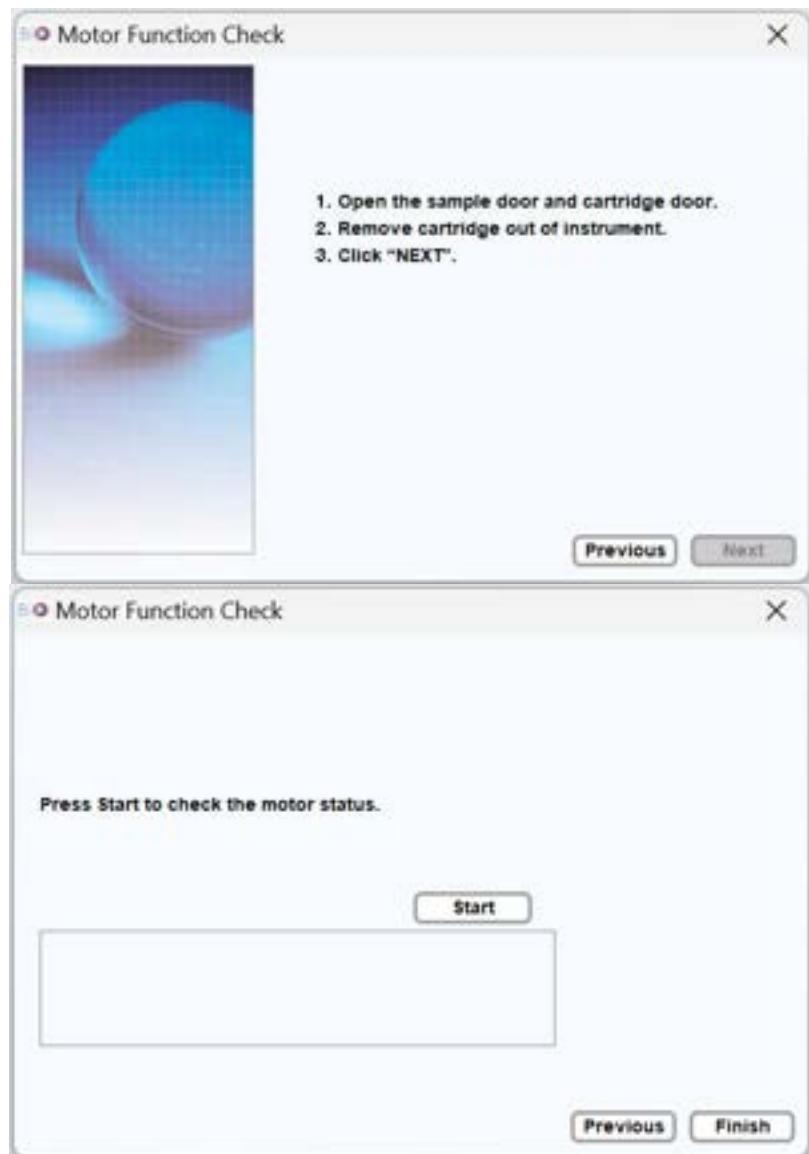
Figure 30   Motor Function Check

The initial step involves conducting sensor checks, and you will be prompted to respond to questions after each action. After the sensor checks are completed, you will be prompted to close the sample door to proceed with the motor check. The motors will move the tray holder in different directions.

If the Motor Function Check reveals any issues, report the findings to BiOptic Inc. with a detailed description of the problem and any error messages that may have appeared during the check.

HV Function Check

HV Function Check conducts tests to ensure a stable current is maintained.

Follow the instructions provided on the window to insert and latch the cartridge. Ensure that the cartridge is within its validity period and in good condition to perform HV Function Check.
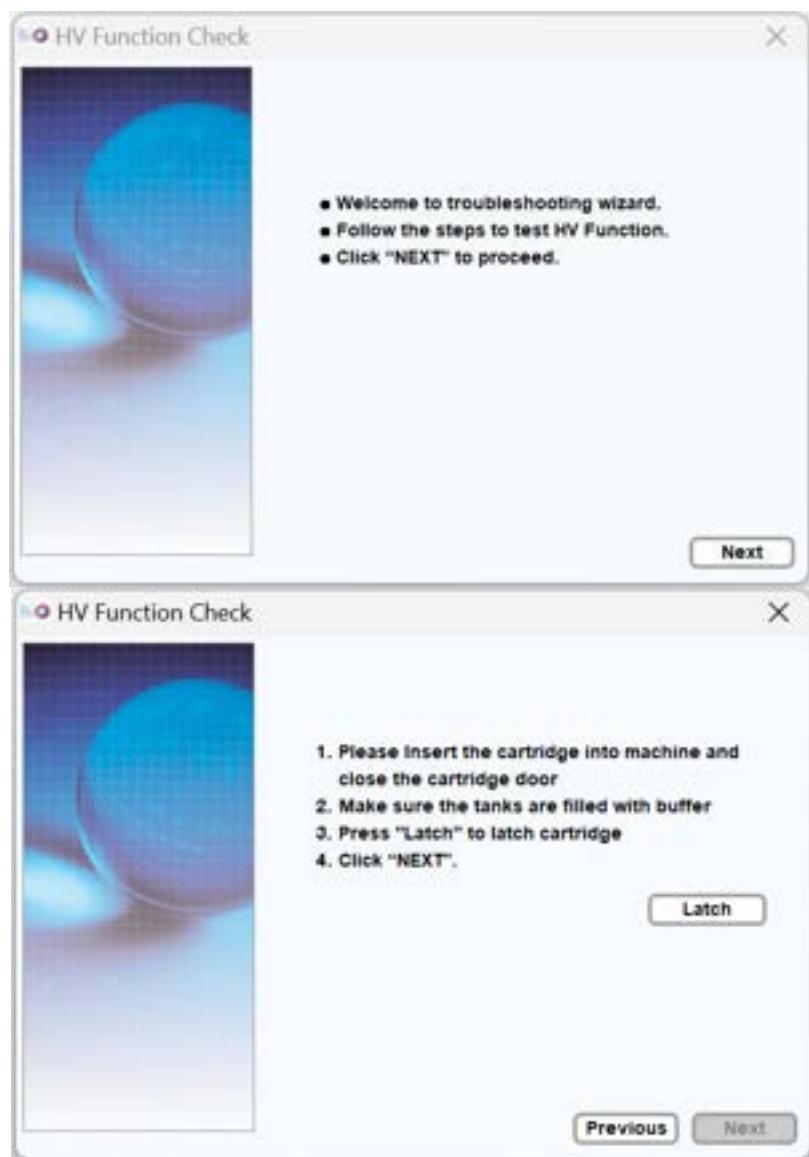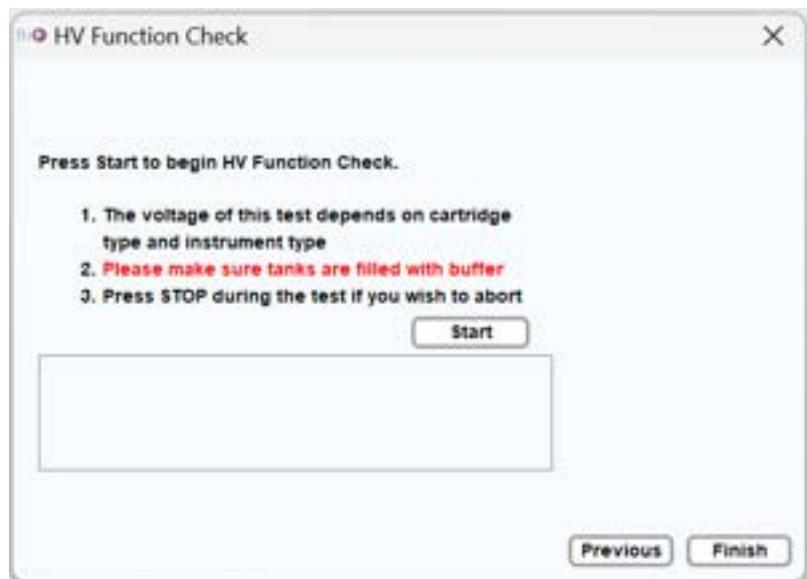
Figure 31   HV Function Check

Lock

The lock function is specifically designed for the preparation of packing the *Qsep₁* Bio-Fragment Analyzer. For detailed information, please refer to section Packing.

Before clicking on the Lock icon, ensure to unlatch and remove the cartridge, as well as remove samples and buffer from the instrument. Clicking on the Lock icon will initiate the movement of the tray holder to the position for EPE foam insertion, securing the tray holder properly. After completion, the tray holder will move to the lock position. Power off the instrument as part of the packing preparation process. Refer to the packing instructions for further guidance.

Real Time

Real Time provides a display of current and signal data in real time, including the current and RFU (capillary electrophoresis signal amplitude).
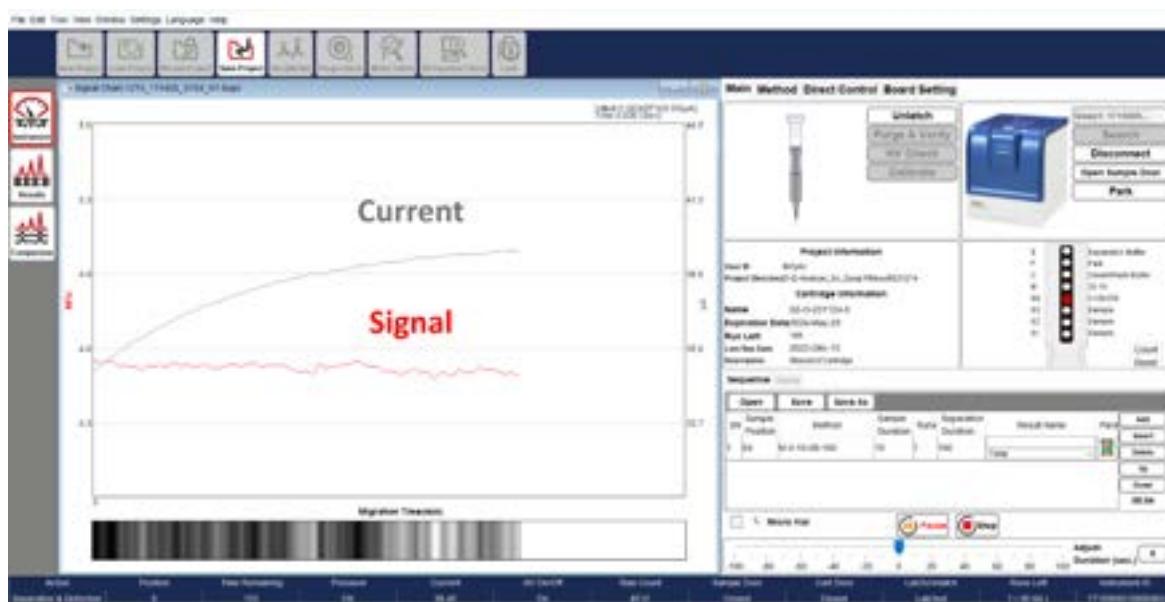
Figure 32   Real Time Display

Results

Results Function is used to display and analyze data from $Qsep_1$ Bio-Fragment Analyzer. It allows you to visualize the signal chart and obtain peak information from the raw data of capillary electrophoresis.

Once the files are loaded into Q-$Analyzer$, various icons in the Toolbar become available, including **Calculate**, **Smear**, **Peak Calling**, **Parameters**, **Show Size/Legend**, **Show Size/Time**, **Invert GelView**, **Show Best/Ori. View**, and **Change Line Thickness**. These icons allow you to edit the raw data as needed. For more detailed information, please refer to section Result for Data Display.
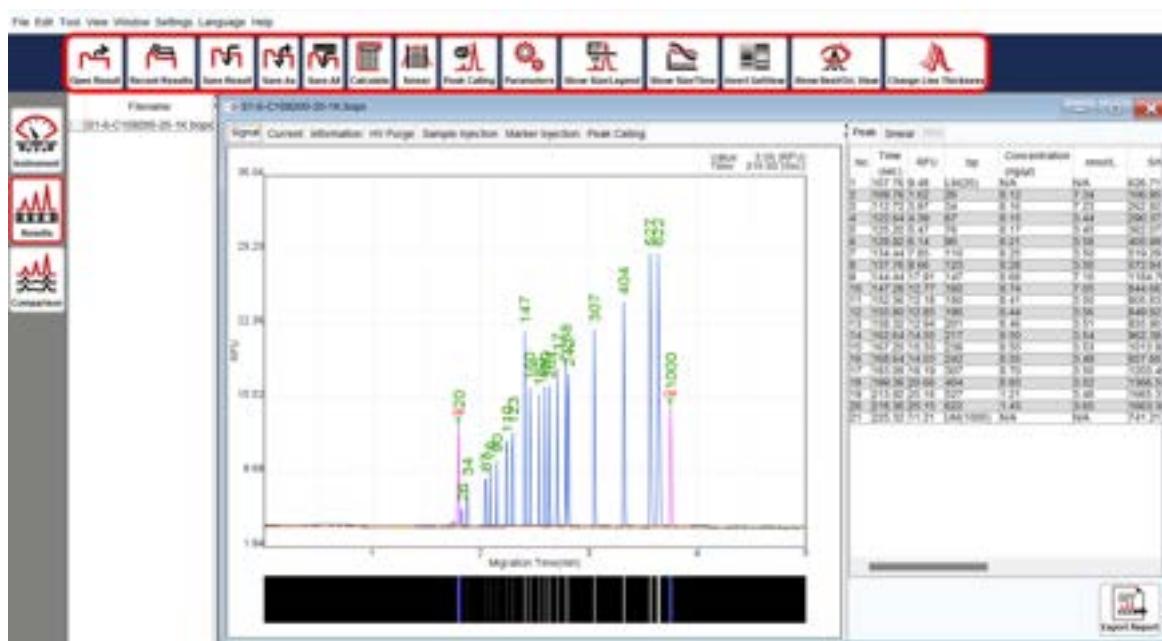
Figure 33   Toolbar Icons in Results

Calculate

The **Calculate** function can be used when the size information of the result is inaccurate or missing. By selecting the Calculate icon in the Toolbar, you can load a suitable reference marker table to calculate the size and concentration values of each peak.

To calculate the size and concentration values, follow these steps:

1. Open the desired result file and ensure that it is selected in the result list.
2. In the Toolbar, select the **Calculate** icon.
3. Click **Load** to load an appropriate reference marker table for the calculation.
4. Choose the reference marker file based on the components used. Each file contains the following four information:
   (1) Cartridge Type (e.g., S1, S2, F3, etc.)
   (2) Operation HV of the Separation (e.g., 6 kV, 8 kV, 10 kV, etc.)
   (3) Size Marker Type (e.g., C109200, C109301, etc.)
   (4) Alignment Marker Type (e.g., 20-1k, 20-5k, etc.)
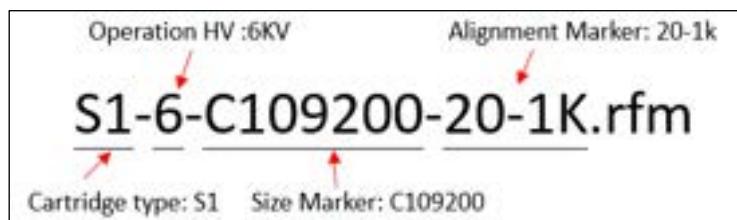
*Note: All reference marker files can be found in the default Load folder: <Workspace>\Reference. The default <Workspace> directory is C:\Users\<username>\Q-Analyzer.

5. Once the reference data file is confirmed, select the necessary setting checkboxes, if applicable, and click **OK**. The peak information will be displayed in the table.
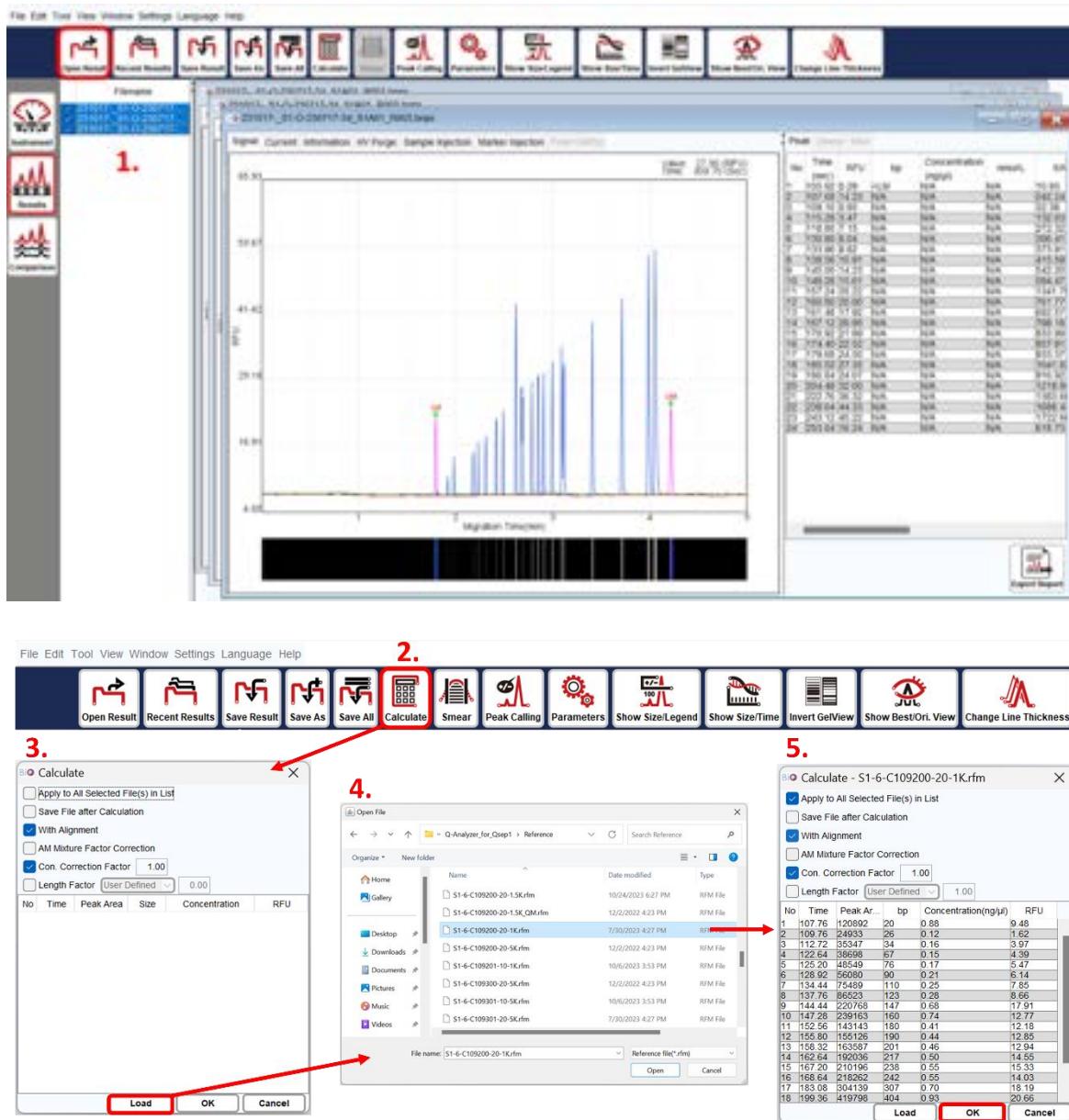
Figure 34   Calculate Results

Additional options available during calculation include:

Checkbox	Description
Apply to All Selected File(s) in List	Multiple files selected in the list will be calculated simultaneously.
Save File after Calculation	The result file will be saved after the calculation.
With Alignment	**This option is selected by default**, allowing the alignment marker to be applied for analysis.
AM Mixture Factor Correction	If the alignment marker is mixed with the sample, the calculation will be based on the mixture factor.
Con. Correction Factor	**This option is selected by default**, basing the calculation on the sample dilution factor.

Smear

The **Smear** function is helpful for understanding the major size and distribution of fragmented genomic DNA. Please note that the result must be calculated with a reference marker to use the Smear function.

*Note: Zone 2 will not be displayed by default. To display Zone 2 on the result file, click "Zone 2" directly.
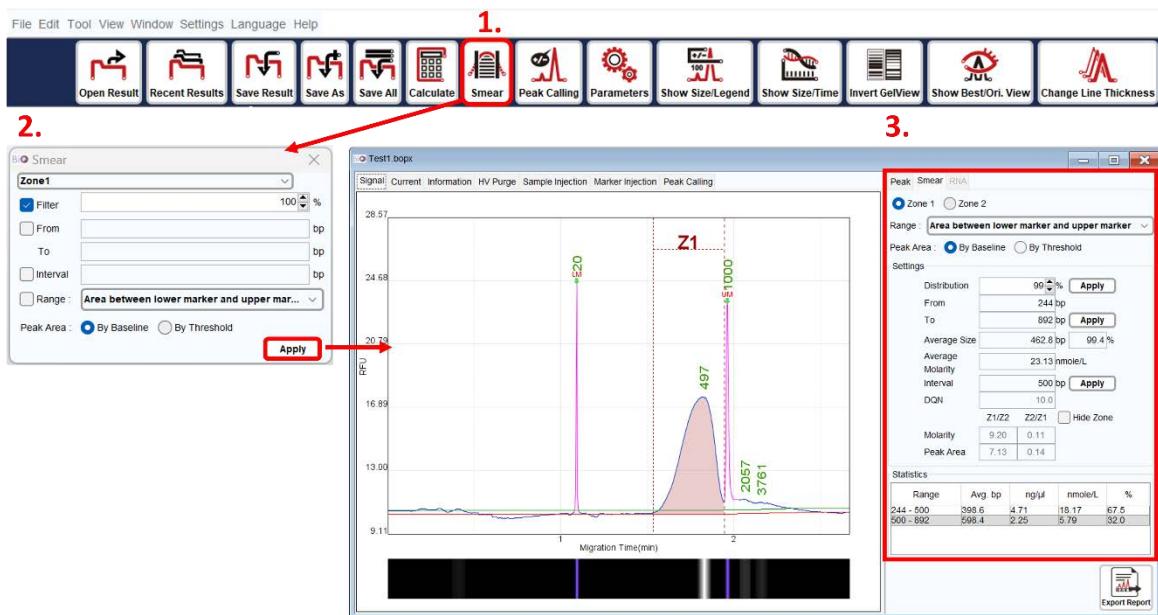
Figure 35   Smear Function

To use the Smear function, follow these steps:
1. Open and select the result file for Smear analysis from the result list.
2. Click on the **Smear** icon in the Toolbar, or alternatively, select **Smear** from Tool on the Menu Bar and click the **Smear** tab after configuring the settings.
3. Enter the percentage of the target distribution (e.g., 50%) or specify the size range to assign the region. If necessary, modify the interval of the size range. You can also select the target range to be either **Area between lower marker and upper marker** or **Area from lower marker to end**. Additionally, the peak area can be selected for analysis either **By Baseline** or **By Threshold**.
4. To view or edit the settings, click on the **Smear** tab on the result file. For more detailed information, please refer to section Smear DNA Analysis (gDNA QC for NGS).

Peak Calling

The **Peak Calling** function assists in distinguishing the target peaks. You can load a saved peak calling table, and the software will follow the rules in the table and generate a report in the peak calling tab window. The report displays "**+**" when peaks are found and "**-**" when peaks are missing. For instructions on creating a peak calling table, please refer to section Edit.
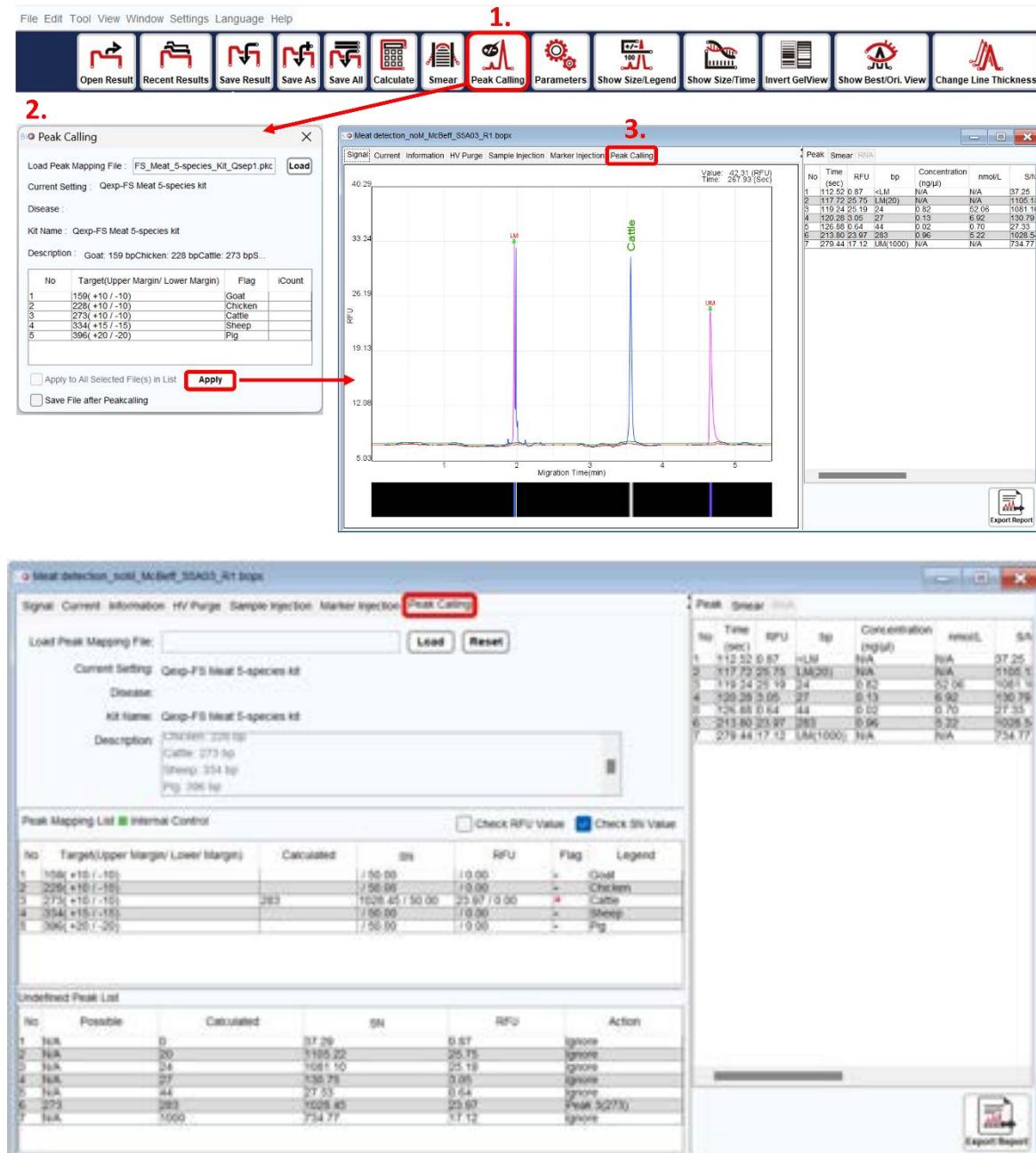
Figure 36   Peak Calling Function

Parameters

The **Parameters** function allows you to modify system-generated baselines and define peaks. By selecting this function, you can adjust parameters to meet their requirements.

Parameters such as Baseline Factor, Peak Smoothing, Peak Threshold, and S/N Peak Definition can be modified to customize the results. The changes can be applied to all selected files in the list, and the results can be recalculated and saved accordingly. The Default button can be used to restore the original settings.
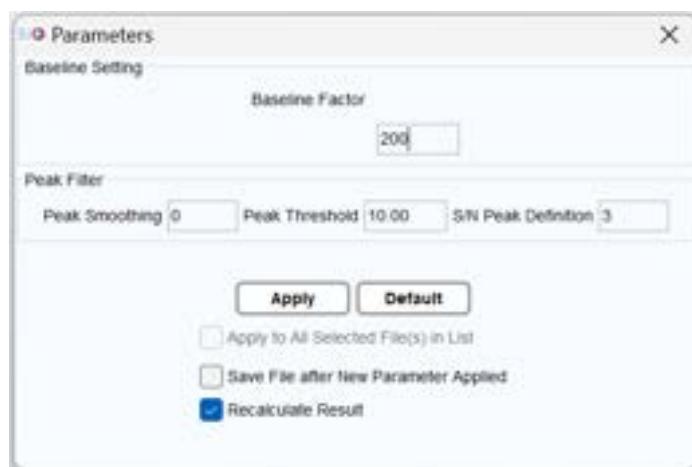
Parameter	Description
Baseline Factor	Baseline Factor affects the smoothness of baseline. The default value is based on the chosen method. The larger the value, the smoother the baseline.
Peak Smoothing	Peak Smoothing affects the smoothness of peak. The larger the value, the smoother the peak. This function may be utilized to reduce the number of peaks.
Peak Threshold	Peak Threshold changes the criteria of defining a peak. The larger the value, the stronger the signal must be for recognition. The peaks with low signal strength can be recognized by lowering the Peak Threshold value. Meanwhile, the noise may be recognized as signal peaks.

	S/N Peak Definition changes criteria of defining a peak. The larger the Peak Definition value, the more data points of the ramping signal must be for recognition. You may lower the Peak Definition value to define peaks with less points of the ramping signal. Meanwhile, the noise may be recognized as signal peaks.
S/N Peak Definition	
Apply to All Selected File(s) in List	Apply to All Selected File(s) in List changes the parameter of the file(s) selected in list. Make sure to select this function for multiple files modification.
Save File after New Parameter Applied	The result file will be saved after the new parameter is applied to it.
Recalculate Result	Recalculate Result recalculates the result and shows the peak size automatically after applying new parameter.
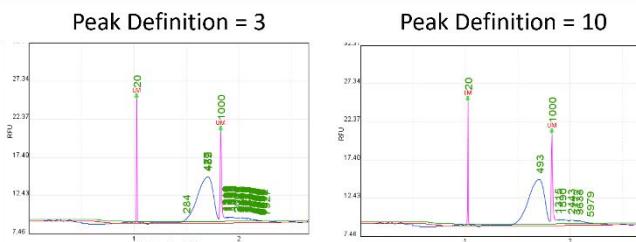
Show Size/Legend

The **Show Size/Legend** function switches the index of the peak between size and legend in the chart. To display the size, the result data must be calculated with a reference marker. To display the legend, data from the **Peak Calling** function must be applied.
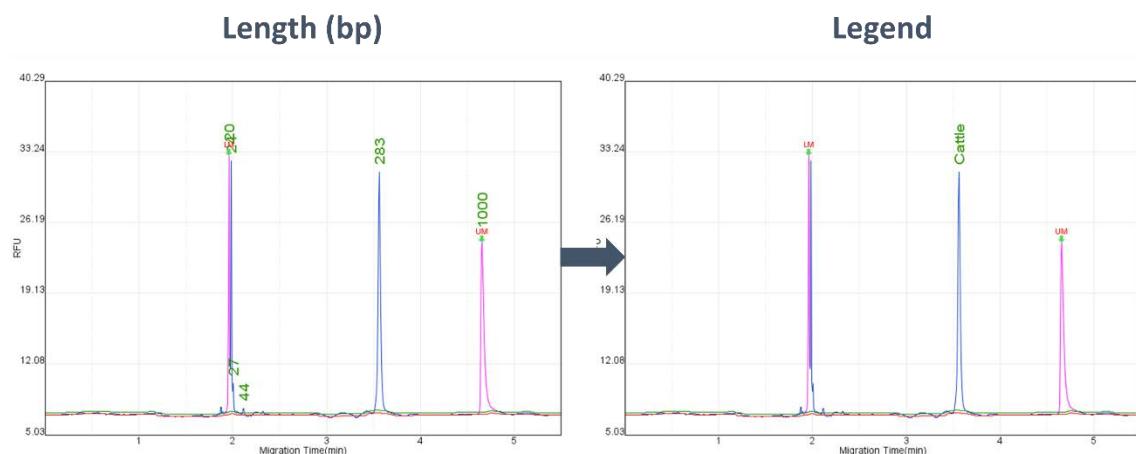
Show Size/Time

The **Show Size/Time** function switches the x-axis between size and migration time (in minutes). To display the size, the result data must be calculated with a reference marker.

Length (bp)

Migration Time (min)
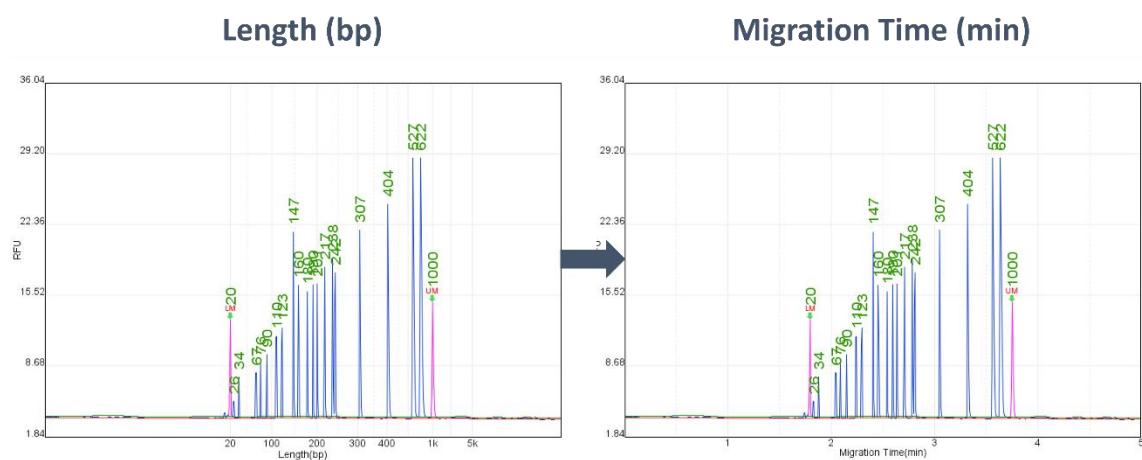
Invert GelView

The **Invert GelView** function inverts the color of the **Gel View**.
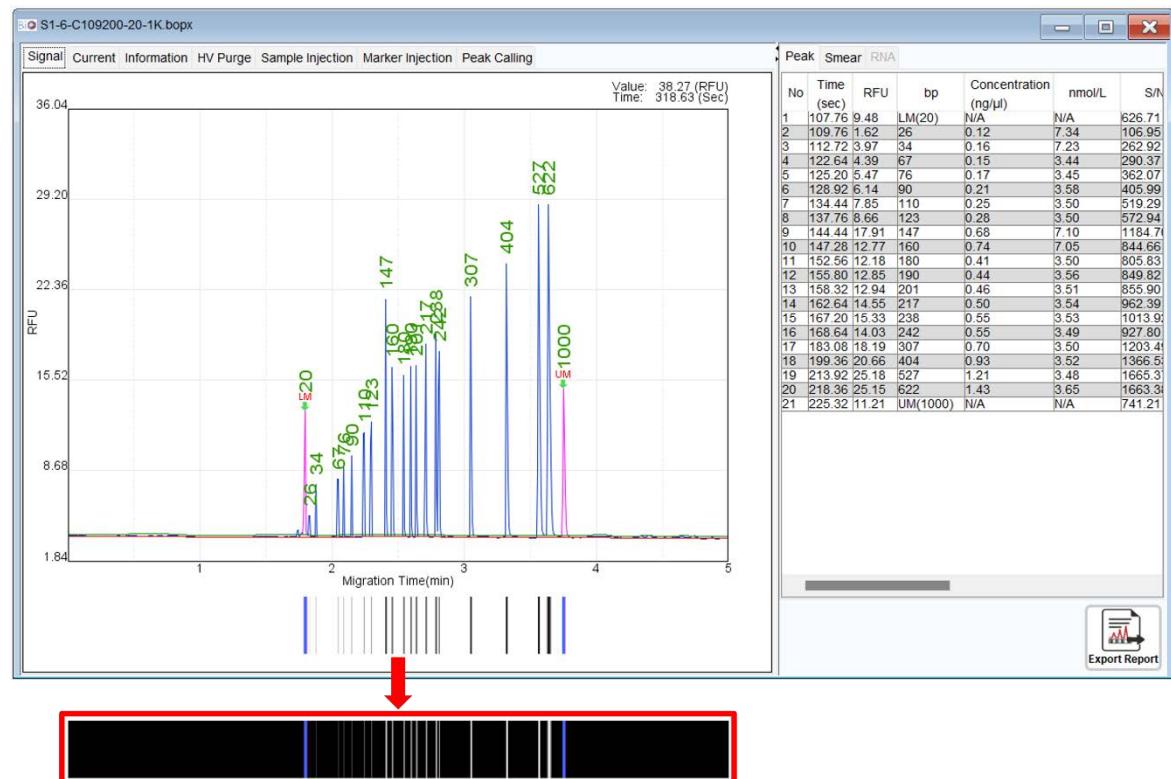
Figure 37   Invert Gel View

Show Best/Ori. View

The **Show Best/Ori. View** function switches between the best view and original view of the signal pattern.

Original View   **Best View**
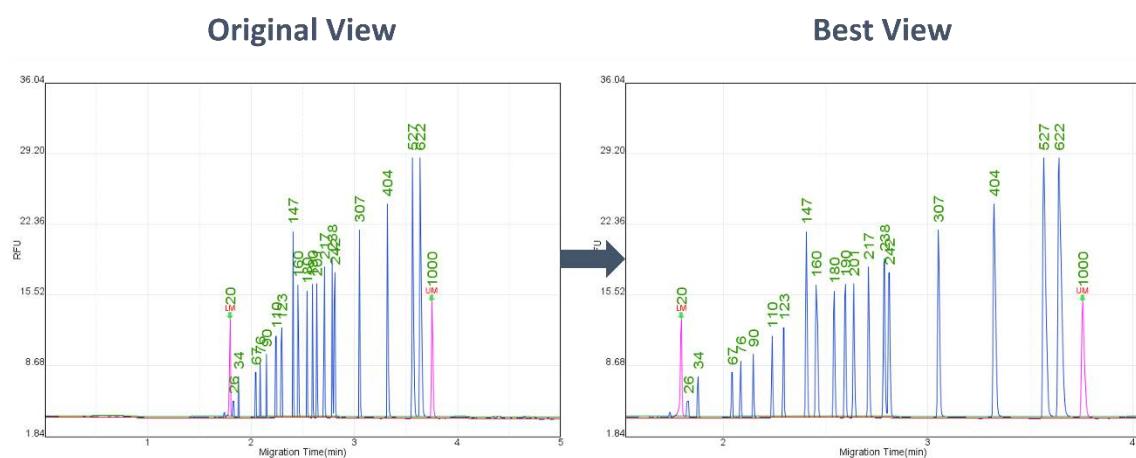
Figure 38   Show Best or Original View

Change Line Thickness

The **Change Line Thickness** function adjusts the thickness of the peaks in the chart.

Original   **Thickened**
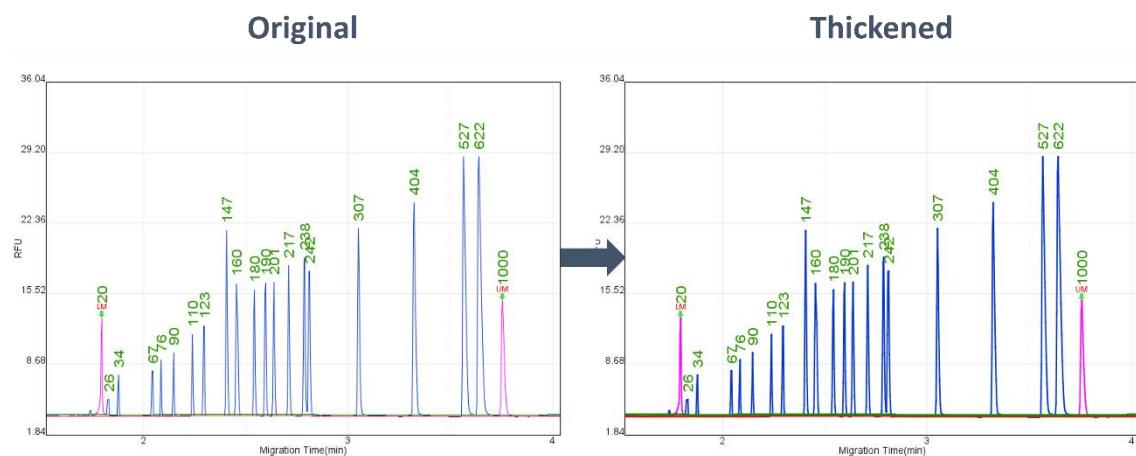
Figure 39   Change Line Thickness

Comparison

The **Comparison** function enables you to compare and analyze multiple results. This function allows you to load several signal charts and examine sample variances.

To utilize Comparison function, follow these steps:

1. Click on the **New Folder** icon in the Toolbar to create a Folder.
2. Drag the result files from the Filename panel into the created Folder window. The results will be displayed within the Folder window.
3. Edit the chart settings for easy viewing and simplified comparison.
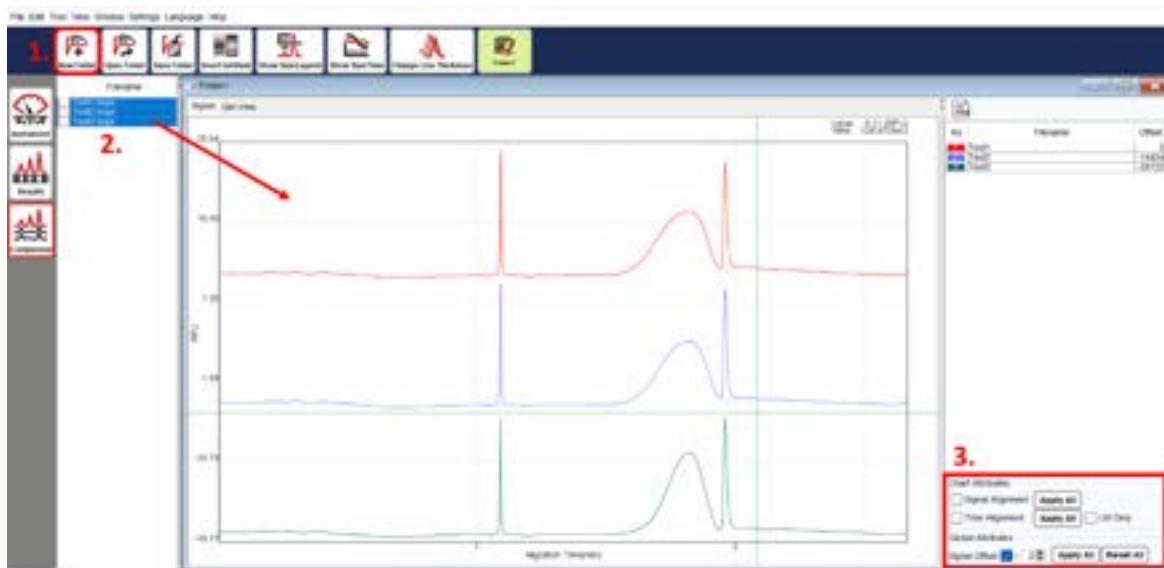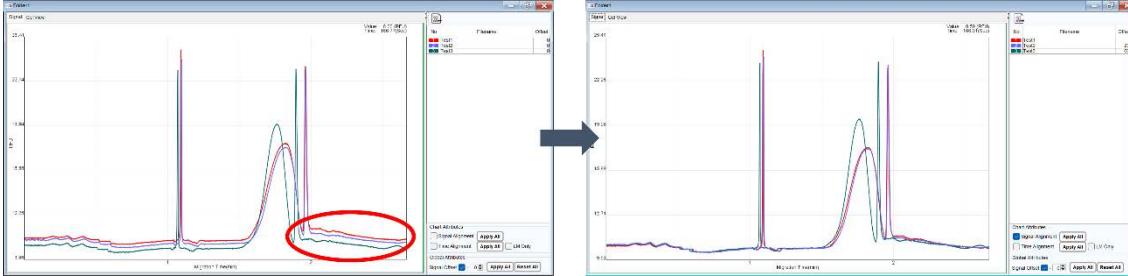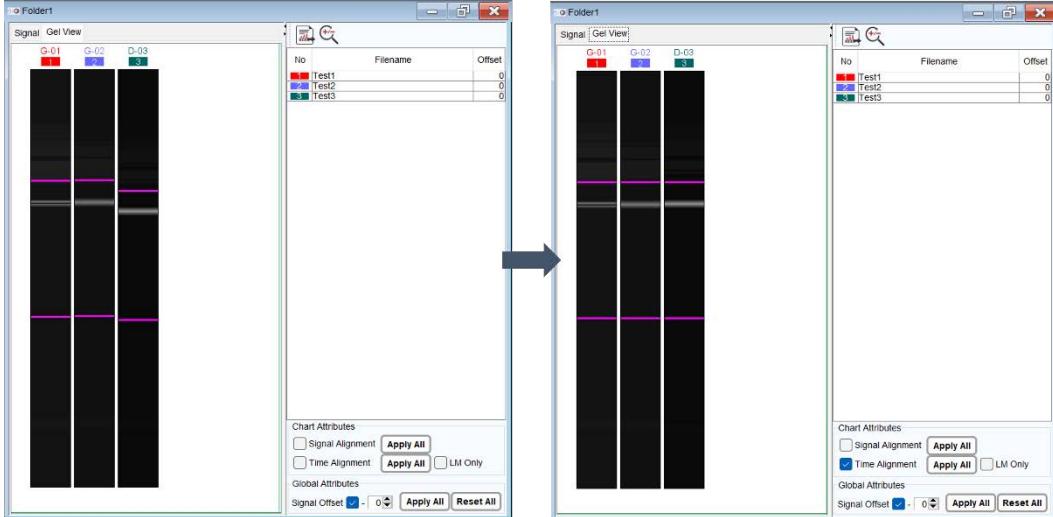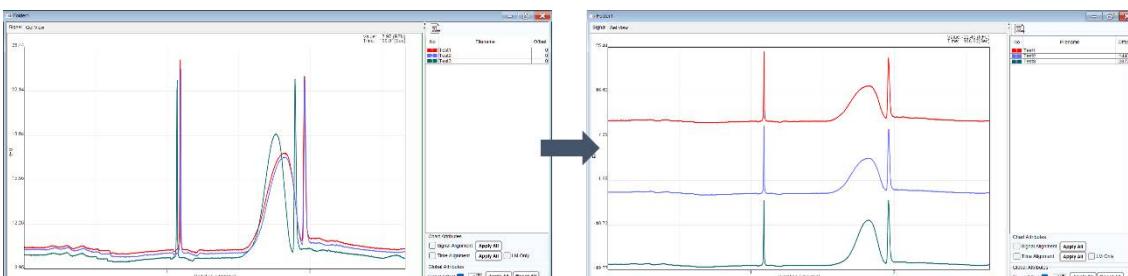
Figure 40   Multiple Results Comparison

Figure 41   Folder Settings
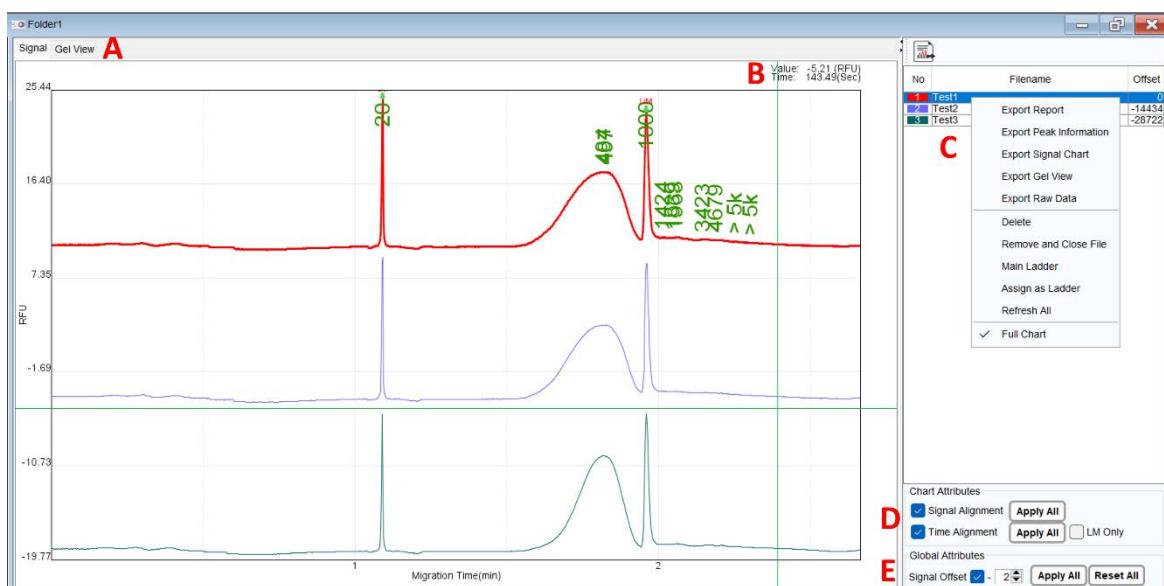
To view the electropherogram or gel image, select the tab in Figure 41 A. Figure 41 B displays the corresponding spot information. By choosing a file name in Figure 41 C, the corresponding chart data will be highlighted. You can change the line color by clicking on the color block in the number column. Alignment functions (Figure 41 D) can be used to normalize test results and align data due to variations in experimental conditions.

Parameter	Description
Signal Alignment	This adjusts the baseline of each file at the same height (RFU) as the baseline of the first file or main ladder (if set).

Time Alignment	This scales the data using the Alignment Marker of each file to align with the first file or the main ladder (if set).

Signal Offset (Figure 41 E)	This helps separate stacked data. Simply set the value in Signal Offset and click Apply All. You can restore the original result using the Reset All function.

To invert or adjust the contrast/brightness/maximum level of the gel view, go to Menu Bar → View → Gel View Setting → Invert/Contrast/Brightness/Max lvl.
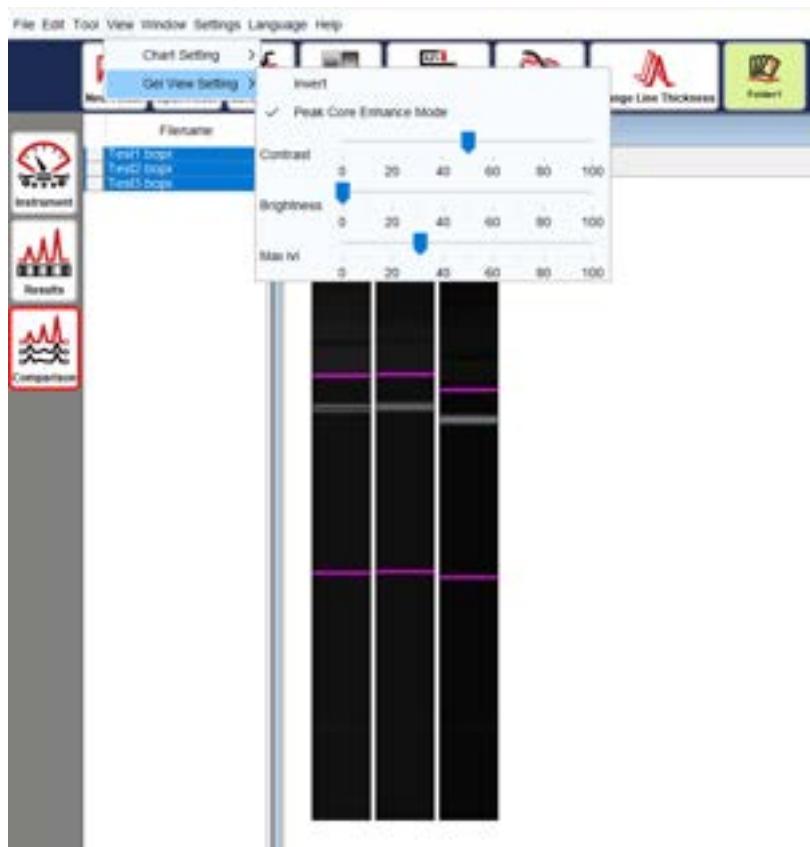
Figure 42   Adjust the gel view

To export the Folder result, right-click and select **Export Report** or click on the export report icon located at the top of the list. Choose the desired file format, enter the file name, and save it to the designated folder.
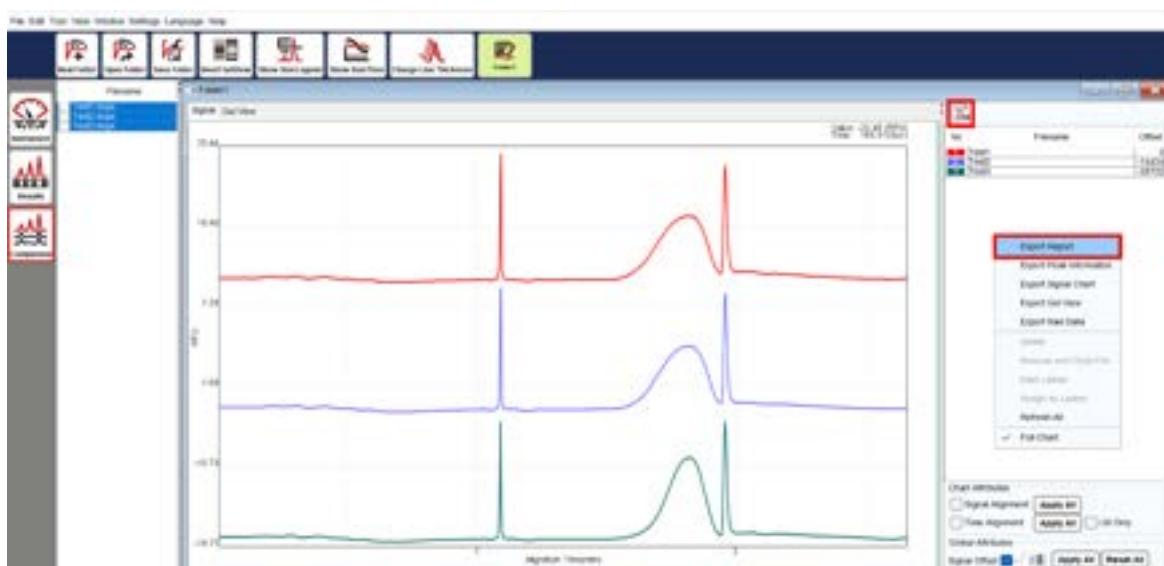
Figure 43   Export Folder Report
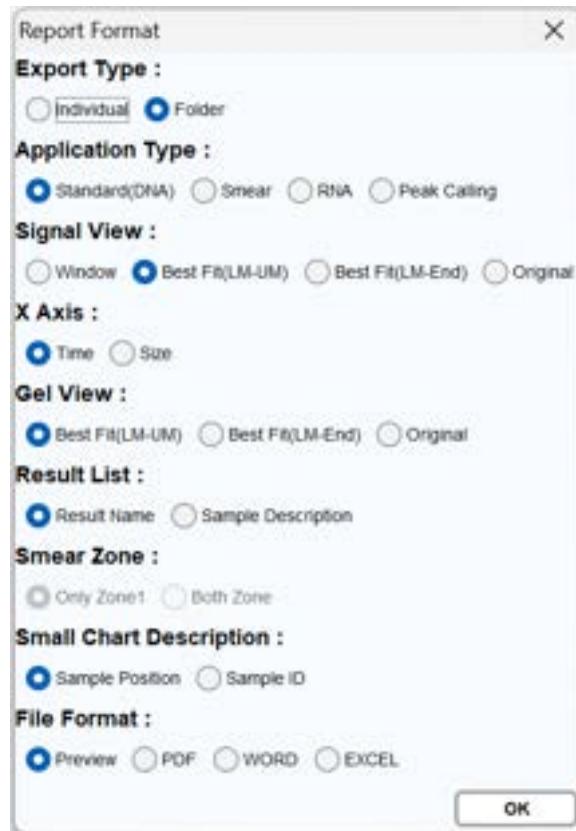
Figure 44　Folder Report Format

By right-clicking on the result list in the folder window, you can export the signal chart or gel view with or without the sample description and result name.
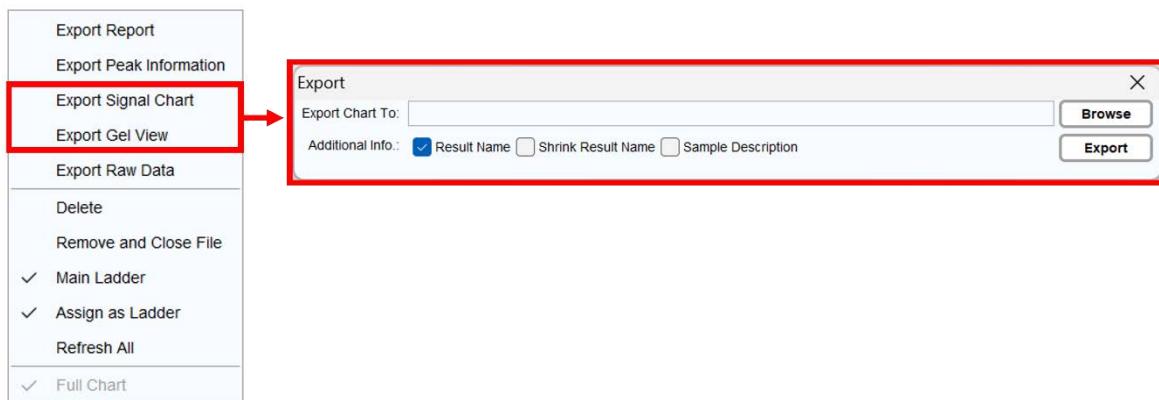
Figure 45　Export Folder Chart

Menu Bar

File

Function	Description
New Project	Create a new project to differentiate experiment conditions. Results will be saved in the corresponding "Result" directory, with the default saving folder at C:\Users\<username>\Q-Analyzer\ Result\<Project name>. Set up the new project before connecting to the instrument.
New Folder	Open a new folder to compare the results. For more information, refer to section [Comparison]().
Open Result	Open result files from the project.
Open Folder	Open folder files from the project.
Save Result	Save and overwrite modified result.
Save As	Save modified results as new files.
Save All	Save and overwrite all modified results.
Save Folder	Save the created or modified folder.
Load Project	Load a saved project before connecting to the instrument.
Save Project	Save the current project, including methods used in the sequence; sample position, duration, sample information, and result names.
Recent Project	List the latest projects opened for loading.
Recent Results	List the latest result files opened for loading.
Close	End and exit Q-Analyzer.

Edit

Sample File

To edit the information of each sample, click on **Edit** on the Sample File window (Figure 46), and the Sample Loader window will appear (Figure 47). Select the sample position and enter the sample information, including sample/patient name, ID, date, and description. Click **OK** to apply the changes, **Clear All** to delete, and **Save** or **Save as** to save the sample file. You can **Load** previously saved sample files for inquiry or editing. Multiple sample description can also be edited by importing an Excel file.
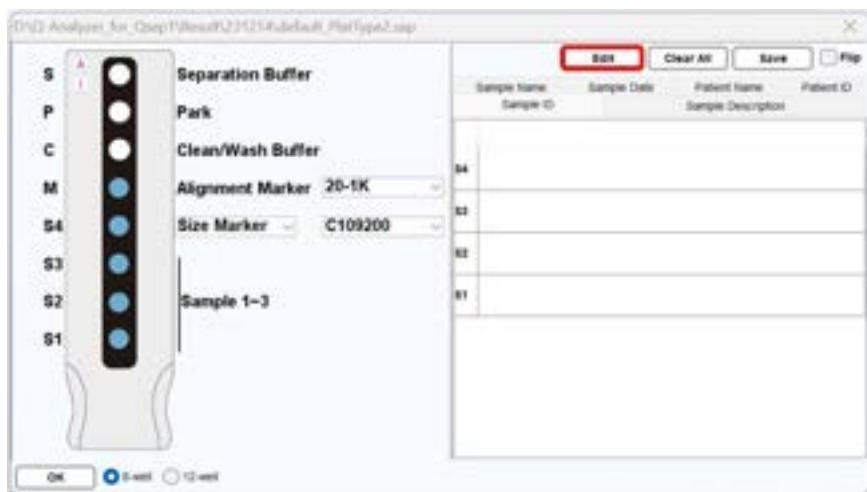
Figure 46   Sample File Window
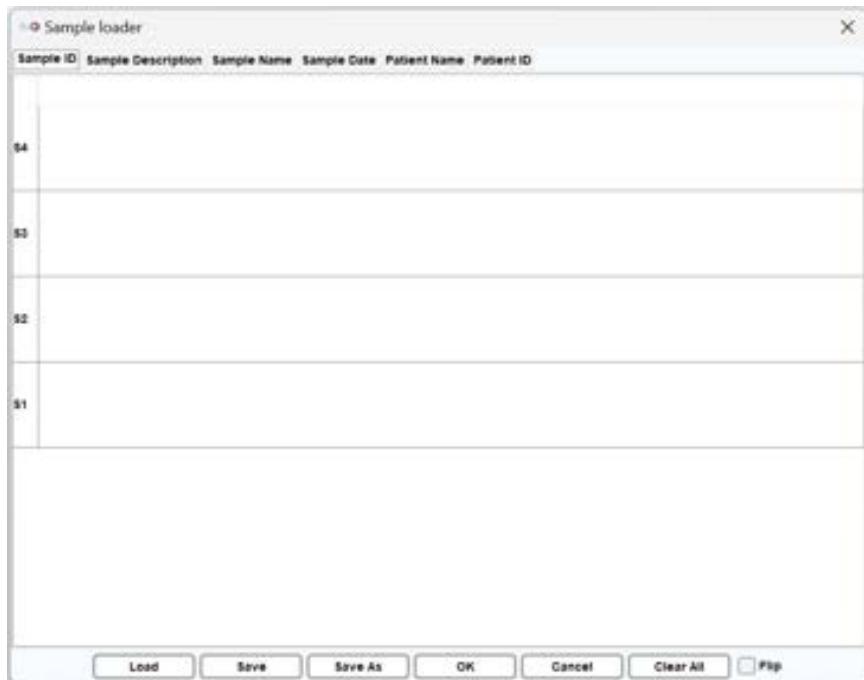
Figure 47   Sample Loader Window

Reference Marker

The **Reference Marker** function allows modification of the saved reference marker table or creation of a new one. To create a new reference marker table, run a specific size marker run, remove redundant peaks, right-click, and select **Copy Peak Info for Creating Reference**. Then, go to "**Edit → Reference Marker**" and right-click on the blank area and to choose **Paste Reference Marker Data**. The data can be edited if necessary.
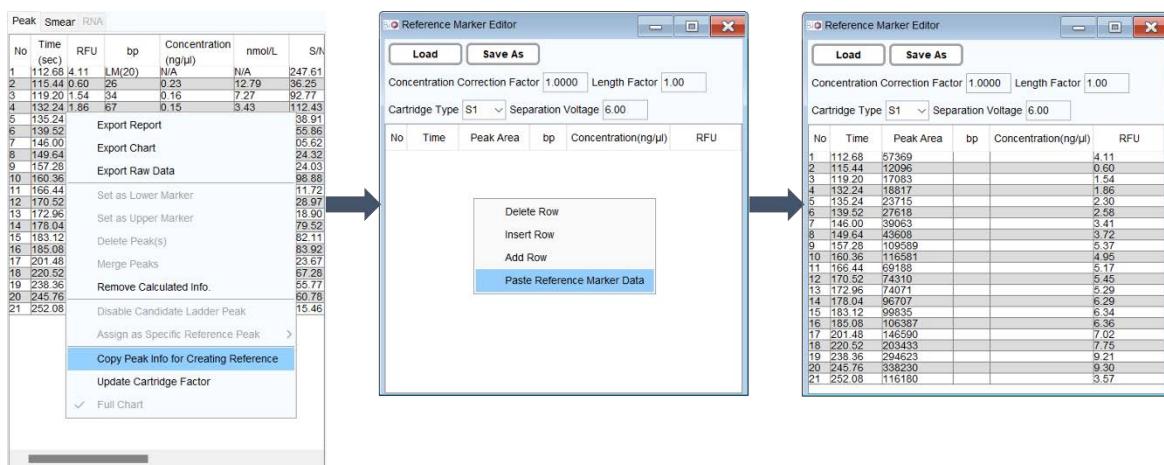
Figure 48   Edit Reference Marker Table

To renew a saved reference file (.rfm) shown in Figure 48, click **Load**.
- **Time**: Migration time of each peak.
- **Peak Area**: Measurement of the area within the peak.
- **bp**: Fragment length.
- **Concentration (ng/µl)**: Concentration of each peak.
- **RFU**: Relative fluorescence units (RFU) of each peak.

Time and **Peak Area** are automatically generated from the test result, so their values should not be changed unless specific requirements dictate otherwise. Enter **bp** and **Concentration** of a known size marker and add or delete rows as needed. After completing all modifications, save the reference marker table for future use.

*Note: When pasting exported data from the result file, ensure that **the row number** of the pasted data **matches** the recalled reference marker table. Additionally, make sure the data is recognized based on the identical base pair.

Parameters

Modify the parameters in the calculation settings. For more information, refer to section Results.
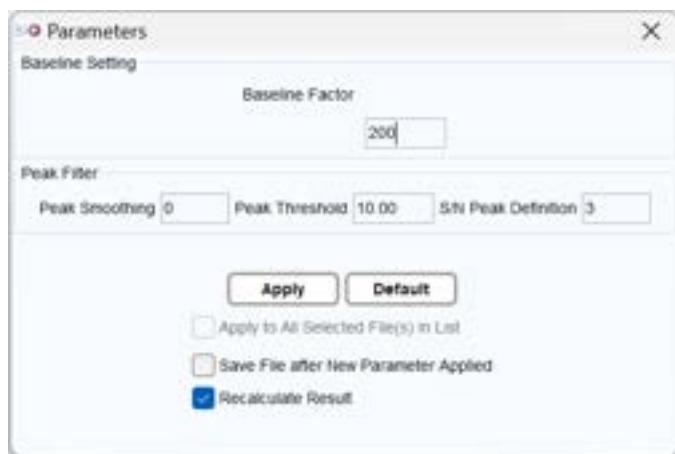
Figure 49   Parameter Settings

Peak Calling Table

Peak Calling creates panels for quick scanning. To utilize the Peak Calling function, set up the Peak Calling Table, which includes target peak information. Customize the peak calling table by adding a new row and assigning the target, upper margin, lower margin, S/N, RFU, Legend, and IC (Internal Control).
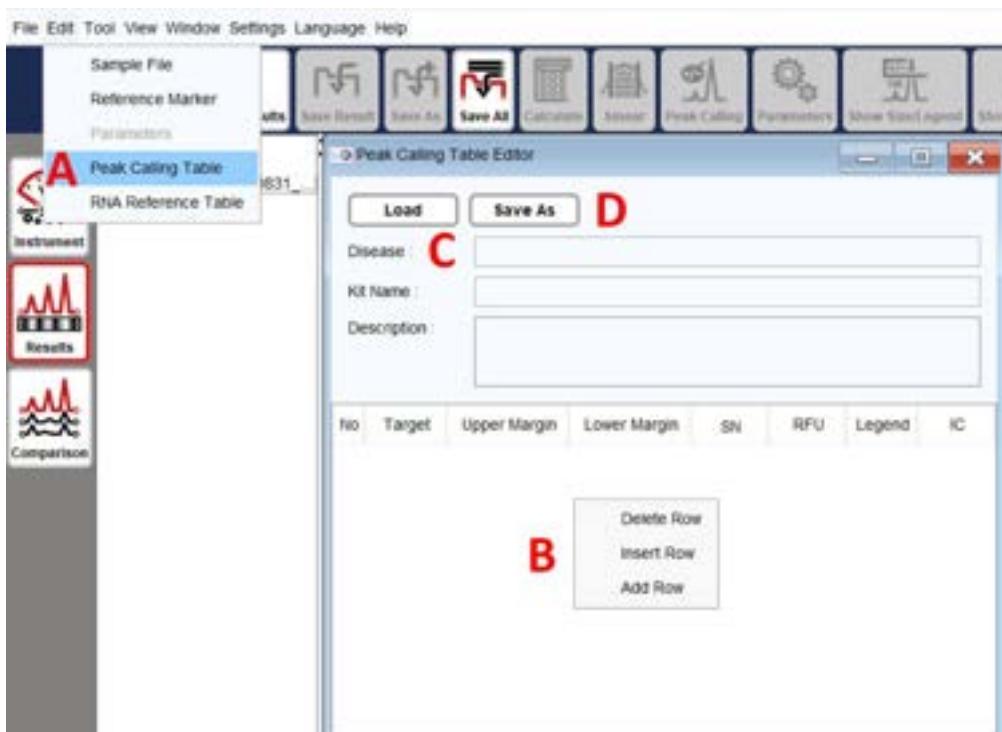
Figure 50   Peak Calling Table

Follow these steps for **Peak Calling**:

1. Click on **Edit → Peak Calling Table** to open the Peak Calling Table Editor (Figure 50 A).
2. Right-click and select **Add Row** to add a new panel (Figure 50 B).
3. Set up the new table by entering criteria in the respective columns (Figure 50 C) and save it as a new file (Figure 50 D) in the default folder.
   - **Target**: Center value of the peak.
   - **Upper/Lower Margin**: Size range of the target peak assigned as the Legend.
   - **SN**: Signal-to-noise ratio criterion for the peak (optional).
   - **RFU**: Signal amplitude criterion for the peak (optional).
   - **Legend**: Name of the Legend.
   - **IC**: Internal control for peak calling.
4. Return to the result files, calculate the fragment size, and select the **Peak calling** tab or click **Tool → Peak Calling** (Figure 51 A-B).
5. Browse and load the desired Peak Calling Table file (Figure 51 C).
6. Click the **Signal** tab to return. The software will automatically scan the peak signals. Click the **Show Size/Legend** button in the Toolbar to display the legend of the target peak. Right-click and select **Export Report** or click on the Export Report icon at the bottom to export the report. (Figure 52)

*Note: Incorrect fragment size may affect the Peak Calling result. Ensure proper calibration and correct bp values are displayed.
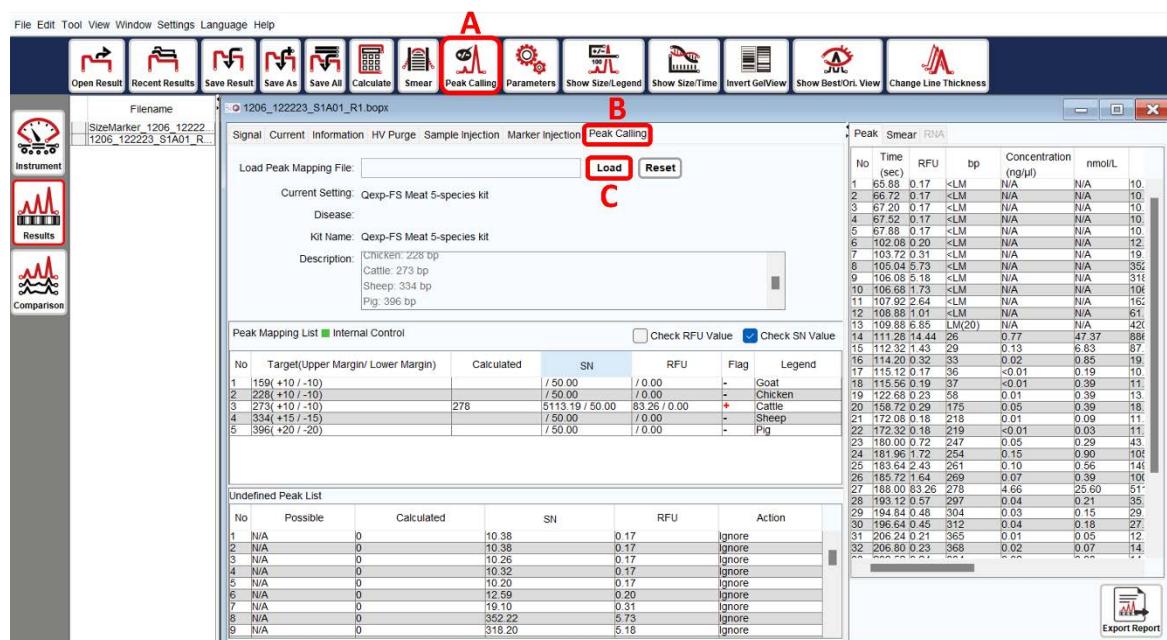
Figure 51   Fragment Size Calculation and Peak Calling Selection
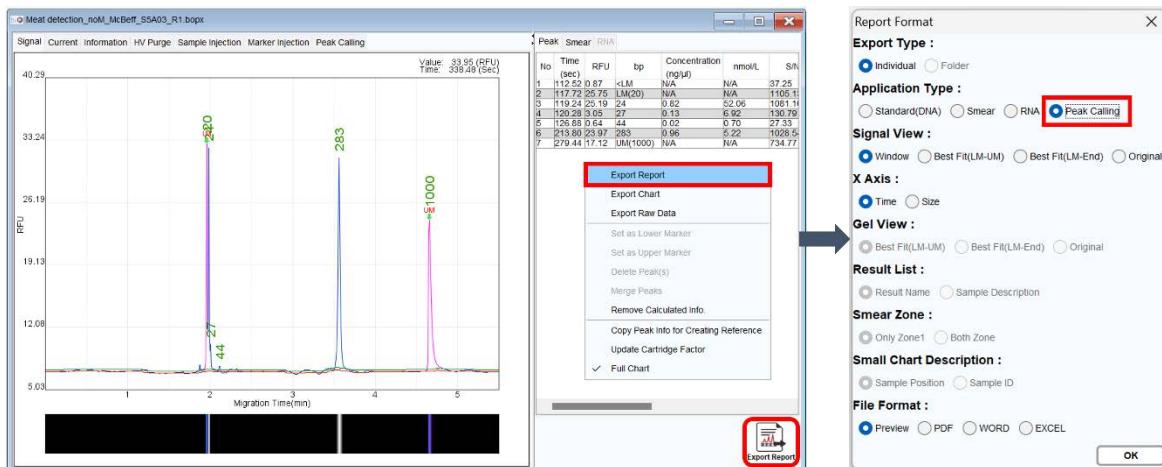
Figure 52   Loading Panel and Exporting the Report

RNA Reference Table:

The RNA factor table allows modification of RNA signal parameters for result analysis (18S and 28S). The values of 18Ss and 18Se are the start and the end of 18S. To modify the values, **Load** the .rfa file from the default directory. For effective use, the built-in reference tables, RNA-4K.rfa and/or RNA-6K.rfa, must be overwritten. For RNA analysis, please refer to section Total RNA Quality Analysis.

Figure 53   RNA Factor Table

Tool

Calculate

Use the reference marker table to perform base pair calling and quantitative result analysis. **Load** the reference marker table and click **OK** to initiate the calculation. The result file will display the corresponding values of size and concentration. For more information, please refer to section <u>Results</u>.
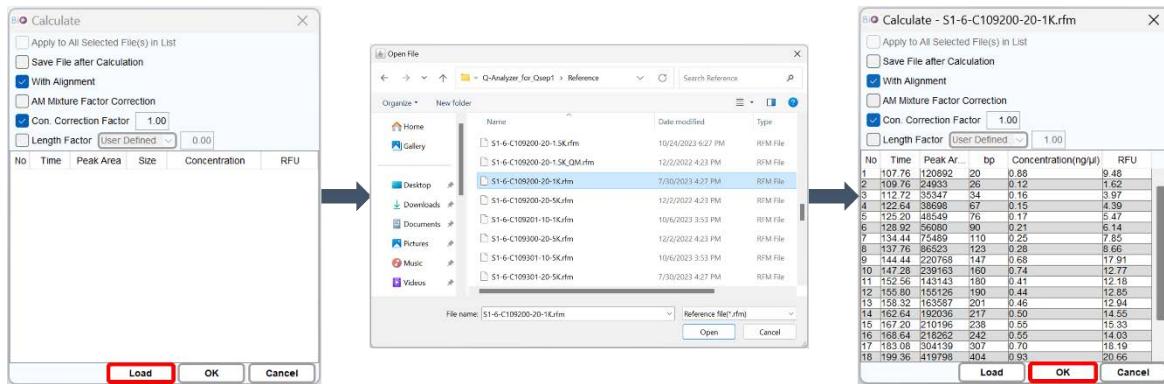
Figure 54   Calculate Workflow

Recalibrate

Enhance the accuracy of alignment marker (upper and lower) identification and update the cartridge factor.

Perform **Recalibrate** in the following situations:

- The alignment marker has been replaced.
- The cartridge has been stored for more than two weeks since the last execution.
- The software fails to identify the alignment marker correctly.

To recalibrate, select the voltage and alignment marker that match the "Method" to be used (Figure 55). For more information, please refer to section <u>Instrument</u>.

Figure 55   The message box of recalibration

Smear

The **Smear** function helps understand the size distribution and average sample size. It can be applied to DNA, RNA, and protein analysis.

Peak Calling

The **Peak Calling** function helps to distinguish the target peaks. Refer to section Edit for instructions on setting up the Peak Calling table.

Analyze

The **Analyze** function automatically generates a summary report for Nucleic Acid Amplification Test (NAAT). It is specifically designed for certain applications. For more information, please contact BiOptic Inc.

*Note: **Analyze** is used to interpret multiple **Peak Calling** results by utilizing built-in kit information.
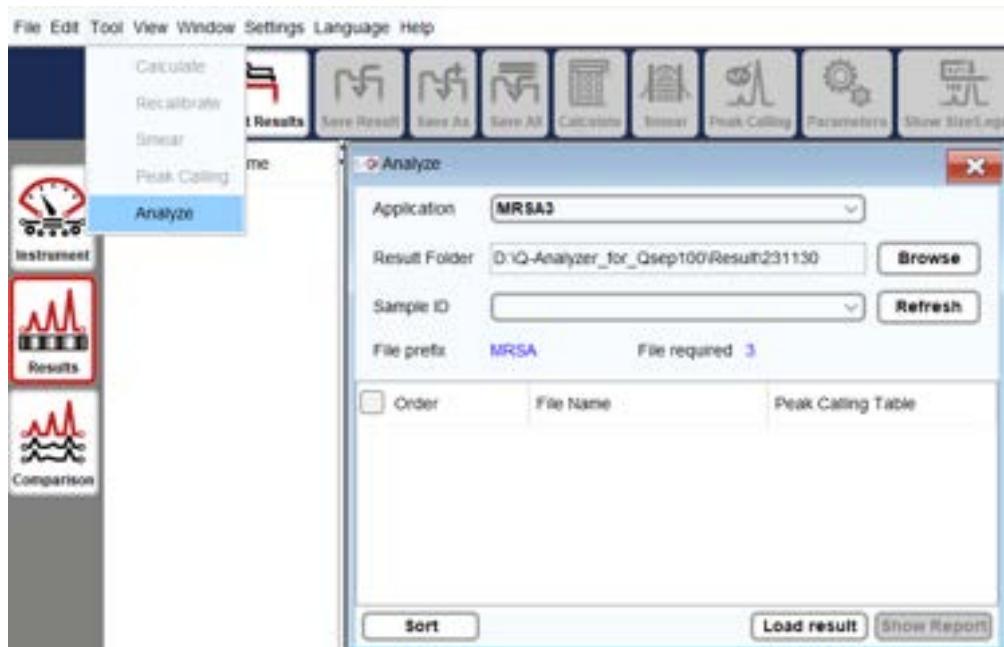
Figure 56   Analyze Function on Toolbar

View

Chart Setting: Modify the configuration of the electropherogram display.

Chart Setting allows user to adjust settings such as Line Color, Size/Legend Color, Grid Color, Background Color, and other data displays.
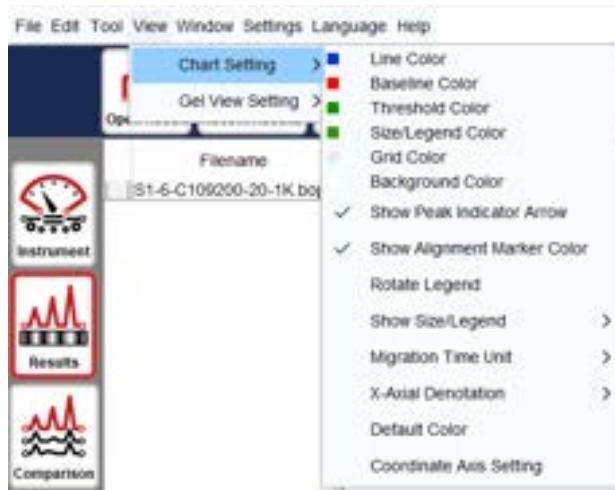
Figure 57   Chart Setting

Gel View Setting: Customize the settings of the gel image simulation.

You can choose to invert the gel image, display the peak line, display alignment marker indicator, or adjust the contrast of the gel image.
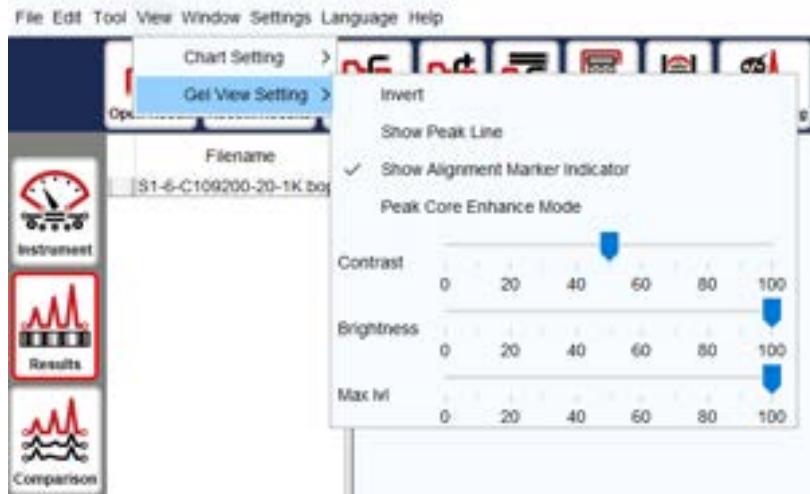
Figure 58   Gel View Setting

In Figure 59, A represents the original gel image in the system. By adjusting the contrast, you can enhance visibility of weak peaks, as demonstrates in B. C shows the inverted gel image, and D displays the inverted image after contrast adjustment to C.
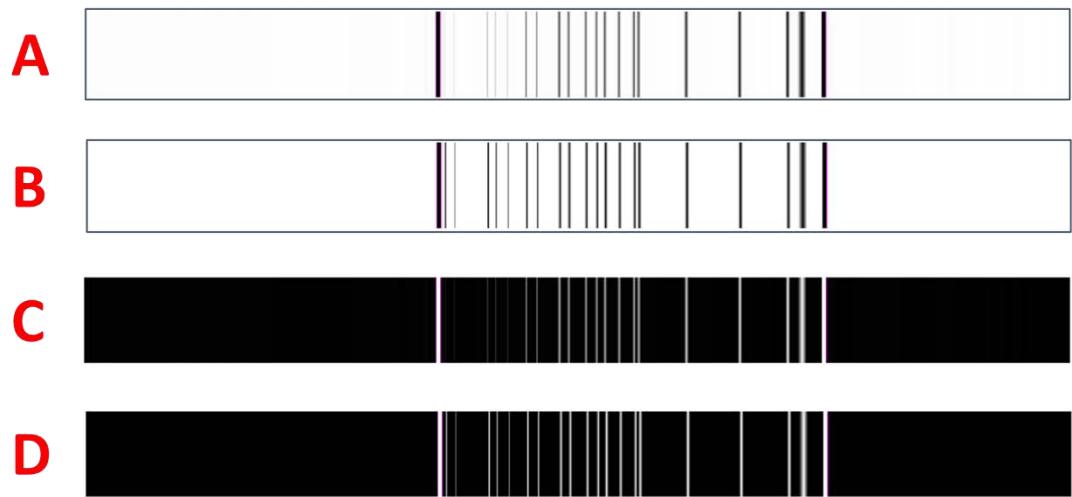
Figure 59   Gel Image

Window

All opened files will be listed under the Window menu.

Cascade

The **Cascade** option arranges the opened windows in an array format for easier viewing.

Figure 60   Cascade

Close All

The **Close All** option closes all opened windows.

Setting

Change Password

The **Change Password** feature enables you to update the password associated with the software key.

Figure 61   Change Password

To modify the password, input the information into the fields for Old Password, New Password, and Password Confirmation. The password must consist of **8-12** characters, inclusive of both numbers and letters, and is **case-sensitive**.

Preference

The **Prefernece** page is used to configure the default settings of *Q-Analyzer*, such as the view method, calibration time, motor settings, and more. Please note that some preference settings may modify the default value of the instrument and can affect the experiment results. It is recommended to undergo professional training from BiOptic Inc. before making any changes to these settings.

Figure 62   Preference Settings

Language

You have the option to choose between three languages to operate *Q-Analyzer*: English (Default), Chinese (Simplified), Chinese (Traditional). To apply the selected language, you need to relaunch *Q-Analyzer*.

Help

<u>**Version**</u>

The **Version** option displays the software and firmware versions. To obtain firmware information for *Qsep₁* Bio-Fragment Analyzer, the instrument needs to be connected.

Figure 63   Version Window

For technical support, follow the instructions to **Export** the file in .tecsupport format and send it back to BiOptic Inc. The license file will be provided in .bioptic format for you to Import.
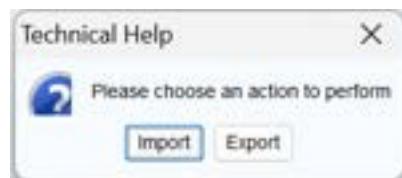
Figure 64   Technical Help Window

Help

The **Help** option opens *Qsep₁* Bio-Fragment Analyzer User Manual.

Report

The **Report** function collects error information and generates a report file for further evaluation. When encountering difficulties, use this function to generate an error report and send it to BiOptic Inc. via service@bioptic.com.tw for technical support.

Toolbox

The Toolbox is a comprehensive troubleshooting feature, guiding you through a step-by-step process to address potential issues. It encompasses the following functions:

- **HV Function Check**: Conducts tests to ensure a stable current is maintained.
- **Purge Function Check**: Initiates purging while the cartridge door is open, allowing you to verify the proper release of air during the process.
- **Motor Function Check**: Validates the motor position and settings by moving the tray holder in different directions, facilitating the observation of the motor status at each step.
- **Computer Compatibility Check**: Ensures the software settings align with computer specifications, including hardware and software requirements.

For more detailed information, refer to section Instrument.

Control Panel

Upon launching *Q-Analyzer*, the Control Panel will automatically appear, providing access to three main functions: **Main**, **Method**, and **Direct Control** (Figure 65), unless entering Advanced mode, where **Board Setting** will also be visible. The extent of access granted to these functions is determined by the software key in use. For a comprehensive comparison of the functions and their corresponding access levels, please refer to section [Software Key](#).

Main

Main page is the major control to operate *Qsep₁* Bio-Fragment Analyzer.
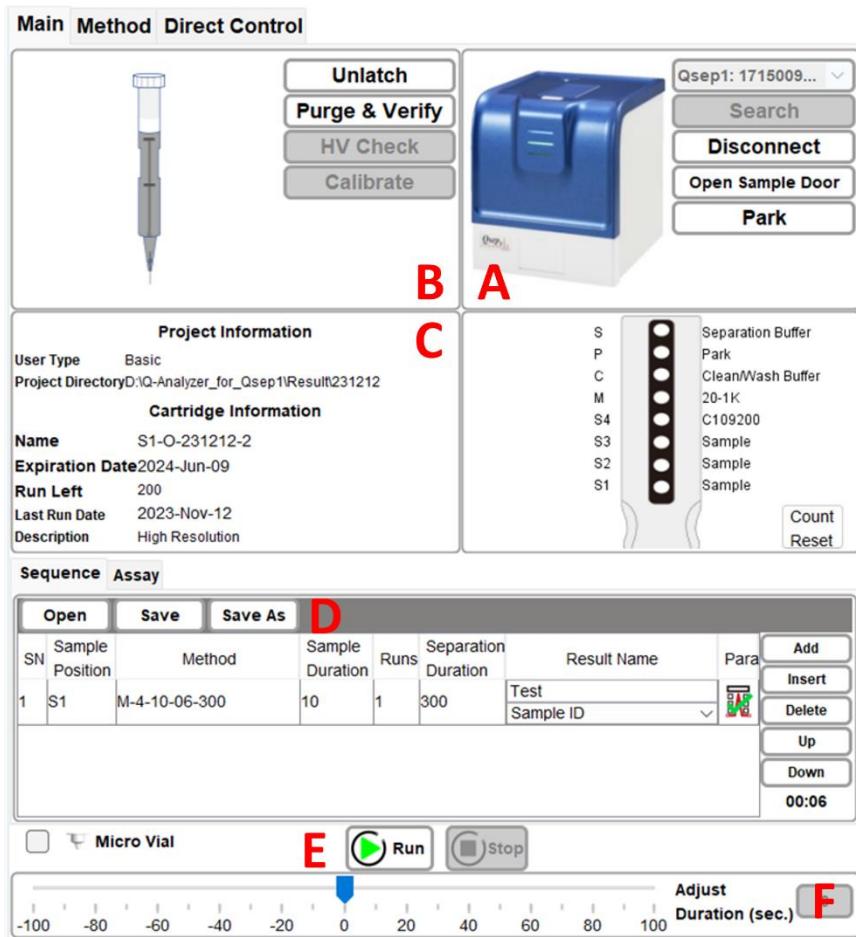
Figure 65   Main Page

| Function	Description
A: Instrument Control	
Search	Retrieve the connected $Qsep_1$ Bio-Fragment Analyzer by conducting a search. The instrument's serial number will be displayed for identification purposes.
Connect/Disconnect	Establish or terminate the connection with $Qsep_1$ Bio-Fragment Analyzer. Once successfully connected, the image of the instrument will display in color, and the Connect button will change to Disconnect for manual disconnection.
Open Sample Door	Move the tray holder to the door, facilitating the loading or unloading of buffers, markers, and samples.
Park	Position the P well of the buffer tray below the cartridge tip to prevent the cartridge tip from drying out during periods of non-use.
B: Cartridge Control	
Latch/Unlatch	Secure or release a cartridge by latching or unlatching it. To execute a sequence, the inserted cartridge must be latched. The cartridge information will be displayed, and the picture will appear in color. Click Unlatch and wait a few seconds before removing the cartridge.
Purge & Verify	Initiate a five-minute purging process for the cartridge. The last 60 seconds of the purge will be High Voltage (HV) purge to check the stability of the current.
HV Check	Perform an HV Check on a new cartridge before running the sequence. For more information, please refer to section Calibration.
Calibrate	Complete the Calibration process for a new cartridge before running the sequence. For more information, please refer to section Calibration.
C: Project and Cartridge Information	
Display User ID and Project Directory. After latching, the cartridge information such as Cartridge Number, Expiration Date, Runs Left, Last Run Date and Description will be shown.	
D: Sequence Settings	
Build the sequence by editing Sample Position, Method, Sample Duration, Runs, Separation Duration, Result Name, and Parameters. For more information, please refer to section Creating A Sequence.	
E: Sequence Control	
Run	Start a sequence. During a test, the **Run** button allows starting the test, **Pause** to pause the running test, and **Continue** to resume the unfinished test.
Stop	End a sequence.
F: Slide Bar (Reduce and Extend)	
Adjust the remaining time of an ongoing action by dragging the slide bar at the bottom of the window. For example, if the Separation & Detection time is running out, but the sequence is not yet completed, dragging the slide bar to the right (e.g., 40) will extend the time. The amount of the adjusted time will be displayed on the button. Click the button to confirm the change, and the Separation & Detection action will be extended by 40 seconds.	
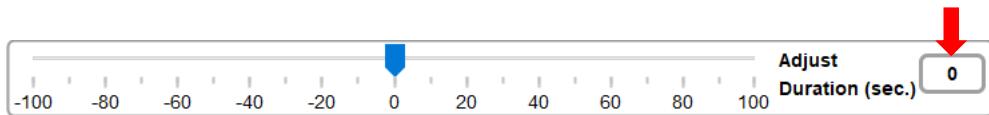
*Note: Only the remaining time of the ongoing action can be extended or shortened.

Method

Method tab provides functionalities for viewing, editing, and creating methods. Please note that only Advanced users can create and edit methods.

Figure 66   Method page

To create a method, click on **New Method** in the Method tab. Click **Add** to add an action. Select the desired action and enter the duration time. For each action row, select a position except for **Sample Injection**, as the sample position is controlled by the Main tab.

A **Method** consists of various steps, including:

Function	Description
Purge	Refill the capillary using air pressure.
High Voltage Purge	Refill the capillary using air pressure with high voltage.
Sample Injection	Inject the sample into the capillary electrokinetically. *Note: Only one Sample Injection action is allowed in a method.
Marker Injection	Inject the alignment marker into the capillary electrokinetically.
Separation & Detection	Initiate the capillary electrophoresis analysis. *Note: Only one Separation & Detection action is allowed in a method.
End	Declare the final commend of a Method.
Pause	Park the cartridge tip at the Park position.
Purge Check	Verify that the air tube is not clogged before use or clean the gel if accidentally sucked into the air tube after use.
Wash Step	Perform a wash on the cartridge tip at the Wash position.
Clean Step	Clean the cartridge tip at the Clean position.

Direct Control

Under **Direct Control**, all actions of the method can be executed manually and independently . The tray holder can also be moved to the designated position.

Figure 67   Direct Control page

Function	Description
Motor Initialize	Initialize the motor position.
Position	Move the desired well below the cartridge tip.
Purge	Set the duration for purging.
HV Purge	Set the duration and voltage for purging.
Action	Select Sample Injection, Marker Injection, and Separation & Detection, and specify the duration and voltage for each. ***Note**: Each execution of Separation & Detection will decrement the number of remaining runs for the cartridge.

Board Setting (Advanced User Only)

In **Board Setting**, you can find the following information for the *Qsep₁* Bio-Fragment Analyzer:

Figure 68   Board Setting Page

Function	Description
Instrument ID	Display the unique identifier for the connected instrument.
Raw Count/Current	Show raw data during data collection.
PMT Voltage	Inspect PMT by adjusting the voltage.
Sampling Rate	Change the frequency of collecting raw data.
Data Collect	Collect raw data for a specified duration. The table below will show the average, standard deviation, maximum/minimum of the raw counts and current.
CH_01	Represent the channel of the connected *Qsep₁* instrument used for analyzing the results.

Result/Data Analysis

Result for Data Display

By selecting the **Result** function, you can analyze data from *Qsep₁* Bio-Fragment Analyzer using capillary electrophoresis. The Result function enables you to display charts and edit the size from the raw data.

To open the result file, you can click the **Open File** icon on the Result window tab or access it from the **File** on the Menu Bar. The browser allows you to select multiple files and view the chart of the collected signal data. By default, the result file is saved as a "**.bopx**" file, and the *Q-Analyzer* stores the result directory at: <Workspace>\Result\<Project Name>. The default <Workspace> location is C:\Users\<username>\Q-Analyzer.
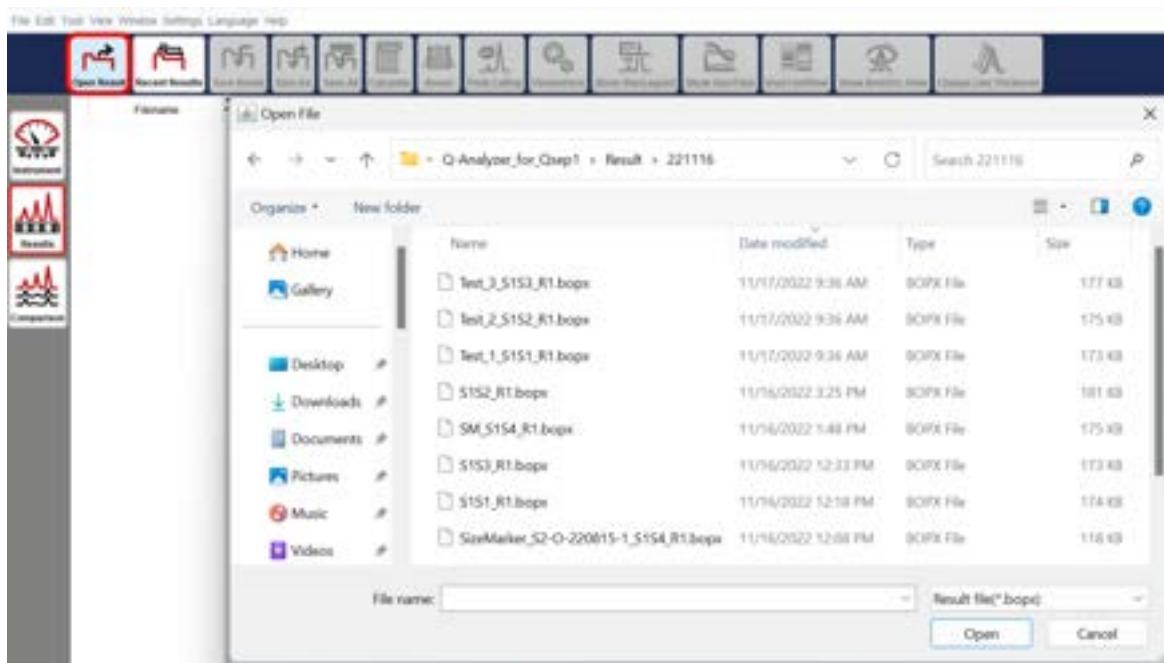

Figure 69   Open File

After completing each method, the latest result will be shown on the Main Panel, and the previous one will be automatically closed.

When multiple results are opened, you can change the file order by right-clicking on "Filename" and re-arranging the order based on "**Sample, Runs, Test Time, Cartridge, Channel**". Clicking the first row allows you to change the order.

Figure 70   Result Display

Function	Description
A: Tabs on Result Window	The Result Window includes several tabs: • Signal: Displays the full electropherogram image. • Current: Shows the current of separation. • Information: Provides detailed result information, including time, cartridge, sample, method, etc. • HV Purge: Displays the current of HV purge. • Sample Injection: Shows the current of sample injection. • Marker Injection: Shows the current of marker injection. • Peak Calling: Displays the peak calling result if it was set.
B: Peak Table	The table includes various columns like Time, RFU, bp, Concentration (ng/μl), nmol/L, S/N, Area (%), Peak Area, Peak Start (sec), Peak End (sec), and FWHM (sec). The values of bp and

	concentration will only be displayed after the calculation is performed.
C: Export Report	The shortcut for promptly exporting the report in your preferred format.
D: Electropherogram	The green arrows indicate the alignment marker(s) in the result, and the red arrow indicates the selected peak in the table.
E: Gel View	The simulation gel view of the result is presented.
F: Raw Data Point	The raw data of the selected point is displayed.

Right-clicking on the peak table provides additional functions:
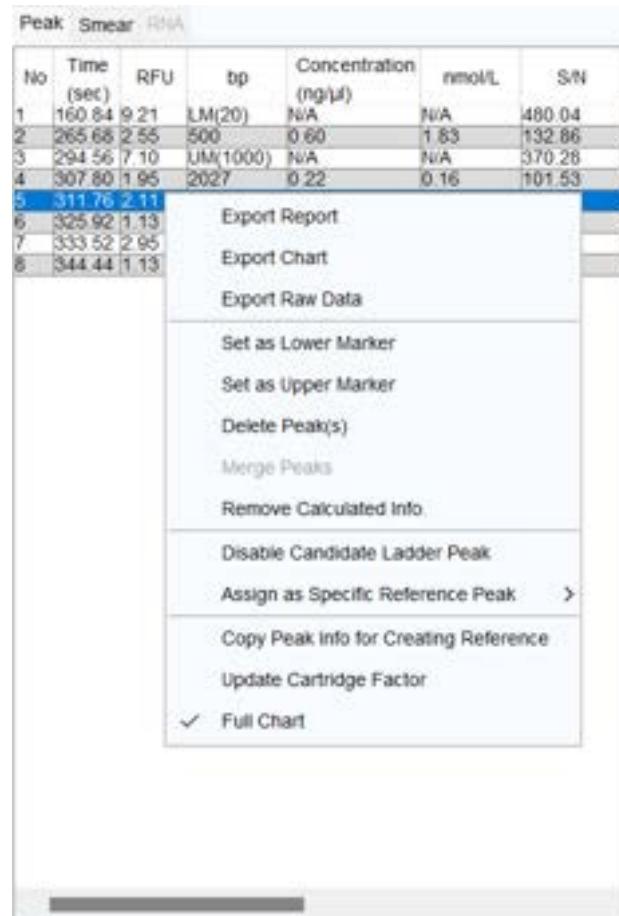
Function	Description
Export Report/Chart/Raw Data	Allows you to export the result after analysis, with exported raw data saved in .csv format.
Set as Lower/Upper Marker	Assign the selected peak to be a lower or upper marker. Correctly assigning these markers ensure accurate size and concentration results.
Delete Peak(s)	Remove redundant peak(s).
Merge Peaks	Combine two or more peaks into one or merge minor peaks with the major ones. The peak area will be added up, and peak migration time will be further defined.

	*Note: The Calculate function must be executed after merging peaks to obtain more accurate results.
Remove Calculated Info	Clear the original calculated information. Calculating with a saved reference marker will obtain the information again.
Copy Peak Info for Creating Reference	Copy data to create a reference marker table.
Update Cartridge Factor	Renew the following running results after assigning the correct lower and upper markers.
Full Chart	Display the full electropherogram image.
For Size Marker Only	
Disable for Ladder Peak Candidate	Ignore the peak not included in the size marker.
Assign as Specific Reference Peak	Correct wrongly identified peak sizes by assigning them as specific reference peaks. Size markers for the following runs will be reassigned, and the information will be updated accordingly if listed in the file list.

Smear DNA Analysis (gDNA QC for NGS)

The **Smear** function is designed to provide insights into the major size and distribution of fragmented genomic DNA. However, it requires that the result be calculated with a reference marker.

Additionally, it is important to note that Zone 2 will not be shown by default in the result file. To view Zone 2 on the result file, you need to click directly on the "Zone 2" button. This step will enable the display of Zone 2, providing you with more comprehensive information about the analyzed data.
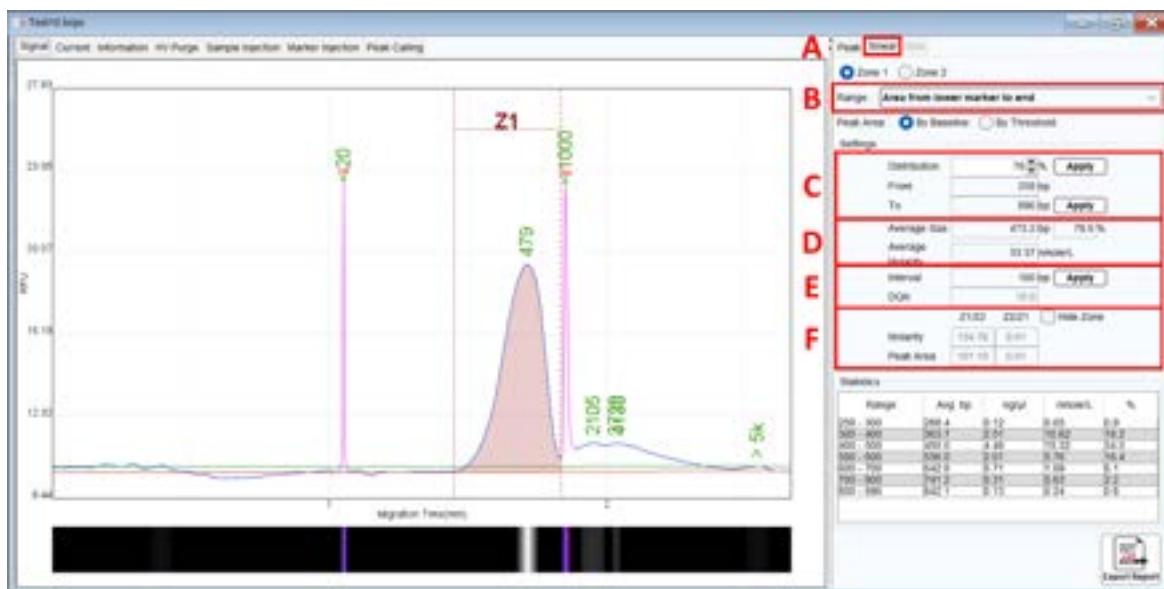

Figure 71   Fragmented Genomic DNA Sample Result

To utilize the Smear function, follow these steps:

1. Click on the Smear tab (Figure 71 A) or select Smear from the Toolbar.
2. Define the peak range, which can be set between the lower and upper markers or from the lower marker to the end (Figure 71 B).
3. Enter either the percentage of the target distribution (e.g. 50%) or the size range of the target region (Figure 71 C), then click Apply.
4. Detailed information about the assigned region, such as average size and concentration (nmol/L) will be displayed (Figure 71 D).
5. If necessary, modify the Interval of the size range and click Apply to update the setting and obtain more distribution details (Figure 71 E).
6. The Smear function also allows you to edit the other zone by selecting Zone 2. The editing rules remain the same as mentioned above. This feature helps you understand the ratios between Zone 1 and Zone 2, providing more information about these two

zones (Figure 71 F).

7. All distribution-related information will be presented in the table below.

8. To export the report, right-clicking on the right panel or the Export Report icon at the bottom. You have the option to export a report with information on both zones by selecting "Both Zone" for Smear Zone.
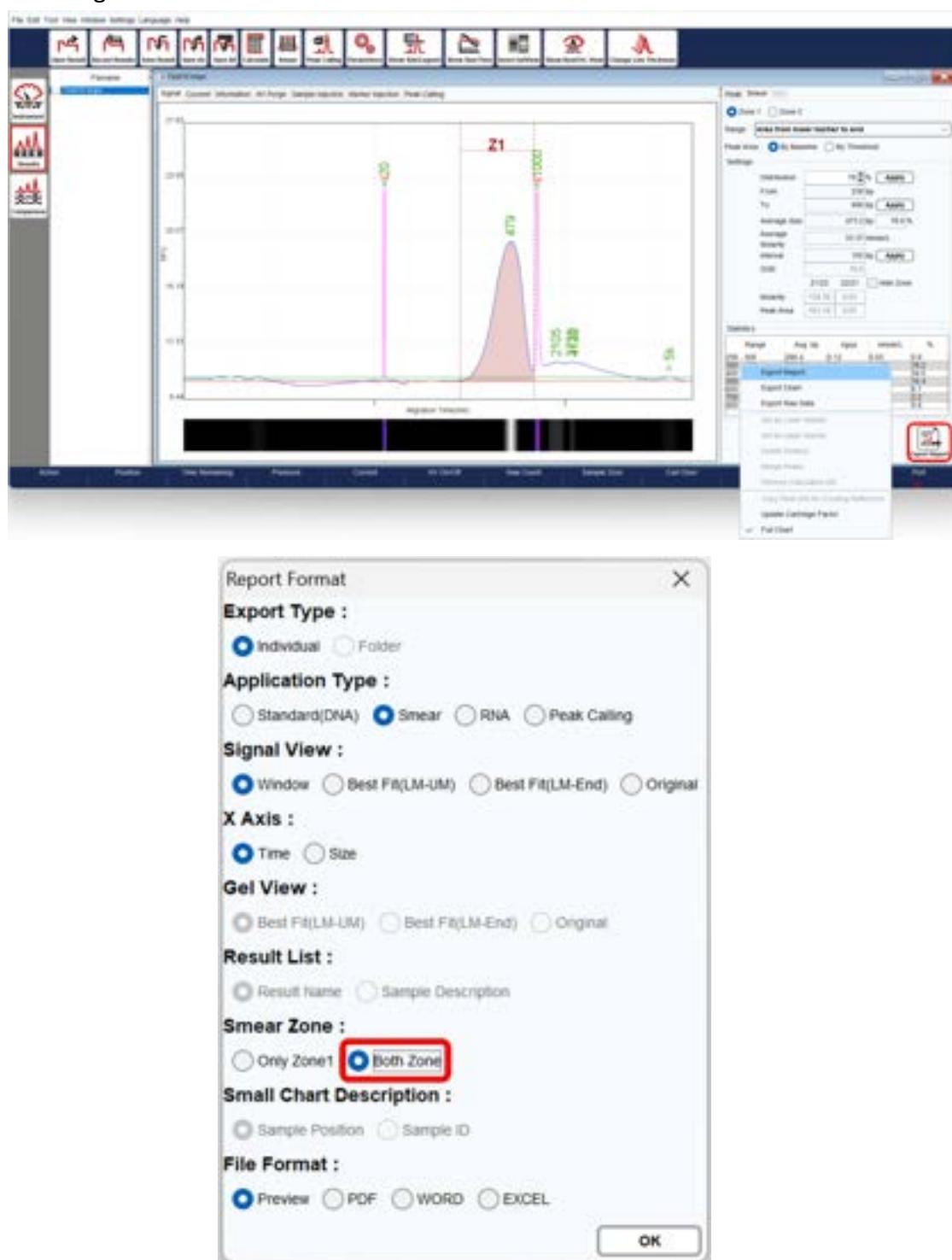
Total RNA Quality Analysis

To assess the quality of the RNA sample and determine the RNA quality number (RQN), follow these steps:

1. Ensure that the sample was run using the RNA cartridge and selected the RNA method. This will enable the RNA tab in the result file. Please note that to obtain the RQN, the alignment marker must be applied. If the RNA integrity is compromised, the system may not interpret SSU/LSU accurately.
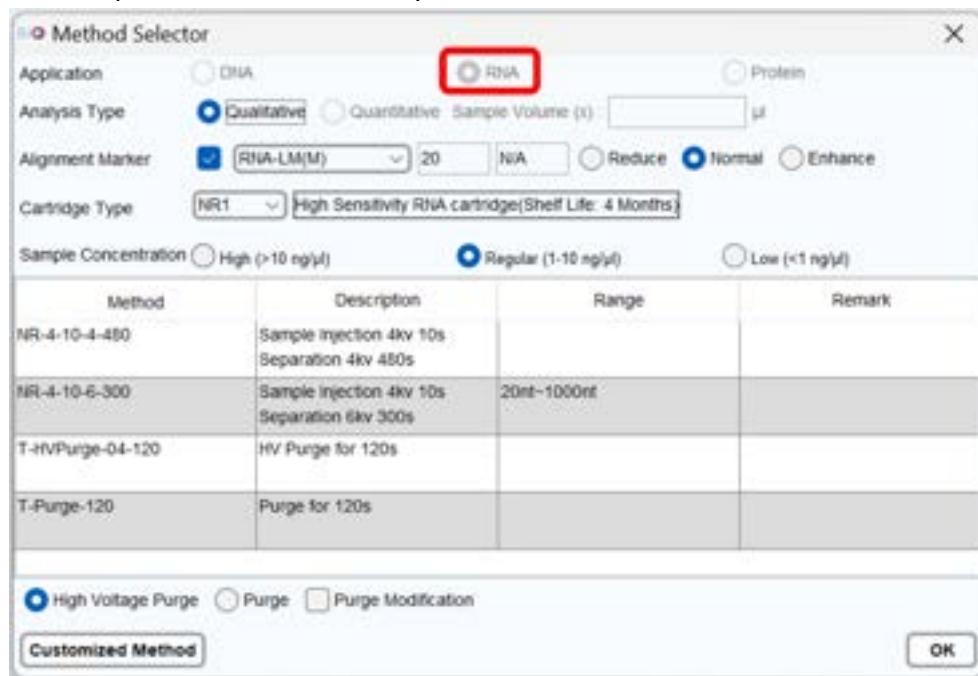

2. **Auto Assign rRNA** will be automatically selected in Para. This enables you to choose between Eukaryotic and Prokaryotic options or user-defined SSU/LSU based on their specific requirements. Any user-defined SSU/LSU assignments will be included in the exported files.
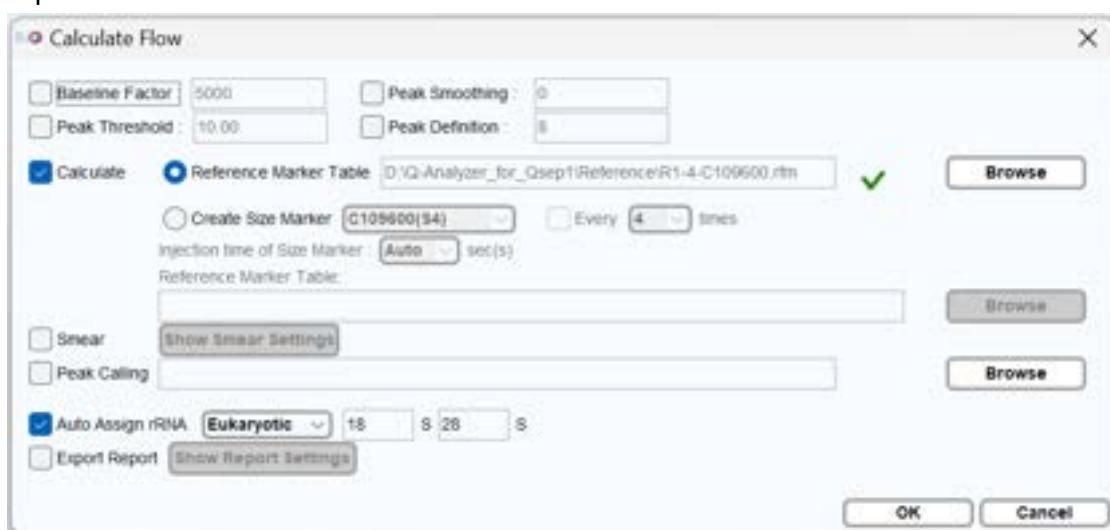
3. After the analysis is complete, select the **RNA** tab to obtain the RQN and other relevant information.
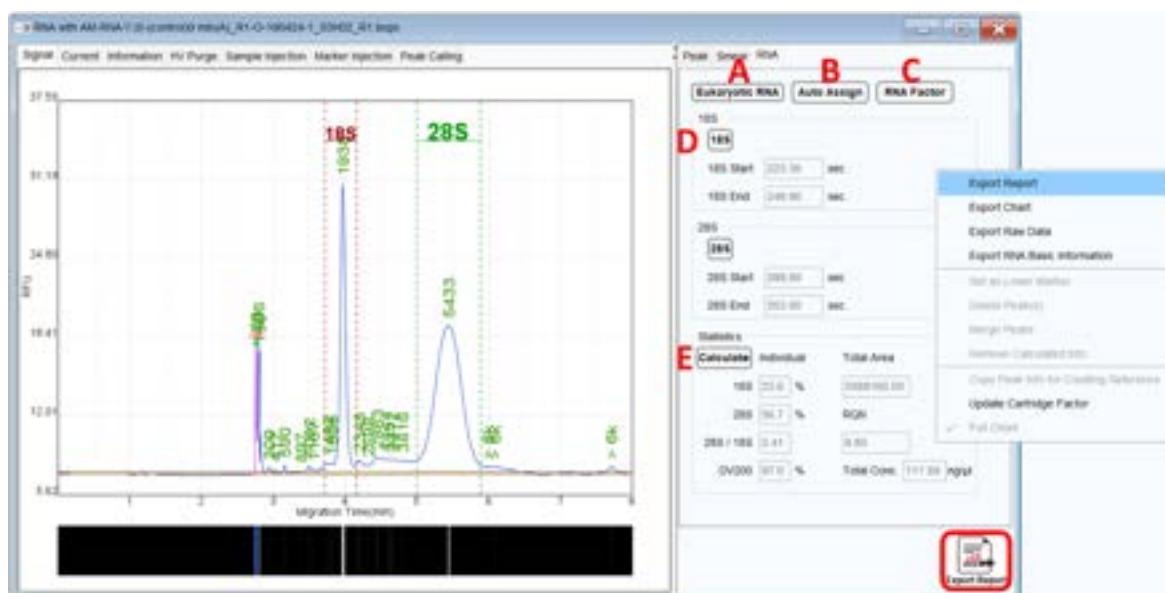

Figure 72　　RNA Quality Check Panel

4. To choose either Eukaryotic RNA or Prokaryotic RNA, click on the **Eukaryotic RNA** button (Figure 72 A)
5. The systems will **Auto Assign** 18S/28S RNA (Eukaryotic) or 16S/23S RNA (Prokaryotic) based on your selection (Figure 72 B). If the margins do not properly cover the RNA area, you can manually drag them to the correct positions.
6. To save the settings, click on the **RNA Factor** button to edit and save the RNA factor table (Figure 72 C).
7. By clicking the 18S and 28S or 16S and 23S buttons, you can either hide or show (by default) the rRNA assignation on the electropherogram (Figure 72 D).
8. Click on the **Calculate** button, and the 28S/18S or 23S/16S ratio, along with the RQN (RNA Quality Number) will be displayed (Figure 72 E).
9. To export the report, right-click on the gray panel or the Export Report icon at the bottom.

The criteria for obtaining a better RQN are based on the experimental design. Generally, a higher RQN indicates better RNA integrity.
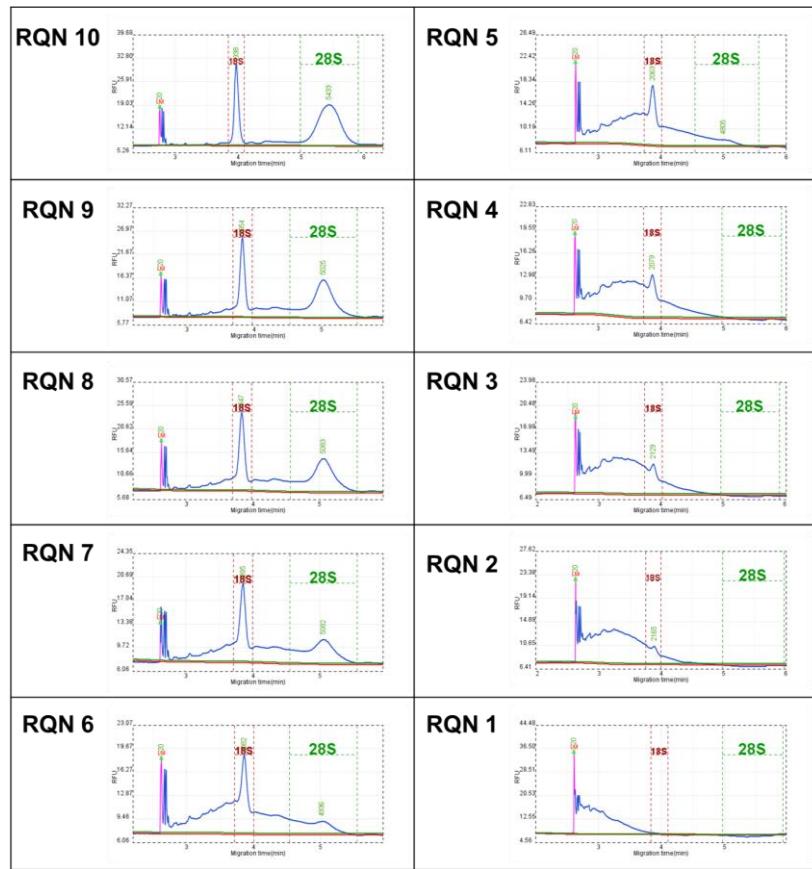
Figure 73   RQN

For more detailed operational information, please refer to the RNA Cartridge User Guides.

Maintenance

Ensure the instrument's optimal performance with routine care. Clean the instrument daily using a dry cloth after use. Conduct weekly maintenance by inspecting the condensation trap on the air pump for any signs of condensation; if present, empty the water. For comprehensive instrument care, consider scheduling yearly preventive maintenance through your local distributor or by reaching out to BiOptic Inc.

FAQ

This section offers guidance on addressing questions or errors encountered while using the $Qsep_1$ Bio-Fragment Analyzer. If you need to contact BiOptic Inc. regarding an error, it is recommended to document the steps leading to the error, share result files in .bopx format, and provide log report in .zip or any files mentioned in the error window if necessary. These details will assist in a more effective resolution of the error.

Instrument

Q. What do the $Qair_{box}$ lights indicate?

A.

Blue light: Power on
Green light: Pressure OK
Red light: Alert

Q. The $Qair_{box}$ is working too frequently (> 3 times/min). How can I identify the issue with air leakage or the filters?

A. Here are some steps to troubleshoot the problem:

1. Check for Air Leaks:

    Examine the system's airway for any signs of air leakage. To do this, you can use soapy water and observe if there are any air bubbles escaping from the connections or tubing. If you find any air leaks, they need to be addressed to ensure proper functioning of the $Qair_{box}$.

2. Filter Strength:

    Over time, the filter's effectiveness may diminish, necessitating periodic replacements

to maintain optimal performance.

3. Cartridge Cap Lock:

The tightness of the cartridge cap lock can also affect air leaks. If the cap is too tight, it can cause an enlargement in the distance between the cartridge cap and the purge piston, leading to air leakage. Conversely, if it is too loose, it can cause an enlargement in the distance between the cartridge cap and gel reservoir, resulting in air leaks.

To resolve these issues, adjust the cartridge cap lock to ensure it is neither too tight nor too loose, preventing air leaks and maintaining smooth operation of the $Qair_{box}$. By addressing these potential causes, you can improve the performance of the $Qair_{box}$ and reduce the frequency of its operation. If the problem persists, contact BiOptic Inc. for further assistance.

Q. What steps should be taken if the PC is unable to connect with $Qsep_1$ Bio-Fragment Analyzer via Wi-Fi?

A. If you encounter difficulties connecting your PC to the $Qsep_1$ Bio-Fragment Analyzer through Wi-Fi, follow these troubleshooting steps to address the issue:

1. Confirm that no other PC is currently connected to the $Qsep_1$ Bio-Fragment Analyzer. Only one connection should be established at a time.

2. Verify the LED light status on the $Qsep_1$ Bio-Fragment Analyzer after turning on the power. Different LED patterns may indicate specific conditions; consult section External Features of $Qsep_1$ for guidance.

3. Confirm that the $Qsep_1$ Bio-Fragment Analyzer appears in the Wi-Fi connection list.

   a. If the $Qsep_1$ Bio-Fragment Analyzer is visible on your mobile device's Wi-Fi list but not on your PC/laptop, try the following:

      i. Reboot the network card on your PC/laptop.

      ii. Attempt the connection with another PC/laptop.

      iii. Reboot the instrument.

   b. If there is another instrument but not the $Qsep_1$ Bio-Fragment Analyzer after restarting the instrument, please report the issue to BiOptic Inc.

4. If you still cannot connect the $Qsep_1$ Bio-Fragment Analyzer, check the firewall to authorize Q-$Analyzer$ in private and public network.

Q. The sample tray cannot move smoothly. How can I resolve this?

A. If the tray holder of $Qsep_1$ Bio-Fragment Analyzer is not moving smoothly or is experiencing any issues, refer to Motor Function Check in section Instrument to identify potential problems.

Cartridge

**Q. What if the cartridge has expired but still has runs left?**

A. BiOptic Inc. does not address issues arising from an expired cartridge. Regarding the expired cartridge, users can utilize them for pre-analysis or testing samples with unknown buffer conditions. It's important to note that unknown buffers may potentially damage the capillary.

Q. What if the wrong buffer is used?

A.

- If dilution buffer is used for separation, the peaks pattern becomes abnormal, and the current increases.
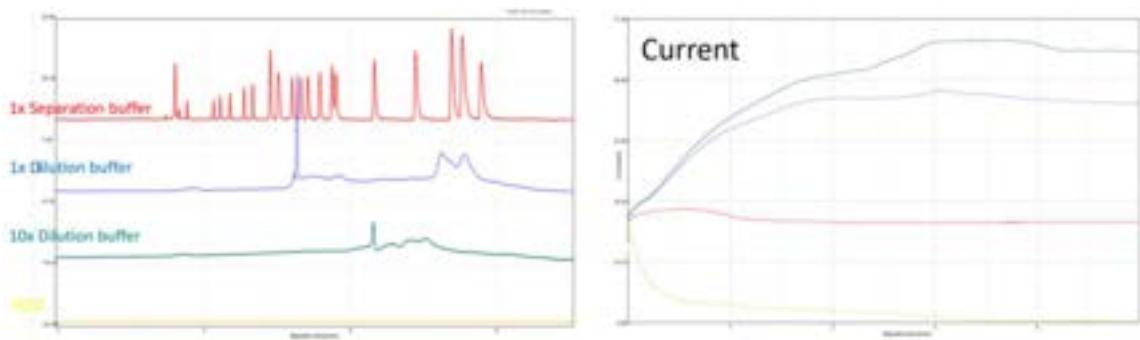

- If separation buffer is used to dilute the sample, the current is acceptable, but the pattern becomes abnormal.
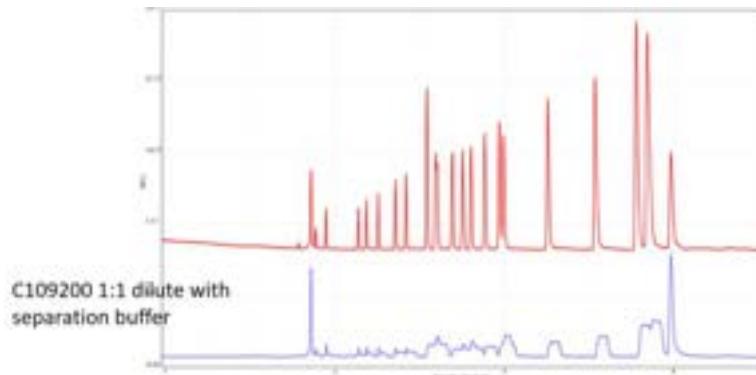
Software

**Q. The current is unstable. How can I resolve this?**

A. Unstable currents are often related to issues with samples and cartridges. Begin by inspecting samples and cartridges to rule out any problems. Then, check the current at each stage to identify potential causes.
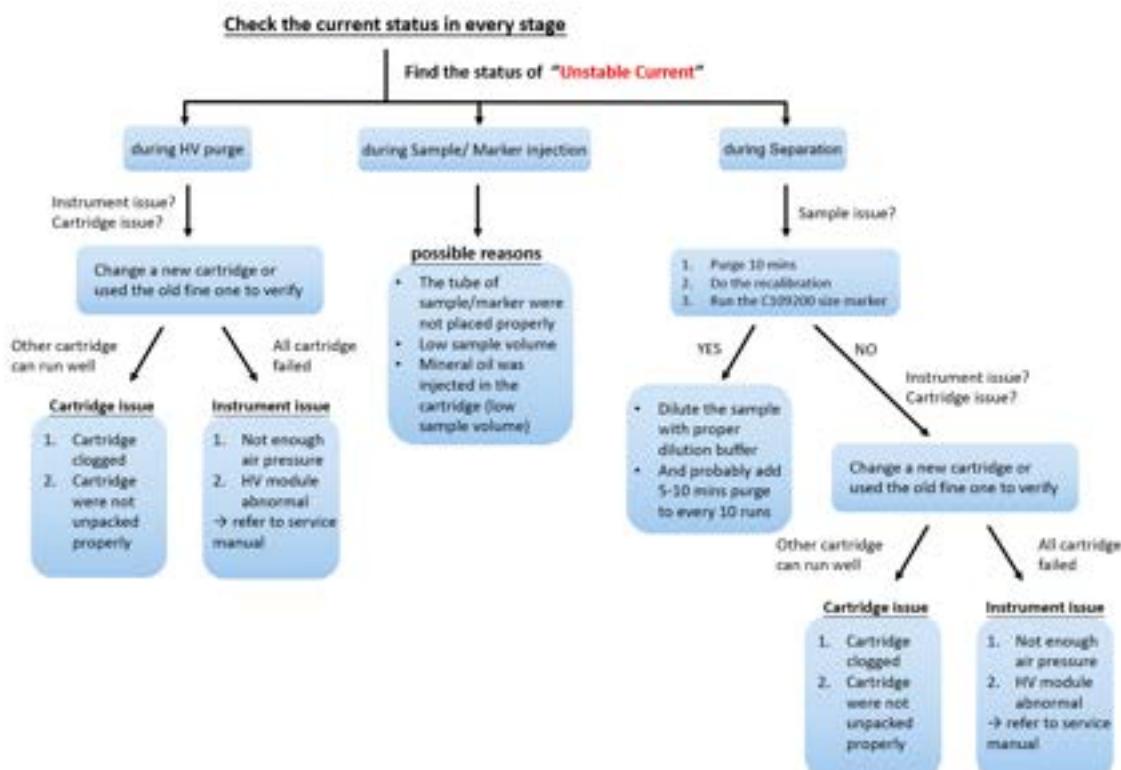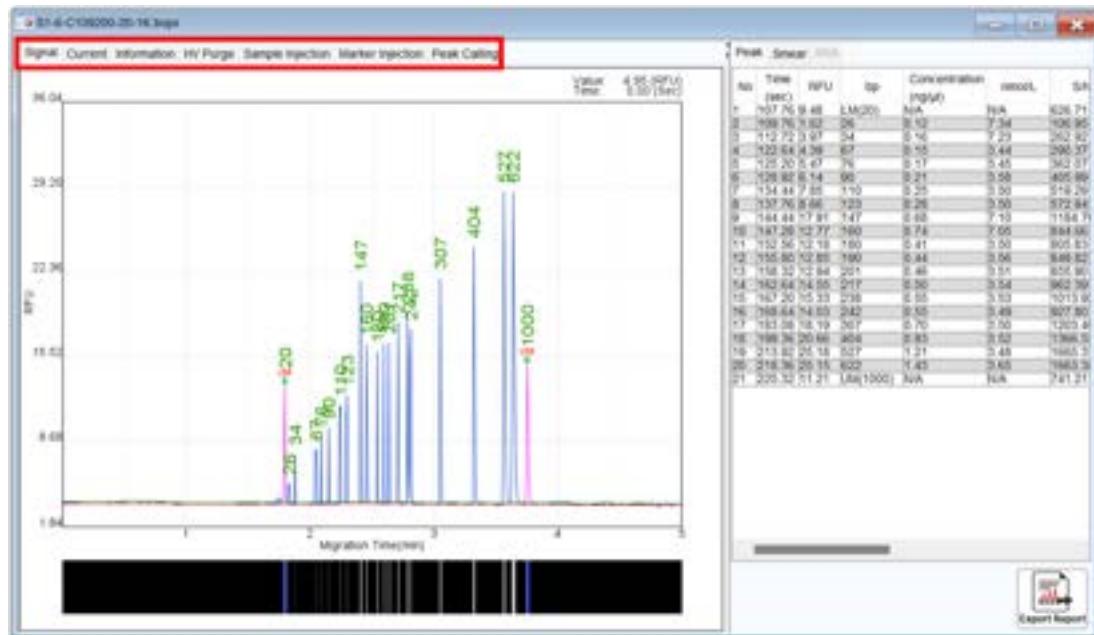
Q. Peaks are migrating later than usual, and the upper alignment marker is missing. How can I resolve this?

A. To address the issue, recalibrate the cartridge to update the cartridge factor. This will adjust the separation time accordingly. Additionally, verify the selected method and check the stability of the current. If the current is stable, the problem may be due to environmental factors, such as temperature. The migration time changes depending on the temperature:

- 10°C: Separation current speeds up 1.2X
- 10-19°C: Separation current speeds up 1.1X
- 20-29°C: Migration time remains the same at room temperature
- 30+°C: Separation current slows down 0.9X
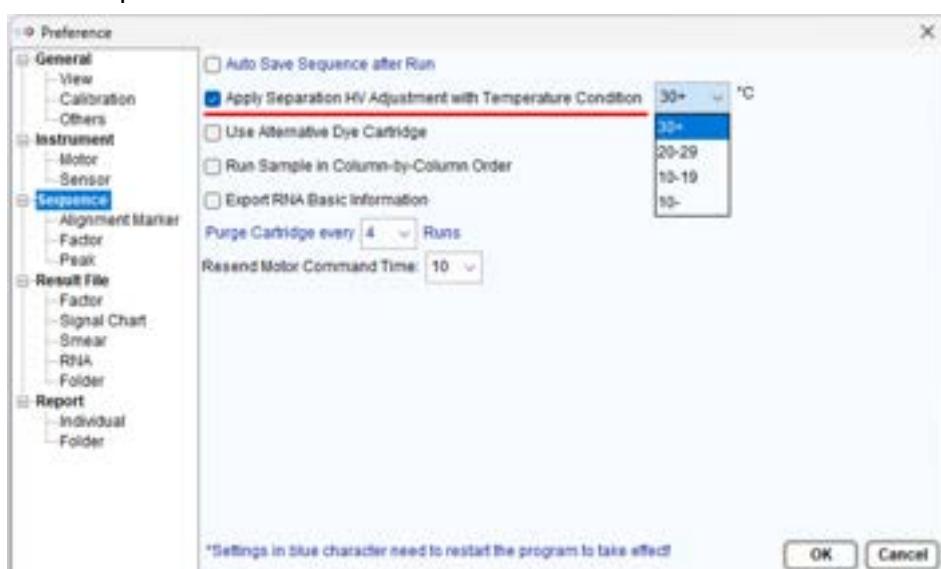
If the current is low or erratic, it could be due to a clogged cartridge. You can perform a long purge to unclog the cartridge and restore normal operation.

Q. The current is stable, but there is no signal or alignment marker shown, and the RFU is ~ 0. How can I resolve this?

A. If the RFU is close to zero and no signal is detected, it may indicate a major issue with the light pathway (Ex/Em). In such cases, it is recommended to contact your local distributor or BiOptic Inc. for further assistance. Before contacting them, you can try using another cartridge to confirm the situation.

Q. The current is stable, but there is no signal except alignment marker shown. How do I resolve this?

A. Begin by performing a size marker to confirm if the issue is related to the samples. Check the sample concentration and sample injection current. If the sample concentration is too low, select "Low (< 0.1 ng/µl)" on the Method Selector or use the N1 High sensitivity

cartridge to analyze samples.
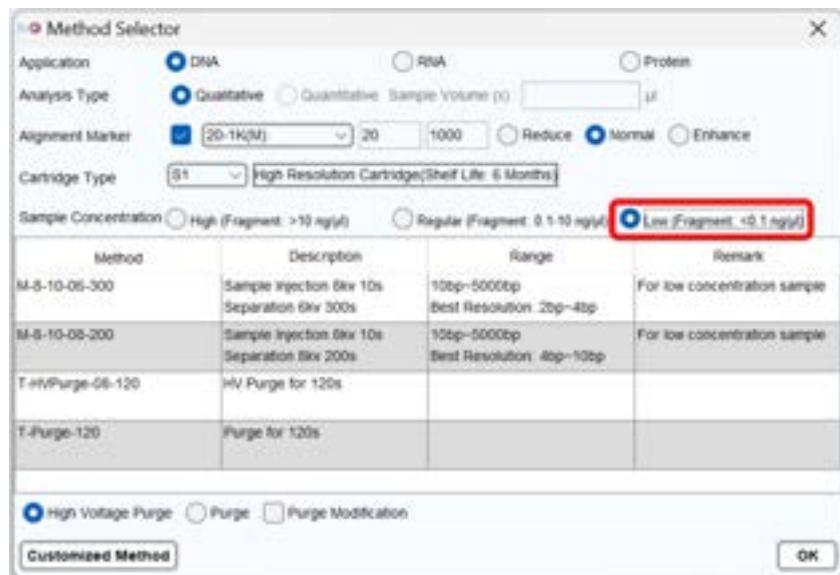
Additionally, check the electrolyte salt concentration of the samples. If the salt concentration is too high, please dilute the samples with a 0.1X dilution buffer.

Q. The alignment marker and/or size marker were not found or assigned correctly. What can I do?

A. Properly assigning alignment and size marker is crucial for accurate sizing results. You can recalibrate the cartridge to update the cartridge factor for future runs or manually assign the marker(s) as follows:

1. Load a built-in reference marker to confirm if the software identifies it correctly.
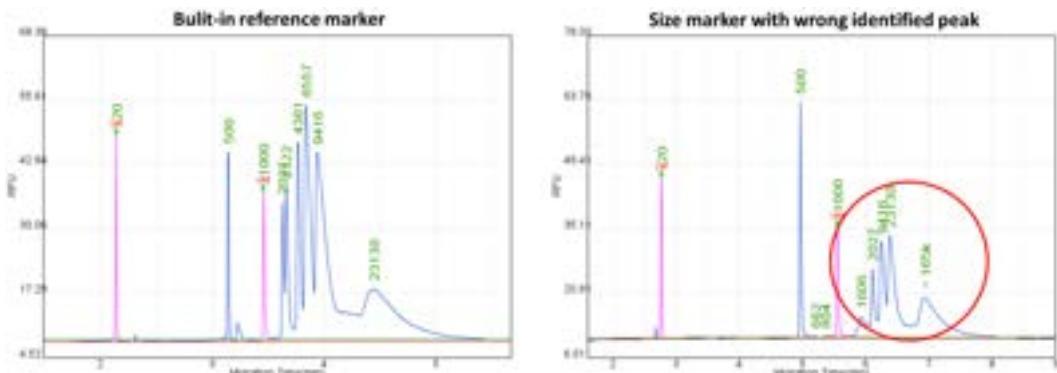
2. Right-click on the peak and choose to disable/assign as a ladder peak:

   Disable for Ladder Peak Candidate: Use this option to ignore peaks not included in the size marker.

   Assign as Specific Reference Peak: Correct peak size if the software identifies the size marker wrongly.
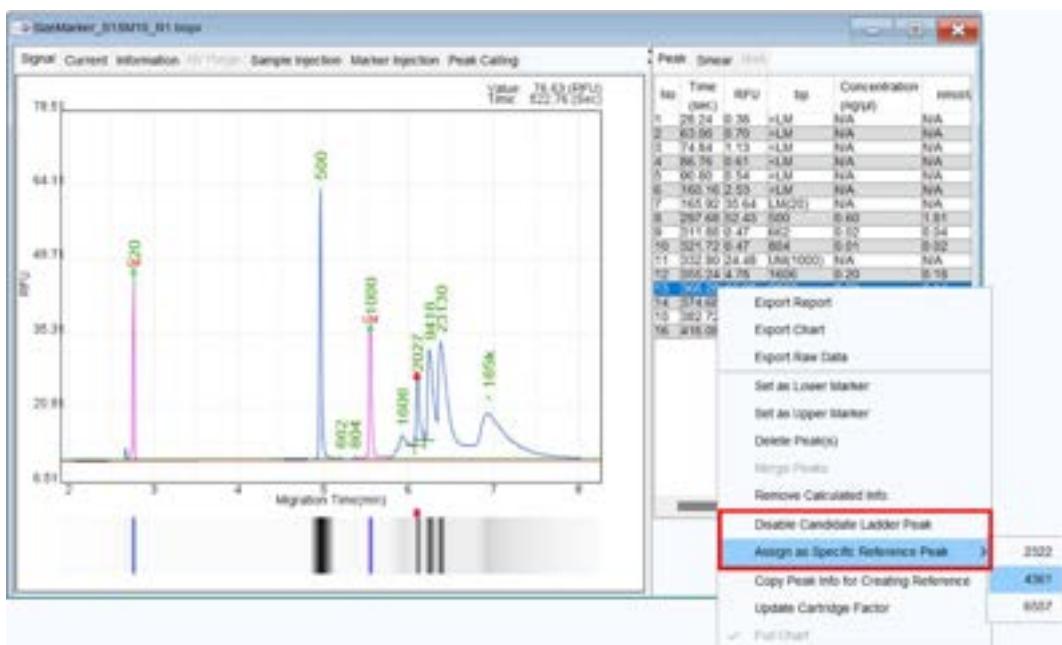
After re-assigning all the peaks, the software will recalculate the results and update the reference marker file automatically. If you like to export the reference marker file for result calculation, go to the information tab of the size marker file, right-click on the Reference Marker table, and select **Export Reference Marker Data** to export the reference marker file for later use in calculations.
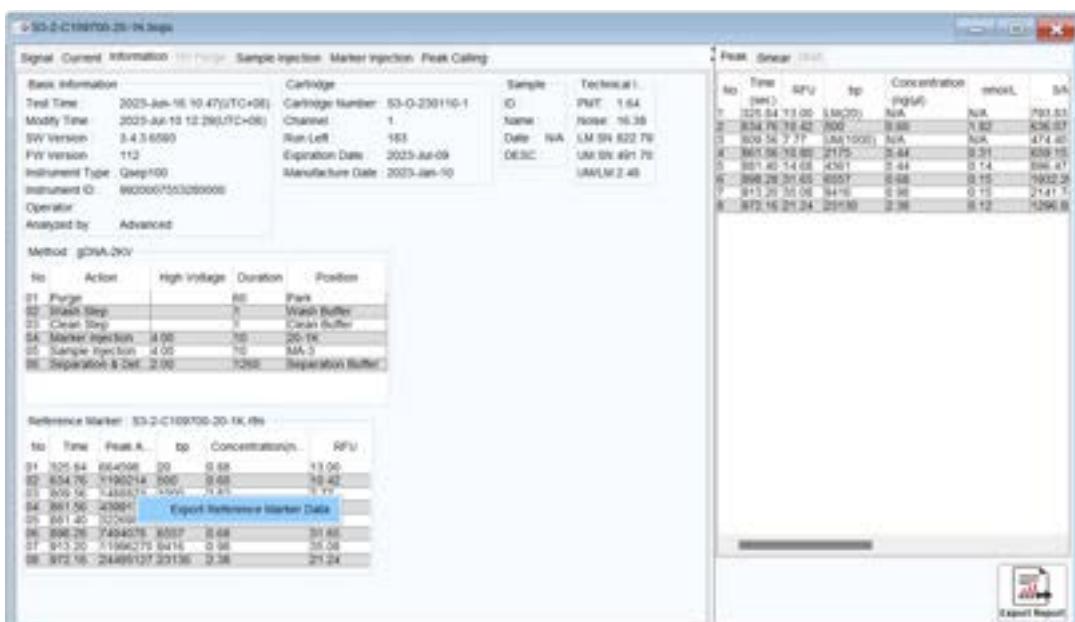
**Q. Is the concentration displayed in result table reliable?**

A. The peak concentration is calculated based on the "Peak Area" and the corresponding parameters related to the Size Marker (SM) used for estimation. However, the electrolyte salt concentration in the sample can affect the injection of charged molecules. Therefore, if the electrolyte salt concentration differs from the size marker, the concentration estimated by the Peak Area may not match the exact concentration. You can refer to the table to compare samples in similar buffer conditions.

Q. The signal and current are looking abnormal. How can I know if I accidently used the wrong buffer?

A. Using water or dilution buffer for separation can lead to abnormal peak patterns and unstable current.
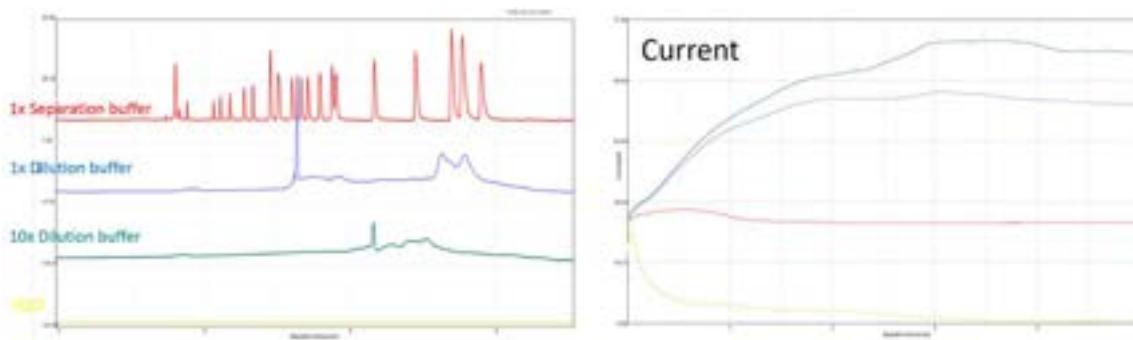
If you used separation buffer to dilute samples, the current may appear normal, but the peak pattern will still become abnormal.
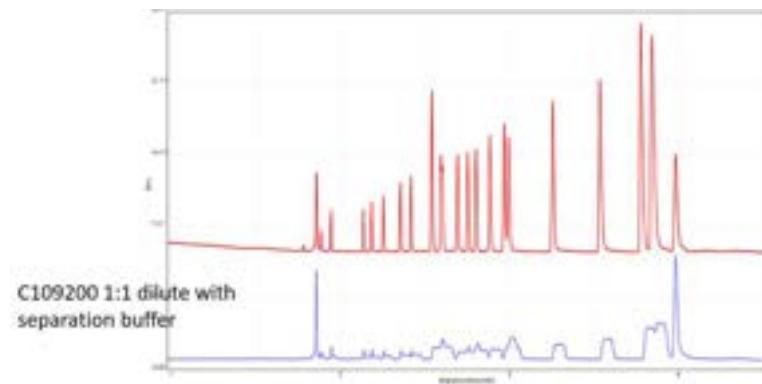
Always ensure that the correct buffer is used for separation and sample preparation to avoid these issues.

Report to BiOptic Inc.

Please provide a report to BiOptic by following these steps:

1. Complete the "Troubleshooting Form".
2. Clearly indicate the time when the issue occurred.
3. Include sample information, such as sample type, buffer condition, and bp or MW.
4. Describe the issue in detail using text, figures, videos, or any other relevant materials, such as result files or reports (.rar format).