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Start Guide

Thank you for purchasing *Qsep* series capillary electrophoresis system. To ensure you a quick and convenient use of this high-performance, fully-automated and easy-to-use system, please read through these instructions carefully prior to use.

Notices

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Symbols

Symbol	Description
CE	CE mark for European conformity marketing
FC	FCC mark of the United States Federal Communications Commission

*FCC ID for RFID Module: 2AUQB

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Limitation of Liability

Qsep series bio-fragment analyzer, software and all related reagents are designed for the use of electrophoresis analysis in general biochemistry laboratory for Research Use Only.

BEFORE ATTEMPTING TO OPERATE THE INSTRUMENT, READ ALL PRODUCT MANUALS AND FOLLOW THE INSTRUCTIONS.

BiOptic Inc. assumes no liability whatsoever for any personal injury, property damage, or other losses resulting from not complying or familiarizing with the manuals, or improper operation of the devices.

System Components

Each *Qsep* series instrument package comes with the followings: *Qsep* CE Instrument *Qsep* Power Cord with Adapter Standard and High Resolution Cartridge Kit (C105200) *Qair_{box}* or Portable Air Pump *Qair_{box}* Power Cord with Adapter Ethernet Cable or USB Cable Software Key* Quick Start Guide

*Note: The administrator software key works as a USB drive and contains setup application of Q-Analyzer CFR Mode and *Qsep* series Operation Manuals, including Hardware and Software versions.

Safety Information

Carefully read through and comply with the following instructions to maintain the integrity of the equipment, the reliability of results, and the safety of operation. Keep *Qsep* series away from other electronic devices and voltage sources. To operate the system safely, please review the operation manual and laboratory safety guidelines. For operation and safety questions, please contact BiOptic Inc. at the official website or with your local BiOptic representatives.

<u>Warnings</u>

- 1. Both the instrument and *Qair_{box}*/portable air pump must be connected to an appropriate power source.
- 2. Use the MAINS power cord within adequate rating.
- 3. Do not operate the system if any of the power cords are damaged.
- 4. The *Qsep* series is based on capillary gel electrophoresis separation driven by high voltage. Do not remove covers to perform any maintenance of the electrical components.

Cautions

- 1. Only the components and consumables provided by BiOptic Inc. shall be used.
- 2. **<u>DO NOT</u>** perform the following actions:
 - Disassemble *Qsep* series instrument and its associated parts.
 - Bump or jolt *Qsep* series.
 - Move or disconnect in action *Qsep* series from external computer or air pump.
 - Open the sample door or cartridge door while *Qsep* series is in action.
 - Remove the cartridge while *Qsep* series is in action.
 - Power off in action *Qsep* series.
- 3. Unlatch and remove cartridge from the instrument and store it in the clam-shell container before shutting down or disconnecting *Qsep* series. Failure to do so may cause dryness of the gel at the cartridge tip and damage the cartridge.
- 4. N₂ 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* series.

1. System Overview

Qsep series is an automated CE system developed by BiOptic Inc., which uses pen-shaped disposable gel-cartridges to improve efficiency. Time-consuming procedures such as gel preparation, sample loading, and capillary changing are no longer required. Furthermore, the results will be obtained easily with the automated *Qsep* series. *Qsep*₁, *Qsep*₁-Plus, *Qsep*₁₀₀ and *Qsep*₄₀₀ are designed to accommodate well trays with different sample capacities. They are 8-well, 12-well and 16+3-well trays, and the standard 96-well plate.

Qsep series with its compact design helps to set up and operate the instrument intuitively. The disposable gel-cartridge with integrated pre-programmed test methods makes capillary electrophoresis experiments no longer a complicated procedure that requires well-trained operators. Worry no more about the operation and human errors caused by different operators which affect the accuracy and reproducibility of the results.

The following sections will describe the product overview, the functions of *Qsep* series instruments and the gel-cartridge. Please read through this section and get ready to be amazed by what *Qsep* series can do.

Caution: The operator of this instrument is advised that if the equipment is operated in a manner not specified in this manual, the protection provided by the equipment may be impaired.

1.1 Pre-programmed Method and Cartridge

Qsep series is designed to work with the special-designed cartridges manufactured by BiOptic Inc. The modularization design of cartridges makes the time-consuming gel preparation no longer necessary. For cartridges, various testing protocols, so-called "Method", integrate the sequential steps to accomplish the test. The various cartridges and pre-programmed methods are utilized in CE experiment that corresponds to the size range and concentration of the sample. Additionally, users can create their own Methods based on their needs.

In the Method Selector (Figure 1-1), user can view the methods corresponding to the suitable size range, alignment marker, and the cartridge type. If the latch action has been taken, *Qsep* series will automatically detect the type of the cartridge in use and only display the suggested methods for the cartridge type.

Application (*	DNA C RNA	C Glycan	C Protein
Analysis Type 🛛 🕫	Qualitative C Quantitative Sampl	e Volume(x) : µl	
Alignment Narker 🛛 🖓	20-1K(MA-1) = 20	1000 C Reduce @ Normal	C Enhance
Cartridge Type	+ High Resolution Cartridge(She	If Life: 6 Months	
Sample Concentration	High (Fragment: >10 nglul)	Regular (Fragment 0.1 - 10 ng/ul)	Low (Fragment <0.1 ng/ul)
Method	Description	Range	Remark
M-4-10-05-300	Sample Injection 4kv 10s Separation 6kv 300s	10bp-1000bp Best Resolution: 2bp-4bp	1
M-4-10-08-200	Sample Injection 4io 10s Separation 8io 200s	10bp-5000bp Best Resolution: 4bp-10bp	
M-4-10-10-120	Sample Injection 4ity 10s Separation 10ky 120s	10bp-5000bp Best Resolution: 10bp-50bp	
T-HVPurge-08-120	Gel Refill with HV on for 120	9	7
T-Purpe-120	Gel Refill without Hy for 120	9	
(* High Voltage Purge C	Purge Purge Modification		

Figure 1-1 Method Selector Tab

1.2 System Installation

1.2.1 Computer Requirements

	Minimum	Recommended
Operating System	Windows 10 32 bit	Windows 10 64 bit
Processor (CPU)	Intel Core i5	Intel Core i7
Memory (RAM)	8G RAM	16G RAM
Hard Disk Drive	500 GB	1 TB
Monitor	1280 x 800	1920 x 1080
Port	RJ45 x 1	RJ45 x 2
	USB 3.0 x 1	USB 3.0 x 2

2. Q-Analyzer CFR Mode Installation

CFR stands for Code of Federal Regulations. Part 11 of Title 21 of the CFR (21 CFR Part 11) establishes Food and Drug Administration (FDA) regulations on which electronic records and signatures are trustworthy and equivalent to paper records.

To generate and store data comply with the guidelines, Q-Analyzer CFR Mode features with limited system access for authorized users, detailed audit trail, etc.

A suitable operating environment is essential to ensure the best performance of *Qsep* series. The conditions of transportation and storage should be -30°C to 60°C for temperature and 20 to 80% RH, non-condensing for humidity.

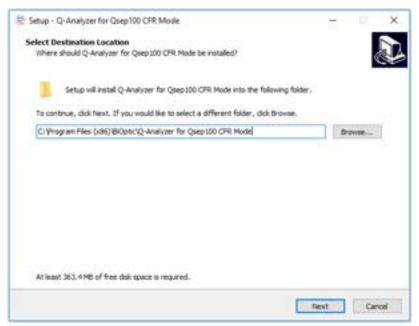
2.1 Software Installation

Q-Analyzer CFR Mode is the software that is specially designed to operate *Qsep* series while compliant with 21 CFR Part 11. The software key for installation could be found in the package. Click "**Q-Analyzer CFR Mode-Setup.exe**" to begin the installation.

***Note**: Please close all the other applications before installing Q-Analyzer CFR Mode. In addition, **DO NOT** connect *Qsep* series to the computer during the installation.

The installation steps are as follows:

- 1. Click Q-Analyzer CFR Mode-Setup.exe to start the installation.
- 2. Specify the default data folder.



3. Select the program destination.

🔅 Setup - Q-Analyzer for Qsep 100 CFR Mode	7	×
Select Start Henu Folder When should Setup place the program's shortcuts?		Ð
Setup will create the program's shortou's in the following Start Menu fulder.		
To continue, dick Next. If you would like to select a different folder, dick Browse.		
BCptic/Q-Analyzer for Qsep 100 CPR Model	Brows	E
Back Ne	nt	Canoni

4. Create a desktop shortcut and select the drivers for installation.

Setup - Q-Analyzer for Qsep 100 CFR Mode	- 10	×
Select Additional Tasks		
Which additional tasks should be performed?		Ċ,
Select the additional tasks you would like Setup to perform while installing Q-Analyzer Mode, then click Next.	for Qsep 100 CFR	
Additional shortcuts:		
Create a desktop shortcut		
Instal Drivers		
Sinstal Software Key Driver		
🗹 Install US8 Driver		
		-
Back	Next	Cancel

5. Verify the settings of the installation and click Install to start the process.

ety to Install etup is now ready to begin installing Q-Analyzer for Qsep 100 CPR. Mode on your computer. Destination location: C: Virogram Frides (x86)(BIOptic)Q-Analyzer for Qsep 100 CPR. Mode Start Menu folder: BIOptic)Q-Analyzer for Qsep 100 CPR. Mode Additional Shortcuts: Create a desitop shortcut Install USB Driver Install USB Driver	-
Ide Install to continue with the installation, or dick Back if you want to review or change any setter Destruction location: C: 'Program Piles (x86)'(BiOptic)Q-Analyzer for Qsep100 CPR Mode Start Menu folder: BiOptic)Q-Analyzer for Qsep100 CPR Mode Additional tasks: Additional shortcuts: Create a desktop shortcut Instal Onivers Instal USB Driver	
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Additional shortcutti Create a desitop shortcut Install Software Key Driver Install USB Driver	
*	ų.
Back Justal	2.

6. Q-Analyzer CFR Mode installation is in progress.

left Setup - Q-Analyzer for Qsep100 CFR Mode	-		×
Installing			
Please wait while Setup installs Q-Analyzer for Qsep 100 CFR Mode on your computer.			<u>አገለ</u>
Heuse ware white becap instans & whenyzer for goep 200 errenfoue on your comparent		(
Extracting files			
C:\Program Files (x86)\BiOptic\Q-Analyzer for Qsep100 CFR Mode\Q-Analyzer for Qsep1	00 CFR M	lode.exe	
			· · · ·
		Can	cel

7. The wizard will install the necessary software drivers to complete the installation.



8. Accept the license agreement to continue.

To continue, accept the following license agreement. To read the entire agreement, use the scroll bar or press the Page Down key. IMPORTANT NOTICE: PLEASE READ CAREFULLY BEFORE INSTALLING THE RELEVANT SOFTWARE: This licence agreement (Licence) is a legal agreement between you (Licensee or you) and Future Technology Devices International Limited of 2 Seaward Place, Centuron Business Park, Glasgow G41 1HH, Sootland (UK Company Number SC136640) (Licensor or we) for use of driver software provided by the Licensor(Software). BY INSTALLING OR USING THIS SOFTWARE YOU AGREE TO THE V OI accept this agreement	license Agn	eement		<u>IN</u>
INSTALLING THE RELEVANT SOFTWARE This licence agreement (Licence) is a legal agreement between you (Licensee or you) and Future Technology Devices International Limited of 2 Seaward Place, Centution Business Park, Glasgow G41 1HH, Scotland (UK Company Number SC136640) (Licensor or we) for use of driver software provided by the Licensor(Software). BY INSTALLING OR USING THIS SOFTWARE YOU AGREE TO THE V O Laccept this agreement. Save As Print	×			the entire
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Chaccept this agreeners		BY INSTALLING OR USING THIS	SOFTWARE YOU AGRE	E TO THE V
O I cont accept this agreement		I accept this agreement	Save As	Print

9. The installation of drivers is complete.

Device Driver Installation Wiza	rd
	Completing the Device Driver Installation Wizard
	The drivers were successfully installed on this computer.
	You can now connect your device to this computer. If your device came with instructions, please read them first.
	Driver Name Status ✓ FTDI CDM Driver Packa Ready to use ✓ FTDI CDM Driver Packa Ready to use
	< Back Finish Cancel

10. The installation of Q-Analyzer CFR Mode is complete.



After launching Q-Analyzer CFR Mode, the administrator must activate the software key and set up users to begin (Section 2.2.1). **Main Window** will appear on the screen. The instrument page is used to communicate with the device (Figure 2-1). For different instruments, the corresponding pictures will show when connected to *Qsep* series.

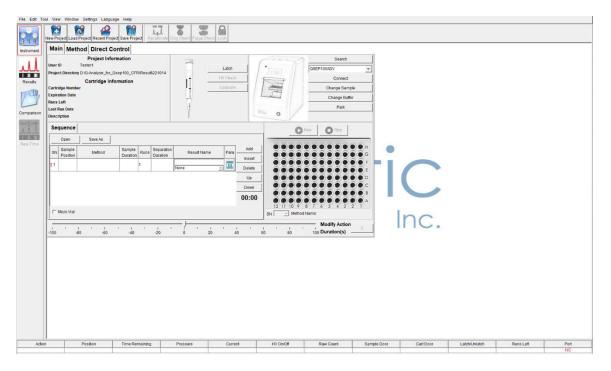


Figure 2-1 Software Overview

2.2 CFR Mode

CFR Mode limits system access to authorized individuals and keep track of system operation to hold individuals accountable for actions initiated under their electronic signatures. In the following sections, different user types will be introduced and discussed.

User Type Function	Administrator	Operator	Basic Analyzer	Advanced Analyzer	Quality Assurance Specialist (QA)
Set Up User Authority	1				
Audit Trial Viewer	1				1
Run Sequence		1	1	1	
Post Analysis			1	1	
Export Report			1	1	
Set Up Preference				1	
Create/Edit Method, Reference, and Peak Calling Tables				1	
Instrument Function Check				1	1
View Result Only					1
All Other Functions in Manual		✓	1	1	

Table 1. Authorization Setting Table

2.2.1 Administrator

The Administrator has the authority to set up different levels of users and audit trials. After installing the Q-Analyzer CFR Mode, click on the icon to register the key. The password must contain at least one uppercase letter, one lowercase letter and one number, and it must be 8-12 characters long.

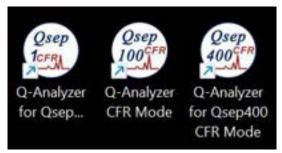


Figure 2-2 Launch Q-Analyzer CFR Mode

Software Key Acti	vation_Adm_FS X	
User Information		
Company/Organization N	ame	
User ID		
Password	Must contain one uppercase one lowercase letter and one nu	
Password Confirm	and must be 8-12 characters lo	
	ок	
10 m		

Figure 2-3 Software Key Activation

The Administrator must activate the key for the first-time use. If the authorization needs to be transferred, the administrator can restore the data with exported .db file saved previously.

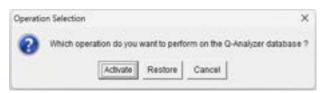


Figure 2-4 Operation Selection

Select the checkbox to agree to the terms and fill in the password (Fig. 2-5) to enter the administrator page (Fig. 2-6).

• V3.4_CFR_Part11_Mode	×
Bioptic	
Please read these Terms of Use carefully before using Q-Analyze Effect Date: May 61, 2020	r .^
By using this system (including the parts to which you will get access through this system), you signify your assent to the terms of use of this system. If you do not agree to them, do not accept these terms o use the site.	
Use of Electronic Signatures	
By applying an electronic signature to information within this system, or by using any of the electronic signature features of this system, you agree that: (a) the electronic signatures used within this system as the legally binding equivalent of your own traditional handwritten signatures, unitals, or other general signings:(b) you have not shared your system identification or password information, and that signature applied using your identification and password an your own; (c) you will immediately change any compromised or suspected compromised passwords; (d) you will immediately report in an urgent mass	* *
any compromised pasewords, suspected compromised pasewords, or unauthorized attempts to access	he ¥
evidee to your reseivation's control administration's)	
User ID: BOplic	
Password	
Accest: Cancel	

Figure 2-5 Administrator Login

_	Nen Uter	Relatives (10)	Mathematic	Aut Tra	Tel
No.	liter (D	Level	Faultiane	Leditoria	Aster

Figure 2-6 Administrator Page

Click on "New User" and enter user ID, password (can be reset by user), first and last names, and select the user level to create a new user. The ID cannot be the same as Administrator or any other users; it must be 3-10 characters long and can only consist of letters (not case sensitive) and/or numbers. As for the password, it must contain one uppercase letter, one lowercase letter and one number, and be 8-12 characters long. If the checkbox of "Must change password at next login" is selected, the new user will need to change the password when logging in the first time.

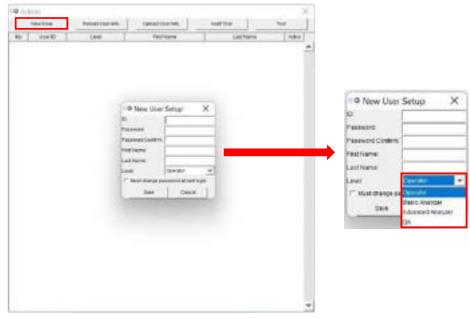


Figure 2-7 New User Setup

The user level can be modified by the administrator later. If certain user is no longer considered an authorized individual, uncheck the box in **Active** column. The user ID will then be inactivated.

	min Auv-User	Mattal User MID.	Upinet Uper Vite	1001708	160
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1.4	Advanced	Advanced Hullpon	Advanced	CFR	2
- 11	Denn	Operator w	Page 1	010	
		Operator			
		Sade Insight			
		Adversed Assister GA			

Figure 2-8 User Level Setup

Once the user is created, click on "Upload User Info." to confirm the changes. If the step is missing, the data will not be recorded. On the other hand, if the administrator decides to undo the action after creating the user, click on "Reload User Info." to refresh the page. The new user will not be recorded and listed. The process will have to start over again.

1ª Confirm	×	
User D Dirate Passent		
		Teel
Level First N	And the second s	
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	Confirm Confirm Confirm Confirm Confirm Confirm	

Figure 2-9 Upload User Info and Reload User Info

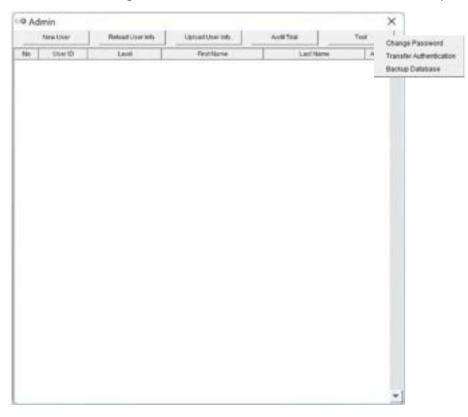
If the user closes the window before uploading the created or modified user information, the system will give a reminder that the action has yet to be completed.



In additional to setting up users, the administrator can audit trial by clicking "Audit Trial". The detailed information including the PID, Date and Time, User ID and Action will then be listed. "Export" to save the record in .csv.

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2012-A-1	H - 2002-Aut-Ni	User ID: 4		6.00	
PD	Title	UserD		Abm	
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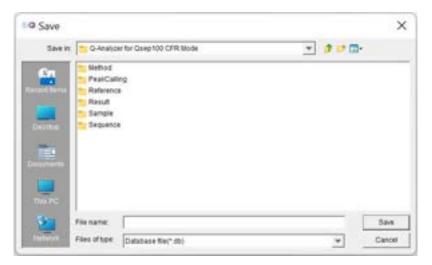
Figure 2-10 Audit Trial and Export



The "**Tool**" consists of Change Password, Transfer Authentication and Backup Database.

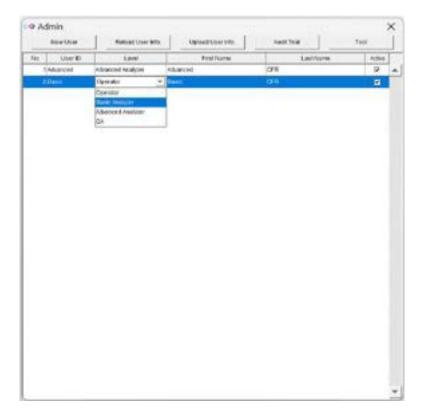
Change Password: Change the Administrator password.

Transfer Authentication: The authentication can be transferred. If the Administrator wish to recover the data later, it is necessary to back up the data before confirming the transfer. **Backup Database:** Backup the data for later restoration. It is recommended to back up the data periodically.



2.2.2 Other User Types

There are four user types with different authorizations for the administrator to create, which are **Operator**, **Basic Analyzer**, **Advanced Analyzer**, and **Quality Assurance personnel**. Please refer to Table 1 in section 2.2 for more information.



Operator

The Operator has the authorization to run sequence and perform all other functions of Q-Analyzer CFR Mode unless specifically specified in the authorization setting table (Table 1). The default settings grant the Operator access to set the sequence and modify alignment marker and size marker. However, if the sequence file (.seq) loaded is pre-made by Basic or Advanced Analyzer, the settings cannot be changed. It also does not have the authorization to export results.

Basic Analyzer

The Basic Analyzer has the authorization to run the sequence, do post-analysis, export result files, and perform some other functions of Q-Analyzer CFR Mode. The main differences between basic and advanced analyzers would be access to preference setup, and method/reference table/peak calling table edit and creation.

Advanced Analyzer

The Advanced Analyzer has the authorization to operate the Q-Analyzer CFR Mode fully. The Advanced Analyzer has access to set up preference and create/edit method, reference table and peak calling table that other users do not have.

Quality Assurance Specialist (QA)

The Quality Assurance Specialist (QA) shall be responsible for monitoring each action related to the *Qsep* series and shall be independent of the personnel involved in the study. Therefore, the Quality Assurance Specialist has the authorization to audit trials, perform instrument function check and view results.

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3. Start to Use *Qsep* Series

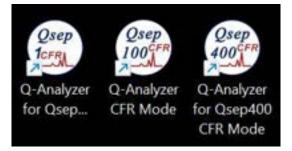
Q-Analyzer CFR Mode is the software that is specially designed to operate *Qsep* Series. To execute, the user needs to connect *Qsep₁*, *Qsep₁*-Plus, *Qsep₁₀₀* or *Qsep₄₀₀* via Wi-Fi or cable (power must be on) and follow the instructions below to initiate the system.

*Note: Before turning on the $Qsep_1/Qsep_1$ -Plus, the SD card needs to be inserted into the instrument.

*Note: For $Qsep_{100}$, sample plate holder is secured by the protecting foam. Lift the sample plate holder to remove the protecting foam before turning on the power or it may cause motor damage.

*Note: For $Qsep_{400}$, sample plate holder is secured by the fixture and the securing screws. Remove the fixture and the securing screws before turning the power on or it may cause motor damage. For more information, please refer to $Qsep_{400}$ Quick Start that comes with the instrument.

1. Click on Q-Analyzer CFR Mode icon.



2. Fill in the user ID and password and check the box to assent the terms of use to operate Q-Analyzer CFR Mode. The ID must be 3-10 characters long.

Bioptic	×
Please read these Terms of Use carefully before using Q-Analyzer Effect Date: May 01, 2020	^
By using this system (including the parts to which you will get access through this system), you signify your assent to the terms of use of this system. If you do not agree to them, do not accept these terms or use the site.	
Use of Electronic Signatures By applying an electronic signature to information within this system, or by using any of the electronic signature features of this system, you agree that (a) the electronic signatures used within this system are the legally binding equivalent of your own traditional handwritten signatures, initials, or other general signings,(b) you have not shared your system identification or password information, and that signatures applied using your identification and password are your own, (c) you will immediately change	
(" I have read and agree to the terms. User ID Password: Agrees Cancel	

3. Connect the instrument and latch the cartridge to continue.

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4. For steps that affect how the result may turn out, the user needs to enter the password to confirm the operation before continuing. The reason shall be addressed automatically. For operation, please refer to Section 3.1.

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3.1 Operation of *Qsep* Series

After launching Q-Analyzer CFR Mode, the **Main Window** will appear on the screen. Users can access the **Instrument** function to communicate with the device (Figure 3-1). This section will only introduce the **Main** tab features and how to operate *Qsep* series (Figure 3-1 A). The other function tabs will be introduced in Section 4.4.

The software will create a default project named after operation date automatically. Users can also create projects for experiments with different purposes by clicking **New Project** in the Toolbar (Figure 3-1 B) before connecting the instrument.

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Figure 3-1 Main Window of the Instrument Mode

3.1.1 Connection Assistance

Please ensure Qsep series is connected to the computer with either the USB cable (for $Qsep_{100}$ and $Qsep_{400}$) or the Ethernet cable (for $Qsep_1$ and $Qsep_1$ -Plus). Turn ON the power of Qsep series instrument, and then click the icon to launch the software. $Qsep_1$ and $Qsep_1$ -Plus also have an option to be connected via Wi-Fi. Please refer to Quick Start for more information.

To connect *Qsep* series with the computer, click the **Connect** button on the Control Panel (Figure 3-2 A). Once the text of the button changes to **Disconnect** and the displayed image of *Qsep* series turns into color form, the connection is established.



Figure 3-2 Instrument Connection

The **Purge Check** message box will pop up after connected and before disconnected (Figure 3-3). Click **Purge Check** and follow the instructions to complete the process.

Use **Purge Check** to ensure that air tube is not clogged before use or the gel is not accidentally sucked into the air tube after use.

*Note: To avoid the permanent damage to the system, it is recommended to execute **Purge Check** whenever the software asks.

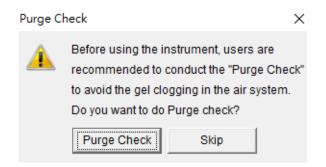
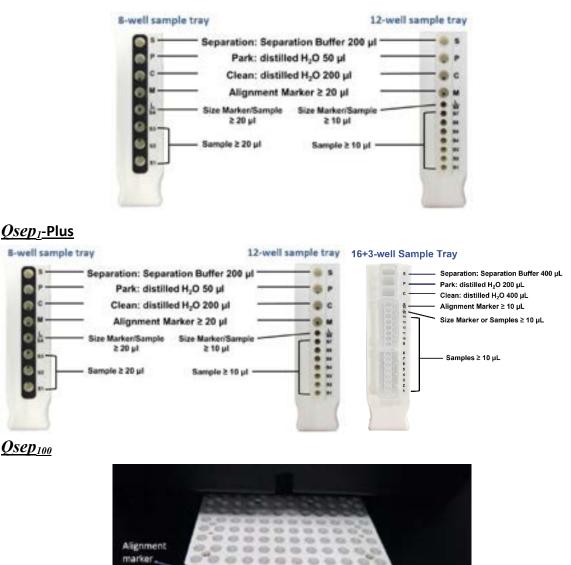


Figure 3-3 Purge Check Message Box

Click **Change Buffer** (for $Qsep_{100}$ and $Qsep_{400}$) or **Open Sample Door** (for $Qsep_1/Qsep_1$ -Plus) to move the buffer tray holder to the door (Figure 3-2 C). Add Separation Buffer into S wells and diH₂O into P/W/C wells of the buffer tray for $Qsep_{100}$ and $Qsep_{400}$. Each well should be 80% full or to the groove of the buffer tray. Overfilling or having droplets on the dividers will conduct the current and make it hard to keep track of the current changes. Once done preparing the buffers, place the buffer tray in the tray holder. If alignment markers are needed, please use individual 0.2 ml PCR tubes for markers (Figure 3-4).

Qsep₁



Park

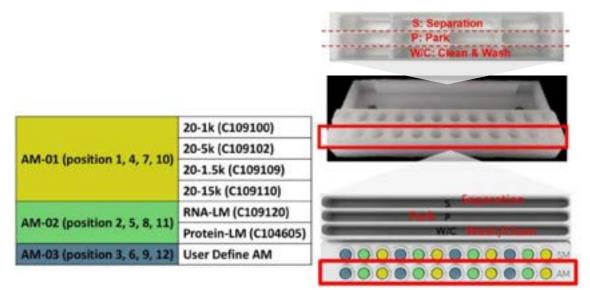
Separation

Wash

Clean

MB MC MD

<u>Qsep₄₀₀</u>

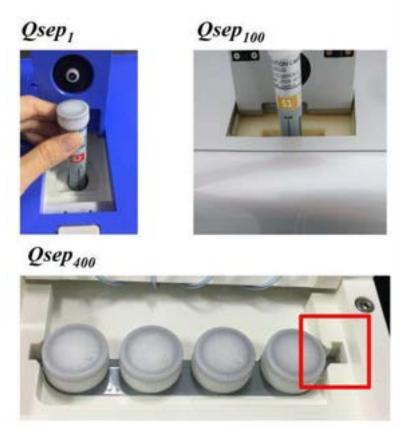


Make sure Alignment Markers are placed in the assigned positions.
 Make sure to use individual 0.2 mL PCR tubes for Alignment Markers and Size Markers.
 Figure 3-4 Change buffer and place solution

Then, click **Change Sample** (for $Qsep_{100}$ and $Qsep_{400}$) or **Open Sample Door** (for $Qsep_1/Qsep_1$ -Plus) to move the tray holder to the door and place the samples (Figure 3-2 B). After these steps, close the sample door by clicking **Park** (Figure 3-2 D).

Open the cartridge door on the top of the instrument. To insert, make sure the guiding groove of the cartridge is facing the front (Figure 3-5). Close the cartridge door to complete the series of actions.

*Note: Before inserting the cartridge, click **Park** to ensure the tray holder is in the park position.



L-shaped connector of cartridge should follow the L-shaped guiding groove inside the instrument.
 Figure 3-5 The guiding groove of the cartridge shall face the front

*Note: Cartridge can only be inserted in one orientation (Figure 3-5).



Figure 3-6 Push the cartridge to the bottom

Click the **Latch** button on the control panel. Once the cartridge is latched, the information of the cartridge will be displayed on the **Cartridge Information** section on the left (Cartridge Number, Expiration Date, Runs Left, etc.) and the image of the cartridge will turn into color form (Figure 3-7). The cartridge must pass the HV check and calibration process for its initial use. For more information, please refer to Section 3.2.

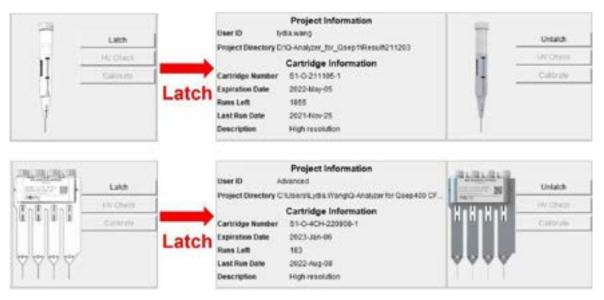


Figure 3-7 Before and after the cartridge latched

3.1.2 Sequence Settings

After latching and verifying the cartridge, follow the instructions and fill in the blanks in **Sequence** (Figure 3-8). Seven setting options provide the operational flexibility for the experiment. Edit the sequence by **assigning the sample position**, **selecting the separation method**, **entering the result name**, and **editing the parameter**. Click **Add** to add another row for the new sequence and **Run** to execute the sequence analysis after confirming.

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Figure 3-8 Edit Sequence

1. <u>Sample Position</u>: Select the sample position on the pop-up window.

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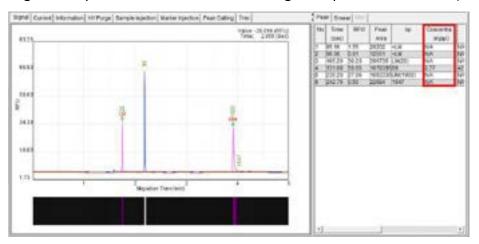
2. <u>Method</u>: Choose the method for the sample used.

A method selector window will pop up and provide the suggested pre-programmed methods based on the cartridge types and the sample conditions. Users can specify the items according to their conditions.

- ✓ Select **DNA**, **RNA** or **Protein** application based on the cartridge used.
- Select the analysis types preferred, qualitative or quantitative (only for S1, S2 and N1 cartridges (Cat. C105201-Q, C105202-Q and C105205-Q)).
 The quantitative function is used to measure the sample concentration more accurately. To measure the concentration, the sample must mix with the quantitative marker (C109109-500Q). The quantitative marker will act as an internal marker to adjust the calculation. To do the quantification, follow the steps below:
 - A. Enter the sample volume that is mixed with the quantitative marker.

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- B. Select the recommended method and start the analysis.
- C. The concentration will display at the result file (the value indicates the original sample concentration before mixing with quantitative marker).

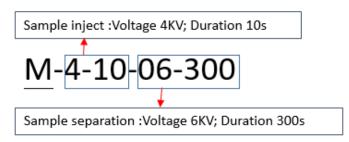


- ✓ Different Alignment Marker must be placed in the right place.
 - (1) When taking 20bp-1,000bp Alignment Marker (C109100) as the alignment marker, please place at MA1 and select **20-1K (MA-1)**.
 - (2) When 20bp-5,000bp Alignment Marker (C109102) is used, please place at MB1 and select **20-5K (MB-1)**.
 - (3) When 20bp-1,500bp or 20bp-15,000bp Alignment Marker (C109110-500A) is used, please place at MD1 and select **20-15K (MD-1)**.
- ✓ If the Alignment Marker checkbox is unselected, the alignment marker sample will not be used in the separation. The calculation will be affected.
- ✓ Select **Sample Concentration** based on the sample conditions.

After all items are selected, the available pre-programmed methods will be listed in the table.

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Sample Concentration (*	High (Fragment = 13 ng/ul)		r (Fragment 0.1 - 50 ng/ul) Range	C Low (Fragment +0.1 nglut)
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8-4-10-08-200	Sample Injection 4kv Separation 8kv 200s	2.020 PA	bp-5000bp stResolution: 4bp-10bp	
##10-10-120	Sample Injection 4kv Separation 10kv 120k		bp-5000bp stResolution: 10bp-50bp	
1-HMPurge-08-120	Gel Refil with HV on t	lor 120s		
f-Purge-120	Get Reftl without HV1	ler 120s		
	Purpe Purpe Modification			

The pre-programmed method will be named under the rules below:



With higher voltage and longer sample injection time, the signal will be enhanced. With higher separation voltage, the separation time will be shortened, and the resolution will

be worsened. The lower separation voltage on the other hand will increase the resolution as the separation time lengthened. The sample separation corresponds to the size range of base pairs.

3. <u>Sample Duration</u>: The duration of the sample injection time.

The selected method will display the **Sample Duration** on the sequence table. The **Sample Duration** affects the quantity of the injected sample. Modify the duration according to the sample condition as per your preference.

4. <u>Runs</u>: The number of runs.

The same sequence will be repeated for 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. <u>Separation Duration</u>: The separation time of the execution.

The separation time may be affected by the experimental condition such as temperature, concentration of separation buffer, etc. The **Separation Duration** of the selected method will be displayed on the sequence table. Modify the duration of the separation according to the condition if needed (optional). The **Separation Duration** time is recommended to be 30 seconds longer after the upper marker appears.

6. <u>Result Name</u>: The result name to be saved.

The result file will be saved with information of sequence, position, and execution such as <*Result Name>_<pull-down option>_*S1A01_R1.bopxr; here, S1, A01 and R1 mean sequence number, sample position and the order of execution, respectively. Move the cursor to the columns of Result Name to preview the result file name.

	Open	Save As						
511	Sample Position	Method	Sample	Runs	Separation	Result Name	Para	Add
		M-4-10-06-300	10	1	300	Demo None e.g.:Demo	7	Delete
						e.g. Demo	STADI_H	Up
								Down
								POWER IN

sn	Sample Position	Method	Sample Duration	Runs	Separation Duration	Result Name
1	A-01	M-4-10-06-300	10	1	300	
1		14 10 00 000	1.8	<u> </u>	000	None

7. Para: The setting configurations for this method.

Baseline Factor Peak Threshold		T Peak Smoothing	0	=			
Calculate	C Reference Ma	rker Table D.VQ-Analyzi	r_for_Gaep10	PReference/31-6-C1	09200-20-1K.rfm	~	Briefe
	Create Size M	arket C109200(MA-2)	· F 6	bery 4 🐨 time	14.		
20	Size marker inject	ion time: Auto 💌 sec	083				
0	Reference Marker	Table:					
0	D10-Analyzer_tox	Oxep100/ReferenceIS1	-6-C109200-20	9-tiKritm		 	Browse
Smear	C Children I	100 <u>-</u> % C Roop		-	38		
Peak Calling							Browse
and a second state	. Income	w 18 8 28					
	- lenvatione	4 10 0 28	19				

Software will calculate the result using the default reference table. If users wish to use their own created reference table, click **Browse** and select the file. Baseline and Peak Threshold can be also adjusted.

Alternatively, if user needs to improve the accuracy of the base pair calculation, **Create Size Marker** will update the reference table periodically with the size marker information. The following sample test will then be calculated based on the updated reference table. ***Note**: The size marker used to **Create Size Marker** is according to the alignment marker selected on **Method Selector** window.

Users can assign their own reference marker instead of using built-in reference marker when:

- (1) The signal pattern is different between the built-in reference marker and the new one, and the software cannot recognize the new pattern correctly.
- (2) The size of upper marker is not the same as used. For example, if using 20bp-1,000bp, the upper marker must be 1,000bp.

To execute **Create Size Marker**, do the followings:

A. Place fresh Alignment Marker and Size Marker in the assigned positions. For different instruments, the positions could be different (Figure 3-5).

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

SN	Sample Position	Method	Sample Duration	Runs	Separation Duration	Result Name	Para
	4.01	M-4-10-06-300	10	4	300		1
	A-01	14 10 00 000	10	<u> </u>	500	None -	1

C. Check Create Size Marker.

User may change the frequency (**Every** <u>times</u>) to execute the size marker if higher accuracy of the sample sizing is needed.

 Calculat 	e Flow				>
IT Baseline Fa	actor: 200 /** Peak Smoothing: 0				
Peak Thres	inold: 10.00 ^m Peak Definition: 3				
Calculate	C Reference Marker Table D.10-Analizer_for_Ocep100Reference/81-6-C109200-30-1K.rtm	~		Briefe	
	Size marker injection time: Auto 💌 sec(s) Reference Marker Table: D10-Artalyzet_for_Osep100/Reference(S1-6-C109200-20-5K-rtm		~	Broase	
Smear	C Distribution 100- % C Rampe - 36	1	-		-
T Peak Callin	9	-		Browse	
	117014) Eukarjobc 🐨 18 8 28 8				
-					
	et				

Smear: If the Smear checkbox is selected, the Smear analysis will be applied to the results.

Peak Calling: If the **Peak Calling** checkbox is selected, the Peak Calling analysis will be applied to the results.

Auto Assign rRNA: If **Auto Assign rRNA** checkbox is selected, user can not only choose between Eukaryotic and Prokaryotic, but also define SSU/LSU based on the needs.

Baseline Factor: 5000 □ Peak Smoothing: 0 Peak Threahski: 10.00 □ Peak Definition: 8 Calculate	
Calculate # Reference Marker Table DIG-Analyzer_No_Deep100ReferenceR1-4-C109800.rtm P C Create Size Marker C10960000C-2) If Even Browse Size marker injection time: Auto Becks Browse Reference Marker Table Even Browse Smear C Distriction: No Props	
C Create Size Marker (C10900000C-2) → IT Entir (4 → Simes Size marker hijection time: Auto → sec(3) Reference Marker Table Smear C Distribution: [100→ % C Hamps - rt	_
Site marker hijedion time: Auto + Bec(3) Reference Marker Table Smear C Distribution: 100-1 % C Range - re	
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Smear C Distriction 100 % C Range - re	
Smear Chatritudius (100-1 % C Range - re	
Peak Calling Browse	
Auto Assign dRNA Eukaryotic 💌 18 S 28 S	
Export Report Protaryatic	
User define OK C	

Export Report: If the **Export Report** checkbox is selected, click on **Show Report Settings**, and set the **Report Format**.

Report Format	×
Export Type :	
@ Individual C Folder	
Application Type :	
In Standard(DNA) ⊂ Smear ⊂ RNA ⊂ Peak Calling	
Signal View :	
Window ← Best Fit(LM-UM) ← Best Fit(LM-End) ← Orig	ginal
X Axis :	
Gel View :	
Ø Best Fit(LM-UM) € Best Fit(LM-End) € Original	
Result List :	
& Result Name C Sample Description	
Smear Zone :	
Gniy Zone1 ← Both Zone	
Small Chart Description :	
& Sample Position C Sample ID	
File Format :	
@ Preview C PDF	
	ок

3.2 Cartridge Calibration

To ensure the quality of the new cartridge, calibration is required before the initial use.

The concept of the verification is to do the **HV Check** by checking if the current of the cartridge is stable under HV condition. The result will show "passed" if the current is stable. The function of **Calibrate** is to confirm the quality of the cartridge by executing the test of the alignment marker/quantitative marker and checking if the signal of the alignment marker/quantitative marker can be detected when using the cartridge.

Prepare $\ge 20 \ \mu$ L fresh alignment marker/quantitative marker in a 0.2 mL PCR tube (adding 10 μ L mineral oil to prevent evaporation) and place the marker at position MA1. Make sure the buffer tray is placed on the buffer tray holder. Insert the new cartridge and close the door.

Click **Latch** to begin the calibration of the new cartridge. The pop-up window will instruct user to finish HV check and calibration first (Figure 3-9). Press OK and begin **HV Check**. The working current of the cartridge should be above 2 μ A. After **HV Check**, click **Calibrate** to verify the cartridge. For some cartridges, the pop-up window will provide users alignment marker options to choose from to initiate the process (Figure 3-10).

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Figure 3-9 Cartridge Performance Check



Figure 3-10 Choose Alignment Marker Type

The cartridge is ready to be used after the "Passed" message appears on the screen. If the "Failed" message appears, please refer to unpacking guide for troubleshooting instructions. If the calibration continuously fails for five times, please contact your local distributor for technical support.

3.3 Edit Sample Position Settings

User can select the corresponding location on the simulation well plate to set the **Sample Position** (Figure 3-11). The selected location will be marked in red. To undo, click it again.

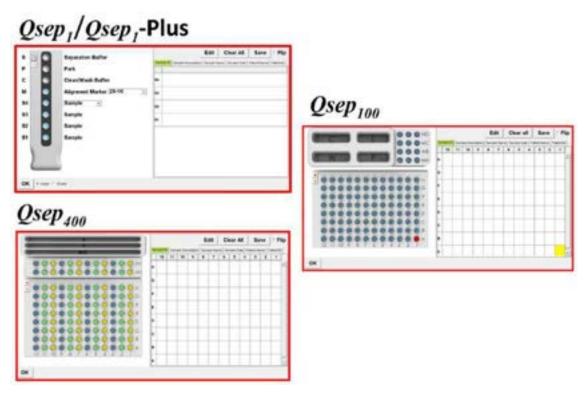


Figure 3-11 Select the sample position

The whole column or row can be selected by clicking the coordinates A-H or 01-12 on the side. Users can also select all the sample positions by clicking the "A" button and invert the selection by clicking the "I" button on the upper left corner. When multiple samples have been selected to execute the method for several times, the system will execute the marked sample positions in numerical order. For instance, if A01, A02, and A03 positions are selected and assigned to execute the method for three times, the sequence will be processed in the following order: A01, A02, A03, A01, A02, A03, A01, A02, A03.

Notes for samples can be edited on the Sample Loader Window (Figure 3-12). After clicking **Edit**, the Sample Loader Window will appear on the screen. **Load** to enter multiple sample description, **Clear All** to delete all the sample information, and **Save** to save the sample information after editing. After entering the information such as time and serial number, click **OK** to start the process.

The information in the excel file (Figure 3-13) can be loaded into the window and saved after clicking **OK**.

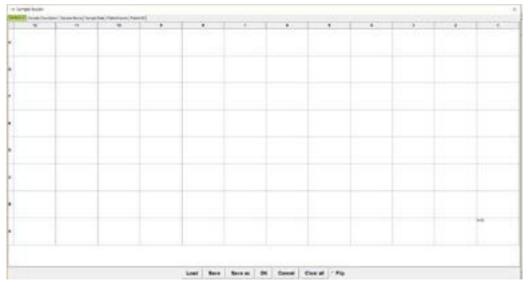


Figure 3-12 Sample Loader Window

14	1	₽	C.	0	£	2	6
D	SamplePosition	SampleID	SuppleSup-	SampleDears lption	SampleDate	Pat Jest 10	FatlestSam
2	4-01	802	608	104	2000/30/30	30	1
3	8.01	202	803	504	2000/10/10	77	2
4	C-01	C02	D03	004	2000/10/10	60	3
3	0-01	202	003	100	2000/10/10	. 111	4
6	E-01	E02	B03	804	2000/00/10	43	5
7	F-01	702	P03	204	2000/10/10	11	5
8	0-01	602	603	\$04	2000/30/30	IX.	7
9	1-01	208	100.5	504	2000/10/10	11.	

Figure 3-13 Excel File for Sample Loader

*Note: The information of the samples in the excel sheet needs to follow the sequence that shows in Figure 3-12. You can find an example file in the installation directory. The default workspace is C:\Users\<username>\Q-Analyzer CFR Mode\Sample.

Each row represents a single sample, and the information of each sample will map into the corresponding tab automatically.

***Note**: "Sample Position" and "Sample Date" need to follow specific formats. Sample Position: "A-H"-"01-12"; Sample Date: yyyy/mm/dd.

4. Q-Analyzer User Interface

4.1 Main Window

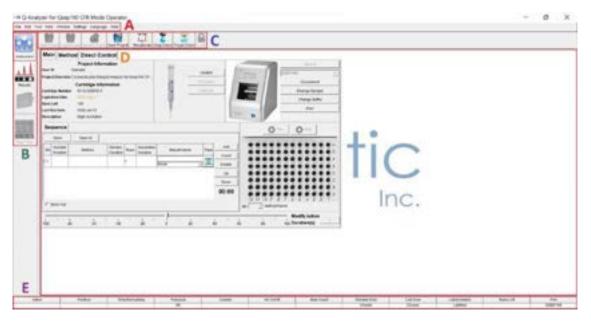


Figure 4-1 Main window

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

4.1.1 System Overview

After launching *Q*-*Analyzer*, the Main Panel (Figure 4-1) will appear and provide the information of *Qsep* series (status, data/results display, and post data analysis).

<u>Menu Bar</u>

All available functions can be found on the Menu bar. For more information, please refer to Section 4.3.

Function Bar

There are four icons on the Function Bar. Select **Instrument** to enter and operate the control panel (Section 4.4), **Results** or **Comparison** to display and analyze the data, and **Real Time** to collect data of the sequence in progress. Refer to Section 4.2 for more information.

<u>Toolbar</u>

The Toolbar provides several icons according to the **Function** selected. The icons listed are the most frequently accessed functions. All detailed information can be found in Section 4.2.

Operation Region

Operation Region is for displaying main window and the corresponding panel after selecting the desired icon on the Function Bar.

<u>Status Bar</u>

The information of the system status will be shown at the bottom of the main panel. Information on whether the pressure is okay or low, the cartridge door/sample door is opened or closed, the cartridge is latched or not, etc. will be indicated.

4.1.2 System Status Bar

Column	Description
Action	The proceeding actions.
Position	The position of the cartridge tip.
Time Remaining	The remaining time for the action to complete.
Pressure	 The status of applied pressure. Fail indicates that the pressure is lower than the system requirement. If the pressure is lowering, the description in the column will change from black to yellow to red.
Current	The amount of current (μA) that flows through the capillary.
HV On/Off	The status of High Voltage power supply.
Raw Count	The original signal.
Sample Door	The sample door is Opened or Closed .
Cart Door	The cartridge door is Opened or Closed .
Latch/Unlatch	The cartridge is Latched or Unlatched.
Runs Left	The remaining runs of the sequence.
Port	The model of the instrument connected to the computer.
Instrument ID	The instrument ID of $Qsep_1/Qsep_1$ -Plus that is connected to the computer.
SD Card	The SD card is detected or not after connecting to $Qsep_1/Qsep_1$ -Plus.

The columns shown on the Status Bar are as follows (Figure 4-1 E).

4.2 Function Bar and Toolbar

Q-Analyzer controls *Qsep* series, collects and processes data, and reports results. The functions are organized into bars and tabs for easy navigation.

The **Function Bar** has four major icons, which are **Instrument**, **Results**, **Comparison**, and **Real time**. By default, the software will begin with the **Instrument** function. Each function corresponds to a different set of **Toolbar**, where most frequently accessed functions are listed. The detailed information will be in the following sections.

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al Time	SN	Position			1				Delete	
			M- <mark>4-10-0</mark> 8-160	10		160	None	- M	Delete	
			M- <mark>4-10-08-160</mark>	10	1	160	None		Up	
			M-4-10-08-160	10	1	160	None		Up	
			M-4-10-08-160	10	1	160	None			

Figure 4-2 Function Bar and Toolbar

4.2.1 Instrument

Instrument is the major control function to operate *Qsep* series. The use of the control panel will be in Section 4.4.

Before connecting to *Qsep* series, the tabs on the Toolbar such as **New Project**, **Load Project**, **Recent Project**, and **Save Project** are enabled. User can create a new project or load a project, if necessary (Figure 4-3). The project can be used to differentiate various experiments. Name the **New Project** and the results will be saved in the designated directory. The default workspace is C:\Users\<username>\Q-Analyzer CFR Mode\Result.

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Figure 4-3 Instrument function before instrument connection

O New Project		×
Project name:		-
Result folder:		Browse
	THE Cancel	

Figure 4-4 New Project

When the instrument is connected, the icons on Toolbar such as **Clog Check**, **Purge Check** and **Lock** will be enabled. Once the cartridge is latched, the **Recalibrate** will be enabled (Figure 4-5).

For $Qsep_1/Qsep_1$ -Plus, when the instrument detects the SD card, the **SDC Data Export** and **SDC Data Clear** will be enabled.

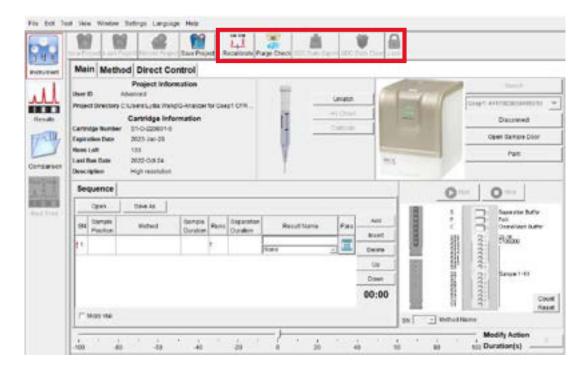
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These functions allow user to perform basic function check on instrument and cartridge.

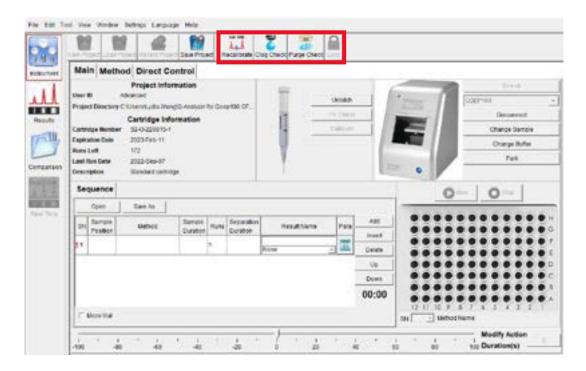
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Figure 4-5 Instrument function before/after latching the cartridge

Recalibration

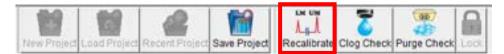
Recalibration helps to identify the alignment marker (lower and/or upper markers) correctly and update the cartridge factor.

Conduct **Recalibrate** if any of the following situations occurs:

- The alignment marker has been replaced.
- The cartridge has been stored for more than two weeks since the last execution.
- The software cannot identify the alignment marker correctly.

To **Recalibrate**, do the following steps:

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



4. Choose the appropriate voltage and alignment marker that match with the **Method** which will be used for the following experiments (Figure 4-6).

Voltage		Alignme	ent Marker Information	,	
· Bio	Alignment Marker	Position	Last Calibrated Date	LM_SN	UM_SN
C Blov	@ 20-1K	MA-1	2019-FEB-20	691.03	551.98
- 10kv	C 20-1.5K	MD-1	NUA	144	184
	С 20-5K	MB-1	Now.	HA	N/A
	Customized	MD-1	NA	NIA	NEA

Figure 4-6 The message box of recalibration

<u>Clog Check</u> **Qsep*₁₀₀ only.

Capillary Clog Check will purge the gel out of the capillary to see if it is clogged or not. If the capillary is not clogged, the droplet will form at the capillary tip.



If the current is too low or unstable (Figure 4-7 and 4-8), user can utilize this function as well. Follow the instructions to complete the check (Figure 4-9).

If the current of separation (Current tab on the result file) is still unstable after performing the Clog Check, the inner air tube might be clogged. **Purge Check** should be done to confirm the issue.

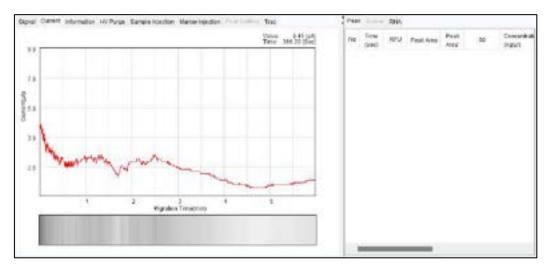


Figure 4-7 Low and Unstable Current

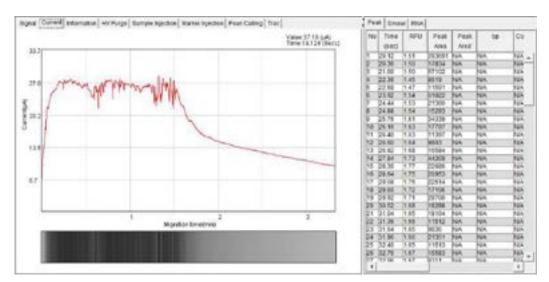


Figure 4-8 Unstable Current

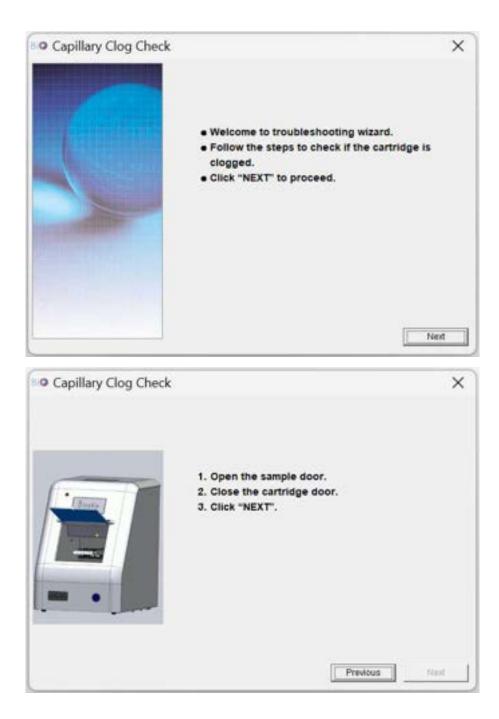




Figure 4-9 Clog Check Windows

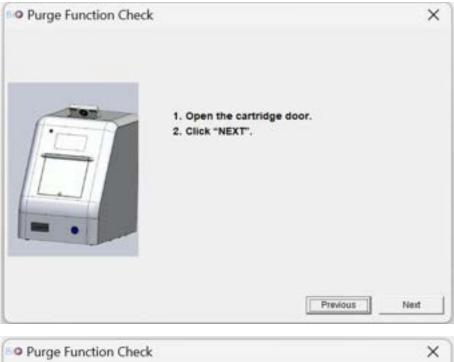
Purge Check

Purge Check confirms that the air is coming out properly from the JET-NOZZLE while purging. A purge check before and after the use of *Qsep* series is recommended.



If there is air coming out, press the JET-NOZZLE down to the end with your thumb. If the JET-NOZZLE can be pressed down easily, the pressure is not enough. If there is no air coming out, the gel may be clogged in the air tube. Cleaning the air tube at the cartridge door is suggested. Please contact BiOptic Inc. or your local distributor for maintenance and repair services.





Purge Function Check		×
1. Please prepare tissues t NOZZLE".	block the "JET-	
2. Click "PURGE" to start th	e 30-second purge.	
	Purge	
	Previous	Finish

Figure 4-10 Purge Check Windows

<u>Lock</u>

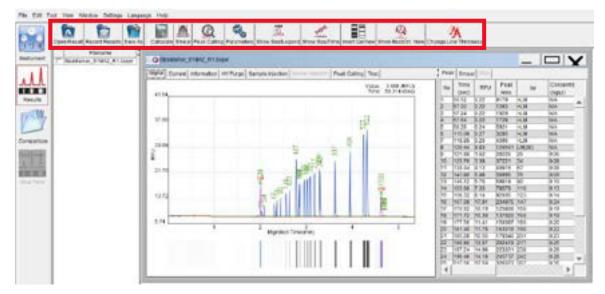
Lock Function moves the tray holder back to its lock position. Before clicking Lock, please remove the samples, buffer, and cartridge in the instrument.

New Project Loa	d Project Recent Project Save Project RecallCrate Clog Check Purge Check Lock					
Model	Description					
<i>Qsep</i> ₁ / <i>Qsep</i> ₁ -Plus	The tray holder will move to the lock position first. Follow the instructions to insert the EPE foam.					
$Qsep_{100}$	The tray holder will move to its lock position. Insert the EPE foam to secure it.					
Qsep ₄₀₀	 The tray holder will move to its lock position. Follow the instructions below to secure it. 1. Take out fixture and securing screws. 2. Place the fixture back. 3. Place the sample plate holder to the left until the screw holes are aligned. 4. Screw back fixture securing screws. 5. Lower the blue frame of the front panel. 					

4.2.2 Results

The Results Function displays or analyzes data from *Qsep* series. User can demonstrate the signal chart or get peak information from raw data of capillary electrophoresis.

Once the files are loaded into Q-Analyzer, the icons on Toolbar such as **Calculate, Smear, Peak Calling, Parameters, Show Size/Legend, Show Size/Time, Invert GelView, Show Best/Ori. View,** and **Change Line Thickness** will be enabled, allowing user to edit raw data. For more information, please refer to Section 5.1.



<u>Calculate</u>

If the size information of the result is inaccurate or missing, user can utilize the **Calculate** function on the Toolbar to get size and concentration value of each peak.



Open the result file for calculation and make sure it is selected in the result list. Select **Calculate** on the Toolbar and click **Load** to load a suitable reference marker table to calculate size and concentration. Please choose the reference marker file based on the components used. Note that each file includes four information:

- 1. Cartridge Type (e.g., S1, S2, F3, ...)
- 2. Operation HV of the Separation (e.g., 6, 8, 10 kV)
- 3. Size Marker Type (e.g., C109200, C109301, ...)
- 4. Alignment Marker Type (e.g., 20-1k, 20-5k, ...)

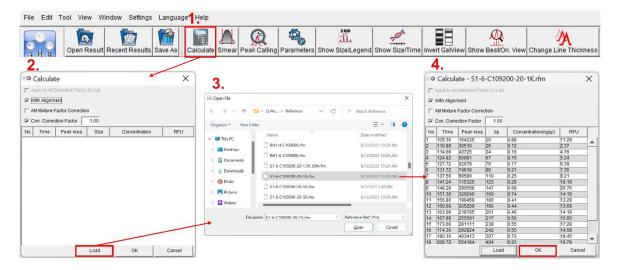
For example:



All reference marker files corresponding to the pre-programmed methods can be found in the default Load folder: <Workspace>\Reference

X Note: Default <Workspace> is C:\Users\<username>\Q-Analyzer CFR Mode

After confirming the reference data file, select the setting checkboxes if necessary, and click **OK**. All the peak information will then be shown in the table.

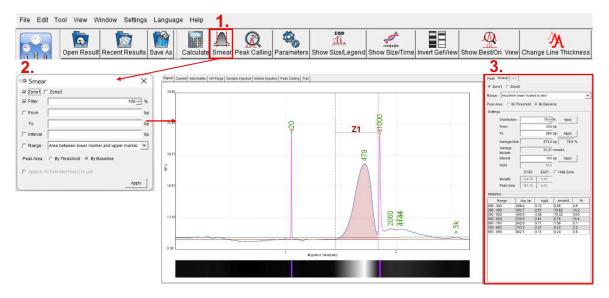


Checkbox	Description
Apply to all selected files in list	Multiple files selected in list will be calculated at once.
With alignment	Selected by default. The alignment marker will be applied for analysis.
AM Mixture factor correction	The calculation will be based on the mixture factor if the alignment marker is mixed with the sample.
Con. Correction factor	Selected by default. The calculation will be based on the sample dilution factor.

<u>Smear</u>

The **Smear** function helps to understand the major size and distribution of fragmented genomic DNA.

*Note: To use the Smear function, the result must be calculated with reference marker.
*Note: Zone2 will not be displayed by default. To display Zone2 on the result file, click "Zone2" directly.

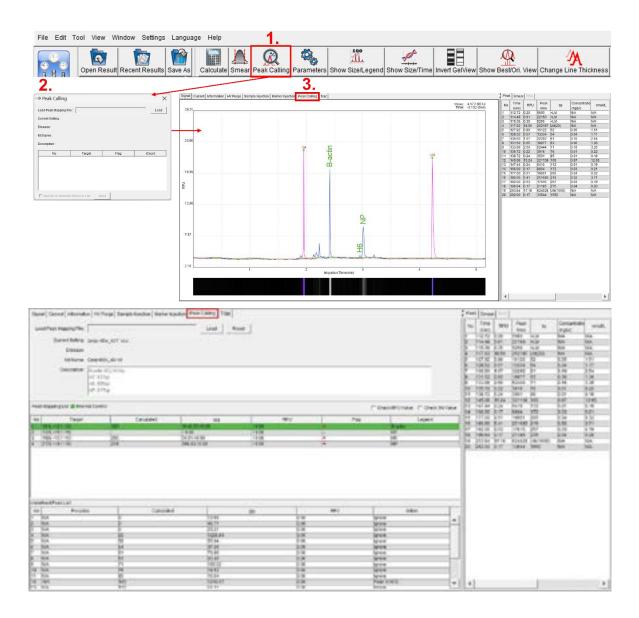


Open and select the result file for Smear analysis in the result list and follow the steps below:

- Click the Smear icon or alternatively select Smear from Tool on Menu Bar and click the Smear tab after settings.
- 2. Enter the percentage of the target distribution (e.g., 50%) or the size range to assign the region and modify the interval of the size range if necessary. User can also select the target range to be **Area between lower marker and upper marker** or **Area from lower marker to end**, and peak area to be **By Threshold** or **By Baseline** for analysis.
- 3. Click the **Smear** tab on the result file to view or edit the settings. For more information, please refer to Section 5.2.

Peak Calling

Peak Calling function helps to distinguish the target peaks. **Load** the saved **Peak Calling Table**. The software will follow the rules in the table and show the report in the peak calling tab window. Report "+" when the peaks are found and "-" when the peaks are missing. To create a Peak Calling Table, please refer to Section 4.3.2.



Parameters

The system will generate the corresponding baseline and define the peaks automatically. User can modify parameters to get result that meets the requirement. For multiple files modification, click the file checkboxes before using the parameter function. (See Parameter Settings in Section 4.2.2)



• Parameters		Х
Baseline Setting		
	Baseline Factor	
	200	
Peak Filter		
Peak Smoothing 0	Peak Threshold 10.00 S/N Peak Definition 3	
	Apply Default	
	🗖 Apply to All Selected File(s) in List	
	🛱 Recalculate Result	

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	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. User 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.	
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 multiple files modification.	
Recalculate Result	Recalculate Result recalculates the result and shows the peak size automatically after applying new parameter.	

*Note: Click **Default** button to retrieve the original settings.

Show Size/Legend

Switch the index of the peak between size and legend in the chart. To display the size, the result data must be calculated with reference marker. To display legend, the **Peak Calling** function data must be applied.



Show Size/Time

Switch the x-axis between size and time (minute). To display the size, the result data must be calculated with reference marker.



Invert GelView

Invert the color of Gel View (as shown below).





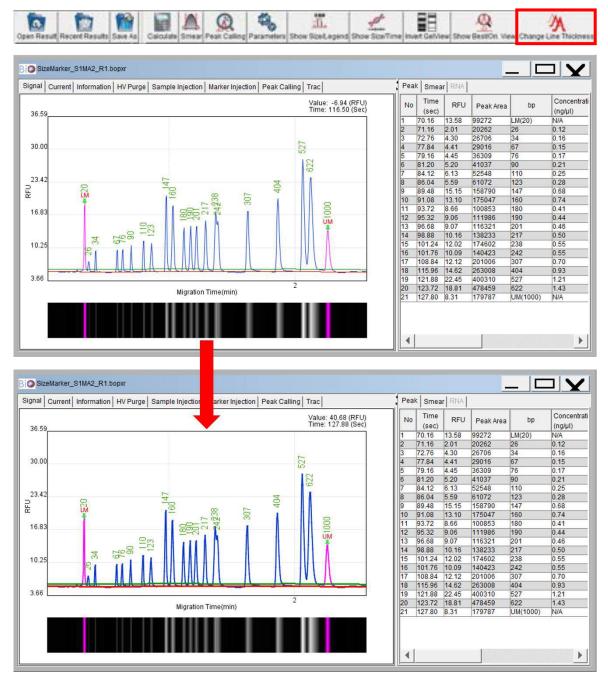
Show Best/Ori. View

Switch between best view and original view of the signal pattern (as shown below).



Change Line Thickness

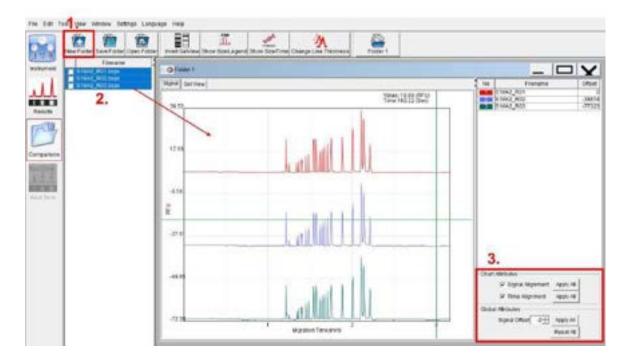
Change the thickness of the peaks in the chart (as shown below).



4.2.3 Comparison

The **Comparison** function allows user to compare or analyze multiple results. With this function, user can load several signal charts and compare sample variances.

Since the **Comparison** function is used to analyze multiple results in a chart, user needs to open them before performing the **Comparison** function. Click **New Folder** and drag the result files to folder window. Then, the results will be displayed on the folder window.



Use the comparison function to compare different result files.

- 1. Click **New Folder** on Toolbar to create a Folder.
- 2. Drag the files from the Filename panel into the Folder created.
- 3. Edit the chart settings for easy viewing and simple comparison.

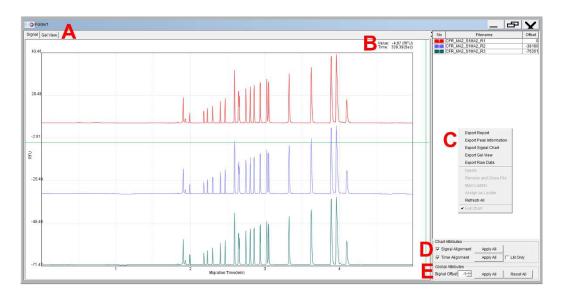


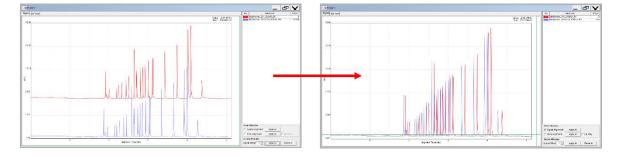
Figure 4-11 Data Comparison in Folder Chart

Show the electropherogram or gel image by selecting the tab in Figure 4-11 A. Figure 4-11 B displays the corresponding information of the spot selected. Select a file name in Figure 4-11 C, the corresponding data in the chart will be highlighted. User can also change the line color by clicking the color block in the number column.

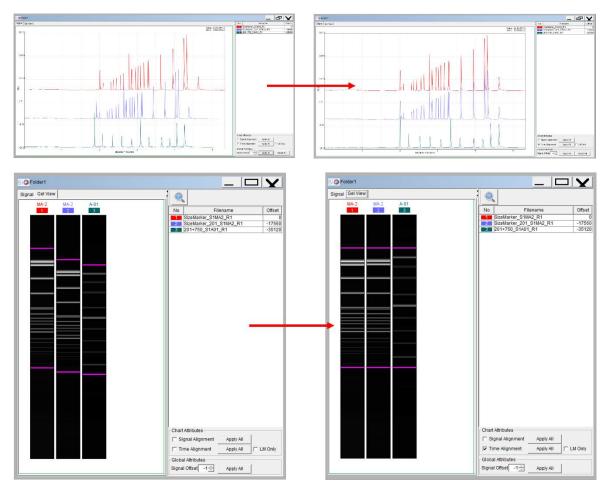
Due to variety of the experiment conditions, user may need to normalize the test results by utilizing the Alignment functions (Figure 4-11 D) to align data.

Parameter	Description	
Signal Alignment	Signal Alignment adjusts the baseline of each file at the same height (RFU) with the baseline of the first file or main ladder if set.	
Time Alignment	Time Alignment uses the Alignment Marker of each file to scale the data and align with the first file or main ladder if set.	
Signal Offset	Signal Offset (Figure 4-11 E) helps to separate the stacked data. Simply set the value in Signal Offset and click Apply All. User may retrieve the original result with the Reset All function.	

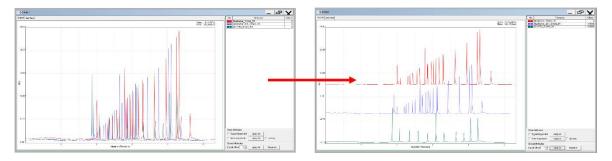
1. Use Signal Alignment to adjust baselines to compare results with different baselines.



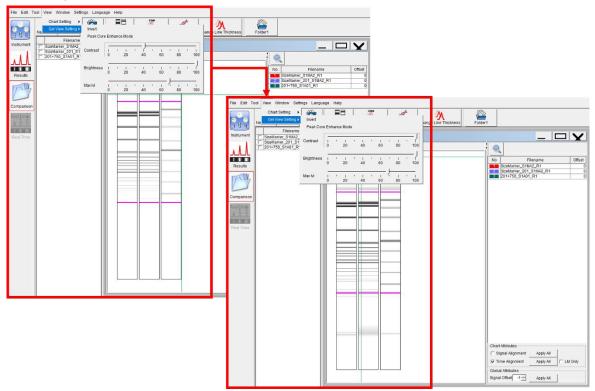
2. Use Time Alignment to scale the peaks and align with the first file or the main ladder if set.



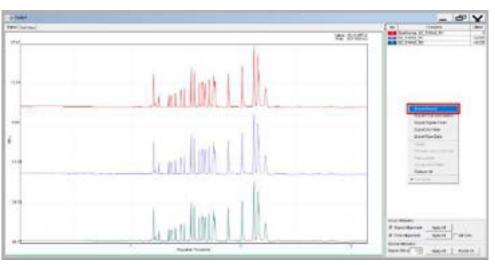
3. Use Signal Offset to unstack results.

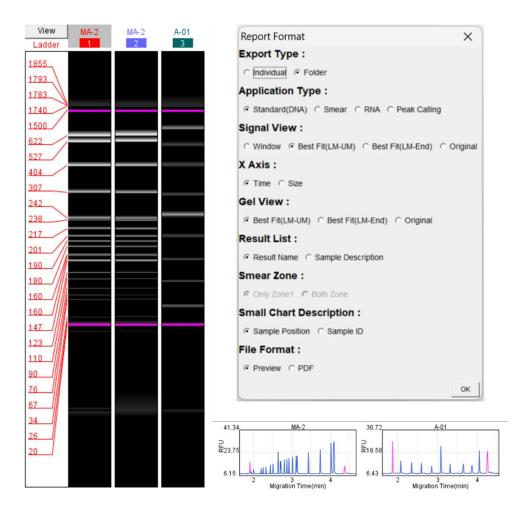


To invert or adjust the contrast of the gel view, go to Main Panel \rightarrow View \rightarrow Gel View Setting \rightarrow Invert/Contrast.



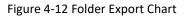
To export the Folder result, right click and select **Export Report**. Choose the preferred File Format, enter the file name, and save the file to the designated folder.





On the folder window, by right clicking on the result file list, user can export the signal chart or gel view with or without the sample description and result name.

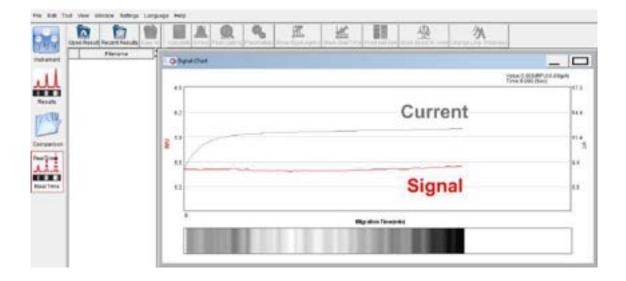
Export Peak Information	faport	
Export Signal Chart	Reproduct's	itizeta
Export Gel View	notifional etc. 12 Resultance (* Brow Result same (* Sames Securates	Cast
Export Raw Data		
Delete		
LANEW.		
Remove and Close File		
Development of the second s		
Remove and Close File Main Ledder		
Remove and Close File		



4.2.4 Real Time

Real Time provides a real time display of the current and signal data, which includes the current and RFU (amplitude of the capillary electrophoresis signal).

*For $Qsep_{400}$, four real time displays will be shown on the same page.



4.3 Menu Bar

4.3.1 File

Function	Description
New Project	Create a new project to differentiate several experiment conditions. The test results will be saved in the corresponding "Result" directory. The default saving folder is C:\Users\ <username>\Q-Analyzer CFR Mode\ Result\<project name="">. The new project setup needs to be done before connecting to the instrument.</project></username>
New Folder	Open a new folder to compare the results. For more information, please refer to Section 4.2.3.
Open Result	Open the result files from the project.
Open Folder	Open the folder files from the project.
Save Result	Save and overwrite the modified result.
Save As	Save the modified results as new files.
Save All	Save and overwrite all modified results.
Save Folder	Save the created or modified folder.
Load Project	Load the saved project before connecting to the instrument.
Save Project	Save the current project. The settings include the methods used in the sequence; sample position, duration, sample information, and result names will be saved.
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 CFR Mode.

4.3.2 Edit

Sample File

Edit the information of each sample (Figure 4-13).

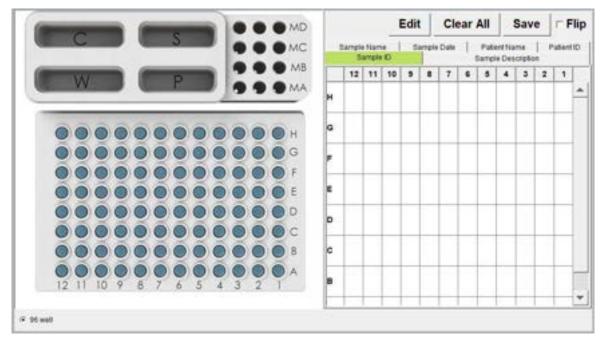


Figure 4-13 Sample File Window

Click **Edit** and the sample loader window will pop up. Select the sample position and enter the sample information, such as 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. User may **Load** the sample files saved earlier for inquiry or editing. An excel file can also be imported to edit multiple sample description.

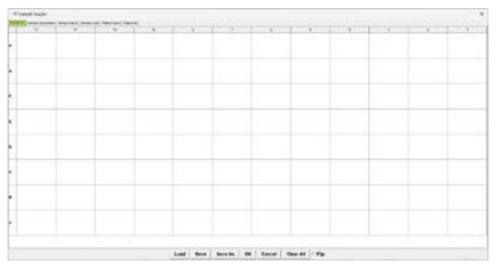


Figure 4-14 Sample Loader Window

Reference Marker

The **Reference Marker** function may be utilized to modify the saved reference marker table or create a new one. To create a new reference marker table, after executing a specific size marker run, remove the redundant peaks, and then right click and select **Copy Peak Info for Creating Reference**. Then, go to "**Edit** \rightarrow **Reference Marker**" and right click on the blank area and select **Paste Reference Marker Data**. User may edit the data if needed.

Peak Sme	ear RNA			Potoronor	Marker Editor		1-		Die	Reference	Marker Editor				~
No Time (sec)) RFU Peak Area	bp Concentrati (ng/µl)		1	Save As						Save As		_		×
1 111.16 2 115.28 3 117.20 4 120.76 5 132.12	Export Report Export Chart				Correction Factor		Length Factor 1.00				orrection Factor		Length Factor 1.00		
6 135.08			No	Time	Peak Area	bp	Concentration(ng/ul)	RFU	No	Time	Peak Area	bp	Concentration(ng/ul)	RFU	T
7 139.36	Cot on Lower Marker								1	111.16	8822			0.85	
8 145.60 9 149.56		<u></u>	_						2	115.28	177620 36986	_		12.63	-
10 157.24			_						3	120.76	52855			5.42	-
11 160.52									1	132.12	59711			6.60	-
12 161.08										135.08	74526			8.06	
13 166.48	Remove Calculated Inf					C	elete Row		1	139.36	87004			9.32	
14 170.04	l					le le	sert Row		8	145.60	117025		6	12.01	
15 173.16		ik Candidate	_				dd Row		9	149.56	134495 338588			13.41 28.94	
16 178.12	2		_				e se recentration		10	160.52	188284			28.94	-
17 183.52			_			- P	aste Reference Marker Data			161.08	198661	-		16.08	-
18 184.92		ult	_						13	166.48	219570			19.05	
19 201.56 20 219.52			_						14	170.04	235381			19.77	
20 219.52 21 235.56			_						15	173.16	256288			20.75	
21 235.50 22 239.80		ating Reference	_						16	178.12	294956			22.83	
23 248.20									17	183.52	328871			24.51	_
25 240.20	Update Cartridge Facto	n –	_						18	184.92	338874			22.94	
	✓ Full Chart		_							201.56 219.52	464979 635243		0	28.04 30.96	_
4	1									235.56	939600			37.01	v

Figure 4-15 Edit Reference Marker Table

Load to renew the saved reference file (.rfm) or reference pack file (.rfmx) shown in Figure 4-15.

- **Time**: The migration time of each peak.
- **Peak Area**: The measurement of the area within the peak.
- **bp**: The fragment length.
- **Concentration (ng/µl)**: The concentration of each peak.
- **RFU**: The relative fluorescence units (RFU) of each peak.

Time and **Peak Area** will automatically be generated from the test result. Please do not change their values unless there are specific requirements. Enter **bp** and **Concentration** of a known size marker and add or delete rows if necessary. After completing all modifications, save the reference marker table for later use.

***Note**: When pasting the exported data from the result file, ensure that **the row number** of the pasted data is **the same** as the recalled reference marker table. Moreover, the data must be recognized from the identical base pair.

Parameters

Change the parameters of the calculation settings. For more information, please refer to Section 4.2.2.

Parameters Baseline Setting		×
	Baseline Factor	
	200	
Peak Filter		
Peak Smoothing 0	Peak Threshold 10.00 S/N Peak Definition 3	
	Apply Default	
	T Apply to All Selected File(s) in List	
	P Recalculate Result	

Figure 4-16 Parameter Settings

Peak Calling Table

Peak Calling creates the panels for quick scanning. To do peak calling, user needs to set up the Peak Calling Table which includes the 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).

Reference Marker nt Results Save As Calculate Smear Peak Calling Parameters Show Size/Legend Show Size/Time Invert GetView Show B Parameters NA Reference Table NA Reference Table Load Save As D Results Load Save As D Disease : C Kit Name : Comparison Description : Description : Description : Description :	File Edit Tool View Window Settings Lang	juage Help
RNA Reference Table Load Save As D Disease : C Kit Name : Description :		I IIIIII TACA db I delete
Real Time	RNA Reference Table Results Comparison	Bio Peak Calling Table Editor Load Save As Disease : C Kit Name :

Figure 4-17 Peak Calling Table

To use the **Peak Calling** function, do the following steps:

- 1. Click **Edit** \rightarrow **Peak Calling Table** to open the Peak Calling Table Editor (Figure 4-17 A).
- 2. Right click and select **Add Row** to add a new panel (Figure 4-17 B).
- 3. Set up the new table. Enter the criteria of the peak in the columns (Figure 4-17 C) and save as a new file (Figure 4-17 D) to the default folder.
 - **Target**: The center value of the peak.
 - **Upper/Lower Margin**: The upper/lower margin in size of the target peak that will be assigned as the Legend.
 - **SN**: Configure the criterion of signal and noise ratio of the peak (optional).
 - **RFU**: Configure the criterion of signal amplitude of the peak (optional).
 - Legend: Enter the name of the Legend.
 - IC: Internal control of the peak calling.
- Return to the result files, calculate the fragment size, and select the Peak calling tab or Tool → Peak Calling (Figure 4-18 A-B).
- 5. Browse the desired file and load the Peak Calling Table (Figure 4-18 C).
- Click Signal tab to return. The software will automatically scan the peak signal. Click Show Size/Legend button on the Tool Bar, the legend of target peak will be displayed on the window. Right click to export the report. (Figure 4-19)

*Note: Incorrect fragment size may affect the result of Peak Calling. Please ensure the experiment is properly calibrated and the correct bp values are shown.

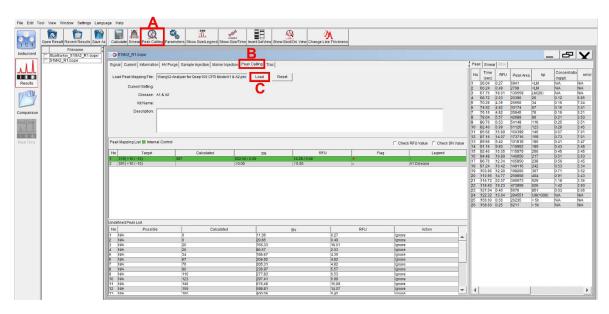


Figure 4-18 Fragment Size Calculation and Peak Calling Selection

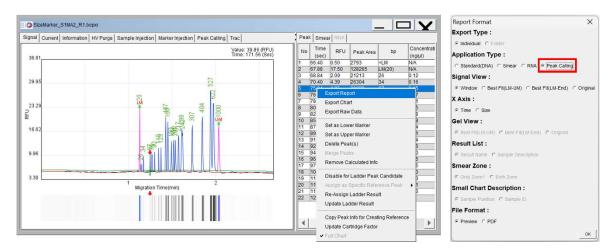


Figure 4-19 Loading Panel and Exporting the Report

RNA Reference Table

RNA factor table modifies the parameters of RNA signal definition for result analysis (18S and 28S). The 18Ss and 18Se are the start and the end of 18S. To modify the value, **Load** the .rfa file from the default directory.

To be effective, the built-in reference tables, RNA-4K.rfa and/or RNA-6K.rfa, must be overwritten. For RNA analysis, please refer to Section 5.3.

RNA Fac	×	
Load	Save As Save	
Item	Time	rt
LM	0	0
189s	0	0
18Se	0	0
2858	0	0
285e	0	0

Figure 4-20 RNA Factor Table

4.3.3 Tool

<u>Calculate</u>

Use the reference marker table to do the base pair calling and quantitative result analysis (Figure 4-21). **Load** the reference marker table and click **OK** to do the calculation. The result file will show the corresponding values of size and concentration. For more information, please refer to Section 4.2.2.

19 Calculate X	Billion file 	- (a 1	* 100-1000	21 mars 34 mars 11 mars	Andator - S Angerman Mane Partor P				×
for Ters Partine Be Forcestate Bij	- Back	North State	An oracle of the second	10100000000000000000000000000000000000		ALCOS NUCLEAR	849 843 843 843 843 843 843 843 844 844 844	NV 100 100 100 100 100 100 100 100 100 10	A COLUMN TO A

Figure 4-21 Calculate Workflow

Recalibrate

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

Conduct **Recalibrate** if any of the following situation occurs:

- The alignment marker has been replaced.
- The cartridge has been stored for more than two weeks since the last execution.
- The software cannot identify the alignment marker correctly.

To recalibrate, choose the voltage and alignment marker that match with the "Method" that will be used (Figure 4-22). For more information, please refer to Section 4.2.1.

Voltage		Alignme	ent Marker Information		
· Big	Alignment Marker	Position	Last Calibrated Date	LM_SN	UM_SN
- 10kv	@ 20-1K	MA-1	2022-OCT-24	587.94	326.09
	C 20-1.5K	MD-1	144	-	104
	⊂ 20-5K	MB-1	NATA	NA	1414
	C Customized	MD-1	NA	NK	FEA.

Figure 4-22 The message box of recalibration

<u>Smear</u>

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

Peak Calling

Peak Calling helps to distinguish the target peaks. To set up the Peak Calling Table, please refer to Section 4.3.2.

<u>Analyze</u>

Analyze is designed to generate a summary report of Nucleic Acid Amplification Test (NAAT) automatically. Use this function for specific applications. For more information, please contact BiOptic Inc.

***Note**: **Analyze** is used to interpret the multiple **Peak Calling** results by using built-in kit information.

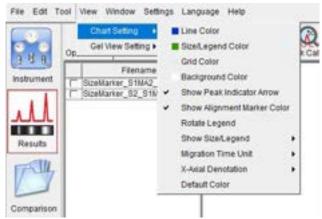


Figure 4-23 Analyze Function on Toolbar

4.3.4 View

<u>Chart Setting</u>: Change the settings of the electropherogram.

Chart Setting is used to set the Line Color, Size/Legend Color, Grid Color, Background Color, and other data displays.



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

Choose to invert the gel image, display the peak line, or adjust the contrast of the gel image.

B B B	Chart Setting + Op Filename SizeMarker_S11442 SizeMarker_S2_S11/	Invert Show Pea Show Alig Peak Core	nme	nt Ma			ator	0		đ	8		
111		Contrast	: 0	5	, 20	1	1 40	- }-	60	ł	ao	1	100
Results		Brightness	10		20	+	1 40	+	60	+	80	×.	100
mparison		MaxM	-	÷			40	*	60		1 80	+	100

In Figure 4-24, A is the original gel image in the system. After tuning the contrast, user may enhance weak peaks as shown in B. C is the inverted gel image, and D is the inverted image after tuning the contrast of C.

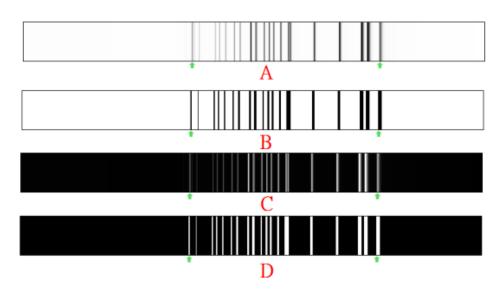
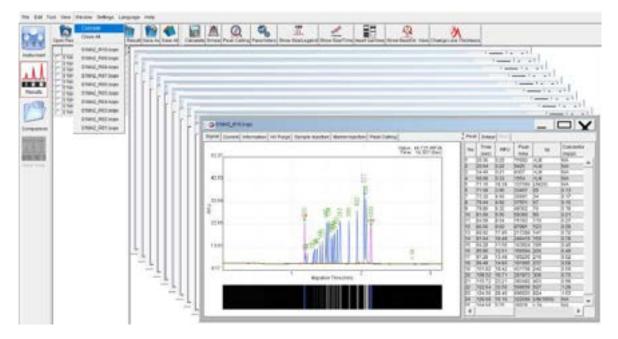


Figure 4-24 Gel Image

4.3.5 Window

All opened files will be listed under Window.

<u>Cascade</u>: Arrange the opened windows in the array format.



<u>Close All</u>: Close all opened windows.

4.3.6 Settings

BIO Change Passwor	d X
Old Password	F
New Password	
Password Confirm	
OK	Cancel

<u>Change Password</u>: Change the password of the operating user.

Figure 4-25 Change Password

Fill in the old password, Password, and Password Confirm fields to change the password. The password must consist of **8-12 characters/numbers (case-sensitive)**.

Preference:

This page is used to configure the default settings of Q-Analyzer such as view method, calibration time, motor settings, etc.

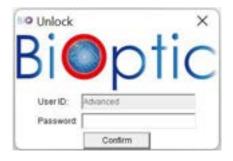
***Note**: Some preference settings might change the default value of the instrument and affect the result of the experiment. It is suggested to complete the professional training from BiOptic Inc. before changing the settings.

Preference	×
General View Calbration Calbration Chers Instrument Motor Sequence Alignment Marker Factor	
Peak ■ Result File ■ Factor - Signal Chart - Smear - RtA - Folder ■ Report - Indvidual	
Folder	OK Cancel

Figure 4-26 Preference Settings

Software Lock:

Software Lock helps to prevent unauthorized access to *Q*-*Analyzer* while user is away. To unlock the software, Login with your user Password.



4.3.7 Language

User can choose between three languages to operate *Q*-*Analyzer*, which are English (Default), Chinese (Simplified), Chinese (Traditional). Relaunch *Q*-*Analyzer* to apply the setting.

4.3.8 Help

Version: Display the version of the software and firmware.

Qsep series needs to be connected to get the information of the firmware (Figure 4-27).

Version Information	X
SW Version: 3.4.3.0.6754 (PID Core Version: 2617 Build Date: 2022/09/27 FW Version: Instrument ID: COM Port: Pressure: Visit: https://www.bioptic.co	
Copyright 2005-2022 (c) BiOpt All rights reserved.	ic Inc.
Technical Support	

Figure 4-27 Version Window

For certain technical support, please contact BiOptic, follow the instructions to Export the file in .tecsupport and send it back. The license file will be provided in .bioptic for user to Import.



Figure 4-28 Technical Help Window

Help: Open *Qsep* series Operation Manual.

<u>Report</u>: Collect error information and generate the report file for further evaluation. When encountering troubles, use the **Report** function to generate the error report and send it to BiOptic via <u>service@bioptic.com.tw</u> for technical support. **Toolbox**: Provide step-by-step troubleshooting process.

- **HV Function Check** will do a series of tests to make sure the current remains stable.
- **Capillary Clog Check** will lower the tray and add pressure to the cartridge. If there is gel drop formed at the cartridge tip, the cartridge is not clogged. For more information, please refer to Section 4.2.1.
- **Purge Function Check** will purge while the cartridge door is opened. Ensure the air is coming out while purging. For more information, please refer to Section 4.2.1.
- Motor Function Check will check motor position and setting condition. Please follow the instructions accordingly. The software will remind user to open the sample door and the cartridge door, and then take out the cartridge from the instrument. Once start, the motors will move the tray holder in different directions, and user can observe motor status during each step.
- **Computer Compatibility Check** will check the software setting environments, including the hardware and software requirements.

4.4 Control Panel

When user launches *Q*-*Analyzer* CFR Mode, the Control Panel displays automatically. There are three major functions on the Control Panel, which are **Main**, **Method**, and **Direct Control** (shown in Figure 4-29).

4.4.1 Main

Main page is the major control to operate Qsep series. The Main pages of other Qsep series, including $Qsep_1$, $Qsep_1$ -Plus and $Qsep_{400}$, are the same as $Qsep_{100}$. Only the picture of the connected instrument will be different for $Qsep_1$, $Qsep_{100}$, and $Qsep_{400}$.

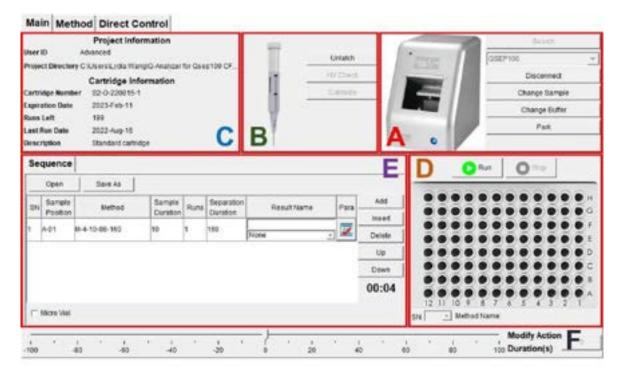


Figure 4-29 Main Page

Function	Description					
A: Instrument Control						
Connect/Disconnect	Connect or Disconnect to <i>Qsep</i> series through the port selected. Once the <i>Qsep</i> series is successfully connected, the image of the instrument will turn into color form, and the Connect button will become Disconnect for manual disconnection.					
Open Sample Door	Open the sample door ($Qsep_{400}$) and move the sample tray to the door ($Qsep_1$).					
Change Sample	Move the 96-well sample holder to the door.					

Change Buffer	Move the holder of the buffer tray and markers to the door.
Park	Position P well of the buffer tray below the cartridge tip to protect the tip from drying out.
B: Cartridge Control	
Latch/Unlatch	Latch or Unlatch a cartridge. To execute a sequence, user must Latch the inserted cartridge. The cartridge information will be displayed, and the picture will turn into color form. Click Unlatch and wait a few seconds before removing the cartridge.
HV Check	The new cartridge must pass the HV Check before running the sequence. For more information, please refer to Section 3.2.
Calibration	The new cartridge must pass the Calibration before running the sequence. For more information, please refer to Section 3.2.

C: Project and Cartridge Information

Show 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 displayed.

D: Sequence Control	
Run	Start a sequence. Run button during a test: Run to start the test, Pause to pause the running test, and Continue to resume the unfinished test.
Stop	End a sequence.

E: 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 3.1.2.

F: Slide Bar (Reduce and Extend)

The remaining time of the proceeding action can be extended or shortened by dragging the slide bar at the bottom of the window. For instance, the time of Separation & Detection is running out, but the sequence has not been completed yet; to extend the time, drag the slide bar to the right (e.g., 40). The amount of the adjustment time will be shown on the button. Click the button to confirm the change. The action of Separation & Detection will be extended 40 seconds more immediately.

-100	'	। -80	1	ا -60	'	ا -40	1	। -20	1) 0	' I 20)	ا 40	ı	۱ 60	1	। 80	1	Modify Action 100 Duration(s)	
**		.					1 ¹	6							la a . a			al a		_

***Note**: Only the remaining time of the action in progress can be extended or shortened.

4.4.2 Method

Method tab is for viewing, editing, and creating the method (Figure 4-30).

*Note: Only Advanced Analyzer can create and edit methods.

- 30 S	i New Method 95 w	-				
Ship	Action	High Voltage	Duration	Pestion		U
					-	Doe
						Ad
						Insi
						Dele

Figure 4-30 Method Page

Create a method by clicking **New Method** in the Method tab. By clicking **Add** on the right, user can add an action. Select the action wanted and enter the duration time. After selecting the action position, add another action row. User needs to select a position for every action except **Sample Injection** since the sample position is controlled by the Main tab.

Method is the combination of several steps such as Purge, High Voltage Purge, Sample Injection, Marker Injection, Separation & Detection, End, Pause, Purge Check, Wash Step, and Clean Step.

Function	Description
Purge	Use the air pressure to refill the capillary.
High Voltage Purge	Use the air pressure with high voltage to refill the capillary.
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	Start the capillary electrophoresis analysis. *Note: Only one Separation & Detection action is allowed in a Method.
End	Declare the last commend of a Method.
Pause	Park the cartridge tip at the Park position.
Purge Check	Ensure the air tube is not clogged before use or clean the gel which was accidentally sucked into the air tube after use.
Wash Step	Wash the cartridge tip at the Wash position.
Clean Step	Clean the cartridge tip at the Clean position.

4.4.3 Direct Control

Perform Purge or HV Purge under Direct Control.

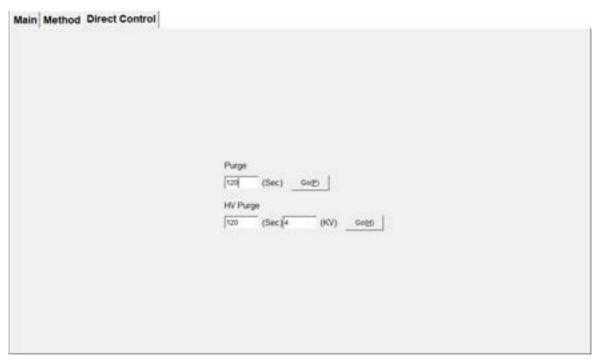


Figure 4-31 Direct Control Page

Purge: Set the time duration for purging.

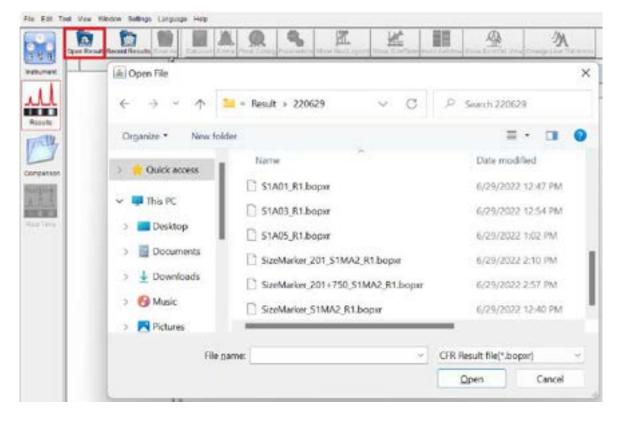
HV Purge: Set the time duration and voltage for purging.

5. Result Analysis

5.1 Result Display

By clicking the **Result** function, user can analyze the data from *Qsep* series. The Result function can demonstrate the chart or edit the size from the raw data of the capillary electrophoresis.

Open the result file by clicking **Open Result** icon on Result window tab or from **File** on the Menu Bar. The browser helps to select multiple files and displays the chart of the collected signal data. According to the default settings, the CFR result file is saved as "**.bopxr**" file, and the result directory of Q-Analyzer CFR Mode is stored at <Workspace>\Result\<Project name>.



*Default <Workspace> is C:\Users\<username>\Q-Analyzer CFR Mode

The latest result will be shown on the Main Panel after each Method complete, and the previous one will be closed automatically.

If the user opens multiple results, the file order can be changed by right clicking on "Filename" (the following pictures) and re-arranging in "Sample, Runs, Test Time, Cartridge, Channel" order. Click the first row to change the order.

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			-	2		
	Sangle	Runs	TestTime	Cattidge	Channel /	Filename
H			TestTinte 2022-May-16 12 25(Cattidge F3-0-404-225415-1	Channel /	
	Sample	Runs 293 294		and the second se	Channel /	Filename F1_S15801_R2_0 F3_S15801_R1_0
	Sample SM-01	293	2022-May-16 12 251	F3-0-4CH-225415-1	Channel / 1 1 2	F3_5158401_R2_0
	Sample SM-01 SM-01	293 294	2022-May-16 12 25(2023-May-16 12 21)	F3-0-404-229415-1 F3-0-404-229415-1	Channel / 1 2 2	F3_S158401_R2 F3_S158401_R1 F3_S158404_R2
LLLL	Sample SM-01 SM-01 SM-04	293 294 293	2022-Maj-16 12 25(2025-Maj-16 12 21(2022-Maj-16 12 21(2022-Maj-16 12 25)	F3-0-40H229415-1 F3-0-40H229415-1 F3-0-40H229415-1 F3-0-40H229415-1	Channel / 1 2 2 3	F3 5158A01 R2 F3 5158A01 R1 F3 5158A01 R1 F3 5158A04 R2 F3 5158A04 R1
LULL	Sangle Sal-01 Sal-01 Sal-04 Sal-04 Sal-04	293 294 293 294	2022-May-16 12 251 2022-May-16 12 251 2022-May-16 12 251 2022-May-16 12 251 2022-May-16 12 251	F3-0-404-228415-1 F3-0-404-228415-1 F3-0-404-228415-1 F3-0-404-228415-1	Channel / 1 2 2 3 3	F3_S158401_R2 F3_S158401_R1 F3_S158404_R2
LELLEL	Sangle S86-01 S86-01 S86-04 S86-04 S86-04 S86-07	293 294 293 294 294 294	2023-Maj-16 12 25(2023-Maj-16 12 21) 2022-Maj-16 12 25(2022-Maj-16 12 25) 2022-Maj-16 12 25(2022-Maj-16 12 25)	F3-0-404-228415-1 F3-0-404-228415-1 F3-0-404-228415-1 F3-0-404-228415-1 F3-0-404-228415-1	Channel / 1 2 2 3 3 4	F3 5158401 R2 F3 5158401 R1 F3 5158404 R2 F3 5158404 R1 F3 5158404 R1 F3 5158407 R2

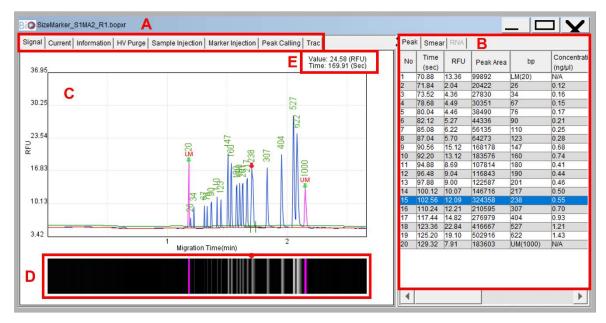


Figure 5-1 Result Display

Function	Description
A : Tabs on Result Window	 Include Signal, Current, Information, HV Purge, Sample Injection, Marker Injection, Peak Calling, and Trac. Signal: View the full electropherogram image. Current: View the current of separation. Information: View detailed information of the result, including time, cartridge, sample, method, etc. HV Purge: View the current of HV purge. Sample Injection: View the current of sample injection. Marker Injection: View the current of marker injection. Peak Calling: View the peak calling result if set. Trac: Keep track of the modification history. Select the version and right click to revert to it.

B : Peak Table	Include Time, RFU, Peak Area, bp, Concentration ($ng/\mu l$), nmol/L, Peak Start (sec), Peak End (sec), S/N, FWHM (sec) and Area (%). The value of bp and concentration will only be displayed after the calculation is performed.
C: Electropherogram	The green arrows indicate the alignment marker(s) of the result; the red arrow indicates the selected peak in the table.
D : Gel View	The simulated gel view of the result.
E: Raw Data Point	Display the raw data of the point selected.

Right click on the peak table for more functions.

N/A S608 Export Report Export Raw Data Set as Lower Marker
N/A S6.08 17.19 18.18 Export Report Export Chart Export Raw Data Set as Lower Marker
N/A N/A N/A N/A N/A N/A N/A 36.23 36.06 17.19 18.18 Export Report Export Chart Export Chart Export Chart Export Raw Data Set as Lower Marker
N/A N/A N/A N/A 36.08 17.19 18.18 Export Report Export Chart Export Chart Export Chart Export Raw Data Set as Lower Marker
N/A N/A N/A 36.08 17.19 18.18 Export Report Export Chart Export Chart Export Chart Export Raw Data Set as Lower Marker
N/A N/A 36.23 36.08 17.19 18.18 Export Report Export Chart Export Chart Export Raw Data Set as Lower Marker
N/A 36.23 36.08 17.19 18.18 Export Report Export Chart Export Chart Export Raw Data Set as Lower Marker
36.03 36.06 17.19 18.18 Export Report Export Chart Export Chart Export Raw Data Set as Lower Marker
36.08 17.19 18.18 Export Report Export Chart Export Chart Export Raw Data Set as Lower Marker
17.19 18.18 Export Report Export Chart Export Raw Data Set as Lower Marker
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Export Chart Export Raw Data Set as Lower Marker
Export Chart Export Raw Data Set as Lower Marker
Export Raw Data Set as Lower Marker
Export Raw Data Set as Lower Marker
Set as Lower Marker
Set as Upper Marker
Delete Peak(s)
Merge Peaks
Remove Calculated Info
Disable for Ladder Peak Candidate
Assign as Specific Reference Peak
Assign as opeulic Reference Peak
Re-Assign Ladder Result
Update Ladder Result
Opdale Ladder Result
Copy Peak Info for Creating Reference

•

Function	Description
Export Report/Chart/Raw Data	Export result after analysis. Exported raw data will be saved in .csv.
Set as Lower/Upper Marker	Assign the selected peak to be lower or upper marker. If the lower or upper marker fails to assign correctly, the size and concentration results will be affected.
Delete Peak(s)	Remove redundant peak(s).
Merge Peaks	Merge two or more peaks to become one or combine minor peak(s) with the major one. The peak area will add up and the peak migration time will then be further defined.
	*Note : Make sure to execute the Calculate function after merging peaks to get more accurate results.
Remove Calculated Info	Remove the original calculated information. Calculate with a saved reference marker will obtain information again.

Copy Peak Info for Creating Reference	Copy the data to create a reference marker table.
Update Cartridge Factor	Renew the following running results after assigning the correct lower marker and upper marker.
Full Chart	View the full electropherogram image.
For size marker only	
Disable for Ladder Peak Candidate	Disable for Ladder Peak Candidate ignores the peak that is not included in the size marker.
Assign as Specific Reference Peak	Assign as Specific Reference Peak to correct the peak size that is wrongly identified.
Re-Assign Ladder Result	Re-assigns the other size markers accordingly after correcting the peak size with Assign as Specific Reference Peak.
Update Ladder Result	Update size marker after re-assigning the peaks.

5.2 Smear DNA Analysis (gDNA QC for NGS)

The **Smear** function helps to understand the major size and the distribution of the fragmented genomic DNA.

*Note: To use the Smear function, the result must be calculated with reference marker.

***Note**: Zone2 will not be displayed by default. To display Zone2 on the result file, click "Zone2" directly.

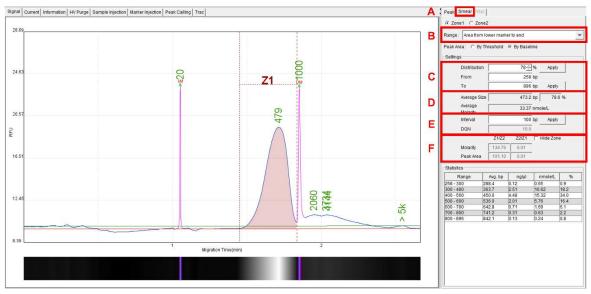
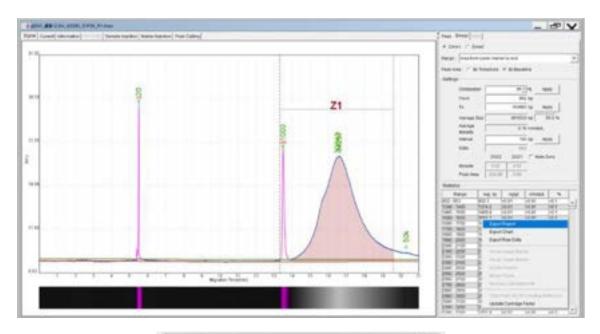


Figure 5-2 Fragmented Genomic DNA Sample Result

To conduct the analysis, follow the steps below.

- 1. Click the Smear tab (Figure 5-2 A) or select Smear on the Toolbar.
- 2. Define the peak range. It could be between lower and upper markers or from lower marker to the end (Figure 5-2 B).
- 3. Enter the percentage of the target distribution (e.g. 50%) or the size range of the target region (Figure 5-2 C), and then click Apply.
- 4. Figure 5-2 D shows the detailed information of the assigned region, including the average size and concentration of the region (nmol/L).
- 5. Modify the Interval of the size range if necessary, and then click Apply to apply the setting and get more details of the distribution (Figure 5-2 E).
- 6. Edit the other zone by selecting Zone2. The editing rules are the same as mentioned above. This function helps user to get the ratios between Zone1 and Zone2 which provide more information about these two zones (Figure 5-2 F).
- 7. All information about the distribution will show up in the below table.
- 8. Right click on the right panel to export the report (Figure 5-3). User can export a report with two zones information, select "both zone" for Smear Zone.



Report Format	×
Export Type :	
4 Individual C Folder	
Application Type :	
← Standard(DNA) I® Smear I ⊂ RNA I ⊂ Peak Calin	9
Signal View :	
Window ← Best FII(LM-UN) ← Best FII(LM-End)	C Original
X Axis :	
R Time C Size	
Gel View :	
€ Best FI(LM-UM) € Best FI(LM-End) € Original	
Result List :	
🕫 Result Name 🔎 Sample Description	
Smear Zone :	
C Only Zone1 🖉 Both Zone	
Small Chart Description :	
# Sample Position /* Sample ID	
File Format :	
Preview C PDF	
	ок

Figure 5-3 Export the Smear Report

5.3 Total RNA Quality Analysis

To understand the quality of RNA sample, assign 18S and 28S to obtain the RNA quality number (RQN). The RNA tab in the result file will be enabled when running sample with RNA cartridge and RNA method chosen (Figure 5-4).

Application	C DNA FROM	C Oypan	C Protein
Analysis Type	Cualitative Countrative Same	ple Volume(x)	
Alignment Marker	P (RNA-LM/MC-1) - 20	NIA C Reduce (F A	iormal (* Enhance
Carthidge Type	R1 + RNA Cartridge(Shelf Life: 4	Months)	
Sample Concentration	" High (>50 ng/ul)	(* Regular (5 - 50 ng/ul)	C Low (<5 nglul)
Method	Description	Range	Remark
R-4-10-04-480	Sample Injection 4kv 10s Separation 4kv 480s		Total RNA QC
R-4-10-06-300	Sample Injection 4kv 50s Separation 5kv 300s	20nt-1000mt	ssRNA & dsRNA Fragment Analysis
T-HVPurpe-04-120	Gel Retill with HV on for 12	98	
T-Purge-120	Gel Refill without HV for 12	0s	
High Voltage Purge	← Purge I [—] Purge Modification		
Customized Method	4		

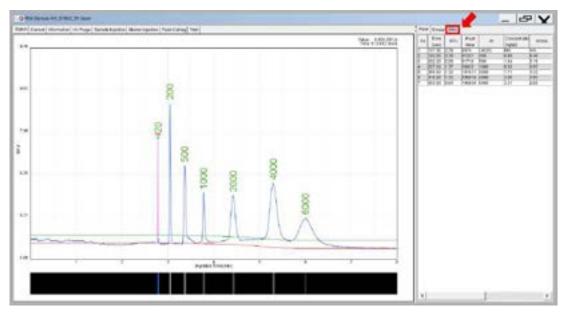
Figure 5-4 Choose RNA in Method Selector

By checking the box of **Auto Assign rRNA** in Para, user can not only choose in between Eukaryotic and Prokaryotic, but define SSU/LSU based on the needs. User defined SSU/LSU will be shown on the exported files.

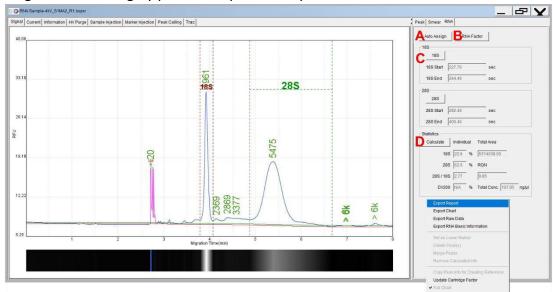
1" Baselite Pado			
	Konnence Namer Faller Conside Size Marker Conside Size M	~	Eronen
		_	- Course -
" Dreat Calling	Constan [02] % Chiefe - -		trees
9 Ada Asaya M 11 Engat Angert	Contraction of the local distance of the loc		er 1 e

Figure 5-5 Define the Small Subunit (SSU) and the Large Subunit (LSU)

1. Select the **RNA** tab to calculate the RQN.



- 2. Click **Auto Assign** to assign 18S/28S RNA (Eukaryotic) or 16S/23S RNA (Prokaryotic) (Figure 5-6 A).
- 3. If the lines do not properly cover the RNA area, drag the lines manually to cover the designated areas.
- 4. To save the settings, click **RNA Factor** to edit and save the RNA factor table (Figure 5-6 B).
- 5. Click 18S/28S or 16S/23S button to hide or show (by default) the rRNA assignation on the electropherogram (Figure 5-6 C).
- 6. Click **Calculate**, the 28S/18S or 23S/16S ratio and the RQN (RNA Quality Number) will show (Figure 5-6 D).



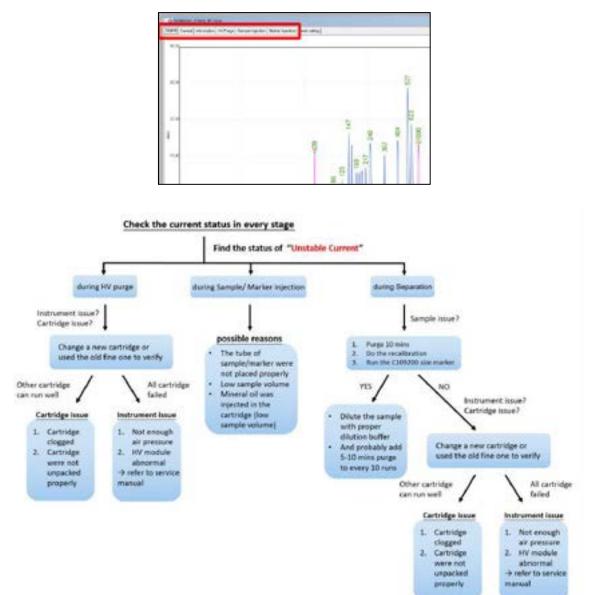
7. Right click on the gray panel to export the report.

Figure 5-6 RNA Quality Check Panel

FAQs

Q. The current is unstable. How do I resolve this?

A. Most unstable currents are due to samples and cartridges. Check samples and cartridges first to rule out the issues. Next, check the current of each stage to verify possible causes.



Q. Peaks are migrating later than usual, and the upper alignment marker is missing. How do I resolve this?

A. Recalibrate the cartridge to update the cartridge factor. The separation time of the sequence will be adjusted accordingly. Other than that, check the method selected and the current of each stage. If the current is stable, it could be the environmental issue such as temperature.

- 10°C: Separation current speeds up 1.2X
- 10-19°C: Separation current speeds up 1.1X
- 20-29°C: The migration time remains the same at room temperature
- 30+°C: Separation current slows down 0.9X

Preference			×
General	C Stop Sequence if Motor Init Fail		
Calibration Others	🗂 Run Sequence in Office Bode if Instrument Support		
Sensor	F Auto Save Sequence after Run		
- Alignment Marker - Factor	F Apply Separation HV Adjustment with Temperature Condition	30+(°C) 🔻	
Peak Result File	Purge Carbidge every 4 💌 Runs	20-29(*C)	
Signal Chart Smear	T Use Alternative Dye Cartridge	10-19(°C) 10-(°C)	
RNA Folder Report Individual	T Run Sample in Column-by-Column Order		
	*Settings in blue character need to restart the program to take effe	ст ок	Cancel

If the current is low or erratic, the cartridge may be clogged. It is recommended to do a Clog Check ($Qsep_{100}$ only) or 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 do I resolve this?

A. Use another cartridge to confirm the situation. RFU near zero indicates that there is no signal detected. The major issue may come from the light pathway (Ex/Em). Please contact your local distributor or BiOptic.

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

A. Perform a size marker run to confirm if the issue was due to samples. Check the sample concentration and sample injection current. If the sample concentration is too low, select "Low (< 0.1 ng/ μ l)" on Method Selector or use N1 High sensitivity cartridge to analyze samples.

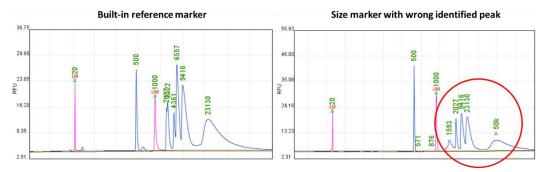
Method Description Range M-0-10-05-300 Sample Injection Bix 10s 193p-1000pp Separation Six 300s Event Resolution 25p-85p M-8-10-06-200 Sample Injection Bix 10s Separation Bix 300s Event Resolution 25p-85p M-8-10-06-200 Sample Injection Bix 10s Separation Bix 250s Event Resolution: 40p-100p	C Probin C Behance LevelFragment -5.1 mptof Remain For low concentration sample For low concentration sample
Algoment Stamer & 20 10034-1) (100 C Reduce A Namue Camtops Type (11) (100 C Reduce A Namue Sample Concentration C regin (Request +10 equil) (100 Regular (Progmant 0.1 - 10 equil) (100 Namue 0.1 - 10 equil) (1	Low (Fragment, =0.1 equal) Remark For low concentration sample
Carthdos Type 51 Fign Recenter Carthdos Shell Like 6 Hanths Sample Concertration C High (Fragment + 10 eg/u) Method Method Method Method Mange Hid-10-00-300 Sample Injunction Tite 10s Method Method Sample Injunction Tite 10s Method Method Method Sample Injunction Tite 10s Method	Low (Fragment, =0.1 equal) Remark For low concentration sample
Bample Concentration Plags (Fragment = 10 mpl/) Plags (Fragment 0.1 =	Remark For low concentration sample
Method Description Hange M-6-10-05-300 Sample injection litis 10s 10sp-1000cp Separation litis 200e Bent Resolution 21p-litip M-6-10-06-300 Sample injection litis 10s 10sp-5000cp Separation litis 200e Bent Resolution 40p-1000cp Separation litis 200e Bent Resolution 40p-100p Separation litis 200e Bent Resolution 40p-100p Separation litis 200e Bent Resolution 40p-100p	Remark For low concentration sample
M-0-10-05-300 Sample injection like 10s 101p-1000tp Separation file 201e Elevel Resolution 2tp-litp M-8-10-06-300 Sample injection like 10s Separation file 201e Beet Resolution 2tp-litp M-8-10-06-300 Sample injection like 10s Separation like 201e Beet Resolution 40p-100p H-6-10-10-120 Sample injection like 10s	For low concentration sample
Separation the 200e Beel Resolution 25p-85p M.6.10.06.200 Sample hydrolen the 100p-5000be Separation the 200e Beel Resolution; 45p-100p H-6-10-10-120 Sample hydrole the 10s 10p-5000bp	
Separation Bis 200x Best Resolution: 4bp-10/bp 849-10-10-120 Sample Injection Bis 10s 10p-5000p	For low concentration sample
	For low concentration sample
T-HVPurge-08-120 Get Rotell with IW/ on Ror 122s	
1 Purge-120 Get Retal without HV Rx 120s	
# High Votage Purge (* Purge Vodification	

Next, check the electrolyte salt concentration of the samples. If the salt concentration is too high, please dilute the samples with 0.1X dilution buffer.

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

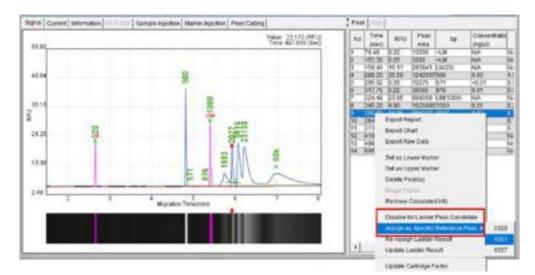
A. Assigning alignment marker and size marker properly is important otherwise the sizing result may be affected. Users can recalibrate the cartridge to update the cartridge factor for the following runs or manually assign the marker(s).

1. Load a built-in reference marker to confirm if the software identifies correctly.

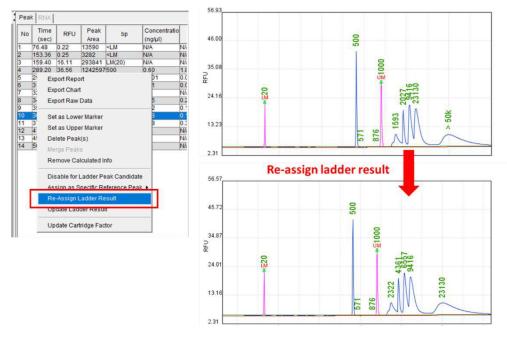


Right click on the peak and disable/assign as a ladder peak.
 <u>Disable for Ladder Peak Candidate</u>: If software assigns a peak that is not included in the size marker, use Disable for Ladder Peak Candidate to ignore the peak.

 <u>Assign as Specific Reference Peak</u>: If the software identifies size marker wrongly, use Assign as Specific Reference Peak to correct the peak size.

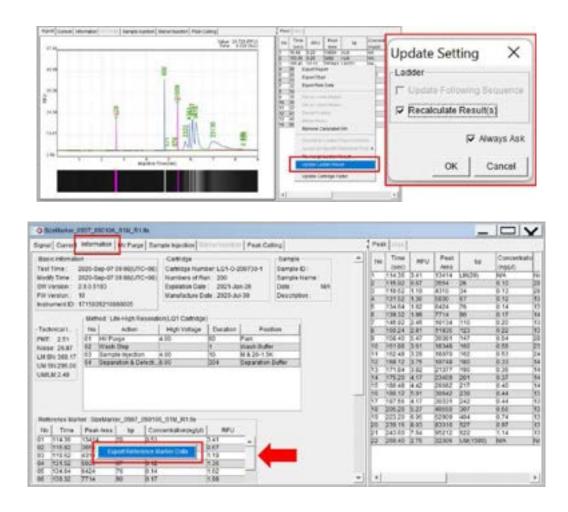


3. Right click on the peak table and select Re-Assign Ladder Result.



4. Right click on the peak table and select Update Ladder Result.

Select **Update Following Sequences** and **Recalculate Result(s)** to update size marker after re-assigning the peaks. After recalculating the size marker file, go to information tab of size marker file, right click the Reference Marker table. Select **Export Reference Marker Data** to export the reference marker file for later calculation.

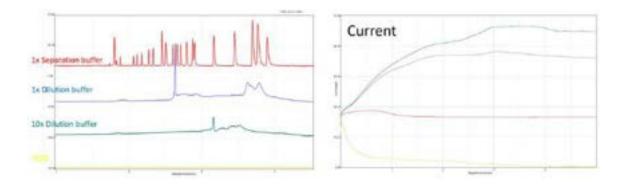


Q. What if one of the 4 Channels shows differently than the others?

A. Make sure all sample volumes are over 20 μ l and check the current of each stage first. Take four 0.2 ml PCR tubes and add 100 μ l separation buffer each. Place them in wells A-01, A-04, A-07 and A-10. Extend the sample injection time to 60 seconds and start the analysis. If the current is unstable, long purge to unclog the cartridge. If the issue remains, the channel may be damaged. To continue using the cartridge, place tubes that are filled with dilution buffer in the corresponding wells for the damaged channel.

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

A. The peak pattern will become abnormal, and the current will be unstable if water or dilution buffer is used for separation.



If separation buffer is used to dilute samples, though the current may be okay, the peak pattern will become abnormal.

