BiOptic Inc.

Qsep Series Operation Manual

--- Software

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Welcome

Thank you for purchasing *Qsep* Series capillary electrophoresis system. Now you can experience this high-performance, fully-automated and easy-to-use system.

Copyright and Trademarks

Qsep™ and *Q-Analyzer*™ are registered trademarks of BiOptic Inc.
Microsoft Windows is registered trademarks of Microsoft Corporation.
Product and company names listed are trademarks or trade names of their respective companies.

Symbols of *Qsep* Series

Symbol	Description
CE	CE mark for European Conformity.
FC	FCC mark of the United States Federal Communications Commission.
UL	Underwriters Laboratories (UL LLC) Safety Certification

Limitation of Liability

Qsep Series, *Q-Analyzer*™ software, and all related reagents are designed for the use of electrophoresis analysis in general biochemistry laboratory. If you use it for profit, diagnosis, or other non-research purposes experiments, the company is not responsible for the accuracy and reliability of the result and the safety of operation.

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 loss resulting from not complying or familiar with the manuals, or improper operation of the devices.

Applications

Genetic Profile Screening
Amplified Fragment Length Polymorphisms
Bacterial/Viral Genotyping and Identification
DNA Fragment/PCR Product Analysis
Rapid Analysis of Genomic DNA Samples
RNA Analysis
Protein Analysis

Packing List

Each *Qsep*™ Series Instrument package comes with the following:
Qsep™ CE Instrument
Qsep™ Standard and High resolution cartridge Kit (C105200)
Power Cord with Adapter
USB Cable
Software Key (Basic edition)
Installation Disc including *Q-Analyzer*™ and *Qsep*™ Operation Manual
***Note**: The Software key contains 8GB storage space, *Q-Analyzer*™ and *Qsep*™ Series Operation Manual, including Hardware version and Software version.

Cautions

Qsep™ Series are capillary electrophoresis instruments driven by high voltage. Carefully read and thoroughly 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 device and voltage sources.

1. Only the components and consumables provided by BiOptic Inc. are suggested to use.
2. **DO NOT** perform the following actions:
   - Disassemble *Qsep*™ Series instrument and its associated parts
   - Bumps or jolts to *Qsep*™ Series
   - Move the instrument or remove any connected external equipment, such as computers and air pumps, while *Qsep*™ Series are in action
   - Open the sample door or cartridge door while *Qsep*™ Series are in action
   - Remove the cartridge while *Qsep*™ Series are in action
   - Power off *Qsep*™ Series before confirming *Qsep*™ Series are inactive
3. Store the cartridge upright; do not lay the cartridge on a table horizontally or place it upside down.
4. Store the cartridge in the calm-shell container (Cartridge needs to be stored in a proper environment as the instructions of cartridge unpacking guide to maintaining its lifetime and quality).
5. Ensure the cartridge is taking out from *Qsep*™ Series before shutting down or disconnecting instrument. Or ensure the cartridge is placed at **PARK** position before shutting down or disconnecting *Qsep*™ Series. Failure to do so will cause dryness of the gel at the cartridge tip, which will damage the cartridge.
6. Before operating *Qsep*™ Series, ensure the buffer tray is placed properly.
7. Dilute the PCR DNA test sample to $\frac{1}{20}$ with our dilution buffer before running the test for obtaining better result and retaining the lifetime of the cartridge. You can adjust the dilution ratio after primary test runs.
8. Ensure that the test sample, alignment marker, cartridge, and buffer tray have been removed from the instrument before transporting or shipping.
9. If the buffer solution spills out on the platform inside the instrument, wipe it with tissue and clean it up immediately.
10. Ensure that the main power of the instrument has been shut down and all

the test materials have been removed before cleaning *Qsep*™ Series.

11. User can also use N_2 gas as an air source to ensure the air quality and prevent damages to the devices caused by dirty air.
12. Make sure to press the plastic connection ring before pulling the air tube out from the back of the instrument.
13. Release the condensation water in the external air pump periodically and check the water level before each use to avoid the mist damage the devices of *Qsep*™ Series.
14. To cut off the power, please disconnect the power plug.
15. Please clean the instrument with a dry cloth. For other maintenance, please contact the local distributor.
16. Please use the MAINS power cord within the adequate rating.
17. The conditions of transportation and storage: Temperature -30 to +60°C. Humidity 20 to 80% RH, non-condensing.

***Note**: Strongly suggest performing this step whenever you start to operate *Qsep*™ Series.

⚠ **Warning:** *Qsep*™ Series are high-voltage Multiple-channel electrophoresis system. Please follow the operation manual and laboratory safety guidelines for system operation. Do not remove covers. For operation and safety questions, please contact BiOptic Inc. at the official website or with your local BiOptic representatives.

1. System Overview

Qsep™ Series are a fully automated CE system developed by BiOptic Inc., which uses pen-shaped disposable gel-cartridges to improve efficiency. Time-consuming manual procedures such as gel preparation, sample loading, and capillary changing are no longer required. Further, the information will be obtained easily with the fully-automated *Qsep*™ Series. $Qsep_1$™, $Qsep_{100}$™ and $Qsep_{400}$™ are designed to accommodate the standard 96-well and 8 & 12 well PCR strip, respectively.

Qsep™ Series's compact design helps you 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 painful procedure that requires well-trained operators. No more worries 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 at amazed with the power of *Qsep*™ Series.

⚠ **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-program Method and Cartridge

1.1.1 Pre-program Method

Qsep™ series are designed to work with the special-designed cartridges provided by BiOptic Inc.. The modularization design of cartridges makes the time-consuming gel preparation no longer be required. For the cartridges, we provide a various specific testing protocol, so-called “Method” which integrates the sequential steps to accomplish the test. The various cartridges and pre-programmed Methods are utilized in performing the CE experiment that corresponds to the size range and concentration of the sample. Additionally, users can create their own Methods according to their preference.

In the Method selector (Figure 1-1), the user can check the Methods corresponding to suitable size range, alignment marker, and the cartridge type. If the latch function is done, *Qsep*™ series will automatically detect the type of the cartridge in use and only display the suggested Methods according to the selected cartridge type.

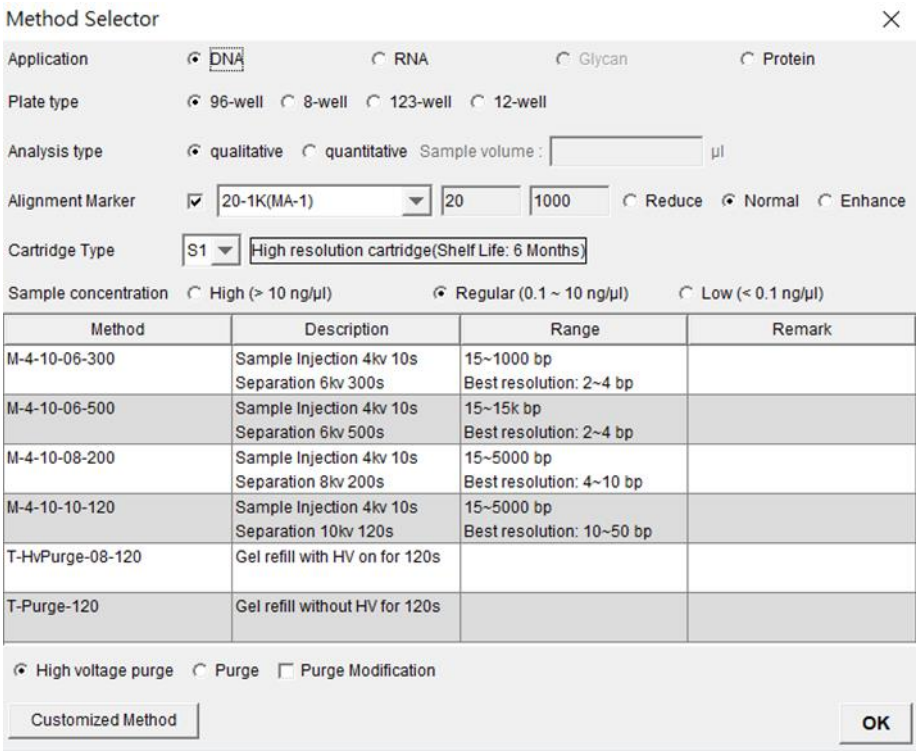


Figure 1-1 Method Selector Tab

1.2 System Installation

1.2.1 Software Requirements

	Minimum	Recommended
OS	Microsoft® Windows 7 32-bit or 64-bit Microsoft® Windows 8 32-bit or 64-bit Microsoft® Windows 8.1 32-bit or 64-bit Microsoft® Windows 10 32-bit or 64-bit	
HDD Space Required	500 MB	1 GB

2. *Q-Analyzer* Installation Instruction

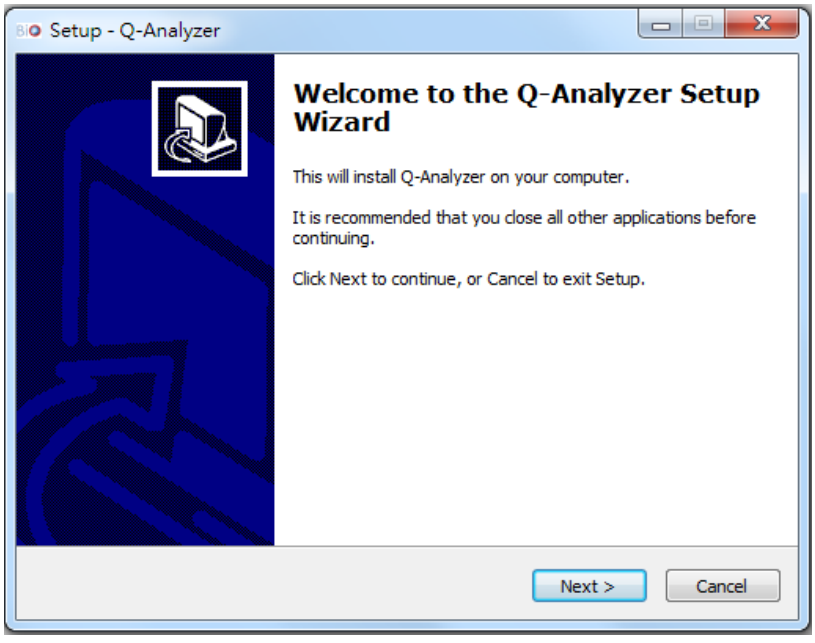
A suitable operating environment is essential to ensuring the best performance of *Qsep*™ Series.

2.1 Software Installation

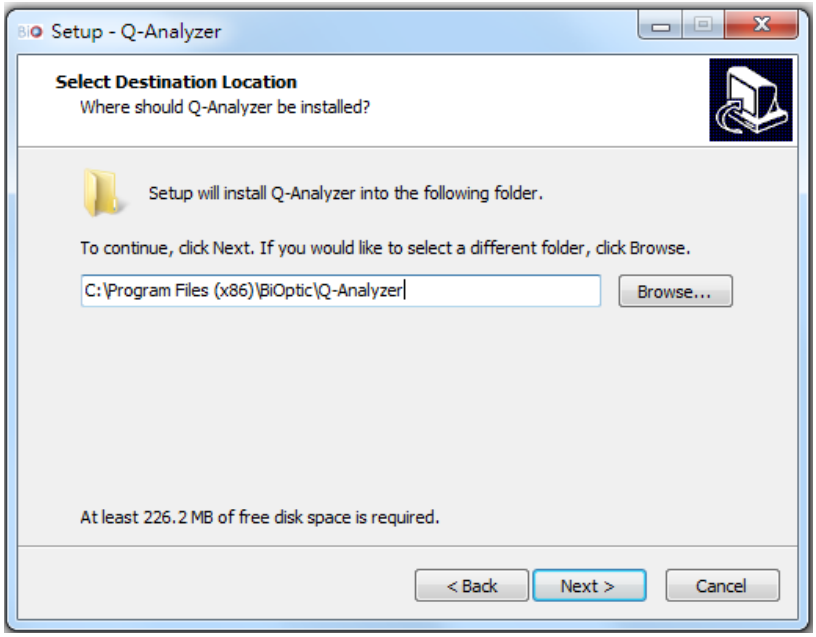
Q-Analyzer™, including Q-Analyzer and Q-Analyzer for Qsep400, are the software that specially designed to operate *Qsep*™ series. The software key will be found in the package. Click "**Q-Analyzer-Setup.exe**" for $Qsep_1$™ or $Qsep_{100}$™ and "**Q-Analyzer for Qsep400-Setup.exe**" for $Qsep_{400}$™ to begin the installation.

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

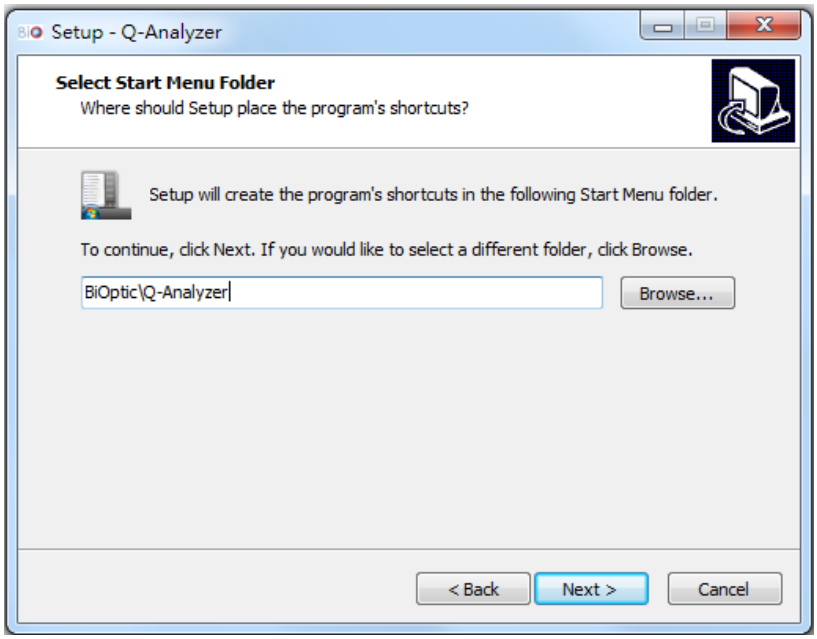
The installation steps are as follow:
Step 1. Click Q-Analyzer-Setup.exe to start Setup Wizard



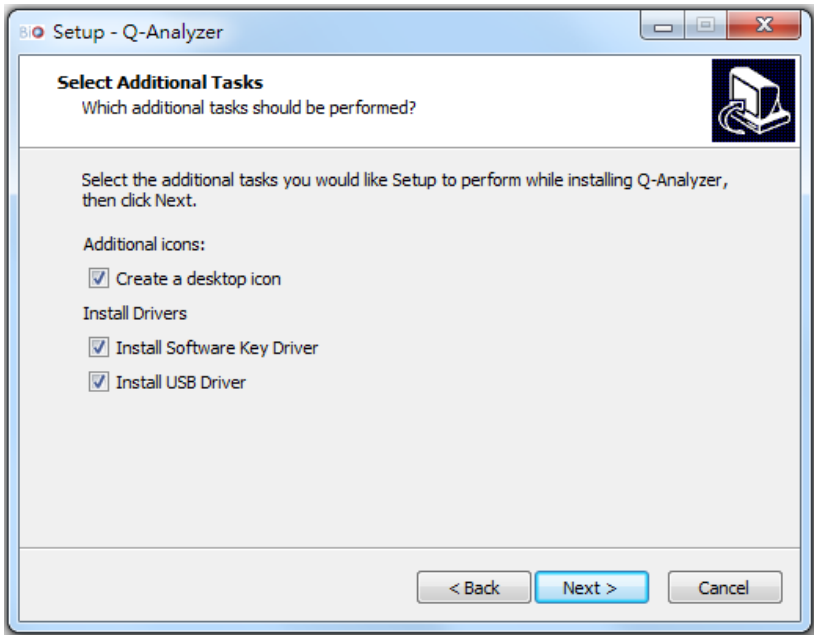
Step 2. Specify the default data archive folder



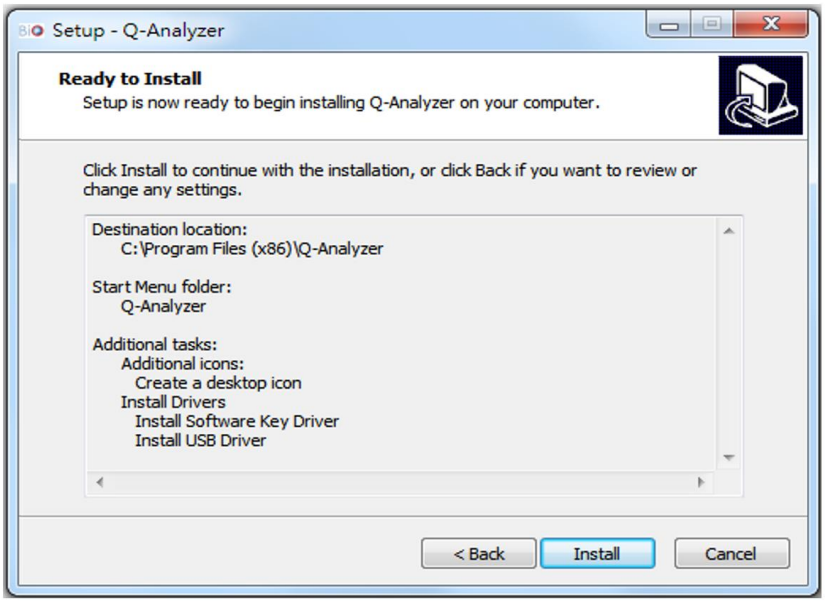
Step 3. Select the program destination



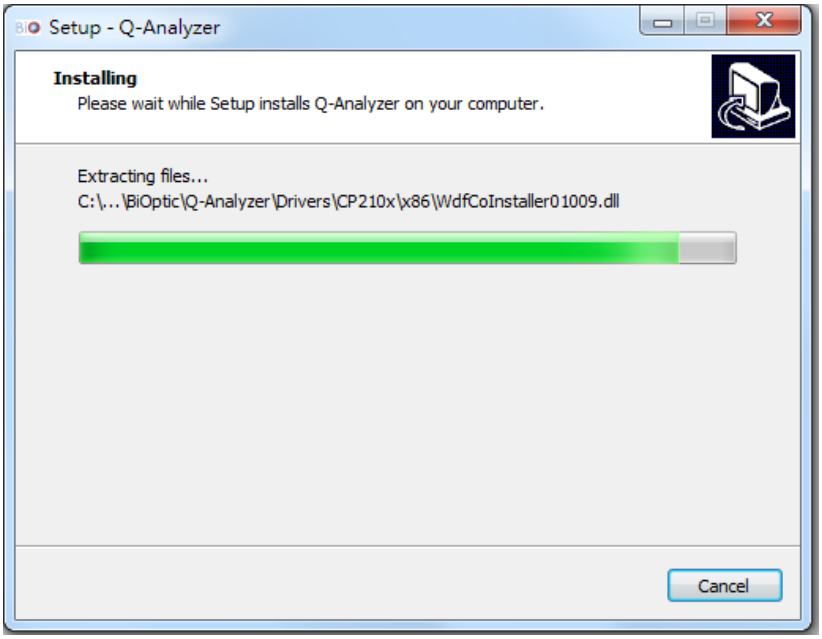
Step 4. Create a desktop icon and select the driver you wish to install



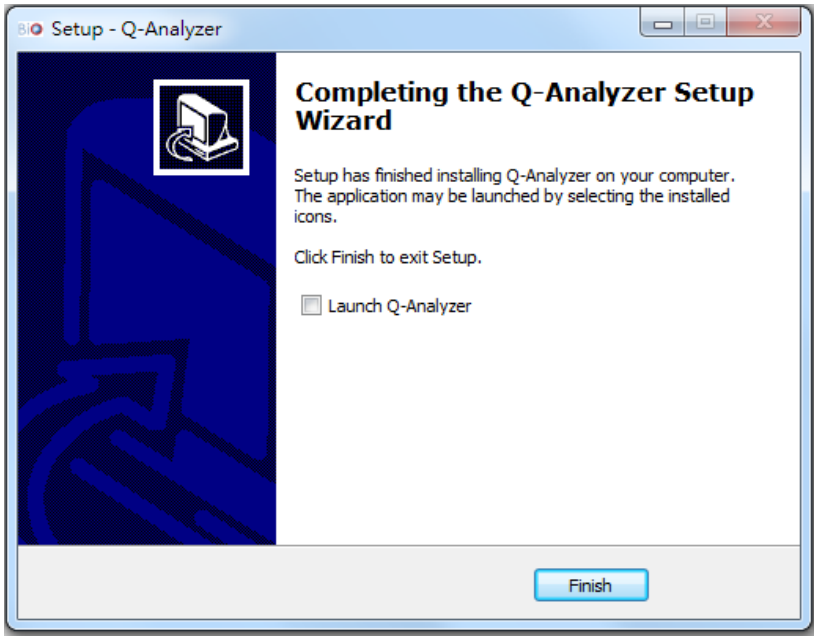
Step 5. Verify the settings of the installation and click Install to start the installation



Step 6. *Q-Analyzer*™ installation is in progress



Step 7. The installation is completed and *Q-Analyzer*™ is ready to be launched



After launching *Q-Analyzer*™, the **Main Window** appears on the screen. The instrument page is used to communicate with the device (Figure 2-1). For the different instruments, the picture of instruments will show the corresponding pictures when connecting *Qsep* Series.

The software will create a default project named according to the date automatically.

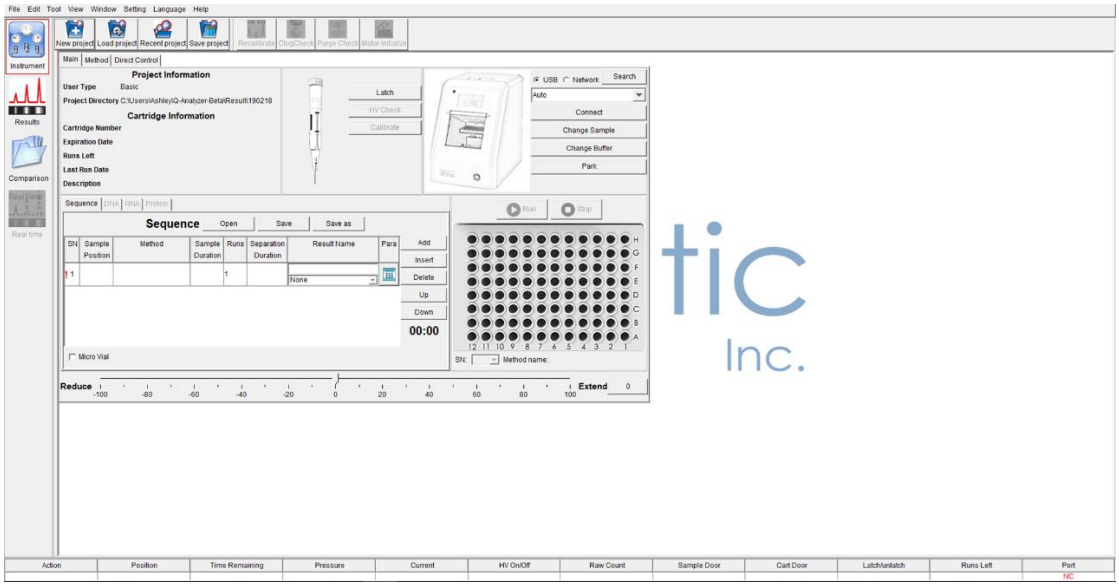


Figure 2-1 Software overview

2.2 Software Mode

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

Q-Analyzer™ Setup has combined with these three programs, so there is no need to install them separately. Please double-click the icon and follow the on-screen instruction to install it.

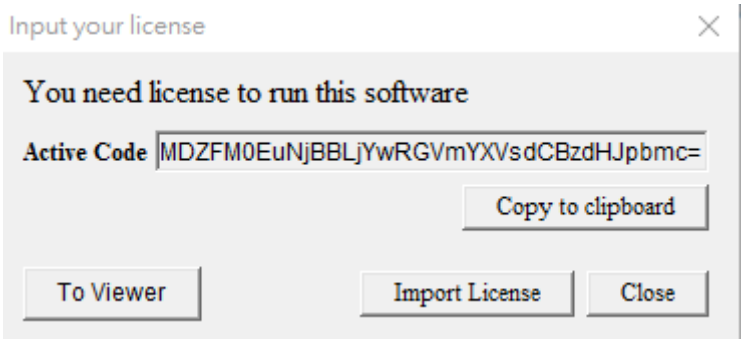
When double clicking *Q-Analyzer*™ icon, the software will automatically detect the status and launch the respective program according to the condition below. (*Q-Analyzer*, *Q-Editor*, or *Q-Viewer*)

Software Level table

Condition / Mode	Software Key (Advance) detected	Software key (Basic) detected	Instrument connected	*Q-Editor* License	None
Password	✓	✓			
Q-Analyzer (Advance)	✓				
Q-Analyzer (Basic)		✓	✓		
Q-Editor				✓	
Q-Viewer					✓

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

Without *Qsep*™ Series or the software key, user can click **To View** to start the *Q-Viewer*, after launching *Q-Analyzer*™. The *Q-Viewer* is only used to display the result file.


Input your license
You need license to run this software
Active Code
MDZFM0EuNjBBLjYwRGVmYXVsdCBzdHJpbmc=
Copy to clipboard
To Viewer
Import License
Close

2.2.1 USB Software Key

- **Activation Software Key**

A software key is required, if the user operates the software in *Q-Analyzer* mode without *Qsep*™ series. The software key must be activated first before its initial usage. Insert the software key into an available USB port on the computer before launching *Q-Analyzer*™.

Figure 2-2 Software Key

The system will automatically lead you to the activation page while starting *Q-Analyzer*™. Enter your Company Name, User ID, and Password. After confirming the password, you will be directed to the Login page.

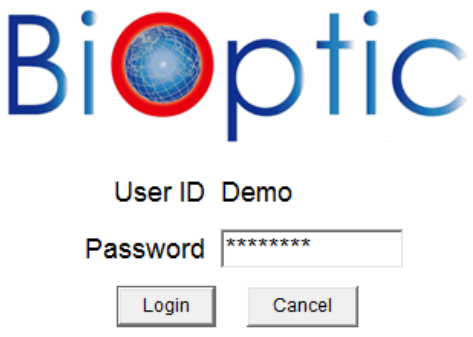


Figure 2-3 Login page

***Note**: Password comprises **8** characters (**6** characters for the previous version of software key) in length contained characters and/or numbers and is case-sensitive.
If the user wants to change the password of the software key, go to **Change Password**

in **Menu→Setting** after the *Q-Analyzer*™ starts.

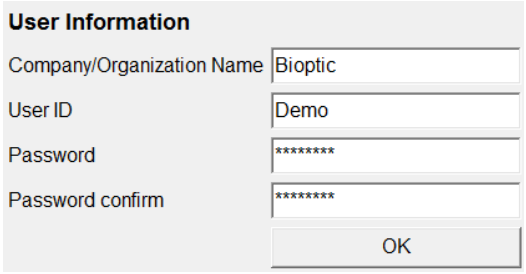
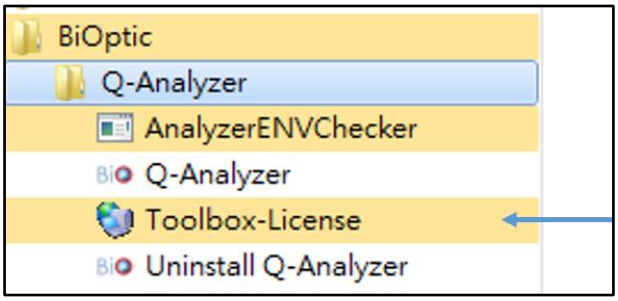


Figure 2-4 Software Key activation

● Forget the password of the Software Key

If the user forgets the password, do the following steps to reset the password.

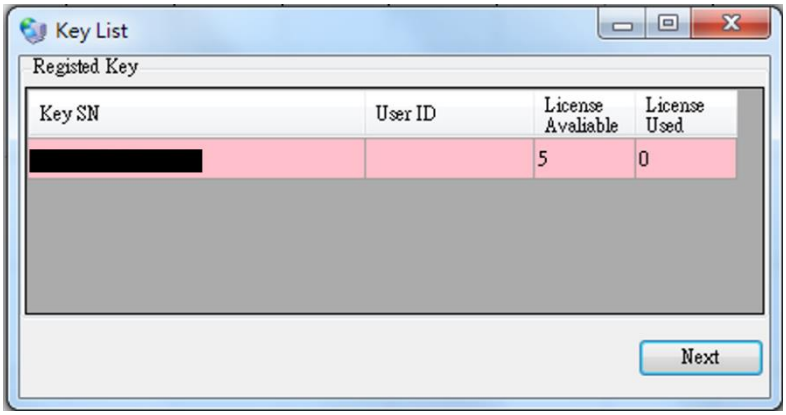
Step 1. Find Toolbox-License in the Microsoft Windows **All Apps (All Programs)**



→ Insert the software key, then click **Toolbox-License**.

→ If the software key is found, the **Next** button will be enabled.

→ Click **Next** to enter the Toolbox window.



Step 2. Click **Unblock software key**

The password of this key will be reset to “00000000” (“000000” for the previous version software key)

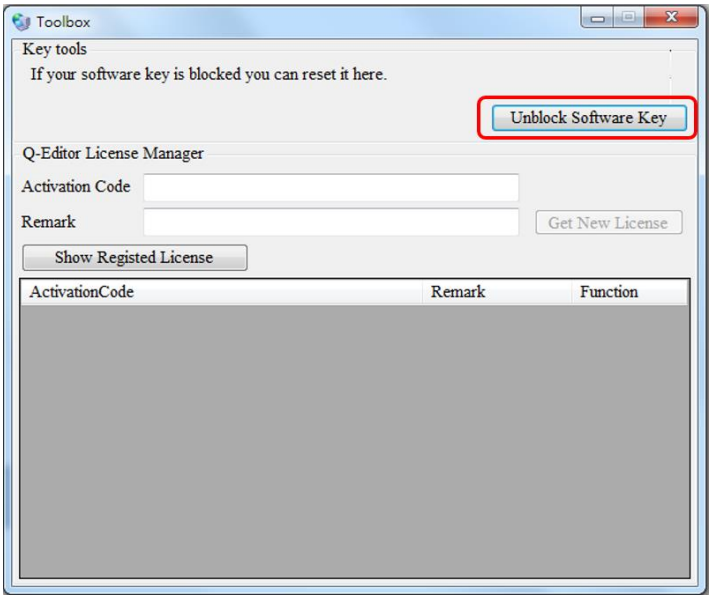


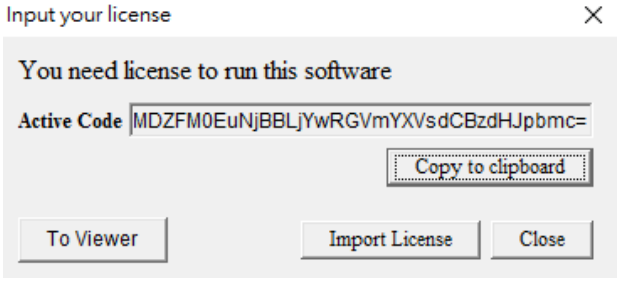
Figure 2-5 Unblocking the Software Key

2.2.2 *Q-Editor* License

The software mode *Q-Editor* helps the user to do the post data analysis offline**.** *Q-Editor* requires a license registered in the computer. One software key provides five *Q-Editor* licenses for different offline computers.

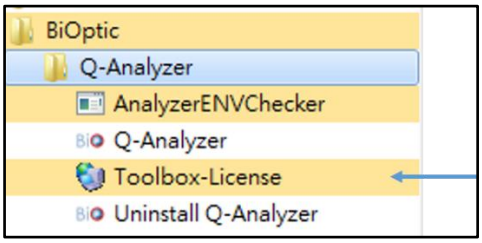
To import the license, do the following:

1. Launch *Q-Analyzer*™ without the instrument and software key connection.
   The message box of **Input your license** will appear on the screen.

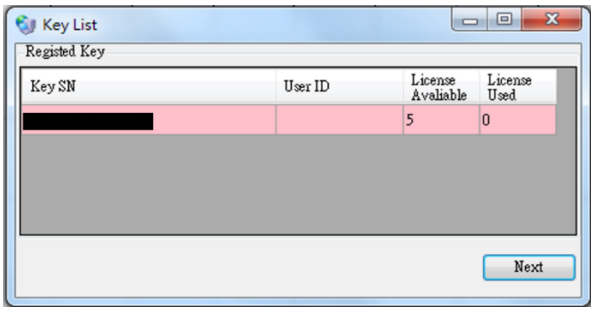


   Click **Copy to clipboard** from **Input your license** message box.

2. Find **Toolbox-License** in the Microsoft Windows **All Apps (All Programs)**



   Insert the software key, then click **Toolbox-License**. If the software key is found, click **Next** to enter the Toolbox window.



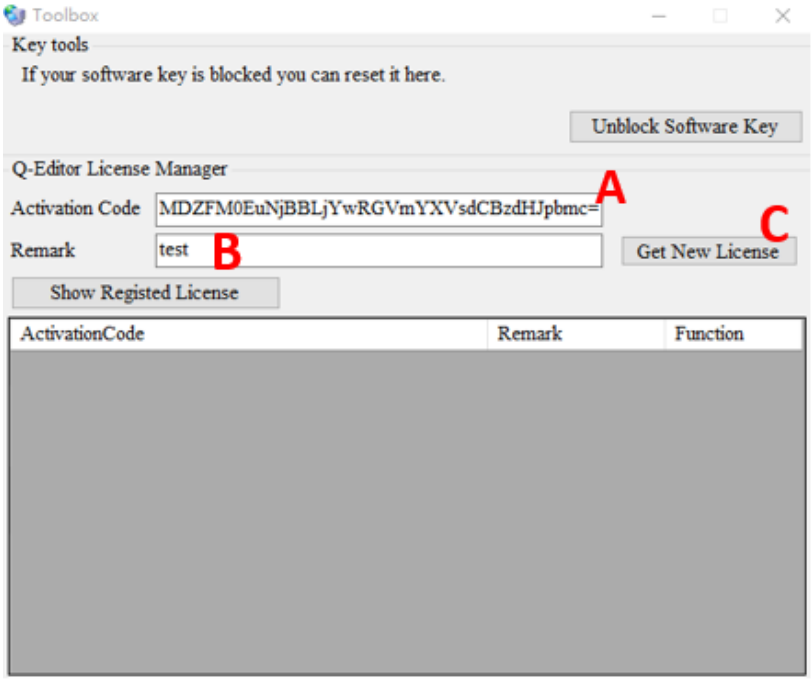
   ***Note**: The number of *Q-Editor* license available and used will be listed on the table.

3. Get *Q-Editor* license file

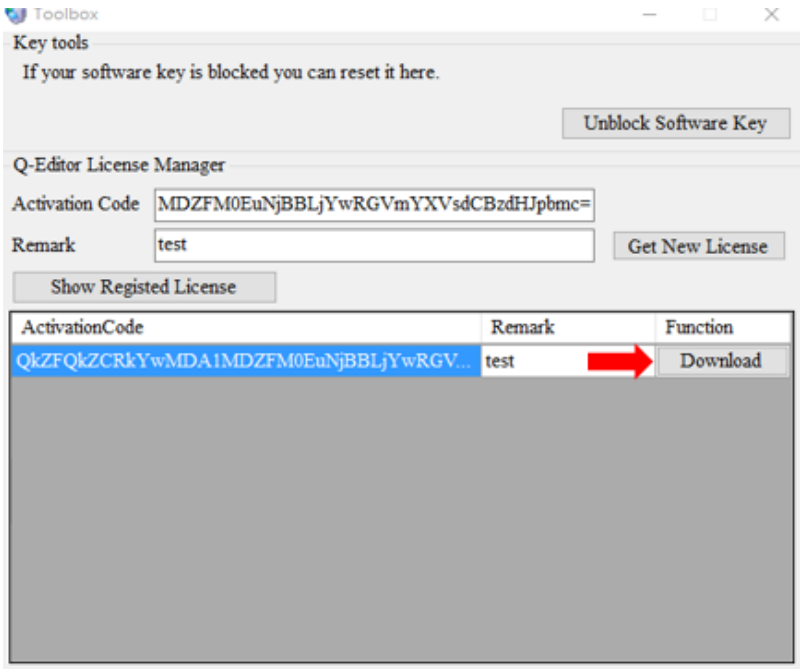
A. Paste the activation code which copied from **Input your License Toolbox** (A).

B. Enter your computer name in the **Remark** field (B).

C. Click **Get New License** (C), then **Get New License** will be enabled.

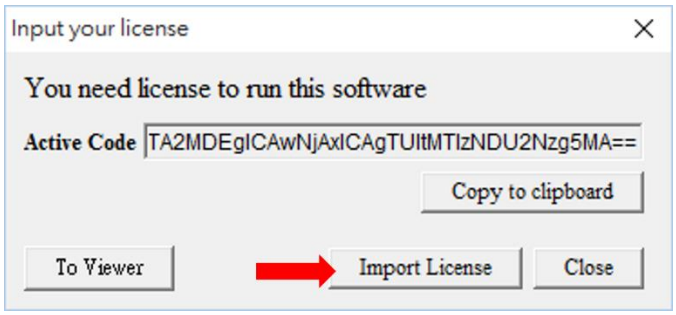


The new License will be generated in the table below. Please click **Download** to save the license as a file which can be used for importing later.



***Note:** If this computer has been registered under this software key, you can retrieve the license by clicking **Show Registered License** (B) to download your license file again.

4. Return to the **Input your license** window and click **Import License** to import the file you just downloaded. The *Q-Editor* is now activated for this computer.



3. Start to Use *Qsep*™ Series

"Q-Analyzer" and "Q-Analyzer for Qsep400" are the software that specially designed to operate *Qsep*™ Series. After installation, double click Q-Analyzer (or Q-Analyzer for Qsep400) icon. To execute the software, the user needs to connect $Qsep_1$™ or $Qsep_{100}$™ or $Qsep_{400}$™ (Power must be on) plug the software key into an available USB port on the computer.

*Note: Before turning on the $Qsep_1$™, the SD card is needed to be inserted in the instrument.
*Note: For $Qsep_{400}$™, sample plate holder is secured by the fixture. Remove the fixture before putting the plug in the socket or it may cause system damage. (For the detail, please see the $Qsep_{400}$™ unpacking guide.)

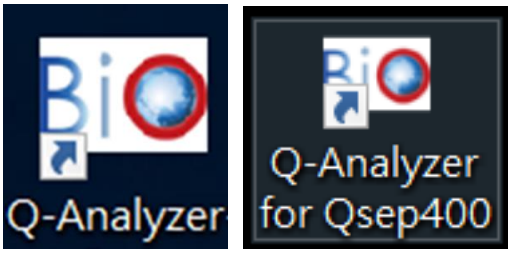


Figure 3-1 Launch *Q-Analyzer*™

There are two versions of the software key, Basic and Advanced. Each version has a different access level to software functions, and each software key is linked to its corresponding user account as well. Basic user can only apply the pre-programmed test Methods. Users with Advanced Software Key will be able to create customized test Method and use other additional functions. Basic Software Key will be included in the instrument package. If you need more information about Advanced Software Key, please contact your *Qsep*™ series distributor or visit BiOptic's official website (https://www.bioptic.com.tw) for further assistance.

3.1 Operation of *Qsep*™ Series

After launching *Q-Analyzer*™, the **Main Window** appears on the screen.
In this window, you can access the **Instrument** function which is used to communicate with the device (Figure 3-2). This section will only introduce the Main tab features and how to operate *Qsep*™ series (Figure 3-2 A). The other function tabs will be described later in the Section 4.4.

The software will create a default project named per date automatically. You can also create another project for experiments with different purposes by clicking **New project** in the Toolbar (Figure 3-2 B).

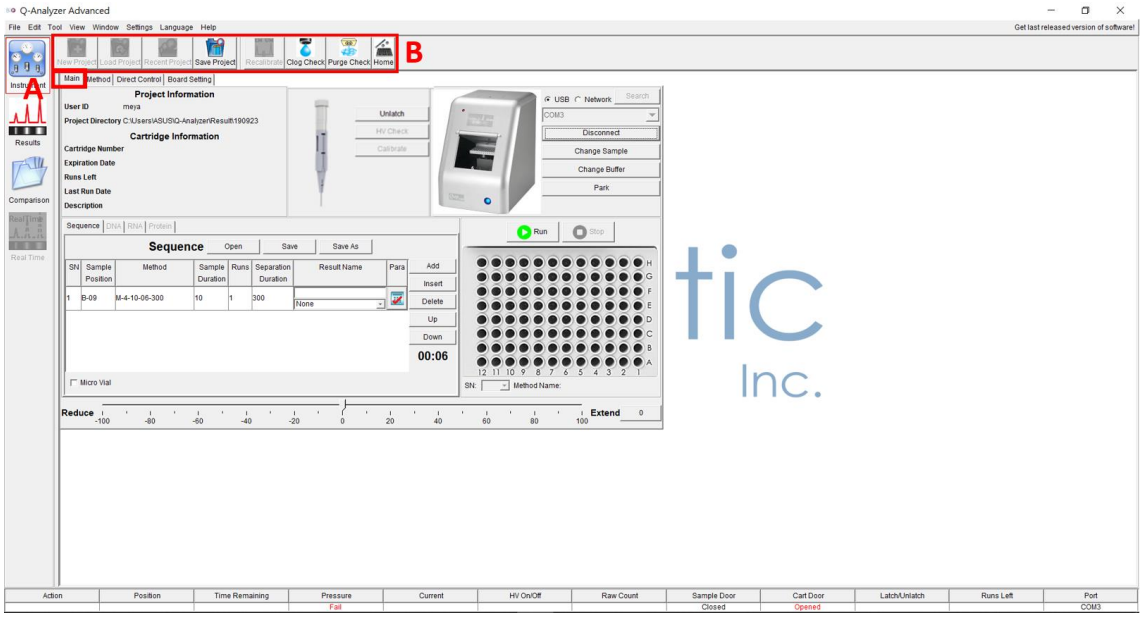


Figure 3-2 Main window of the **Instrument** mode

3.1.1 Connection Assistance

Please ensure *Qsep*™ series is connected to your computer using the USB cable (for $Qsep_{100}$ and $Qsep_{400}$) or the Ethernet cable (only for $Qsep_1$). Turn ON the power of *Qsep*™ series instrument, and then establish the connection of *Qsep*™ series with your computer by clicking the **Connect** button on the Control Panel (Figure 3-3 A). When the **Connect** button changes to **Disconnect** and the displayed *Qsep*™ series picture turns into color form, which indicate the connection is established.

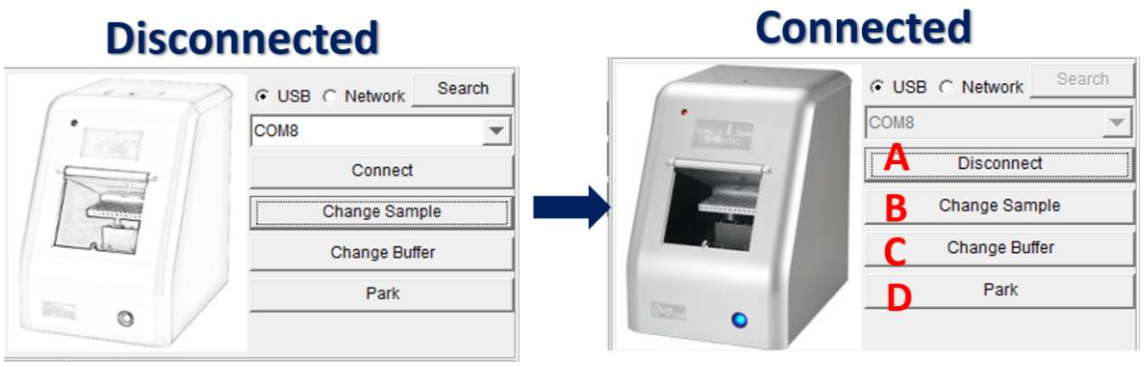


Figure 3-3 Instrument connection

The **Purge Check** message box will pop-up, after connected and before disconnected (Figure 3-4). Click **Purge Check** and follow the instructions to complete the process. Using **Purge Check** function, it ensures that air tube is not clogged before use and clean the gel which was accidentally sucked into the air tube after use.

***Note**: To avoid the permanent damage to the system, user is advised to execute the **Purge Check** every time when the software requires.

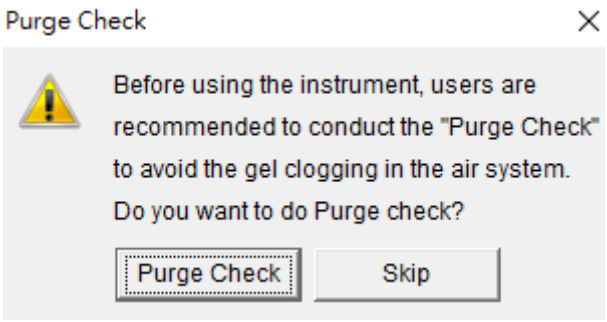
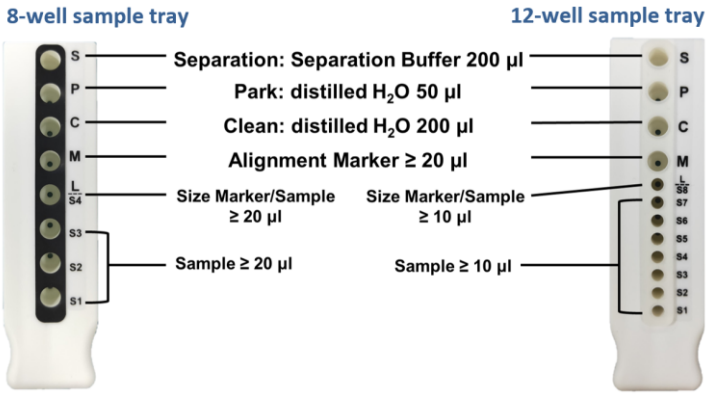


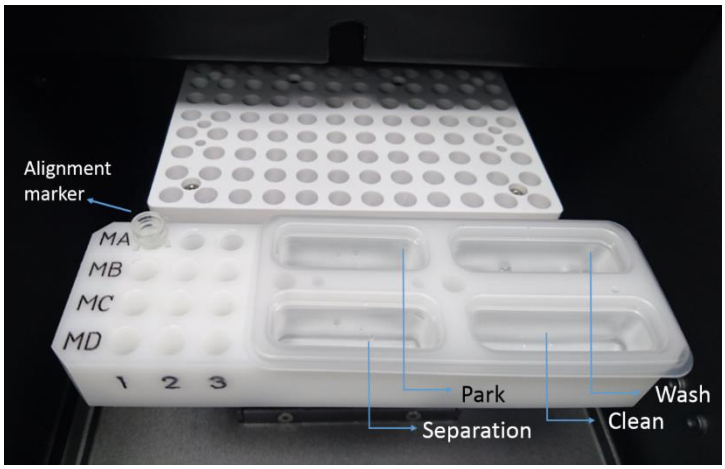
Figure 3-4 Purge Check message box

Click **Change Buffer** to move the buffer tray holder to the door (Figure 3-3 C). Place the solution accordingly in the buffer tray as shown in Figure 3-5. Place the buffer tray properly in the tray holder. Place the suitable alignment marker at MA1~MD1, if needed.

Qsep$_1$

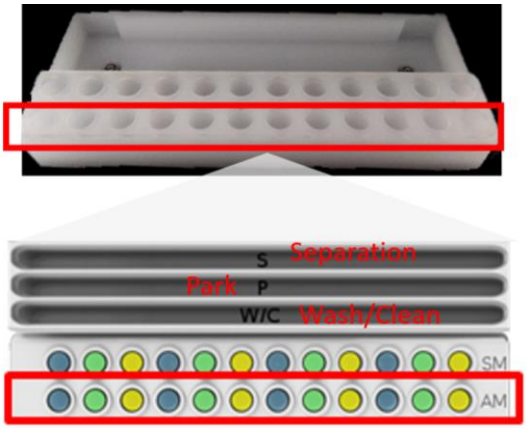


Qsep$_{100}$



Qsep$_{400}$

AM-01 (position 1, 4, 7, 10)	20-1k (C109100)
	20-5k (C109102)
	20-1.5k (C109109)
	20-15k (C109110)
AM-02 (position 2, 5, 8, 11)	RNA-LM (C109120)
	Protein-LM (C104605)
AM-03 (position 3, 6, 9, 12)	User Define AM

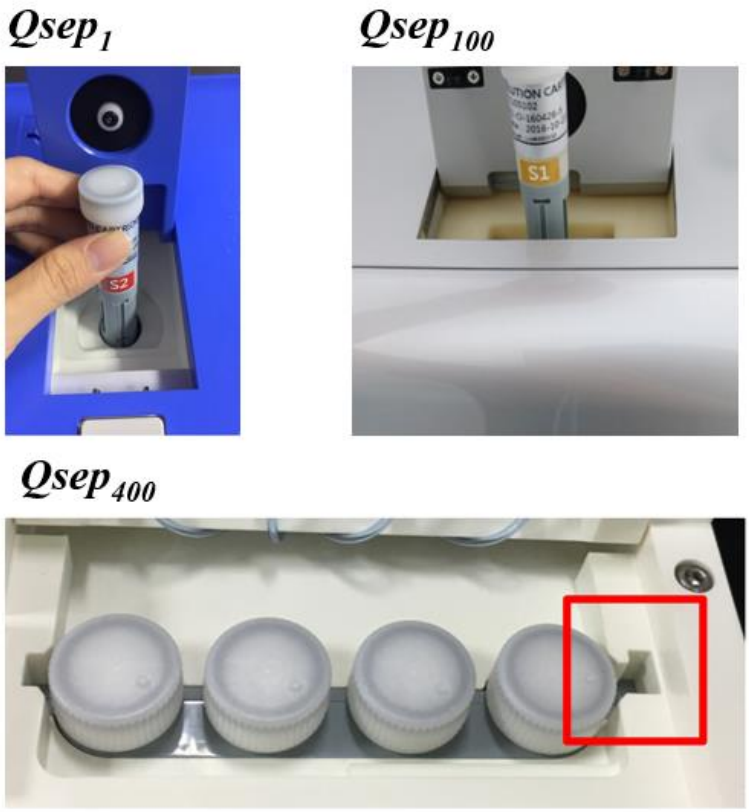


※ Make sure tubes with alignment marker are at the assigned position

Figure 3-5 Change Buffer and place the solution

Then, click **Change Sample** (Figure 3-3 B) to move the tray holder to the door and place the samples. After these steps, close the sample door.

Open the cartridge door on the top of the instrument. Make sure the guiding groove of the cartridge is facing front (Figure 3-6). Then, insert the cartridge and close the door.
***Note:** Before inserting the cartridge, click **Park** (Figure 3-3 D) to ensure the tray holder at the park position.



※ L-shape connector of cartridge should follow the L-shape guiding groove inside the instrument.

Figure 3-6 The guiding groove shall face front

***Note**: Cartridge can only be inserted in one orientation (Figure 3-6).

Figure 3-7 Push the cartridge to the bottom

Then, click the **Latch** button on the Control Panel. The information of the cartridge will be displayed on the **Cartridge Information** section on the left side (Cartridge Number, Expiration Date, Runs Left…) and the cartridge picture will turn into color form after latching (Figure 3-8). The cartridge must pass the calibration process for its initial usage. For the details, please refer to Section 3.2.

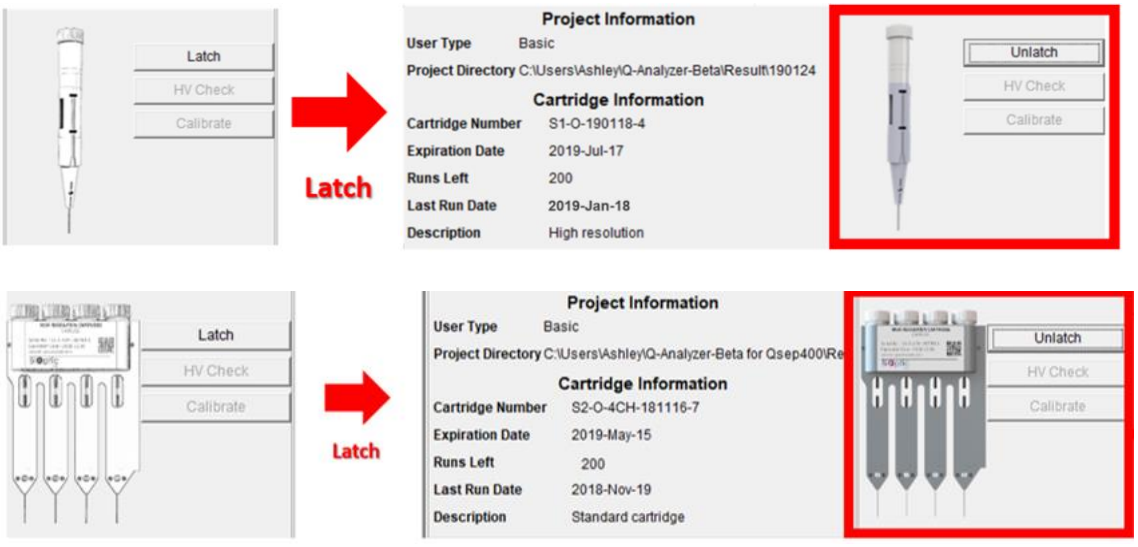


Figure 3-8 Before and After the cartridge latched

3.1.2 Sequence and Method Setting

After latching and verifying the cartridge, you can follow the instructions and enter the blank fields at **Sequence** region (Figure 3-9). Six setting columns provide the operational flexibility for the experiment. Click the **Add** button if you need to create a row for the new sequence. Click **Run** to execute the sequence after the columns are filled.

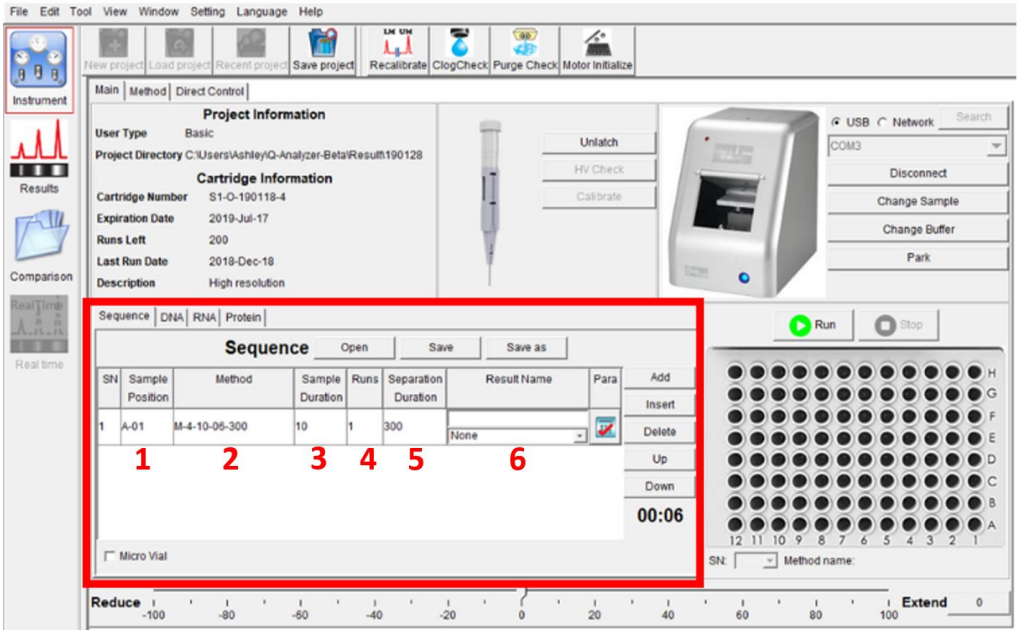
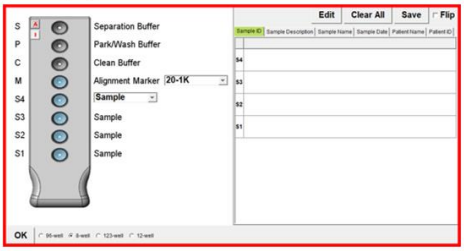


Figure 3-9 Edit sequence

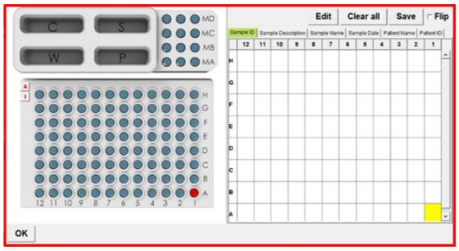
Edit the sequence by assigning the **sample position**, **selecting the separation method**, and **entering the result name**.

Sample Position: Select the sample position in the pop-up window.

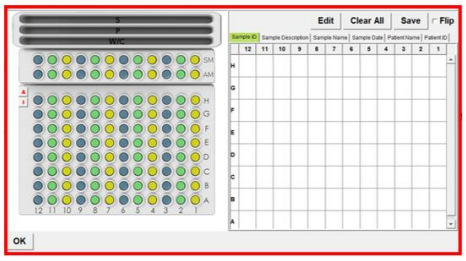
Qsep$_1$



Qsep$_{100}$



Qsep$_{400}$



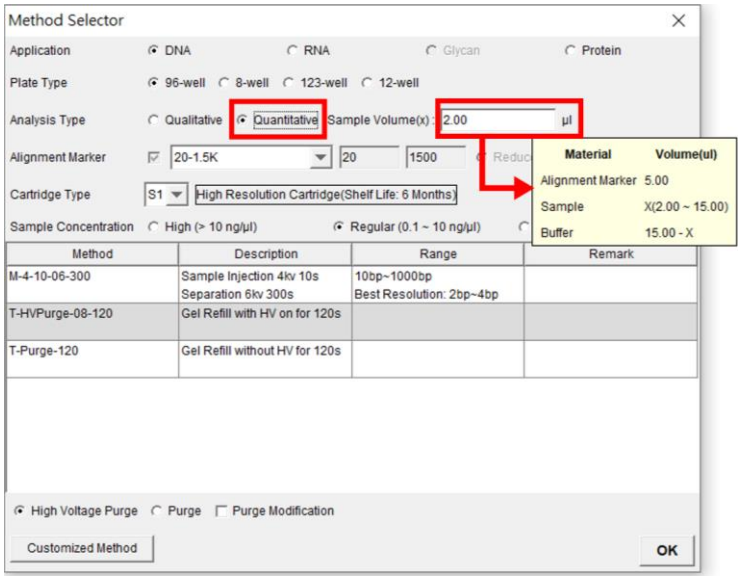
Method: Choose the method for the selected sample.

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

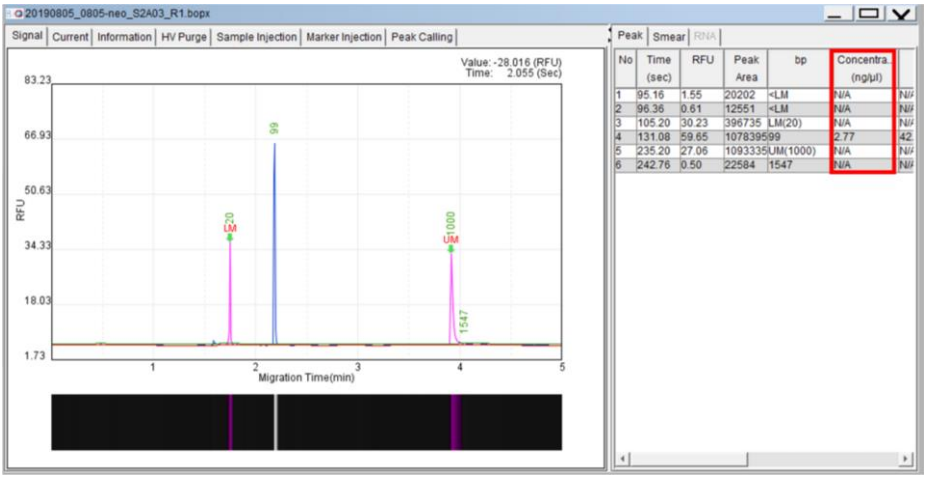
- ✓ Select **DNA, RNA** or **Protein** application respectively to the sample.
- ✓ Select the analysis types you wanted, qualitative or quantitative (only opened in S1 and S2 (Cat. C105201-Q and C105202-Q)).

  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.

  1. enter the volume of sample which mixed with the quantitative marker



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



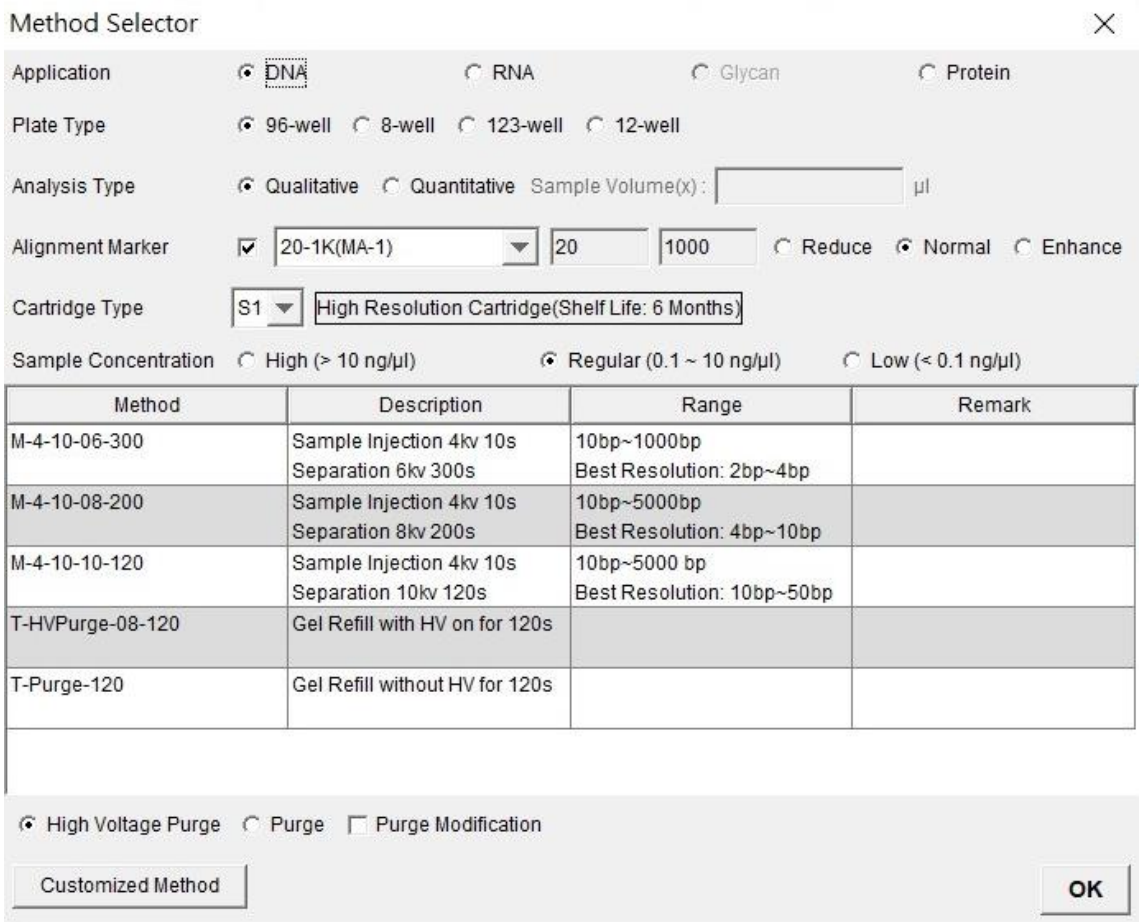
✓ Different Alignment Marker must be placed in the right place

1. When taking 20bp&1000bp Alignment Marker (C109100) as the alignment marker, please place at MA1 and select **20-1K (MA-1)**.
2. When 20bp&5K Alignment Marker (C109102) is used, please place at MB1 and select **20-5K (MB-1)**
3. When 20bp&1.5K and 20bp&15K Alignment Marker (C109110-500A) is used, please place at MD1 and select **20-15K (MD-1)**

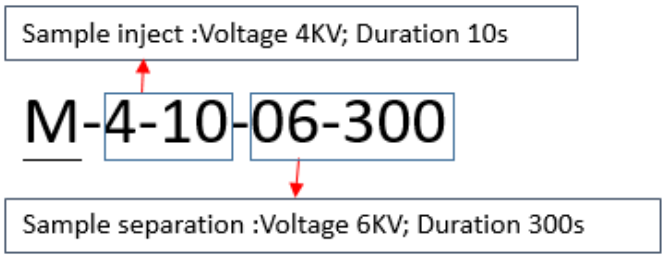
✓ If the **Alignment Marker** checkbox is unchecked, the alignment marker sample will not be used in the separation. The calculation will be affected.

✓ Select **Sample concentration** based on your sample conditions.

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



The pre-programmed method will be named with such rules below:



For example, **M-4-10-06-300** means the voltage of 4 KV for the 10 seconds sample injection and the voltage of 6 KV for the 300 seconds sample separation.
The higher voltage and longer sample injection time, the more sample is injected to enhance the signal.
The higher separation voltage, the separation time will be shortened, but the resolution is worse. The lower separation voltage can help increase the resolution but slower.
The sample separation is corresponding to the size range of base pairs.

Sample Duration: 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 the concentration and the amplitude at your preference.

Run: The number of runs

The repetition of the same samples is required to execute the separation. If the samples are more than one, the sequence will be sample A, B… then sample A, B….

Separation Duration: The separation time during the execution.

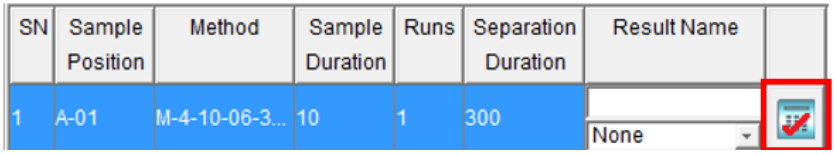
The separation time may be affected by the experimental condition such as temperature, concentration of separation buffer, etc.

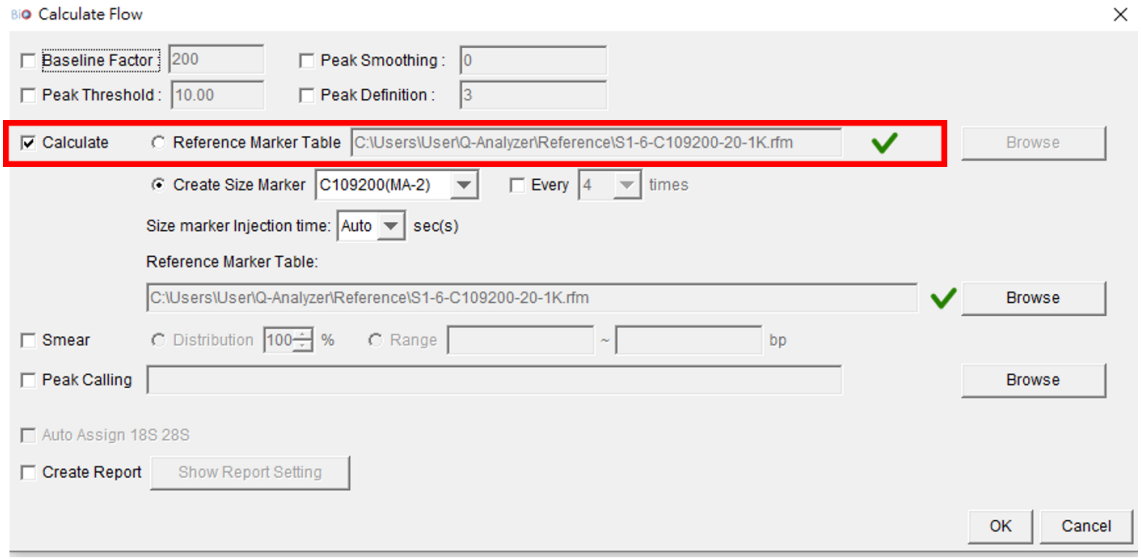
The **Separation Duration** of the selected method will be displayed on the Sequence table. You can modify the duration of the separation according to your condition. The Separation Duration time is recommended to be 30 seconds longer than the time when the upper marker appears.

Result Name: The result name to be saved.

The result file will be saved with information of sequence, position, and execution such as <*ResultName*>_<*pull-down options*>_S1A2_R3.bopx; Here, S1 means first row in sequence and A2 means sample position and R3 mean third execution.

Para.: The setting configurations for this method





Software will calculate the result by using the default reference table.
If users want to use their own reference table, click **Browse** and select the file (See Section 5.1.2).
You can also adjust the Baseline and Peak Threshold in this page.

Alternatively, if user needs to improve the accuracy of the base pair calculation, **Create size marker** is used to update the reference table periodically every n time with the size marker. The following sample test will be calculated based on the updated reference table.
***Note**: The size marker used in the **Create size marker** function is based on the alignment marker you selected at **Method selector**.

The result calculation is based on the built-in reference marker. User can also assign your own reference marker instead of using built-in reference marker when:

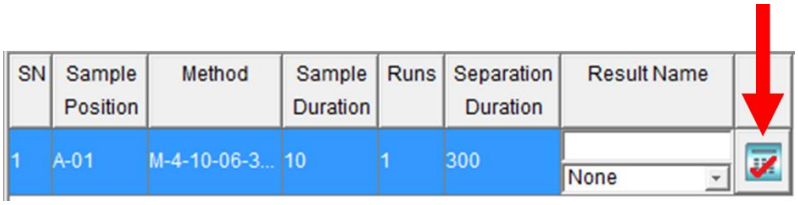
(1) The signal pattern is different between the built-in reference marker and the new one, and the software can not recognize the new pattern correctly.

(2) The size of upper marker is not the same as using. (e.g. If using 20-1K, the upper marker must be 1Kb)

To execute **Create size marker**, do the following:

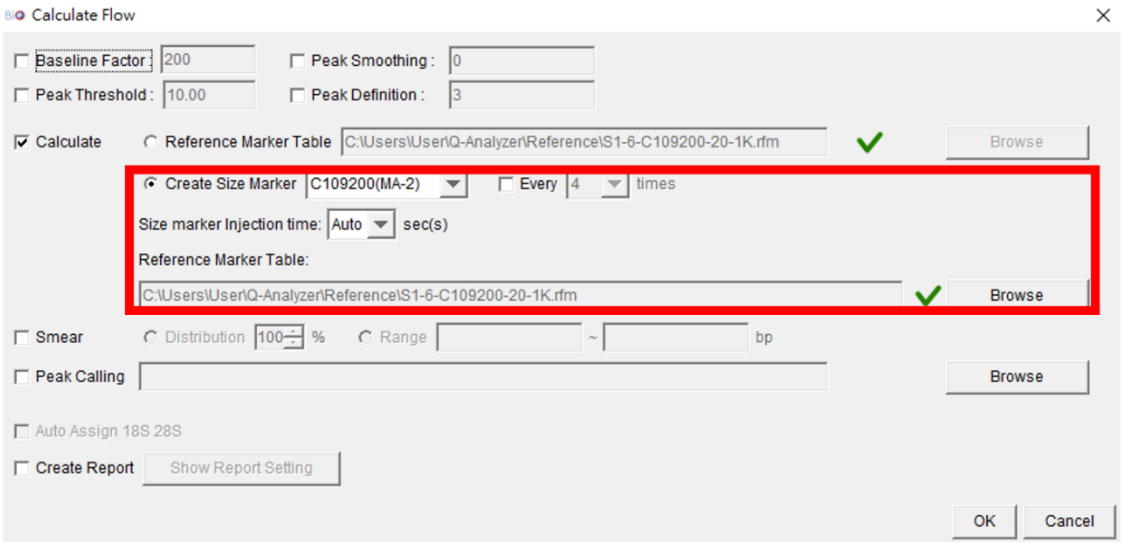
A. Place fresh Alignment Marker (C109100: 20-1K Alignment market) at MA1 position and Size Marker (C109200: Size marker) at MA2 position.
***Note**: For different instruments, please place the right AM in the right positions. (See Section 3.1.1; Figure 3-5)

B. Click the Automatic calculation icon in Sequence.



C. Check **Create size marker**

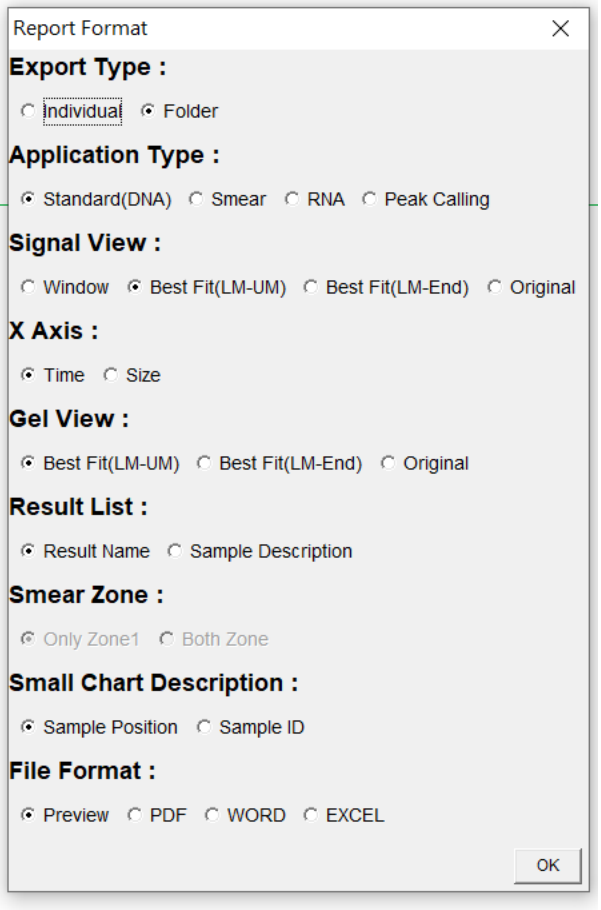
You can select the frequency (☐**Every n times**) to execute the size marker, if you need higher accuracy of the base pair sizing.



Smear: If the **Smear** checkbox is selected, the Smear analysis will be applied to the results. (See the Section 5.4)

Peak Calling: If the **Peak Calling** checkbox is selected, the Peak Calling analysis will be applied to the results. (See the Section 5.5)

Create report: If the **Create report** checkbox is selected, you can select the **Show report setting** and set the **Report format**.



3.2 Cartridge Calibration

In order to ensure the quality of the new cartridge, calibration is required before the initial usage.

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

Please prepare ≥ 20µL fresh alignment marker in a 200µL PCR tube (adding 10µL mineral oil to prevent evaporation) and place the marker at MA1 position. Make sure the buffer tray is placed on the buffer tray holder. Insert the new cartridge and close the door.

First, click **Latch** and then click **HV Check** as shown in Figure 3-10 to check if the cartridge working current is above 2 µA. After **HV Check**, click **Calibrate** to verify the cartridge as shown in Figure 3-11.

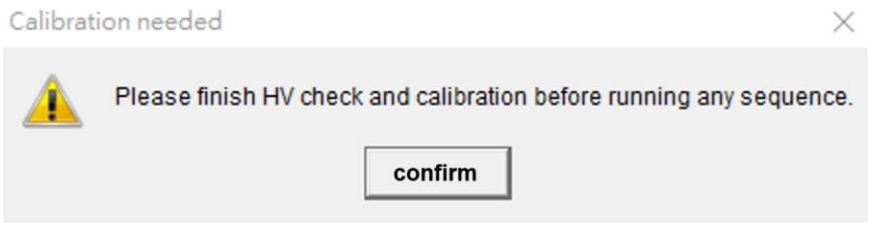


Figure 3-10 Cartridge Verification: HV check

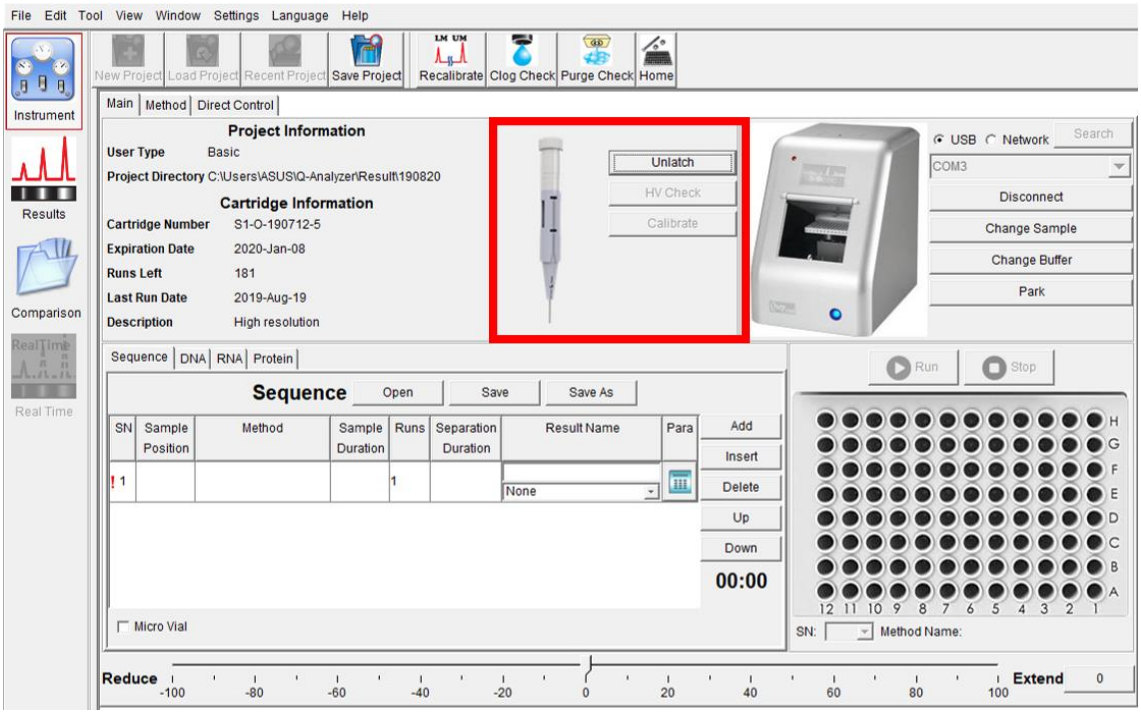


Figure 3-11 Cartridge Verification: Calibration

Qsep™ Series is ready to use after the "Passed" message appears on the screen. If the "Failed" message appears, please check the alignment marker and buffer tray as mentioned earlier. If the calibration continuously fails for ten times, go to **Help** → **Report** to generate the error result file and send the file to your local distributor or visit BiOptic Inc. official website for technical support. https://www.bioptic.com.tw

3.3 Edit Sample Position Settings

In the Sequence panel, user can select the corresponding location of the **Sample Position** on the simulation chart (Figure 3-12), and the selected location will be marked in red. If you want to cancel the selection, just click it again.

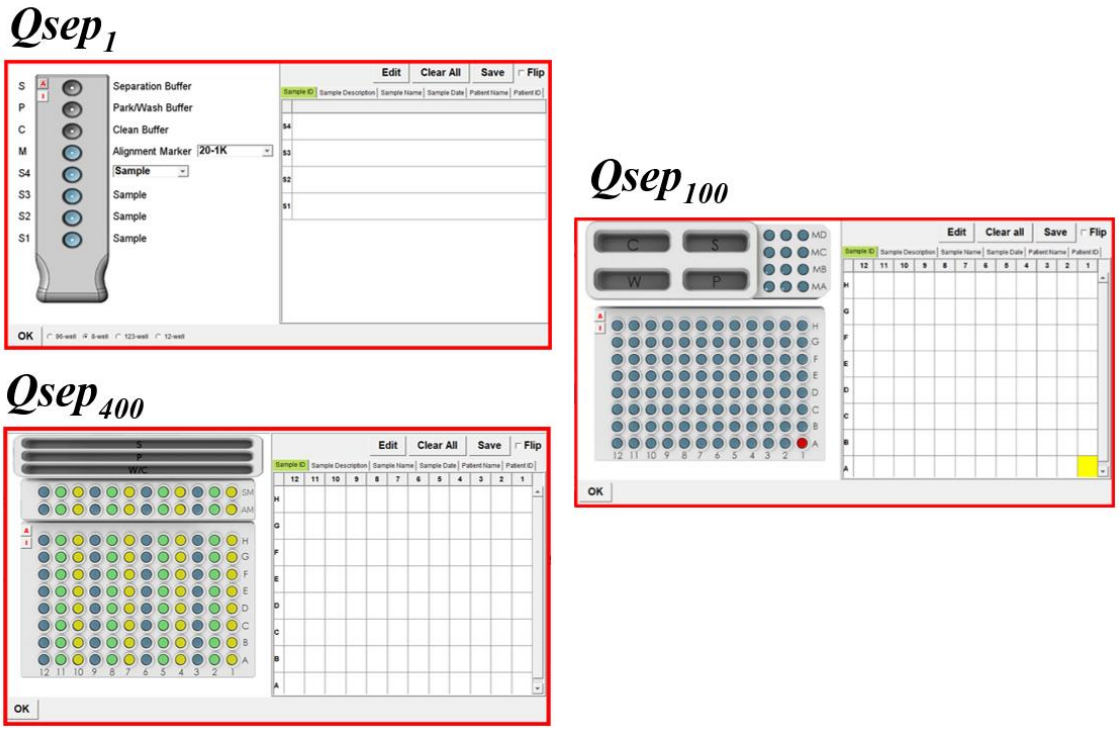


Figure 3-12 Select the Sample Position

The whole column or row will be selected by clicking the coordinates A-H or 01-12 on the side. User can select all the positions by clicking the "A" button and invert the selection by clicking the "I" button on the left side. When multiple samples have been selected to execute the Method for several times, the system will execute the marked sample positions by the 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 in the Sample Loader window (Figure 3-13). **Clear All** button in the window is used to delete all the sample information and **Save** button is used to save the sample information after editing. After entering the information such as time and serial number, click **OK** to start the process.

The **Load** function is used to enter multiple sample description. After clicking **Edit** button, the Sample loader window will appear on the screen (Figure 3-13). The information in the excel file (Figure 3-14) can be loaded into the window and saved after clicking **OK.**

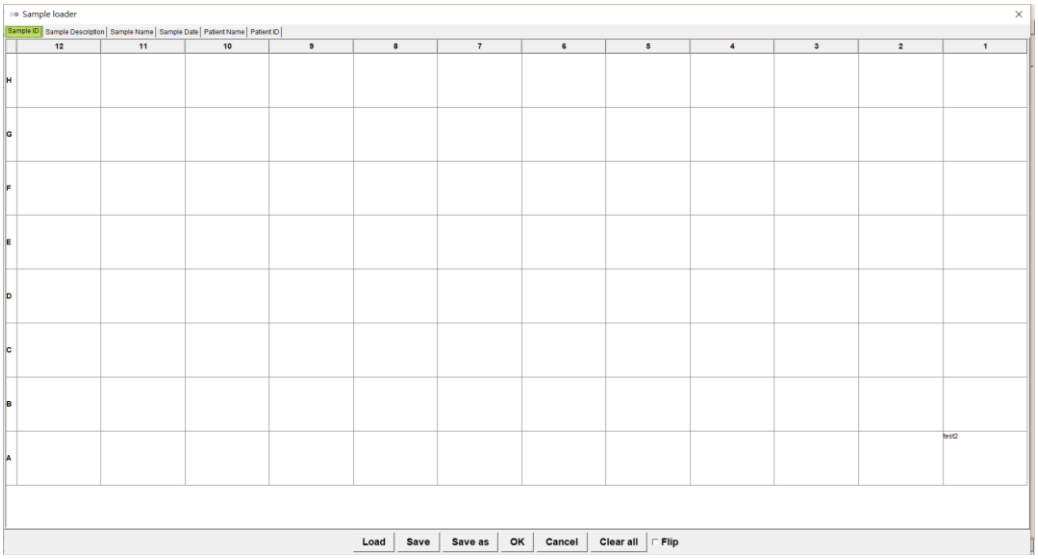


Figure 3-13 Sample loader window

	A	B	C	D	E	F	G
1	SamplePosition	SampleID	SampleName	SampleDescription	SampleDate	PatientID	PatientName
2	A-01	A02	A03	A04	2000/10/10	DD	1
3	B-01	B02	B03	B04	2000/10/10	FF	2
4	C-01	C02	C03	C04	2000/10/10	GG	3
5	D-01	D02	D03	D04	2000/10/10	HH	4
6	E-01	E02	E03	E04	2000/10/10	II	5
7	F-01	F02	F03	F04	2000/10/10	JJ	6
8	G-01	G02	G03	G04	2000/10/10	KK	7
9	H-01	H02	H03	H04	2000/10/10	LL	8

Figure 3-14 Excel file for Sample loader

***Note**: The information of the samples in the excel sheet needs to follow the sequence that shows in Figure 3-13. You can find an example file in the installation directory C:\Program Files (x86)\BiOptic\Q-Analyzer\ExcelSampleExample.xlsx

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 the specific format. Sample Position: “A-H”-“01-12”, Sample Date: yyyy/mm/dd

3.4 Recalibration

Recalibration can help to identify the alignment marker (upper marker and lower marker) correctly.

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

- 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:

Step1. Make sure the fresh Alignment Marker (e.g. 20-1K) is placed at the right position (e.g. MA1)

Step2. Make sure all buffers are in good condition

Step3. Click **Recalibrate** on the Toolbar and follow the on-screen instructions (Figure 3-15)

Figure 3-15 Recalibrate on the Toolbar

To do the recalibrate, choose the appropriate voltage and alignment marker that match with the **Method** which will be used for the following experiments (Figure 3-16).

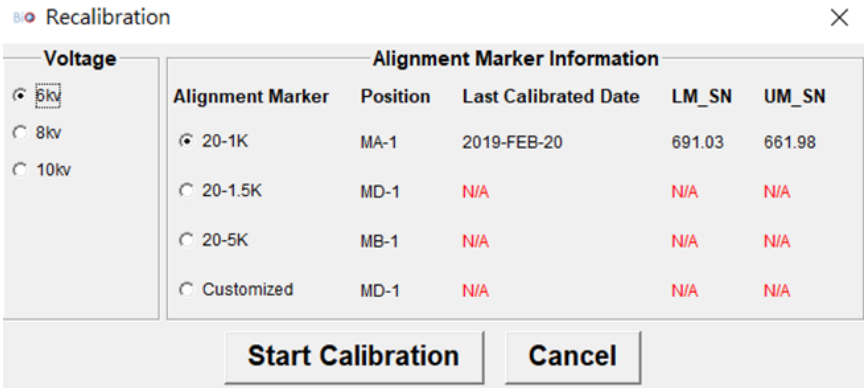


Figure 3-16 The message box of Recalibrate

3.5 Capillary Clog Check

*This function only shows in $Qsep_{100}$.

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

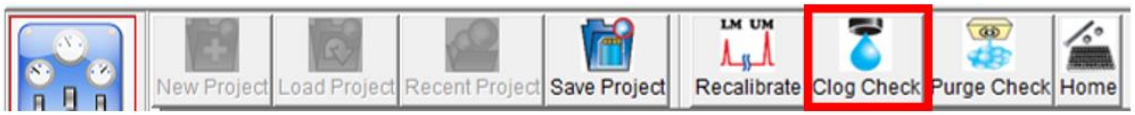


Figure 3-17 Capillary Clog Check function

If the current is too low or unstable, user can use this function to check whether the capillary is clogged or not. The software will guide you through the process (Figure 3-20~ 3-22).

If the current of separation (**Current**) is still unstable after performing the Capillary Clog Check, the inner air tube might be clogged (Figure 3-18 and 3-19). Refer to the **Purge Function Check** function in the next section for more details.

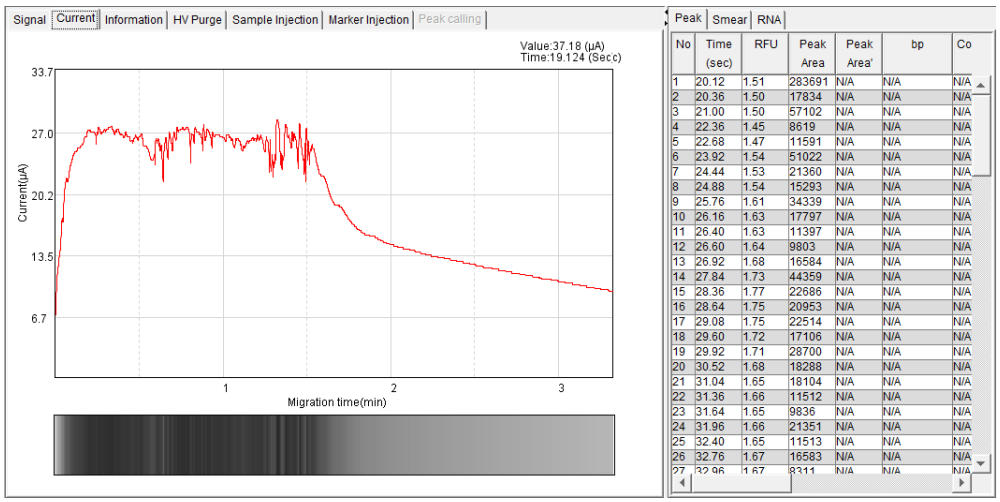


Figure 3-18 Unstable current

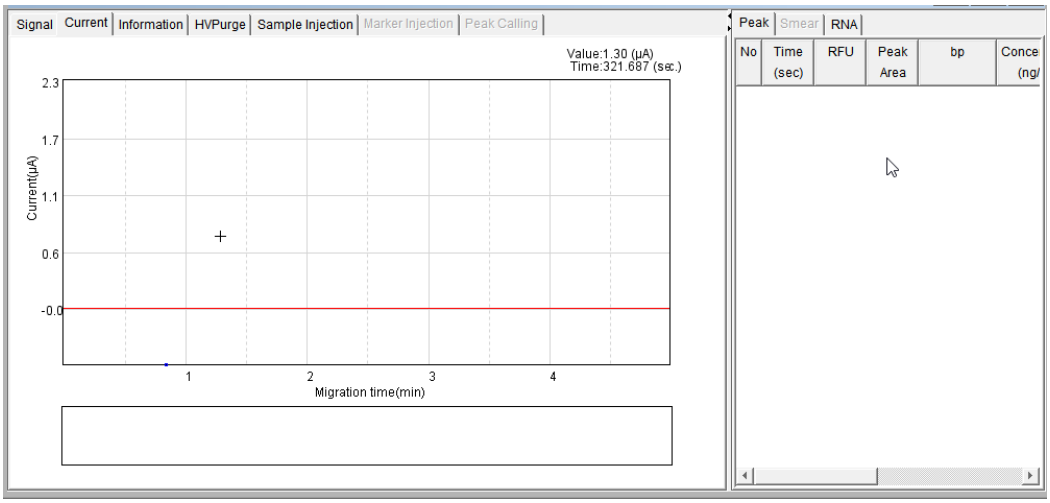


Figure 3-19 Low Current

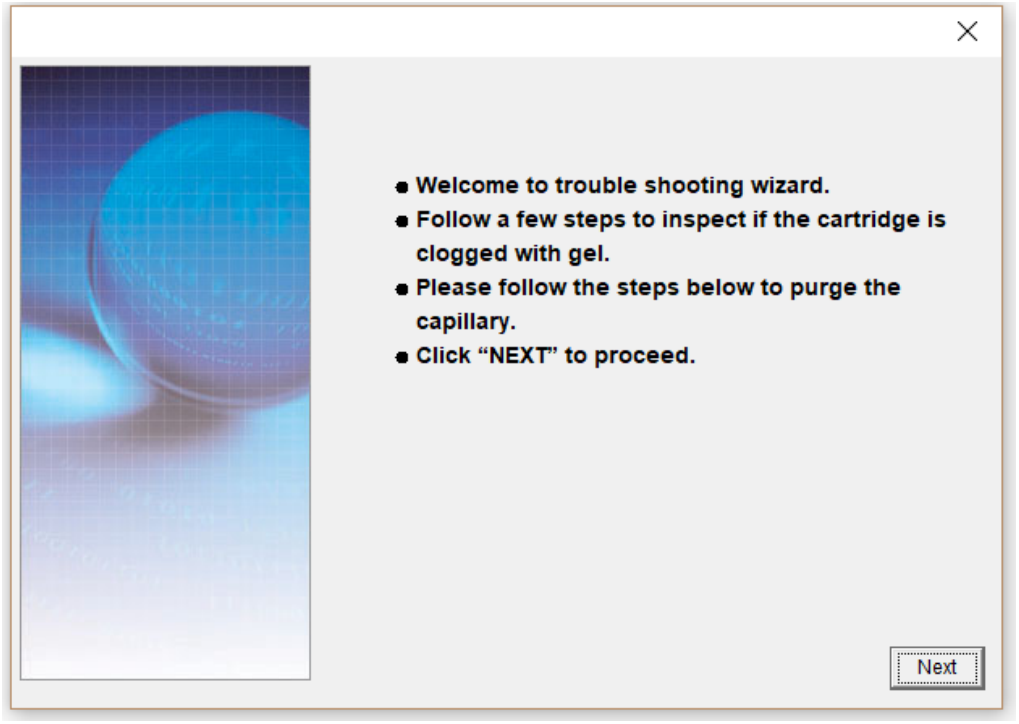


Figure 3-20 Capillary Clog Check window-1

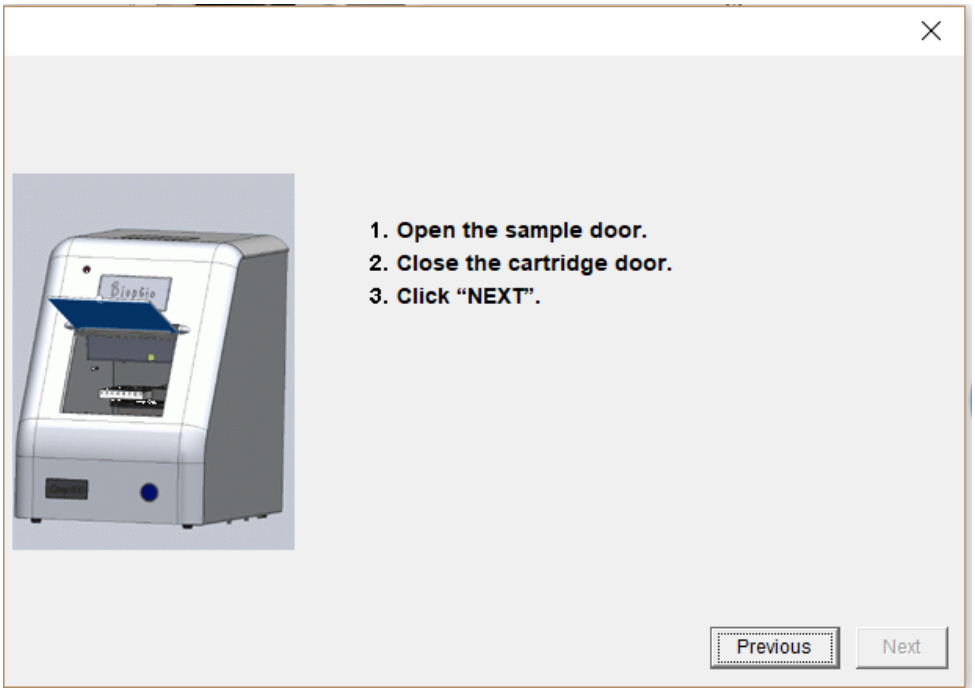


Figure 3-21 Capillary Clog Check window-2

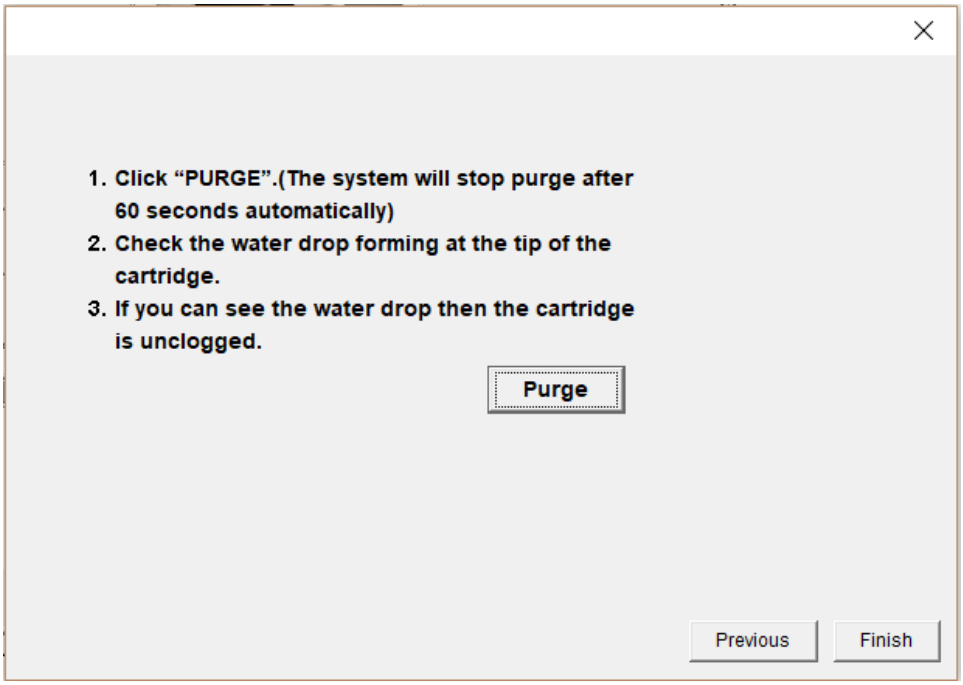


Figure 3-22 Capillary Clog Check window-3

3.6 Purge Check Function

Purge Check Function is used to see if the air is coming out while purging. The system will recommend you to do the purge check function before and after using *Qsep*™ Series.

Figure 3-23 Purge Function Check

Make sure there is air coming out from the JET-NOZZLE. If there is no air coming out, it means the gel is clogged in the air tube. Then, you might need to clean the air tube at the cartridge door (please contact with your distributor for maintenance and repair service). If there is air coming out, please use your thumb to press the JET-NOZZLE to see if the JET-NOZZLE can push to the end or not. If the JET-NOZZLE can be pushed to the end easily, it means the pressure is not enough. Please contact with your distributor for maintenance and repair service.

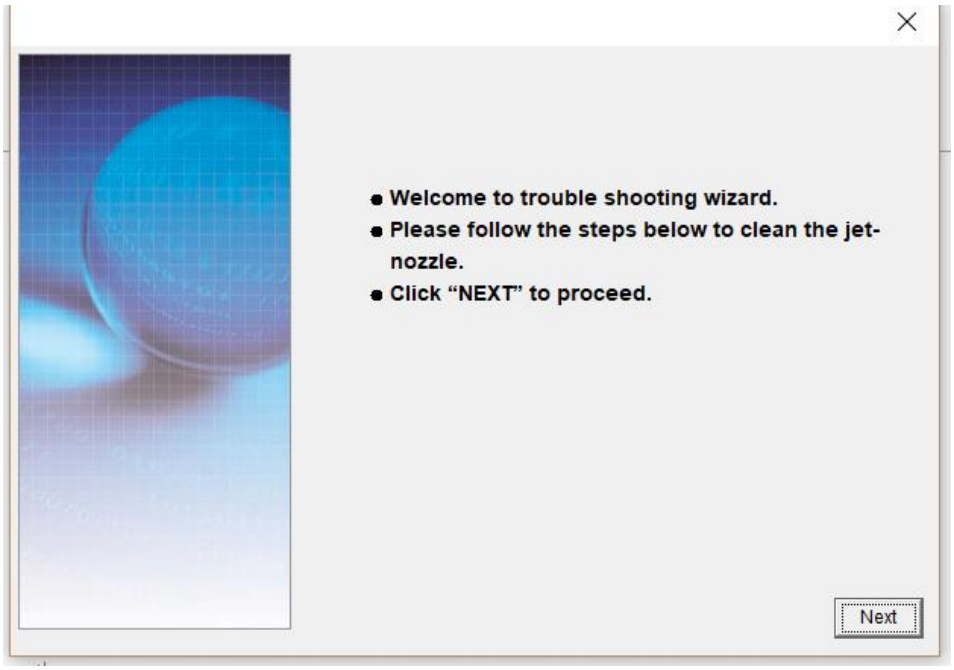


Figure 3-24 Purge Check Function window-1

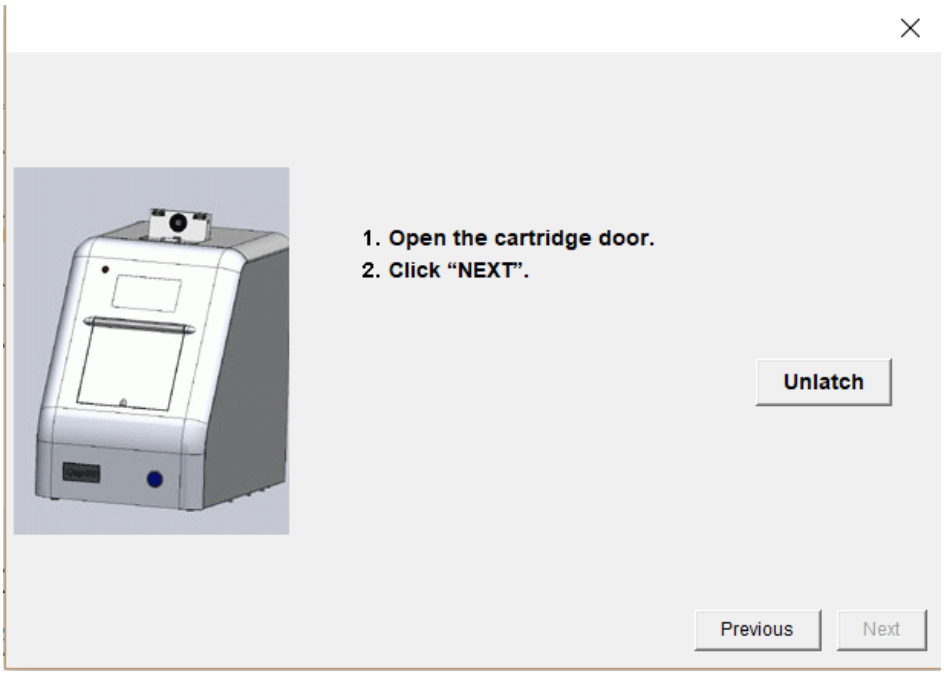


Figure 3-25 Purge Check Function window-2

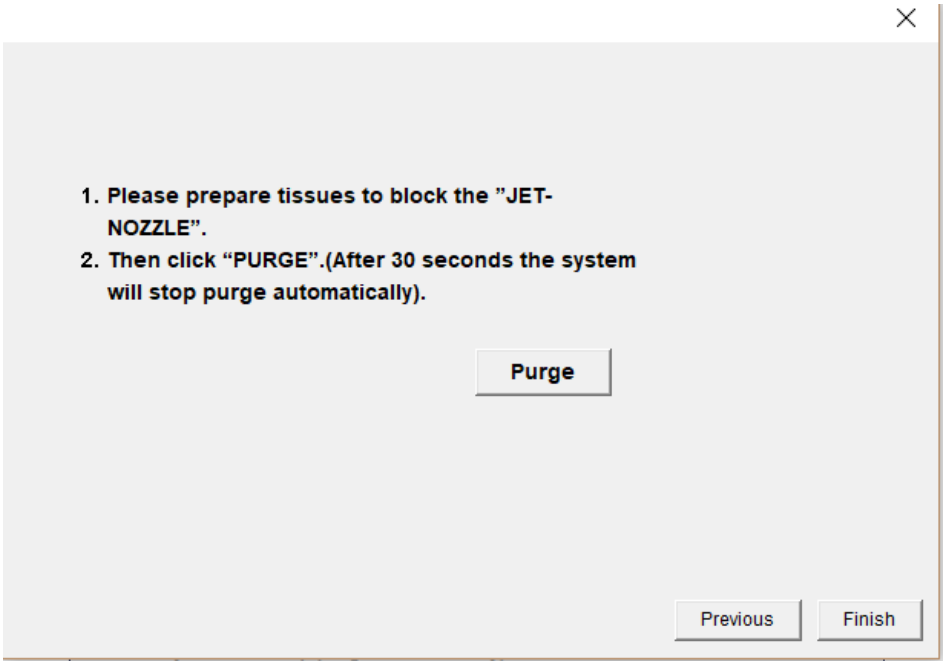


Figure 3-26 Purge Check Function window-3

3.7 Home Function

Home Function will make the tray holder move to the initial place.

For $Qsep_{100}$™, this function can be used to make the tray holder move to the initial place.

For $Qsep_{1}$™, this function can be used to close the sample door when sample tray taken out.

For $Qsep_{400}$™, this function can help you to lock the fixing screws before moving the machine.

Figure 3-27 Home Function

4. *Q-Analyzer*™ User Interface

4.1 Main Window

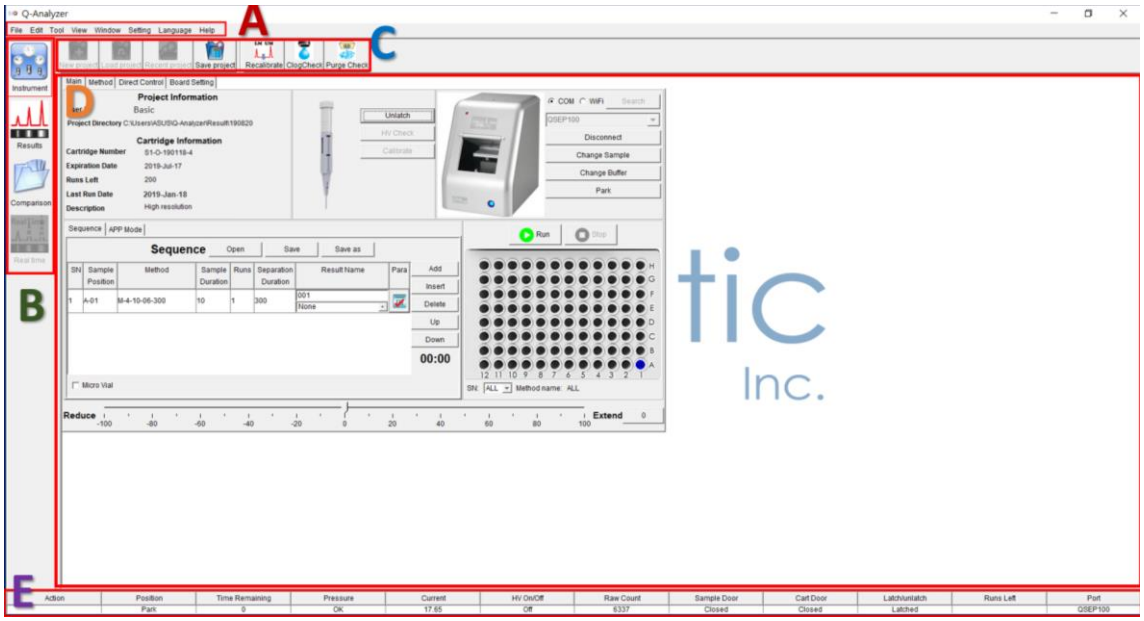


Figure 4-1 Main window

A: Menu
B: Function bar
C: Toolbar
D: Operation region
E: Status Column

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).

Menu:

All available functions can be found in the Menu list. For details, please refer to Section 4.3

Function bar:

There are four icons in the Function bar. You can select **Instrument** to the control panel and the detail of operation can be found in Section 4.4. If you need to display or analyze the data, please click the **Results** or **Comparison** (See Chapter 5). When the execution of sequence is in progress, the collected data will display in the **Real time**.

Toolbar:

The **Toolbar** provides several icons relatively to the **Function** page you have selected.

The icons are the most frequently accessed functions you may need. All the detail of the **Toolbar** relatively to the **Functions** will be described at Section 4.2

Operation Region:

Operation Region is the region that is used to operate or display the main window. After selecting the desired mode, the corresponding panel will display on the operation region. User can switch to the control panel by clicking **Instrument** in Function bar or switch to the display panel by clicking **Result** or **Compare**. When a sequence is in progress, the **Real time** mode will show the real-time data in the operation region.

Status Column:

The information of the system status will be shown at the bottom of Main Panel.

4.1.2 System Status Column

The parameters shown at Status Column are as follow (Figure 4-1 E)

Action: the proceeding action

Position: the position of the cartridge tip

Time Remaining: the remaining time to complete the processing action

Pressure: shows whether the air pressure is applied properly
Fail indicates there is **lower** than the system requirement
If the **pressure is on the low side** of the system requirement, the color of pressure number will **change from black to orange** to remind you.

Current: the current (μA) flow 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: shows whether the cartridge is placed and locked properly

Runs Left: the remaining proceeding test

Port: the serial port connected with the computer

SD card: after connecting $Qsep_1$, the SD card column will show and remain SD is detected or not.

4.2 Function bar and Toolbar

In the Main window, there are four major functions of *Q-Analyzer*™ in **Function bar**, which are **Instrument**, **Results**, **Comparison**, and **Real time**. User can select the desired function either to operate *Qsep*™ series or analyze the data. For easy access, the most frequently used functions are placed at the Toolbar. The detail information will be described in the following section. By default, you will begin with the **Instrument** function.

4.2.1 Instrument

Instrument is the major control function to operate *Qsep*™ series. The usage of the control panel will be described in Section 4.4.
Before connecting with $Qsep_{100}$™, the items on the Toolbar such as **New project**, **Load project**, **Recent project**, and **Save project** is enabled. User can create a new project or load the project, if necessary (Figure 4-2).The project can be used to differentiate various experiments. The results will be saved in the corresponding “Result” directory. “Project name” is the name of the folder where the result files will be saved (i.e. “<*workspace*>\Result\<*Project name*>”).
* Default <*workspace*> is C:\Users\ <*user name*>\ Q-Analyzer

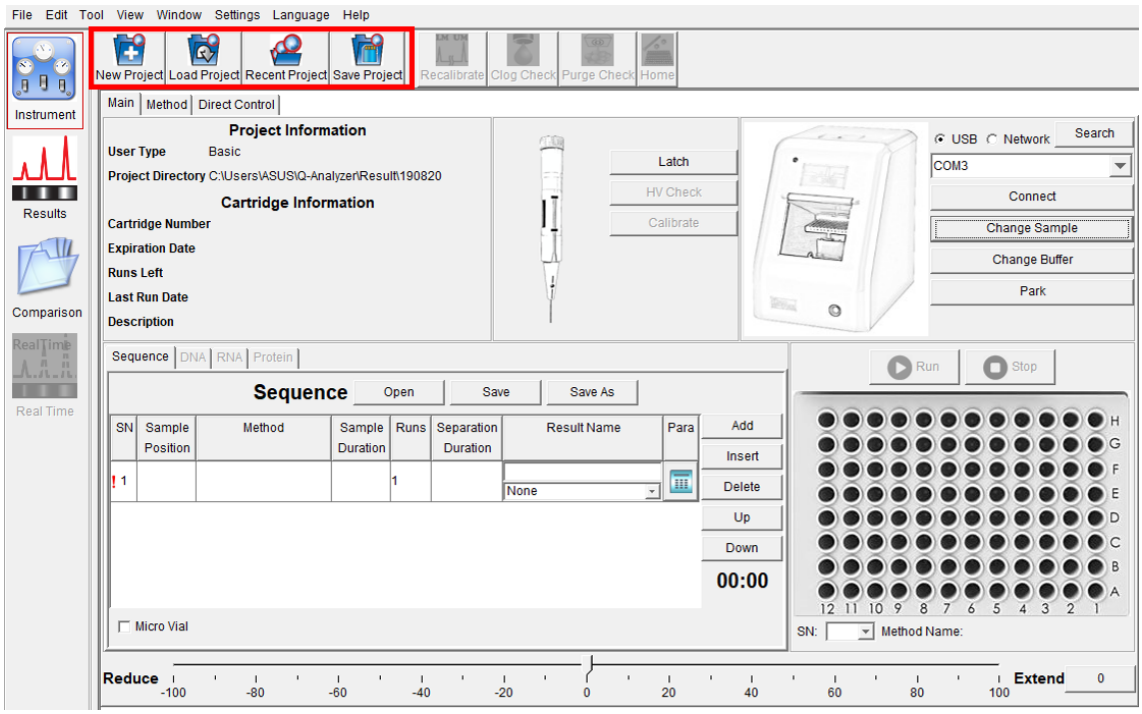
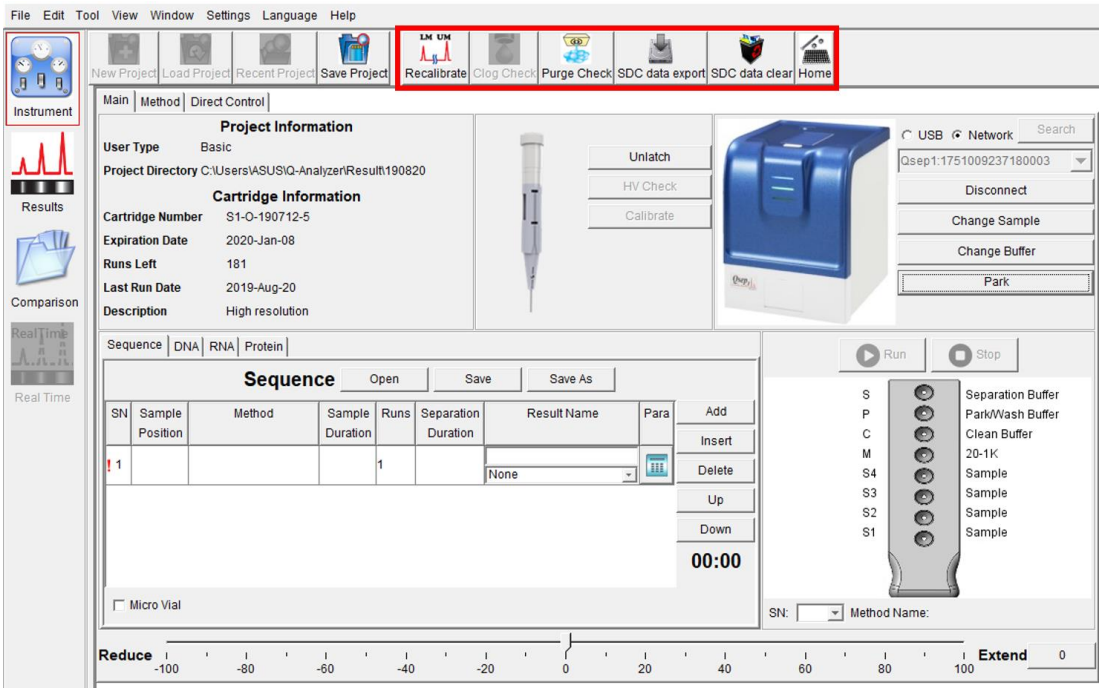


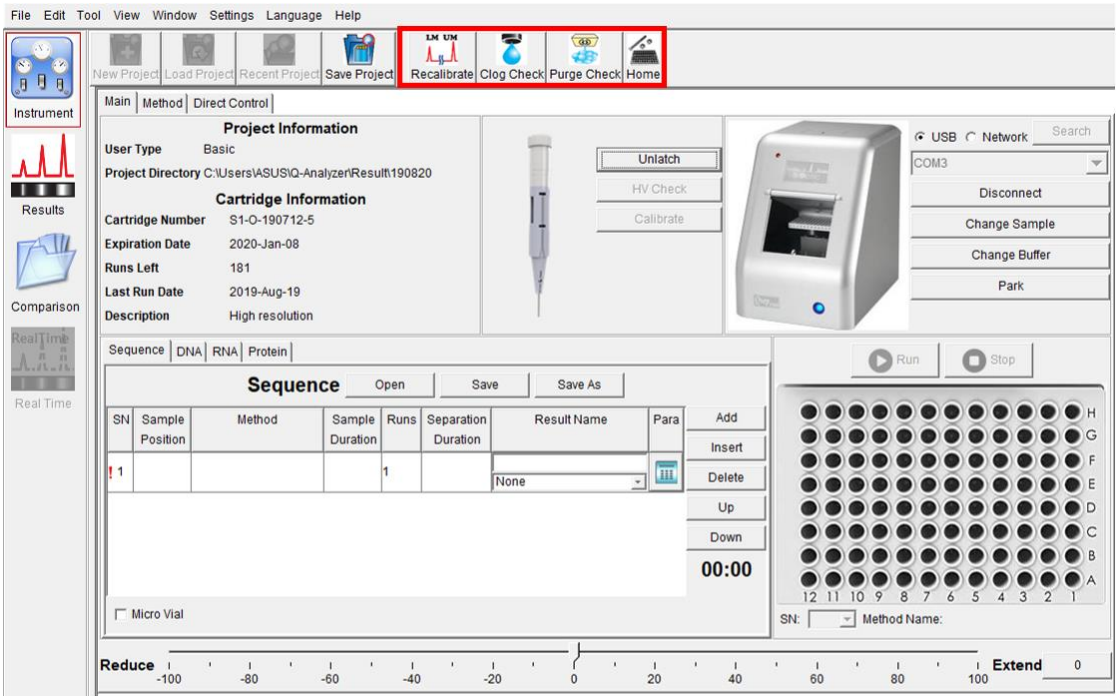
Figure 4-2 Instrument function before instrument connection

After the instrument connected, the items on Toolbar such as **ClogCheck**, **Purge Check** and **Home** will be enabled. After the cartridge is latched with *Qsep*™ Series (Figure 4-3), the item **Recalibrate** is enabled.

For *Qsep*$_1$™, after the instrument detected the SDC, the SDC data export and SDC data clear will be enabled.

These items allow the user to deal with the cartridge issue if the current is not stable during the system operation. (Please refer to Appendix A)


File Edit Tool View Window Settings Language Help
New Project
Load Project
Recent Project
Save Project
Recalibrate
Clog Check
Purge Check
SDC data export
SDC data clear
Home
Instrument
Results
Comparison
Real Time
Main
Method
Direct Control
Project Information
User Type Basic
Project Directory C:\Users\ASUS\Q-Analyzer\Result\190820
Cartridge Information
Cartridge Number S1-O-190712-5
Expiration Date 2020-Jan-08
Runs Left 181
Last Run Date 2019-Aug-20
Description High resolution
Unlatch
HV Check
Calibrate
USB
Network
Search
Qsep1:1751009237180003
Disconnect
Change Sample
Change Buffer
Park
Sequence
DNA
RNA
Protein
Sequence
Open
Save
Save As
SN
Sample Position
Method
Sample Duration
Runs
Separation Duration
Result Name
Para
Add
Insert
Delete
Up
Down
00:00
Micro Vial
Run
Stop
S Separation Buffer
P Park/Wash Buffer
C Clean Buffer
M 20-1K
S4 Sample
S3 Sample
S2 Sample
S1 Sample
SN:
Method Name:
Reduce
Extend
-100 -80 -60 -40 -20 0 20 40 60 80 100


File Edit Tool View Window Settings Language Help
New Project
Load Project
Recent Project
Save Project
Recalibrate
Clog Check
Purge Check
Home
Instrument
Results
Comparison
Real Time
Main
Method
Direct Control
Project Information
User Type Basic
Project Directory C:\Users\ASUS\Q-Analyzer\Result\190820
Cartridge Information
Cartridge Number S1-O-190712-5
Expiration Date 2020-Jan-08
Runs Left 181
Last Run Date 2019-Aug-19
Description High resolution
Unlatch
HV Check
Calibrate
USB
Network
Search
COM3
Disconnect
Change Sample
Change Buffer
Park
Sequence
DNA
RNA
Protein
Sequence
Open
Save
Save As
SN
Sample Position
Method
Sample Duration
Runs
Separation Duration
Result Name
Para
Add
Insert
Delete
Up
Down
00:00
Micro Vial
Run
Stop
H G F E D C B A
12 11 10 9 8 7 6 5 4 3 2 1
SN:
Method Name:
Reduce
Extend
-100 -80 -60 -40 -20 0 20 40 60 80 100

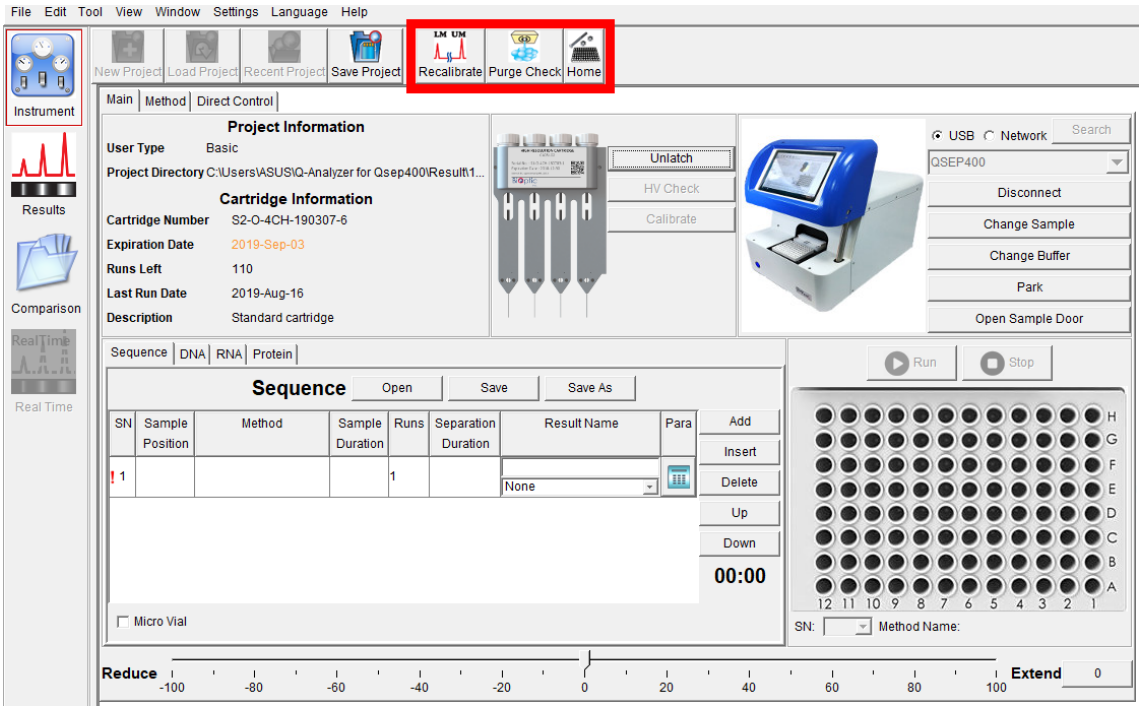


Figure 4-3 Instrument function after latching a cartridge

Recalibrate: 

Recalibration can help the identification of the alignment marker. (See Section 3.5)

ClogCheck: 

Capillary Clog Check will lower the tray and then put the pressure into the cartridge. If there is water drop formed at the cartridge tip, the cartridge is not clogged. (See Section 3.6)

Purge Check: 

Purge Check will purge while the cartridge door is opened. User can ensure that the air is coming out while purging. (See Section 3.7)

Home: 

Home will make the tray holder move to the initial place. For $Qsep_1$™, this function can be used to close the door when sample tray is taken out. For $Qsep_{400}$™, this function can help you to lock the fixing screws before moving the machine.

Slide bar (Reduce and Extend)

The remaining time of the proceeding action can be extended or shortened by dragging the slider at the bottom of the window. For instance, the software is going to running out the time of Separation & Detection, but the result is not completed yet; you can extend the remaining time by dragging the slide bar to the right (e.g.

50). The amount of the adjustment time will be shown on the button. Simply click the button to confirm the changes. Then the action of Separation & Detection will be 50 seconds extended immediately once the value reaches 0.

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

4.2.2 Results

To display or analyze data from *Qsep*™ Series**,** we provide the **Results** function. With the Results function, you can demonstrate the chart or edit the bp value from raw data of capillary electrophoresis.

After the files are loaded in *Q-Analyzer*™, the items on Toolbar such as **Calculate, Smear, Peak Calling, Parameters, Show Size/Legend, Show Size/Min, Invert GelView, Show Best/Ori. View,** and **Change Line Thickness** are enabled. The items allow user to deal with the data. For more details about editing the raw data, please refer to Section 5.1.

Calculate: 

If the size values of the result are wrong or empty, user can utilize the **Calculate** function on the Toolbar to get the respective value of bp and concentration for each peak.

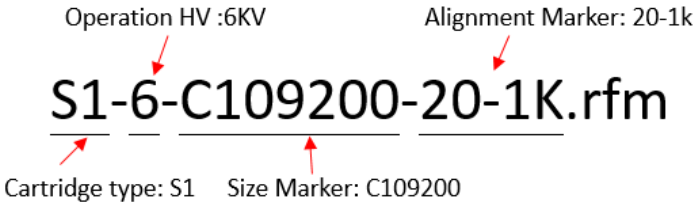
First, open the file you intend to calculate and make sure it is the selected window.

On the Toolbar, select **Calculate** and click **Load** to load a suitable reference marker table for calculating the result.

Please choose the reference marker file according to the condition of your experiments. Note that the files include four information which are

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, C109300…)
4. Alignment marker type (e.g. 20-1k, 20-5k…)

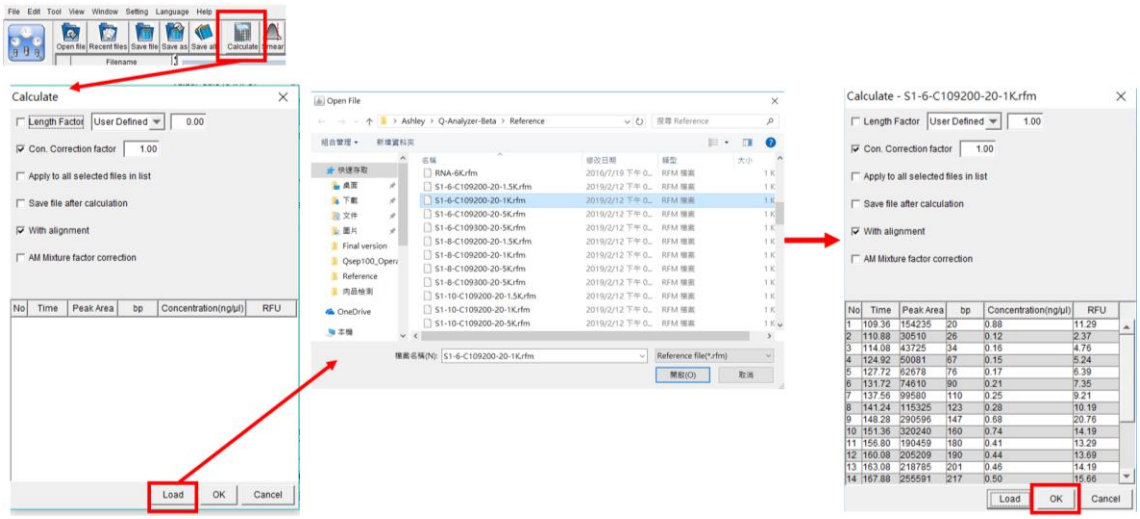
For example:



All the reference marker files corresponding to the pre-program methods can be found at the default Load folder: “<workspace>\Reference”

***Note**: Default <workspace> is C:\Users\<username>\Q-Analyzer

After confirming the reference data file, click **OK**, and the value of size and concentration will be shown in the columns.



Apply to all selected files in list: do multiple result files calculation.

Save file after calculation: save the result file after the calculation completed. Please select this function if you want to have the calculated result every time you open the file.

With alignment: if you apply the alignment marker for analysis, select the checkbox.

AM Mixture factor correction: select the checkbox of this function for proper calculation if the alignment marker is mixed with the sample.

Smear: 

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

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

*** Note**: Zone2 will not be displayed (default setting). If user want to display Zone2 on the result file, click the "Zone2" directly. .

To conduct the analysis, do the following steps:

Step 1. Click the **Smear** tab (Figure 4-4 A). (Alternatively, you can select **Smear** from the Toolbar and click the **Smear** tab after setting.)

Step 2. Select the interested range, **Area between lower marker and upper marker** or **Area from lower marker to end** for analysis (Figure 4-4 B).

Step 3. Enter the percentage of the target distribution (e.g. 50%) and click **Apply**. User can also assign the region by entering the size numbers, and click **Apply** (Figure 4-4 C), then the detail information of the assigned

region, including the **Average size** and concentration of this region (**nmole/L**) shows at Figure 4-4 D

Step 4. Modify the **Interval** of the size range if necessary, and then click **Apply** to apply the setting (Figure 4-4 E). This will help you get more details of the distribution (Shows on the Statistics below).
Also, the **DQN** means the DNA quality number will show below to help you realize the quality of your sample.

Step 5. In addition, you can edit the other Zone by click the **Zone2** and the editing rules are the same as mentioned above. This step will help you get the ratios between Zone1 and Zone2, which help you get more information about these two Zones (Figure 4-4 F).

Step 6. All information about the distribution will be displayed in the below table.

Step 7. Click the right button on the right panel to export the report.

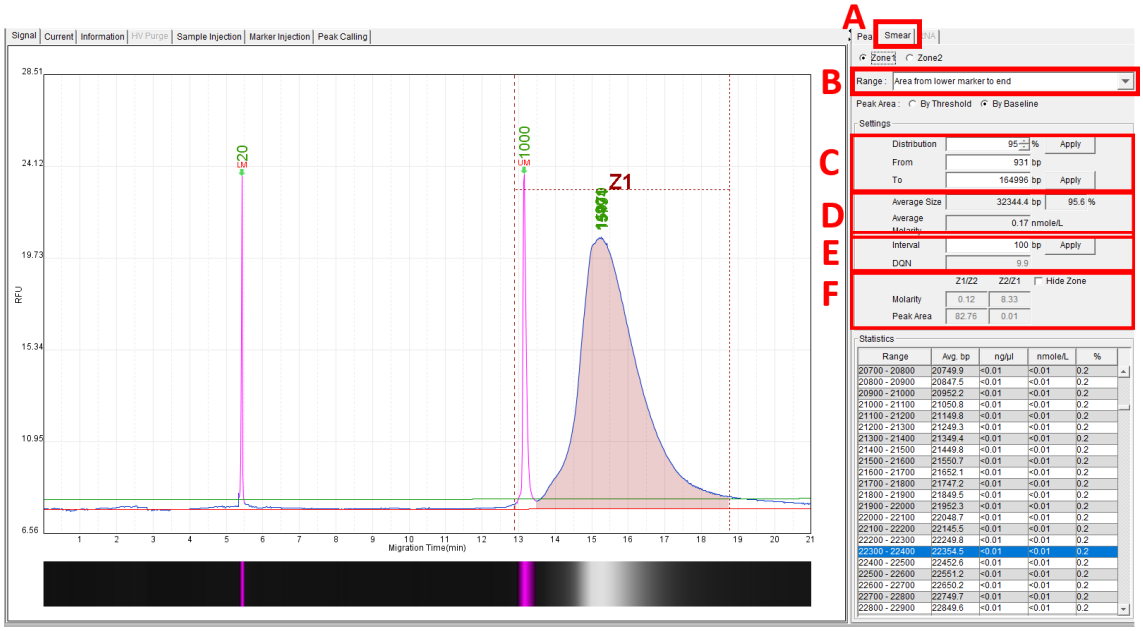


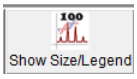
Figure 4-4 The result display of genomic DNA sample

Peak Calling: 

Peak Calling function allows user to distinguish the target peaks.
The software will follow the rules in the **Peak Calling table** and show the report at the Peak calling tab window. Report "**+**" when the peaks are found and show "**-**" when the peaks are not found. (See Section 5.5)

Parameters: 

The system will generate the corresponding baseline and define the peaks automatically. If you are not satisfied with the results, you can modify them with Parameter functions. Make sure the selected result file window is the one you want to modify. Multiple files can be selected by clicking their checkboxes before using the Parameter function. (See Parameter setting in Section 4.3.2)

Show Size/Legend: 

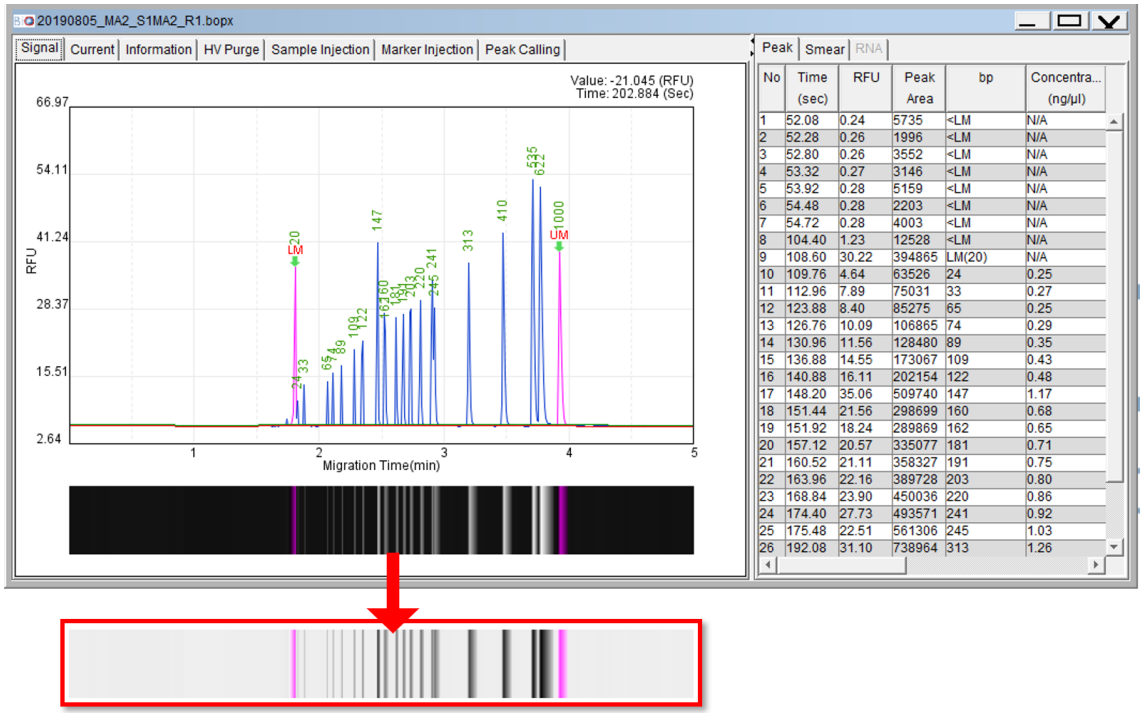
Switch the index of the peak to display the bp value or legend in the chart.
To display the bp value, the result data must be calculated. To display legend, the **Peak Calling** function data must be applied**.**

Show Size/Min: 

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

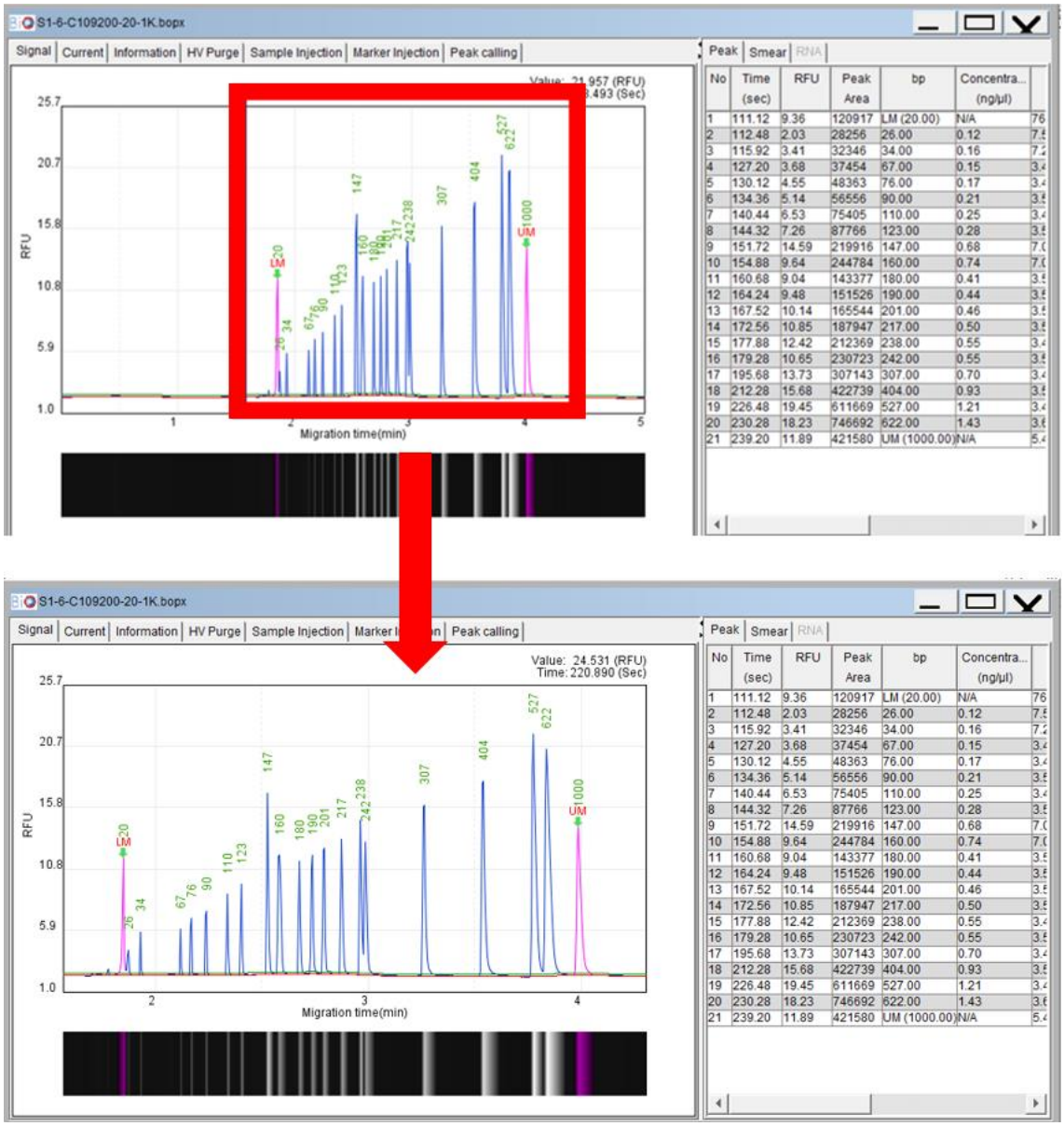
Invert GelView: 

Invert the color of white and black in **Gel View** (as showing below)



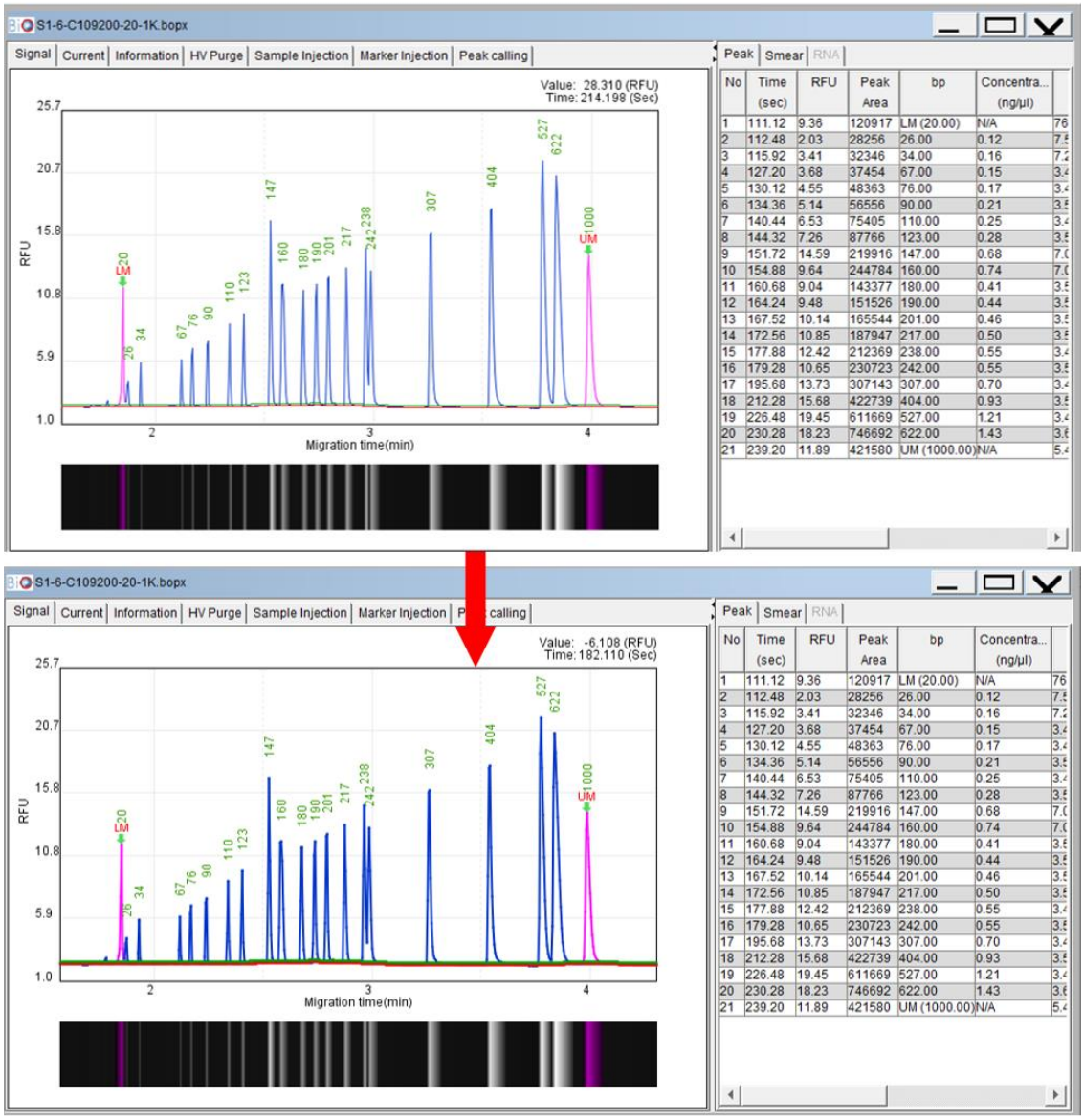
Show Best/Ori.View 

Show the best view of the signal pattern.



Change Line Thickness 

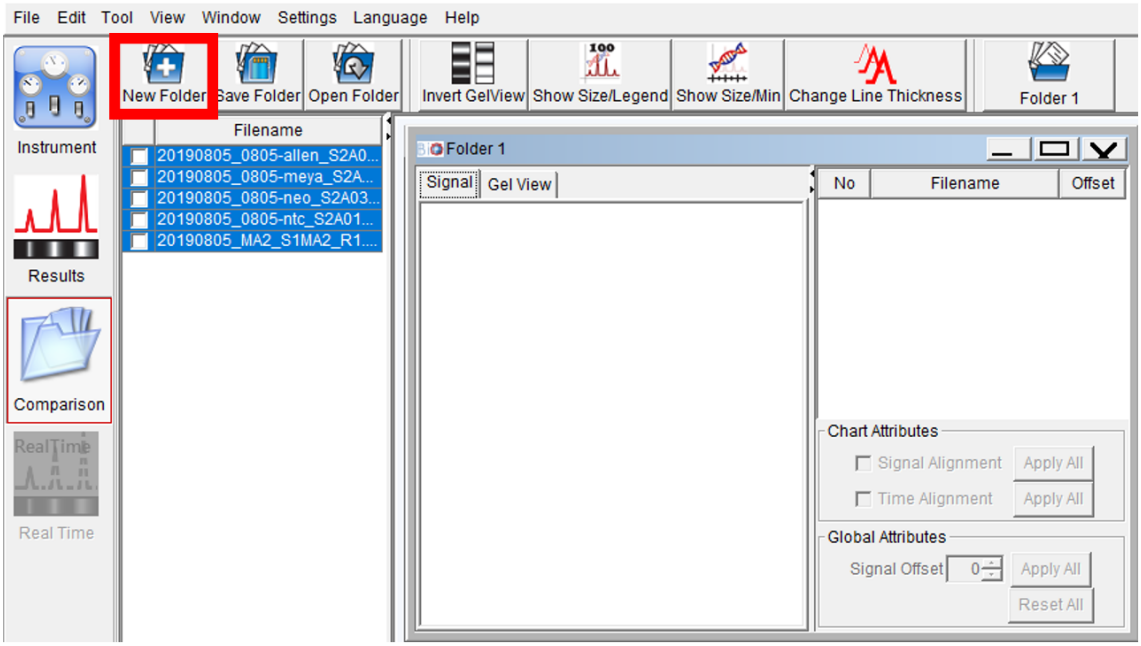
Switch the thickness of the peaks in the chart.



4.2.3 Comparison

Using the **Comparison** function, it allows user to compare or analyze multiple results. With this function, you can put several charts together to compare the variance in different samples or conditions.

Since the **Comparison** function is used to analyze multiple results in a chart, you need to open several results before performing the **Comparison** function. After opening several result files, click **New folder** and drag the result files to the folder window. Then, the results will be displayed on the folder window. The detail functions in the **Folder** such as demonstrating and comparing the results will be described at Section 5.2.



Use the Comparison function to compare different result files.

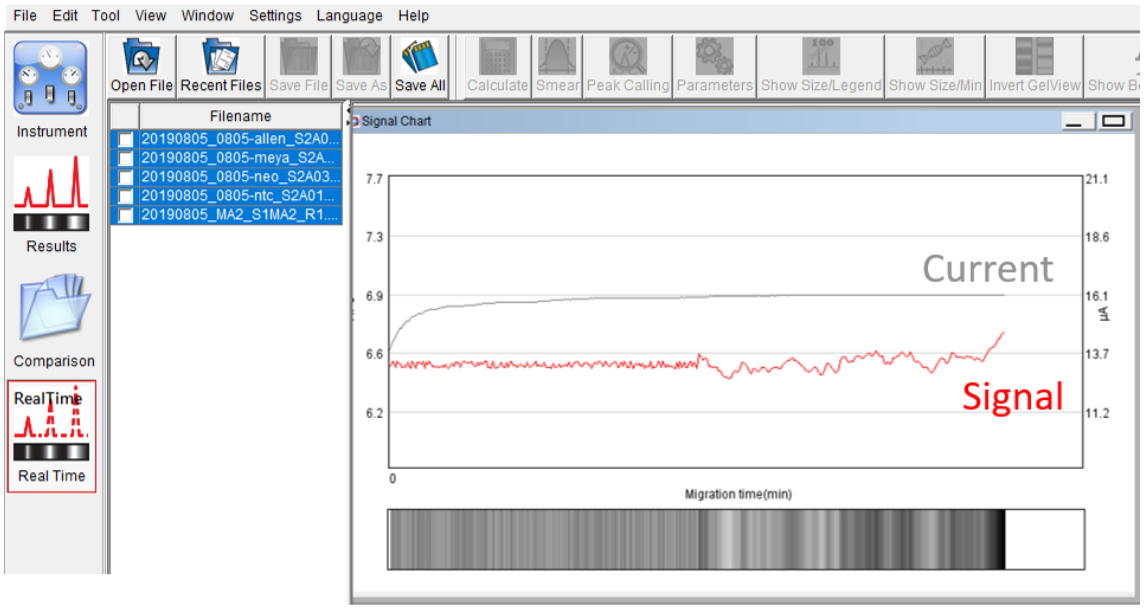
Step 1. Open the files you want to compare.

Step 2. Click **New Folder** in Toolbar to create a Folder.

Step 3. Drag the files from the Filename panel and drop them into the Folder that you have created.

4.2.4 Real Time

The **Real Time function** provides a real time display of the current and collected data which includes the current and RFU (amplitude of the capillary electrophoresis signal). *For the $Qsep_{400}$, it will show four real time displays at the same page.



4.3 Menu

4.3.1 File

File functions are:

New Project: Create a new test project.

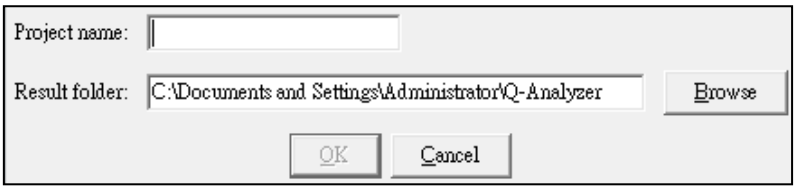


Figure 4-5 New project

Project can be used to differentiate several experiment conditions.

The test results will be saved in the corresponding “Result” directory. “Project name” is the name of the folder where the result files will be saved.

Operating System	Default Saving folder
Windows 7, 8, 10	*<workspace>*\Result*<Project name>*

* Default *<workspace>* is C:\Users\ *<user name>*\ Q-Analyzer

New Folder: Open a new folder to compare the results.

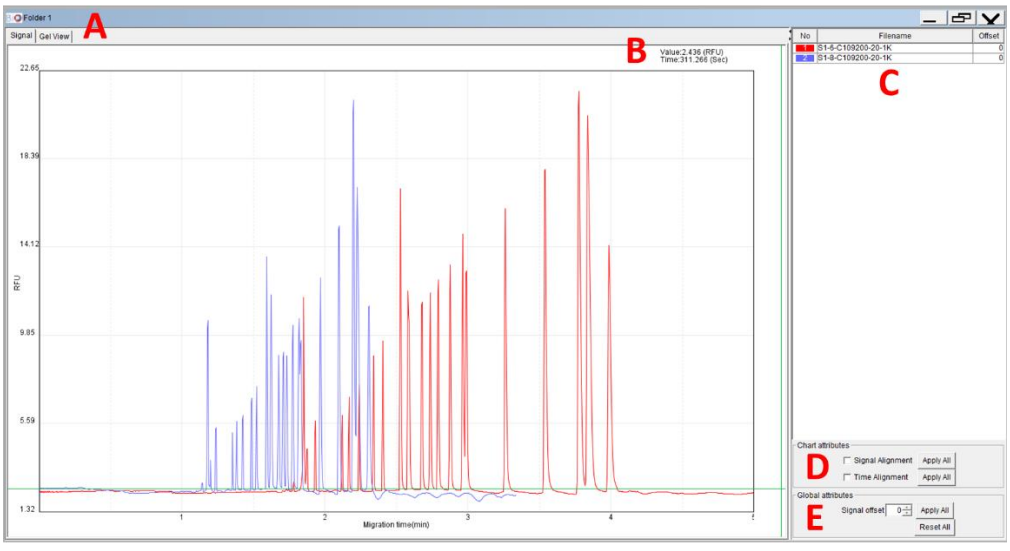


Figure 4-6 New Folder

Folder in *Q-Analyzer*™ is used for comparing results. By clicking the tab at Figure 4-6 A, you may view the result in the peak view or gel view. Figure 4-6 B displays the raw data of the selected point on the chart. Figure 4-6 C displays the imported files. For more details, please refer to Section 5.2.

The results are plotted as RFU (the Y axis) to TIME (the X axis) diagram. The baseline may vary between tests.

The **Signal Alignment** function (Figure 4-6 D) might be utilized to align the baselines to the same level. After the signal alignment is achieved, you can compare the peaks from different results.

The **Time Alignment** function (Figure 4-6 D) might be used to adjust the time variation. The **Time Alignment** function will align the Alignment Marker of each data. The default setting of system is assigning the first file in Figure 4-6 C as the base. You may right click on the file and select **Main ladder** to define your own base. The file name of the base will be presented in red.

Signal Offset function (Figure 4-6 E) helps you separate the stacked data. You may reset the outcome by clicking **Reset All**.

In the folder window, by right clicking in the signal tab, you can export the chart with or without the sample description and result name.

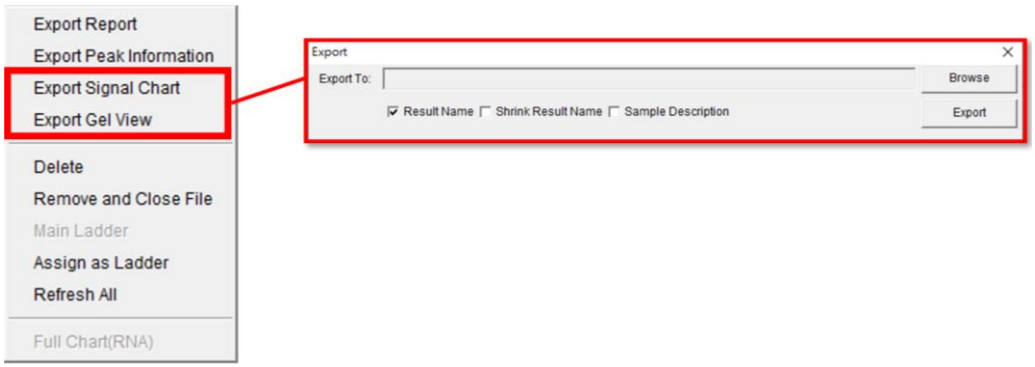


Figure 4-7 Folder Export Chart

Open File: Open the result files from each project.

Open Folder: Open the folder files from each project.

Save File: Save the modified results.
It will only save the selected window.

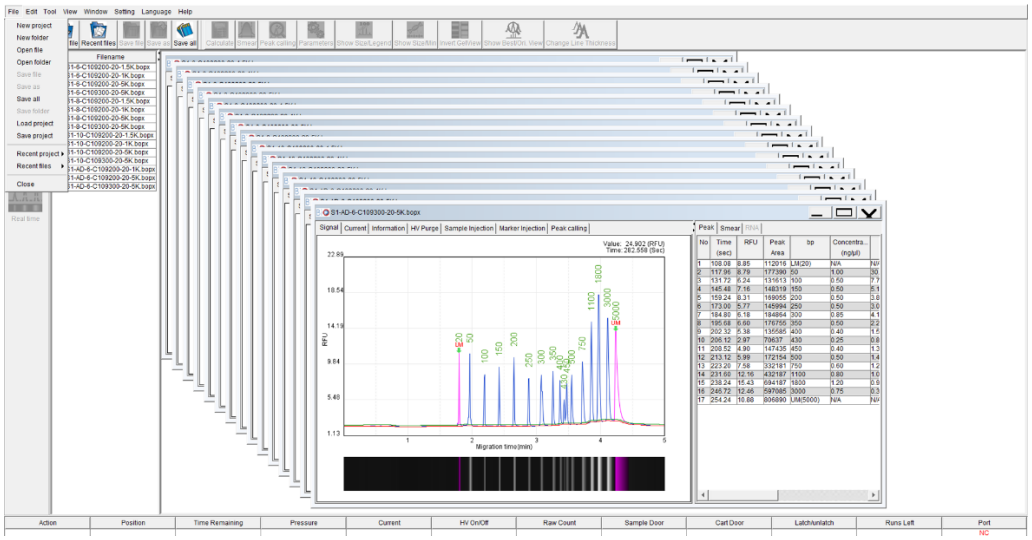


Figure 4-8 Choose the window you want to save

Save As: Save the modified results as another files.

Save All: Save all modified results.

It will save all files open in the operation panel.

Save Folder: Save the created or modified Folder.

The created or modified Folder can be saved.

Load Project: Load the saved project.

Save Project: Save the current project.

The settings including the methods used in the sequence, sampling position, duration, sample information, and result names will be saved.

Recent Projects: List the latest projects you have opened for loading.

Recent Files: List the latest result files you have opened for loading.

Close: End and exit *Q-Analyzer*™.

4.3.2 Edit

Edit functions are:

Sample file: Edit the information of each sample. (Figure 4-9)

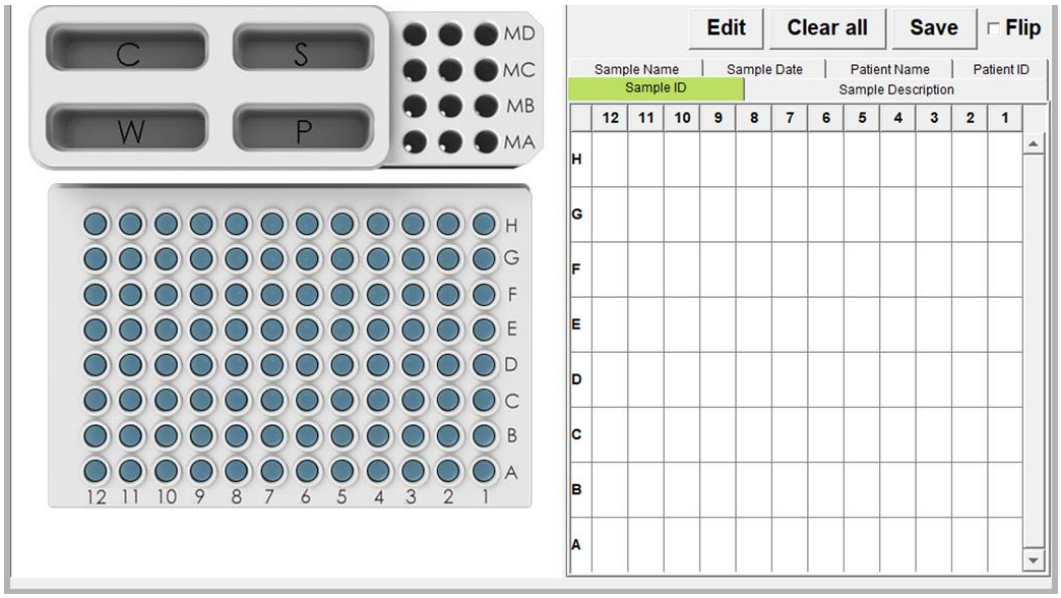


Figure 4-9 Sample file window

Click **Edit** and the Sample loader window will pop-up. Select the sample you want to edit and enter the sample information, such as sample/patient name, ID, date, and description. Click **OK** to apply the changes or **Clear** to delete. Click **Save** or **Save as** to save the sample file. You may import the sample files you saved earlier with **Load** for inquiry or editing. An excel file can also be imported to edit multiple sample description.

Reference marker: Edit your reference marker table.

The **Reference Marker** function might be utilized to modify the saved reference marker table or create a new one. The windows are shown in Figure 4-10.

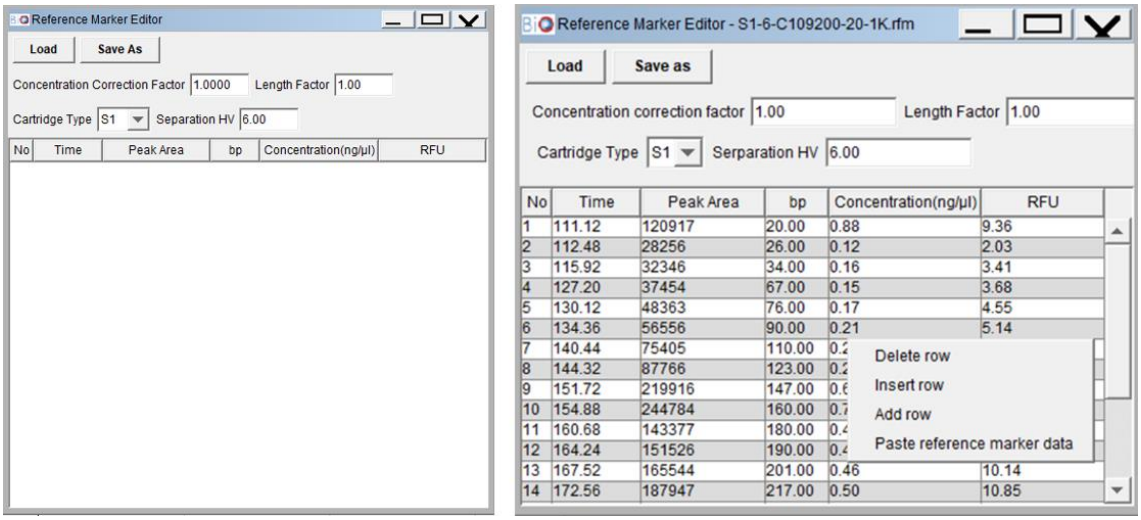


Figure 4-10 Edit Reference Marker Table

Load the saved reference marker table (*.rfm) as shown in Figure 4-10.

- **Time**: the migration time of each peak
- **Peak Area**: the measure of area within the peak
- **bp**: the fragment length
- **Concentration (ng/µl)**: the concentration of each base pair.

Time and **Peak Area** will automatically be generated from the test result. (see Section 5.1.2 for creating reference marker file).

Unless there are specific requirements, do not change the value in **Time** and **Peak Area**. Enter **bp** and **Concentration** of a known size marker and add or delete rows if necessary. After completing all modifications, the data can be saved as a reference marker for later practices. For more details, please refer to Section 5.1.2.

Parameter: Change the parameters of the calculation settings.

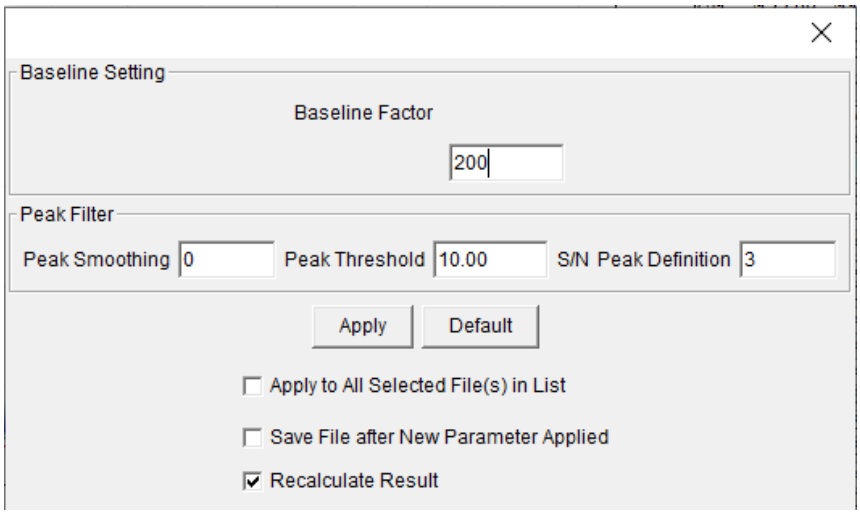


Figure 4-11 Parameter settings

The system will generate the corresponding baseline and define the peaks automatically. If you are not satisfied with the results, the results can be modified by the **Parameter** function. Make sure the selected result file window is the one you want to modify.
You may also select multiple files by selecting their checkboxes before you use the **Parameter** function.

Baseline Factor affects the smoothness of baseline. The default Baseline Factor value is based on the chosen method. The bigger the value is, the smoother the baseline will be, and vice versa.

Peak Smoothing affects the smoothness of peak. The bigger the Peak Smoothing value is, the smoother the result will be. This function might be utilized to reduce the number of peaks.

Peak Threshold changes criteria of defining the peak. The bigger the Peak Threshold value is, the stronger the signal is requested to be recognized as a peak. The peaks with low signal strength can be recognized by lowering the Peak Threshold value. Meanwhile, the noise may be recognized as signal peaks as well.

S/N Peak definition changes criteria of defining the peak. The bigger the Peak definition value is, the more data points of the ramping signal are required to be recognized as a peak. You may lower the Peak definition value to define peaks with less points of the ramping signal. Meanwhile, the noise may be recognized as signal peaks as well.

Apply to all selected files in list will change the parameter of the file you selected in the list. Make sure to select this function if you want to do a multiple result file modification.

Save file after new parameter applied will help you to save file automatically after applying parameter.

Recalculate result will help you to recalculate result and show the peak bp automatically after applying parameter.

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

Peak Calling Table:

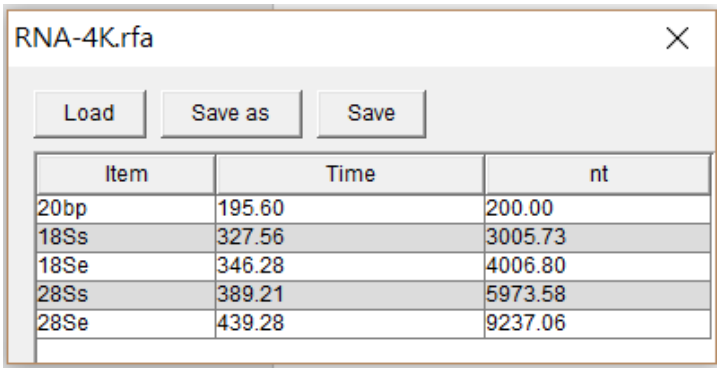
Peak Calling helps user to create the panels for quick scanning. To use peak calling, user needs to set up the Peak Calling Table which including the target peak information. You can customize your own peak calling table by adding a new row and assigning the Target, upper margin, lower margin, S/N, RFU, Legend and IC (Internal Control).

For more details, please refer to Section 5.5.

RNA reference table:

RNA factor table helps user to modify the parameters of RNA signal definition for result analysis (18S and 28S). The 18Ss and 18Se mean start and end of 18S. If you want to modify the value, you can just click **Load** to read the .rfa file from the default directory.

The value will be effective, only if you have overwritten the original file. For more details, please refer to Section 5.3.



4.3.3 Tool

Tool functions are:

Calculate: Use the reference marker table to do the base pairs calling and quantitative analysis of your result (Figure 4-12).

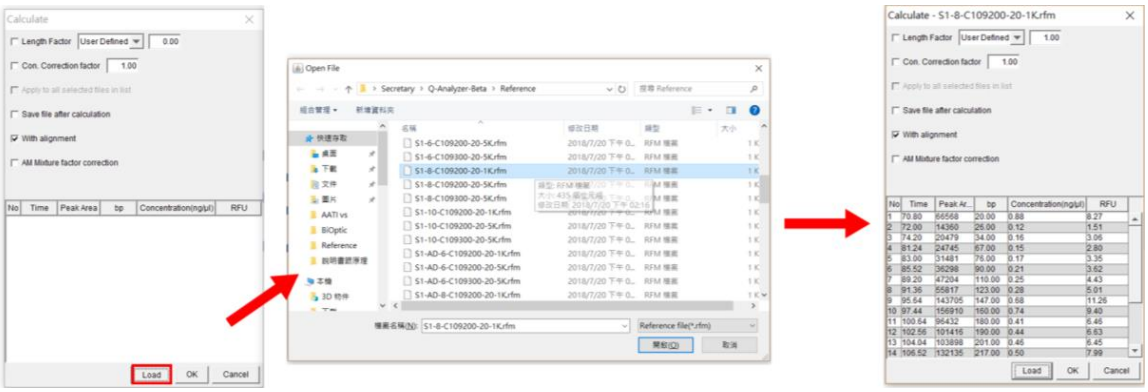


Figure 4-12 Calculate workflow

By clicking Load, the reference marker table will be imported. After loading, click **OK** to do the calculation, and the result file will show the corresponding values of bp and concentration.

Apply to all selected files in list: do multiple result file calculation.

Save file after calculation: save the result file after the calculation is complete. Please select this function if you want to see the calculated result every time you open the file.

With alignment: if you apply the alignment marker for analysis, select the checkbox.

AM Mixture factor correction: select the checkbox of this function for proper calculation if the alignment marker is mixed with the sample.

Click "**File→Save File**" to save the calculated result. You may reset the calculated result of the value of bp and concentration to zero with the **Reset** function.

Recalibrate: Use to improve the accuracy of identifying the alignment marker. (Figure 4-13)

The user can conduct the **Recalibrate** if one of the following occurs:

- Replacement of the alignment marker
- 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:

Step 1. Make sure the fresh Alignment Marker (e.g. 20bp&1000bp Alignment Marker) is placed at right position.

Step 2. Make sure all buffers are in good condition

Step 3. Click "**Tool→Recalibrate**" and follow the instructions

The message box of Recalibrate (Figure 4-13) provides the details in different calibration settings. To do the recalibrate, choose the voltage and alignment marker that match with the “Method” that will be used.

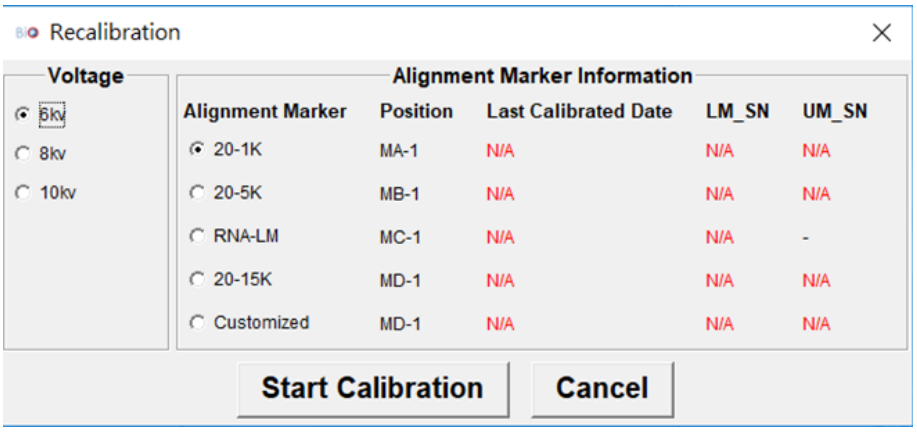


Figure 4-13 Recalibrate message box

Smear:

The **Smear** function can help user to understand size distribution and average size of samples. The function can be applied to DNA, RNA and protein analysis.

Peak Calling:

Peak Calling helps user to create the panels for quick scanning. To use peak calling, you need to set up Peak Calling Table. (**Edit → Peak Calling Table**). For the detail, please refer to Section 5.5.

Analyze:

Analyze is designed to generate a summary report of Nucleic Acid Amplification Test (NAAT) automatically. To use this function, user need to use the kit for specific applications. Please contact BiOptic Inc. (www.bioptic.com.tw) for more information.

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

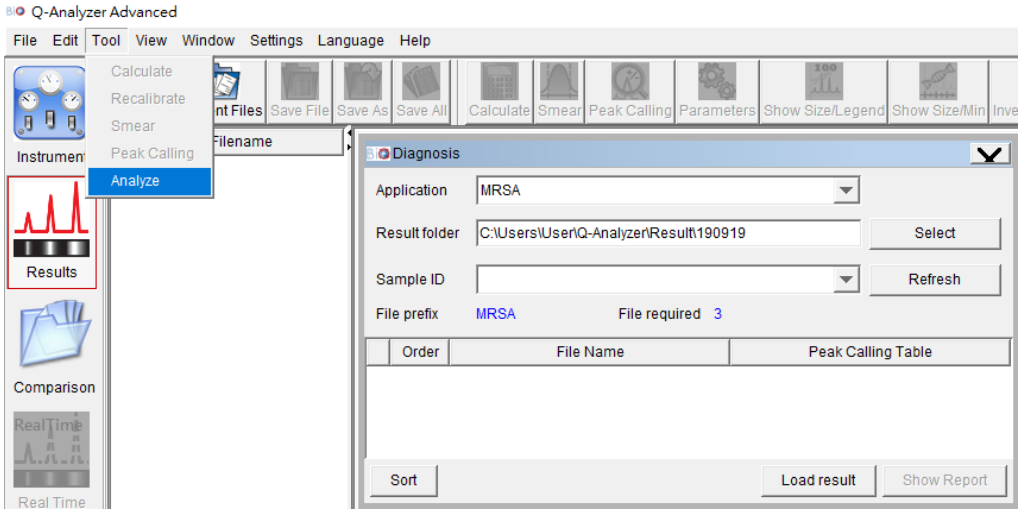


Figure 4-14 Analyze function on Toolbar

4.3.4 View

View functions are:

Chart setting: Change the settings of the electropherogram.

Chart setting functions is used to set the color of line, Size/Legend, grid, background, data display, and whether to show the peak indicator.

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.

In Figure 4-15, A is the original gel image in the system. After tuning the contrast, you may enhance those weak peaks as shown in B. C is the inverted gel image, and D is the inverted image after tuning the contrast of C.

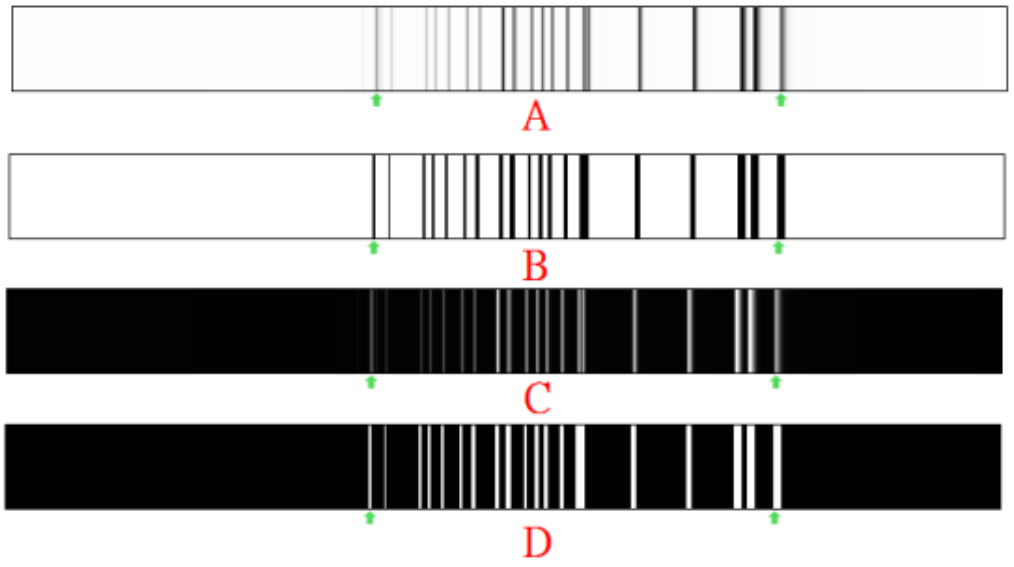


Figure 4-15 Gel Image

4.3.5 Window

Window functions are:
Cascade: Arrange the opened windows in the array format.
Close All: Close all opened windows.

All opened files will be listed below the Window.

4.3.6 Setting

Setting functions are:
Change password: Change your password of the software key.

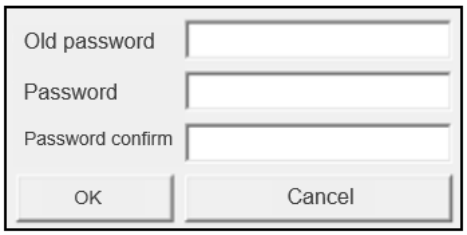


Figure 4-16 Change password

Please fill up the blank (Enter your old password, password, and password confirm). The password consists of **8 characters/numbers (case-sensitive)** (**6** characters for the old software key).
***Note**: If you enter the wrong password over 15 times, your software key will be locked.

Unblock PIN: If you enter the wrong password over 15 times, your software key will be locked. Please contact with BiOptic Inc. at the official website (www.bioptic.com.tw) or with your local BiOptic representatives to **Unblock PIN.**

Figure 4-17 Unblock PIN

Preference:

This page is used to configure the default setting of *Q-Analyzer*™ such as view method, Calibration time, motor setting, etc. We only suggest the professional person to modify these values.

***Note**: Some preference setting might change the default value of this instrument and affect the result of experiment. It is suggested to change this setting after you have a complete professional training from BiOptic Inc..

Figure 4-18 Preference setting

4.3.7 Language

You can select three different kinds of language to use *Q-Analyzer*™, including English (Default), Chinese (Simplified), Chinese (Traditional). After changing the setting, you need to relaunch *Q-Analyzer*™.

4.3.8 Help

Help functions are:

Version:

Display the version of your software and firmware. Technical Support file can also load from this window.

You need to connect to *Qsep*™ series to get the information of the firmware. By clicking **Technical Support**, you can open a "bioptic" technical support file.

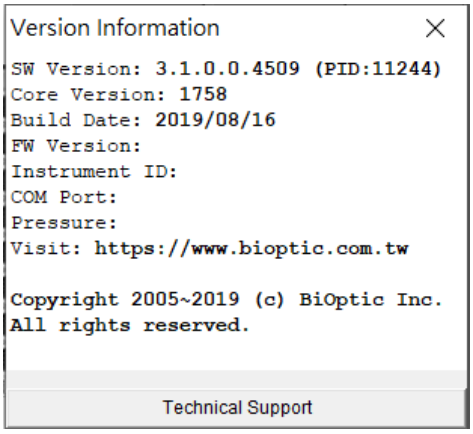


Figure 4-19 Version window

Help: Provide the information of *Qsep*™ series and *Q-Analyzer*™.

Help function can also open this operation manual.

Report: Generate report files.

Collect and save the system errors information into a file. When encountering troubles, you may use the **Report** function to generate the error report files and send them to BiOptic via service@bioptic.com.tw for technical support.

Toolbox: Provide step-by-step trouble shooting process.

Capillary Clog Check will lower the tray and then put the pressure into the cartridge. If there is water drop formed at the cartridge tip, the cartridge is not clogged. User can check whether the cartridge tip is clogged. For more details, please refer to Section 3.6.

Purge Function Check will purge while the cartridge door is opened. User can ensure that the air is coming out while purging. For more details, please refer to Section 3.7

Motor function check is to 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 instrument. After clicking start, the motors will do all kinds of actions to move the tray holder, and user can observe motor status during each step.

Computer compatibility check is to check the software setting environments, including the hardware and software requirements. For more details, please refer to Section 1.3.

4.4 Control Panel

When you launch *Q-Analyzer*™, it will open the Control Panel automatically. There are four major functions in the Control Panel, which are **Main**, **Method**, **Direct Control**, and **Board Setting** (shown in Figure 4-20 F). Different level of access to those functions will be given to the user based on which software key the user has. With Basic software key, the user has access to Main, Method, and Direct Control tabs. Only the user with Advanced software key has access to Board Setting tab.

The functions of those tabs are listed as follows:

4.4.1 Main

Main page is the major control to operate *Qsep*™ series. The Main pages of other *Qsep* series, including $Qsep_1$™ and $Qsep_{400}$™, are the same as $Qsep_{100}$™. It will show different pictures of the connected instrument for $Qsep_1$™ and $Qsep_{100}$™, and $Qsep_{400}$™.

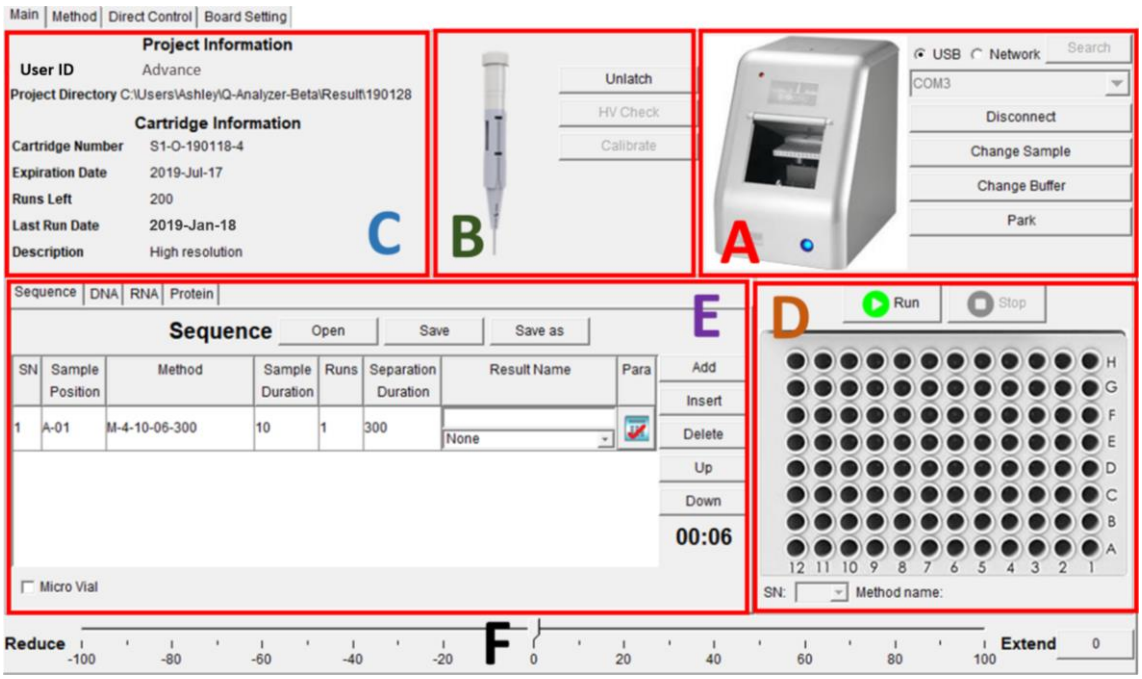


Figure 4-20 Main page

Figure 4-20 A is for instrument control. You can click **Connect** to establish the connection with different *Qsep*™ Series through the port you selected. You may simply

choose **Auto** to let the system to search the port automatically.

After *Qsep*™ Series is successfully connected, the picture of *Qsep*™ Series on the Main Page will turn into color form. **Connect** button will become **Disconnect** for manual disconnection. **Run** and **Stop** buttons are used to start or end a test. There are three different forms of the **Run** button during a test: **Run** to start the test, **Pause** to pause the running test, and **Continue** to resume the unfinished test.

Change Sample moves the 96-well sample holder or the different sample trays (for *Qsep*$_1$) to the door.
Change Buffer moves the holder of the buffer tray and marker to the door.
Park moves the buffer tray under the cartridge tip at the Park position to protect the cartridge tip from dryness.

Latch and **Unlatch** are used to lock or unlock the cartridge(s) in the instrument. Before executing a test, you must **Latch** the inserted cartridge. The cartridge information will be displayed on Figure 4-20 B, and the picture will turn into color. Click **Unlatch** and wait for few seconds before removing the cartridge.

Figure 4-20 C shows **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.

You can build your own Sequence in Figure 4-20 E by editing **Sample Position**, **Method**, **Sample**, **Duration, Runs**, **Separation Duration** and **Result Name** (see Section 3.1.2).

The remaining time of the proceeding action might be extended or shortened by dragging the slide bar in Figure 4-20 F. For instance, the time of Separation & Detection is running out, but the result has not completed yet; you may extend the remaining time by dragging 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. Then, the action of Separation & Detection will have 40 seconds extended immediately.

4.4.2 Method

Method tab is for viewing, editing, and creating the method (Figure 4-21).
***Note**: Only Advanced users can edit and create method.

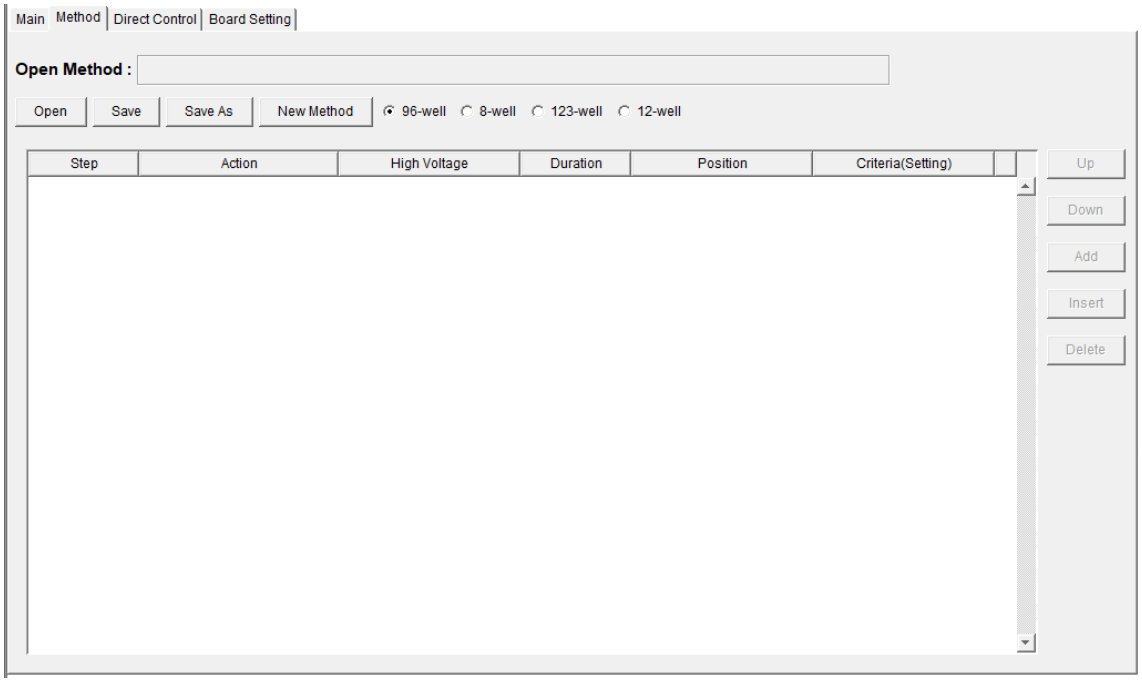


Figure 4-21 Method page

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

The functions of these actions are:
Purge: Use the air pressure to refill the capillary.

High Voltage Purge: Use the air pressure with the high voltage to refill the capillary.

Sample Injection: Inject the sample into the capillary using the electrokinetic.
***Note**: Only one Sample Injection action is allowed in a Method.

Marker Injection: Inject the alignment marker into the capillary using the electrokinetic.

Separation & Detection: Start the capillary electrophoresis analysis.
***Note**: Only one Separation & Detection action is allowed in a Method.

End: Declares the last commend of a Method.

Pause: Parks the cartridge at the Park position.

Purge Check: Purge check ensures that the air tube is not clogged before use and clean the gel which was accidentally sucked into the air tube after use.

You can create a method by clicking **New Method** in the Method tab (Figure 4-21). By clicking **Add** button on the right, you can add an action. Select the action you want and type the duration time. After you select the action position, you can add another action row. You need to select a position for every action except **Sample Injection** because the position of the sample is controlled by the Main tab.

4.4.3 Direct Control (Partial Advanced Only)

All the actions of the Method can be executed manually and independently under Direct Control. You can also move the 96-well tray holder to the designated position.

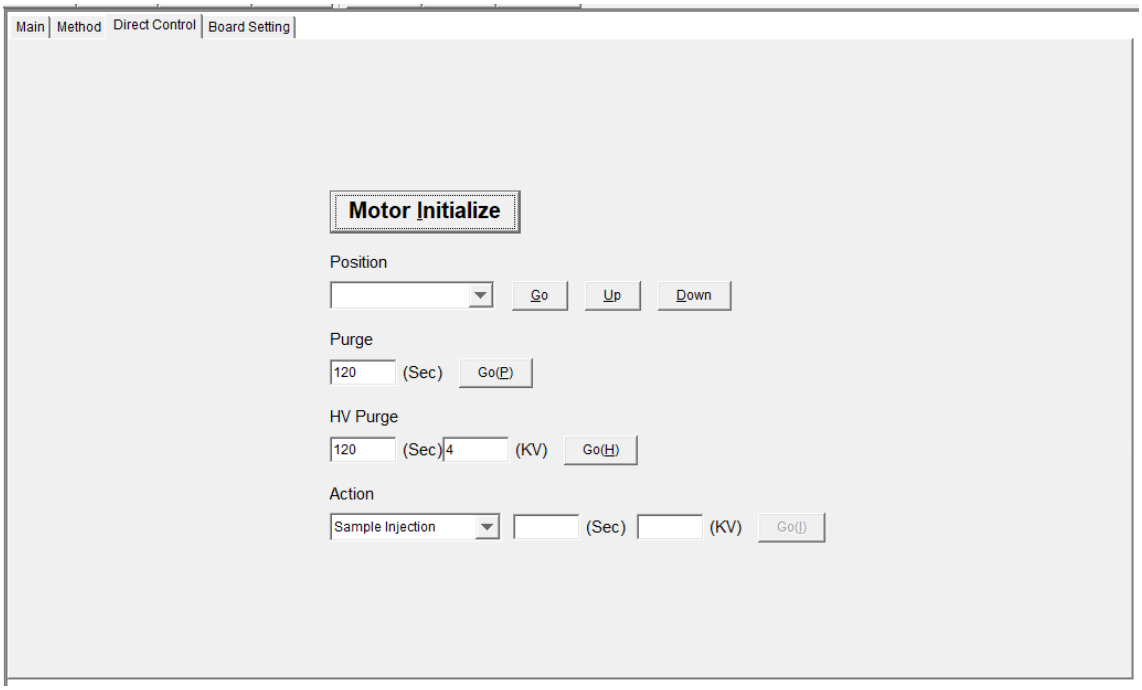


Figure 4-22 Direct Control page

Motor Initialize: initializes the motor position.
Position: can move the ideal well below the cartridge tip.
Purge: set the time used for purging.
HV Purge: set the time and voltage used for purging.
Action: You may select **Sample Injection**, **Marker Injection**, and **Separation & Detection** and assign the duration and voltage.
***Note**: Every execution of **Separation & Detection** will deduct the available runs of the cartridge.

4.4.4 Board Setting (Advanced Only)

Board Setting provides the information of the **Instrument ID**, **Raw Count/Current**, **PMT Voltage**, **Sample Rate**, **Light**, and **Data Collect**.

The **Sampling Rate** function can be used to change the frequency of collecting the raw data. The **Data Collect** function can be used to collect the raw data for an assigned duration. During data collection, the raw data shows at the **Raw Count / Current** table. After executing **Data Collect** function, the average, standard deviation, maximum, and the minimum of the raw counts and current show at the table below. CH_01-CH_04, which means 4 channels, is used in analyzing the results of $Qsep_{400}$™.

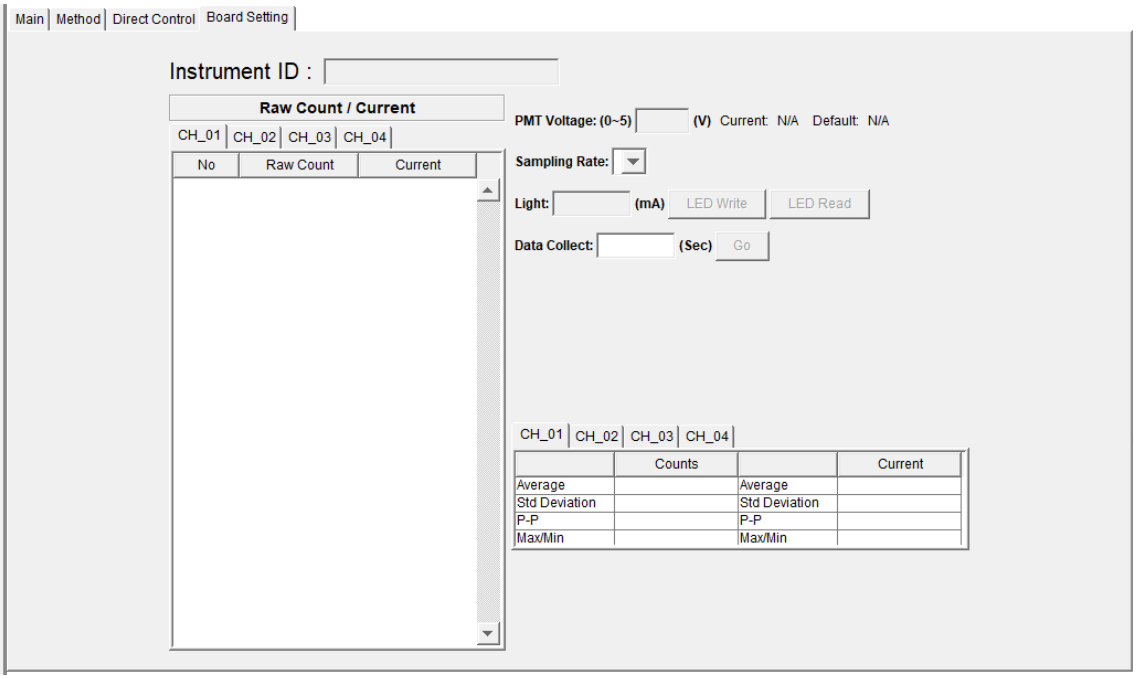


Figure 4-23 Board setting page

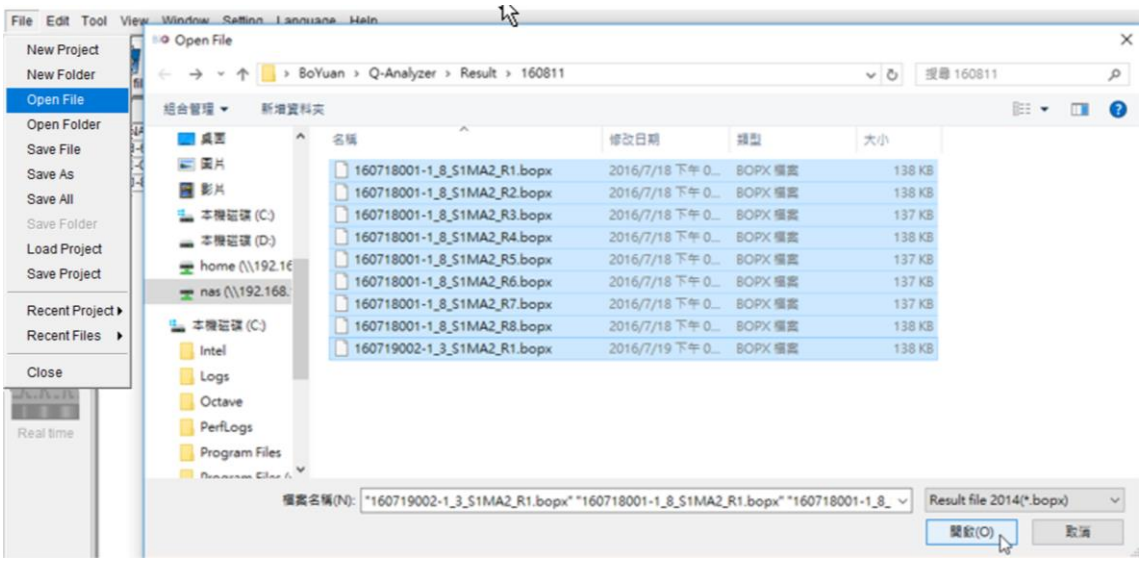
5. Result/Data Analysis

5.1 Result for Data Display

Using the **Result** function, you can analyze the data from *Qsep*™ Series. The Result function can help you to demonstrate the chart or to edit the bp value from the raw data of the capillary electrophoresis.

Open the result file by clicking **Open File** on the Toolbar. The browser helps you to select multiple files and display the chart of the collected data. According to the default settings, the result file is saved as ".**bopx"** file, and the result directory of *Q-Analyzer*™ is stored at: <*workspace*>\Result\<*Project name*>

* Default <*workspace*> *is C:\Users\<user name>\Q-Analyzer*



The operation region will also display the latest result, after each Method has completed. The latest result will be shown in the Main Panel after each test finished, and the previous result window will be closed automatically.

If you open lots of results, the order of files can be changed by clicked the right button in the "Filename" (The following pictures) and user can arrange the files by "**Sample, Runs, Test Date, Cartridge"**.

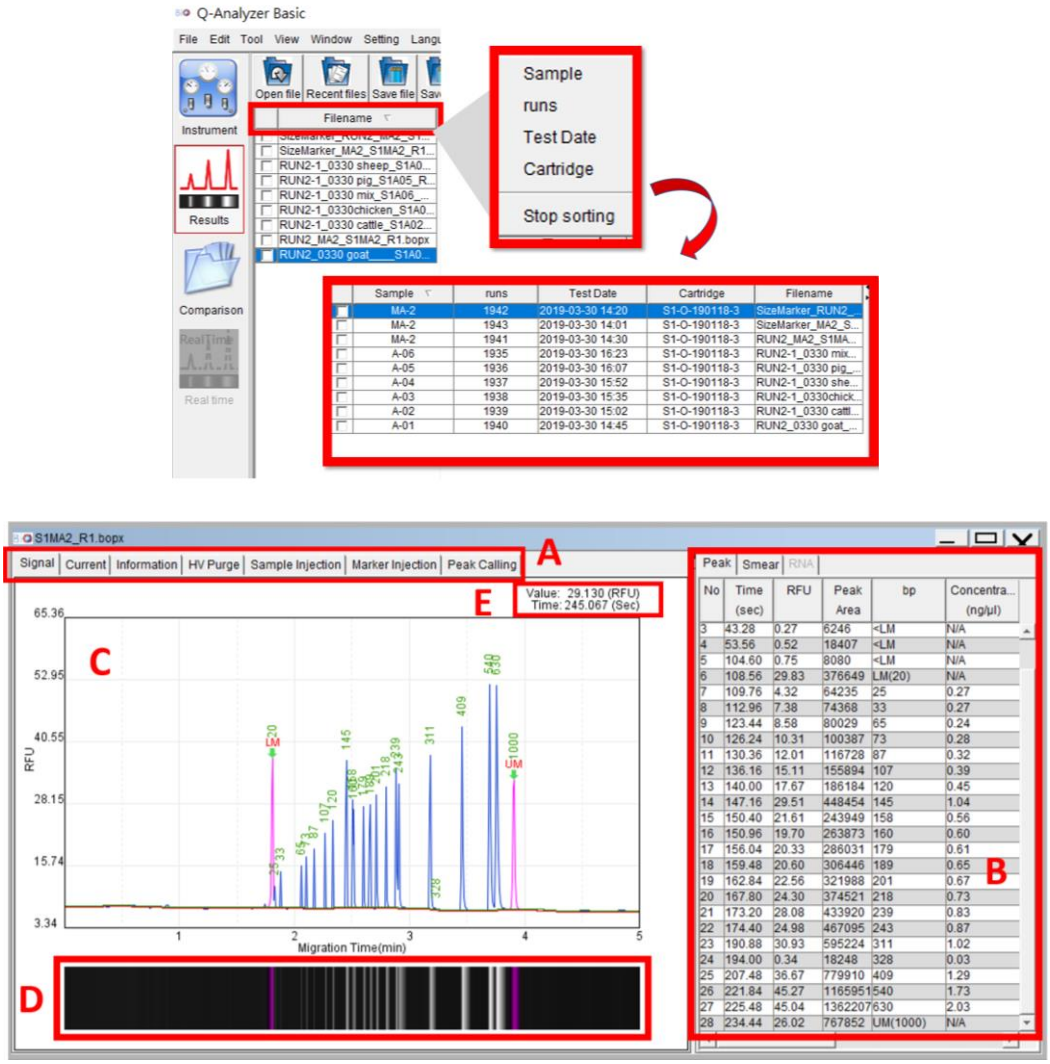


Figure 5-1 Result Display

There are several tabs in the Result file, which are **Signal**, **Current**, **Information**, **HV Purge**, **Sample injection**, **Marker injection**, and **Peak calling** (Figure 5-1 A). On the right side, there is a peak table that including **Time**, **RFU**, **Peak Area**, **bp**, **Concentration (ng/ul)**, **nmol/L, Peak Start (sec), Peak End (sec), S/N, FWHM (sec) and Area (%)** (Figure 5-1 B). The value of bp and concentration will only be displayed after the calculation is performed.

If you right click in B region, several functions are available: **Export Report**, **Export Chart** and **Export Raw Data** to save analysis result after Smear (See Section 5.4) and Peak Calling (See Section 5.5). **Export raw data** to save the data as a ".csv" files.
Set as lower/upper marker can assign the selected peak to be the upper or the lower marker. Make sure to set the correct upper and lower marker in order to calculate bps and concentration. **Delete Peak(s)** to remove unnecessary peaks.

Merge peak helps you combine the minor peaks with the major ones. Select those peaks you would like to combine, and right click the selections and select **Merge peak**. It will add up the peak area of the selected peaks and further define the peak migration time of this merged peak area from the major peak.
***Note**: Make sure you execute the **Calculate** function after merging peaks to get more accurate results.

Remove Calculated Info to remove the original calculated information.

Copy peak Info for Creating Reference to copy the data for creating the reference marker table. **Update Cartridge Factor** to renew the following running results after assigning the correct lower marker and upper marker.

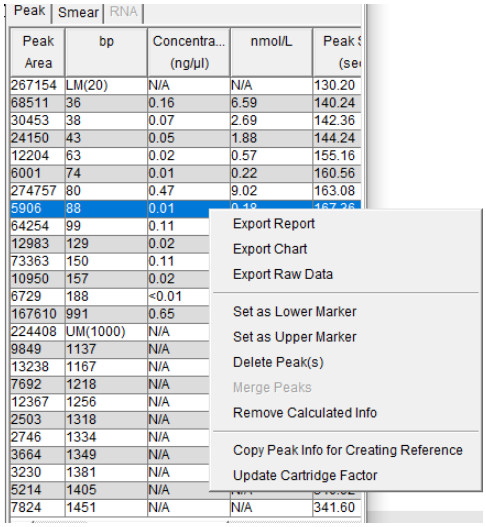


Figure 5-2 Right Click on Peak Table

Figure 5-1 C shows the result of the electropherogram. The green arrows indicate the first and the last peak of the result. The red arrow indicates the selected peak in the table B. Figure 5-1 D shows the gel view image of the result. Figure 5-1 E displays the raw data of the spot you selected.

5.1.1 Calculation

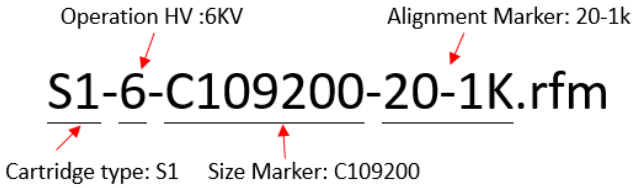
If you find the size values of the result are wrong or empty, you can utilize the **Calculate** function on the Toolbar to get the respective value of bp and concentration for each peak in the result. This function can also be used in RNA samples after running the RNA ladder.

First, open the file you intend to calculate and make sure that it is the selected window. Select **Calculate** on the Toolbar and click **Load** to load a suitable reference marker table for calculating your result.

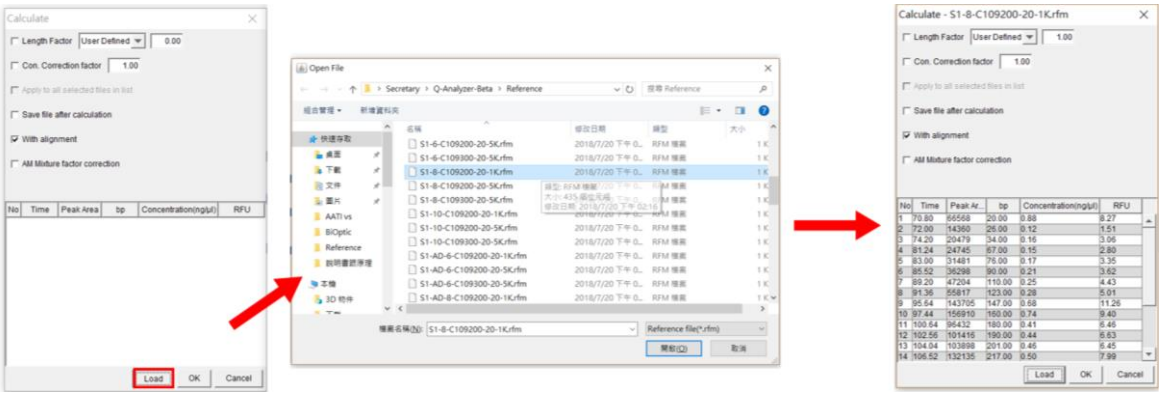
Go **Calculate** on the Toolbar and **Load** a suitable reference marker table for calculating the result.
Please choose the reference marker file according to the test condition. Note that the files include four information which are

1. Cartridge type (e.g. S1, S2, F3…)
2. Operation voltage of the separation (e.g. 6, 8 ,10 KV)
3. Size marker type (e.g. C109200, C109300…)
4. Alignment marker type (e.g. 20-1k, 20-5k…)

For example:



All the reference marker files corresponding to the pre-program methods can be found at the default **Load** Folder: “<workspace>\Reference”
* Default workspace is C:\Users\<username>\Q-Analyzer



Apply to all selected files in list: if you want to do multiple result files calculation.
Save File after Calculation: save the result file after the calculation is complete. Please select this function if you want to see the calculated result every time you open the file.
With Alignment: if you apply the alignment marker for analysis.
AM Mixture Factor Correction: select the checkbox of this function for proper calculation if the alignment marker is mixed with the sample.

Please choose the reference marker file according to your condition.
We prepare a complete set of the reference marker files corresponding to all the pre-program methods. The reference marker files can be accessed by clicking the **Load** in the editor. You also can use another new reference marker you created according to your specific condition (See Section 5.1.2).
Note that the file name includes four information which are cartridge type (e.g. S1, S2, F3), operation HV of the separation (e.g. 6, 8 ,10 KV), size marker type (e.g. C109200, C109300), and the alignment marker type (e.g. 20-1k, 20-5k).

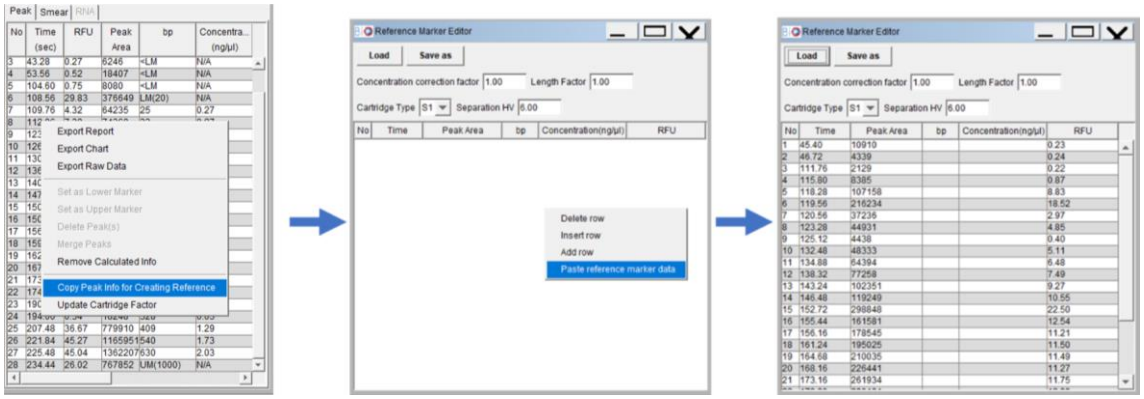
After confirming the reference data file, click **OK**, and then the value of bp and concentration will be shown in the columns. After the qualitative calculation analysis is completed, click **Save File** to save the file.

5.1.2 Reference Marker Table

Create new Reference Marker Table

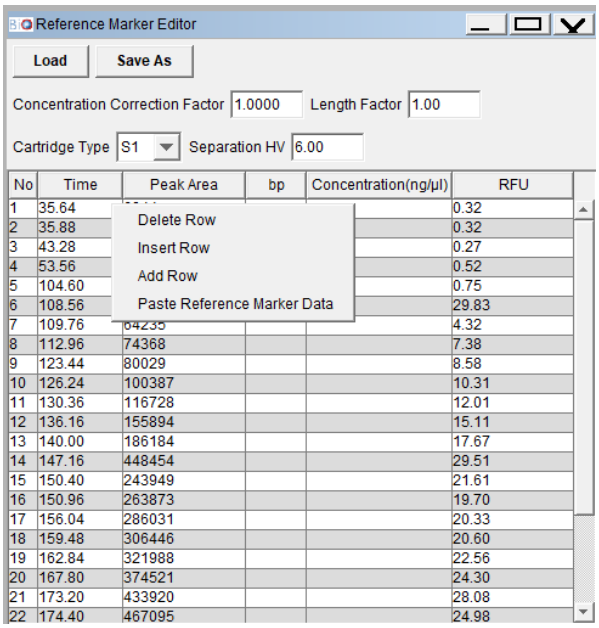
After executing the test of specific size marker, transfer the identified result as a reference marker table to analyze the size and concentration of the unknown samples. First, remove the redundant peaks using **Delete row** in the right click function. Select all rows and right click again to copy the modified data with **Copy peak Info for Creating Reference**.

Then, go "**Edit→Reference Marker**" and right click the blank area to paste the data. You may then start to edit the data.



Since the original data cannot be quantified with the length of base pair and its concentration, so you need to edit the exported data for comparison.

Edit and enter the corresponding values of the bp length and concentration for the peaks.



No	Time	Peak Area	bp	Concentration(ng/µl)	RFU
1	35.64				0.32
2	35.88				0.32
3	43.28				0.27
4	53.56				0.52
5	104.60				0.75
6	108.56				29.83
7	109.76	64235			4.32
8	112.96	74368			7.38
9	123.44	80029			8.58
10	126.24	100387			10.31
11	130.36	116728			12.01
12	136.16	155894			15.11
13	140.00	186184			17.67
14	147.16	448454			29.51
15	150.40	243949			21.61
16	150.96	263873			19.70
17	156.04	286031			20.33
18	159.48	306446			20.60
19	162.84	321988			22.56
20	167.80	374521			24.30
21	173.20	433920			28.08
22	174.40	467095			24.98

Renew Reference Marker Table

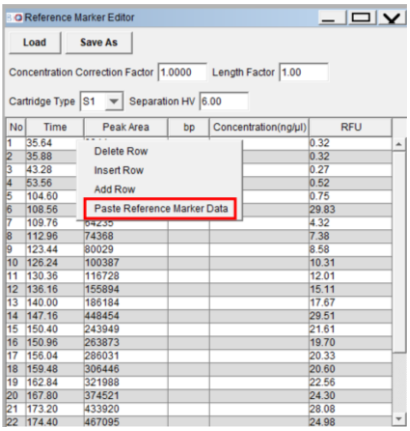
Due to the changes in conditions of each experiment such as test duration, cartridge, temperature, moisture, solutions, and sample nature, the slight differences among the results for the same test sample may occur. In order to maintain an accurate reference marker table file, you may recall the edited file and use **Copy peak Info for Creating Reference** to update the migration time of the peaks and the height of the peaks without entering the data one by one again.

Step 1. Start the Reference Marker editor by "Edit→Reference Marker". **Load** the reference marker file you want to edit.

Step 2. Go to the table of your test result and remove the redundant peaks using **Delete Row** in the right click function.

Step 3. Select all rows and right click again to copy the data with **Copy Peak Info for Creating Reference**.

Step 4. Return to the Reference Marker editor and paste the renew data using **Paste reference marker data** in the right click function on the table.



Step 5. Save as a new file or overwrite the Reference Marker you opened.

***Note**: When you paste the exported data from your result file, ensure that **the number of rows** of the pasted data is **the same** with the recalled reference marker table. Moreover, the data must be recognized from the identical base pair.

After editing your reference marker table, you may save it for later use. Also, you may utilize the **Calculate** function mentioned in Tool to recall the reference marker table for quantitative data analysis. For details, refer to Section 4.3.3.

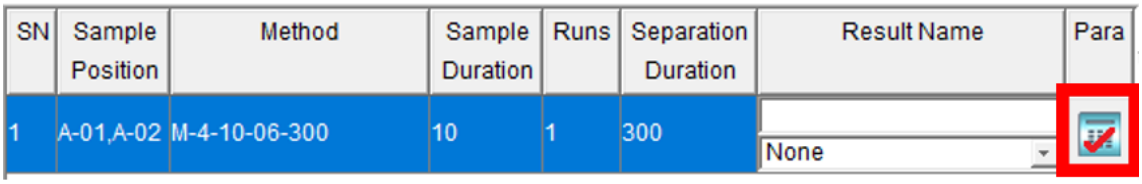
5.1.3 Improve Calculate Accuracy

To improve the accuracy for base pair calculation, user can use the **Create size marker** function to execute a size marker test first and create a reference table for automatic execution. After applying the setting, the following test will be calculated based on a new reference table.

The steps to execute **Create size marker**:

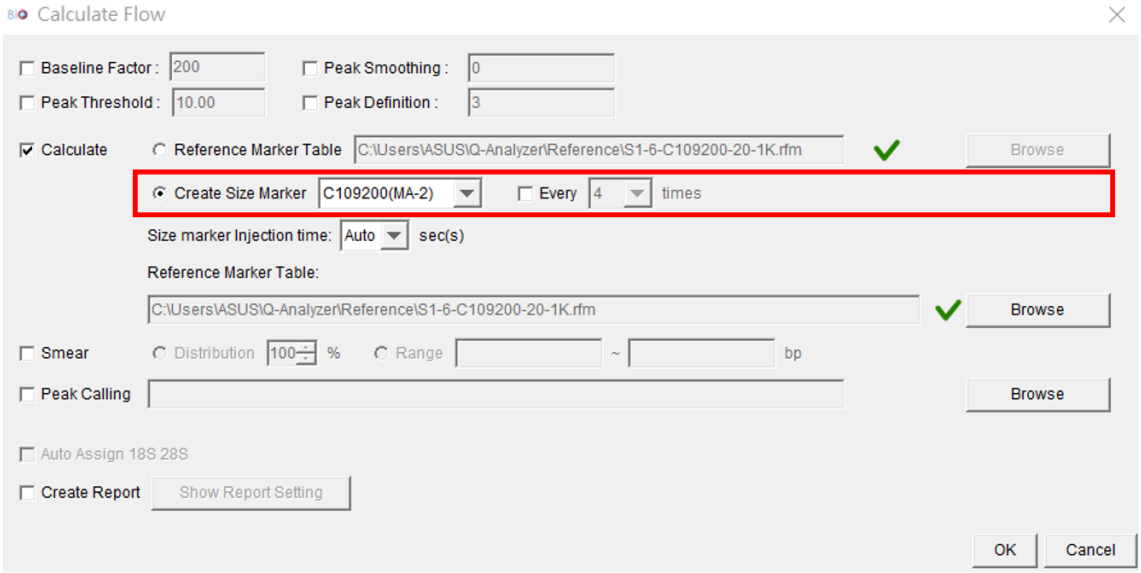
Step 1. Place the fresh Alignment Marker (C109100: 20bp&1000bp Alignment Marker) at MA1 position and Size Marker (C109200: 15-622bp DNA Size Marker) at MA2 position.

Step 2. Click the Automatic calculation icon in Sequence.



Step 3. Select the Create size marker checkbox

You can select the frequency (☐Every n times) to execute the size marker if you need a higher accuracy of the base pair sizing. Then, click **OK**.



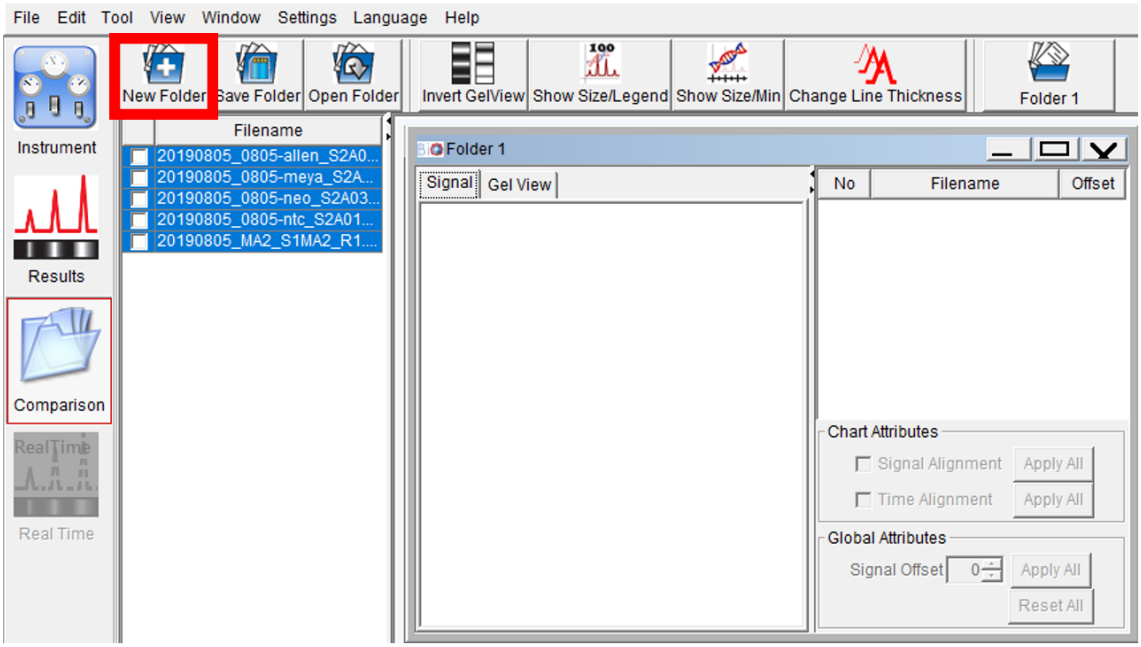
5.2 Comparison

Use **Comparison** to compare the different result files.

Step 1. Open the files you want to compare in **Results**.

Step 2. Click **New Folder** in Toolbar to create a Folder.

Step 3. Drag the files from the Filename panel and drop them into the Folder that you created as shown below:



Show the electropherogram or gel image by selecting the tab in Figure 5-3 A. Figure 5-3 B displays the corresponding information of the spot you have selected. When you click a file name in C, the corresponding data in the chart will be highlighted and you also can change the color of the line by clicking the small color block in the first column.

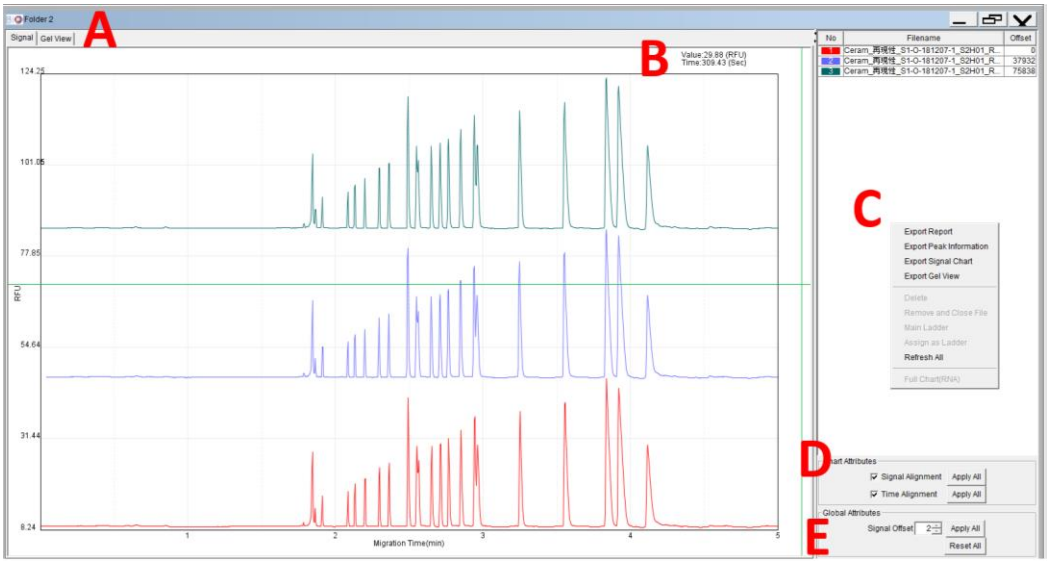


Figure 5-3 Data Comparison in Folder Chart

Due to variety of the experiment conditions, you may need to normalize your test results by utilizing the Alignment functions (Figure 5-3 D) to align your data.

Time alignment uses the Alignment Marker of each file as the mark to scale the data to be aligned with those of the first file (Figure 5-4).

Signal Alignment adjusts the baseline of each file at the same height (RFU) with the baseline of the first file (Figure 5-5).

Signal Offset (Figure 5-3 E) helps you to separate the stacked data. Simply set the value in **Signal Offset** and click **Apply All**, shown in Figure 5-6 and Figure 5-7. You may retrieve the original result with the **Reset All** function.

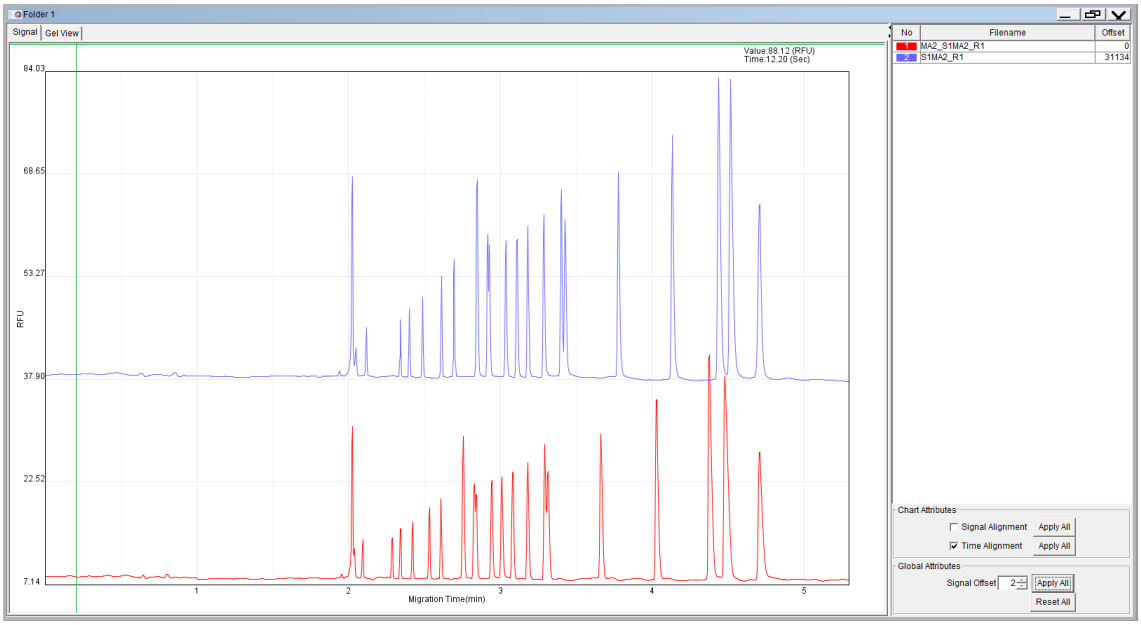


Figure 5-4 Comparison of Result Data with Two Different Baselines

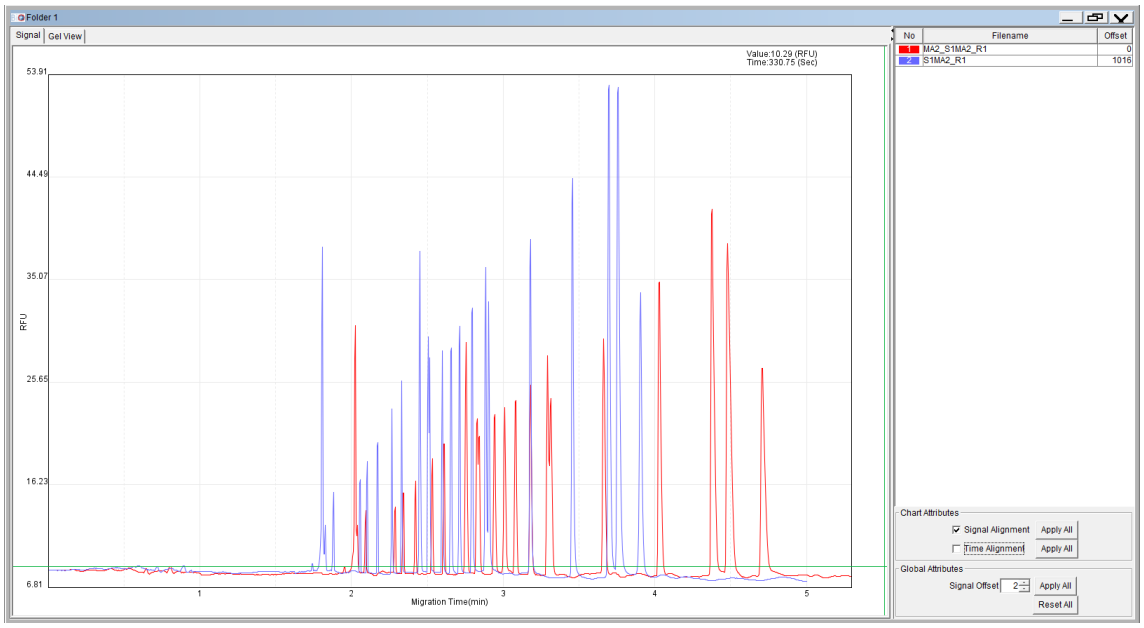


Figure 5-5 Use **Signal Alignment** for Baselines Adjustment

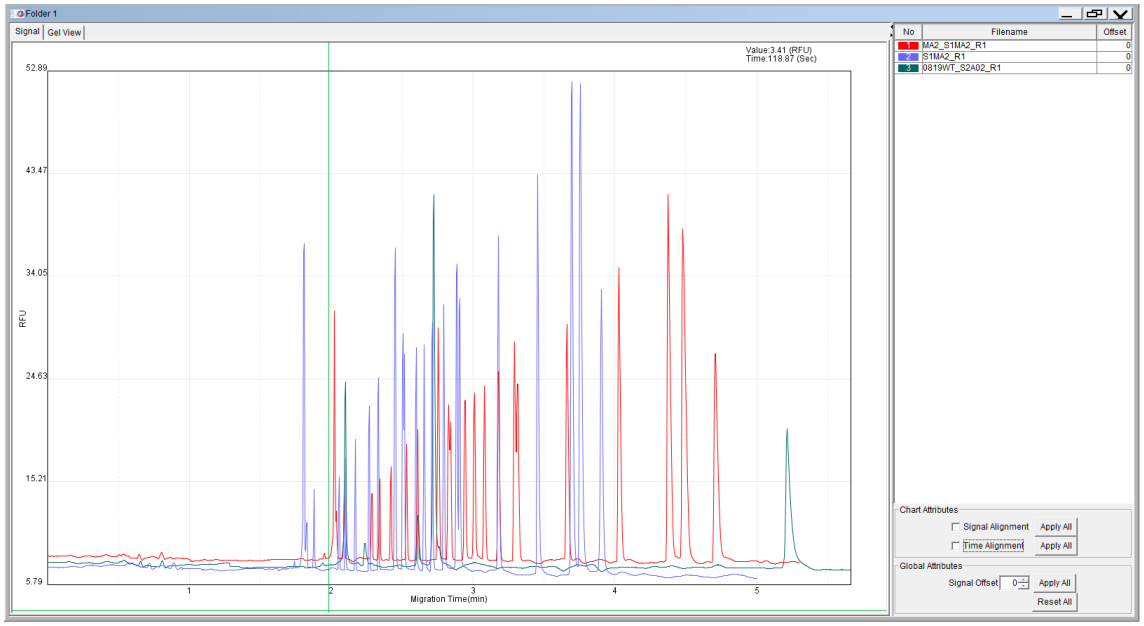


Figure 5-6 Three Stacked Result Data

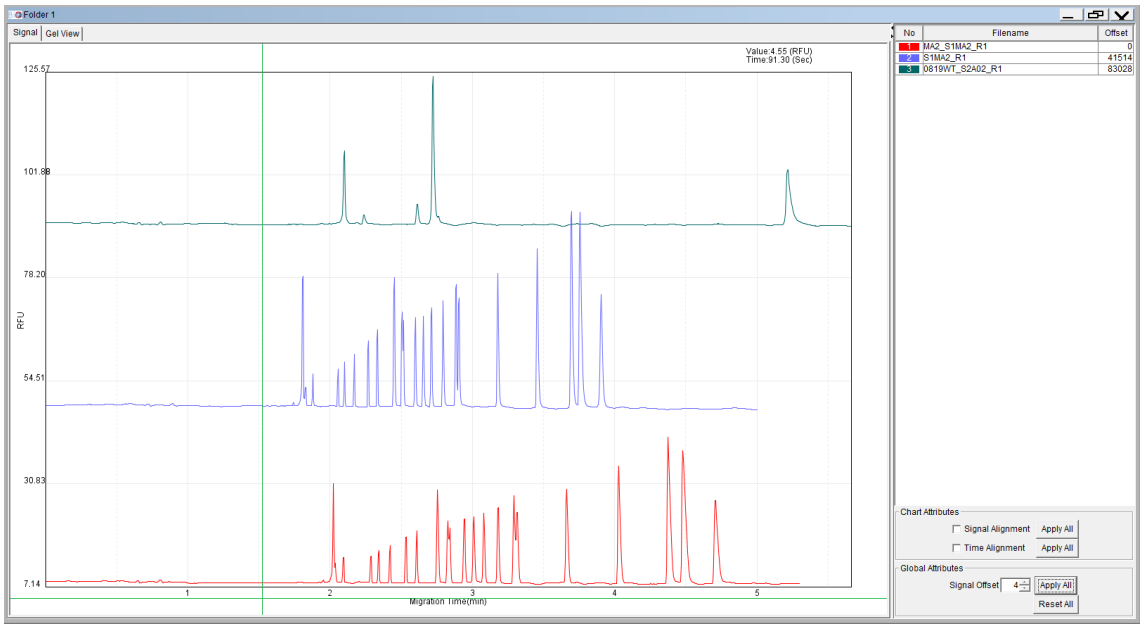


Figure 5-7 Use **Signal Offset** for Distance Modification among Each Result Data

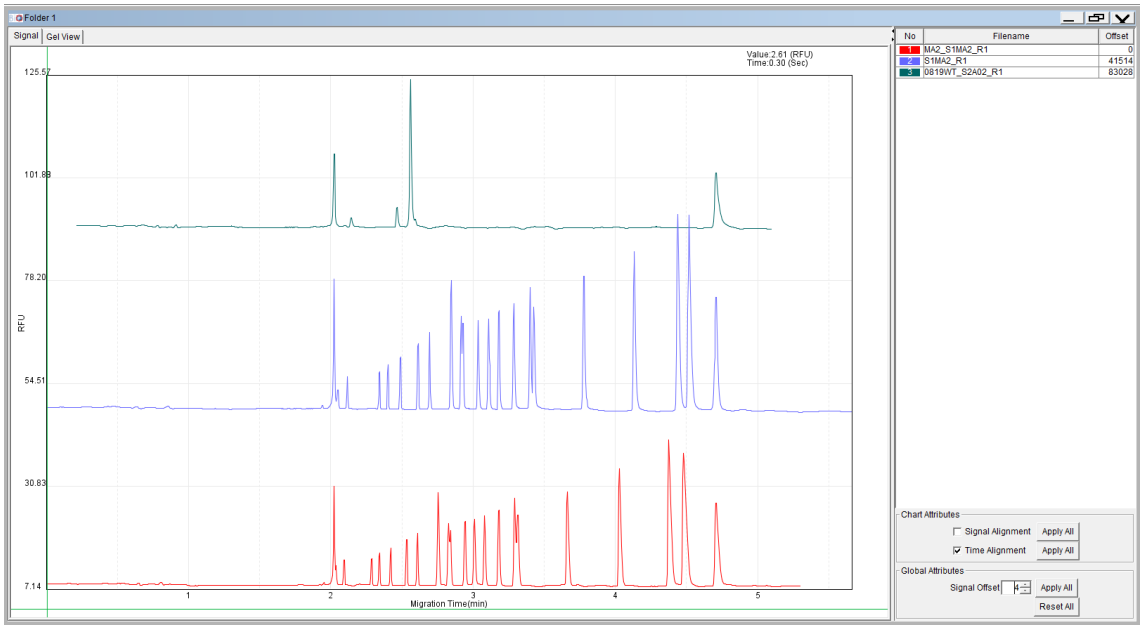


Figure 5-8 Use **Time Alignment** for Scaling the Two Peaks as the Same Time Line

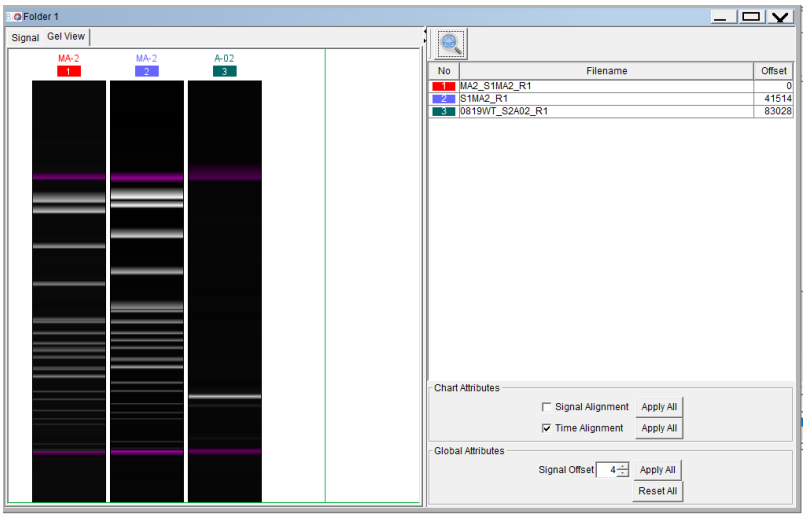


Figure 5-9 The Gel View Based on **Time Alignment** for Scaling the Two Peaks as the Same Line

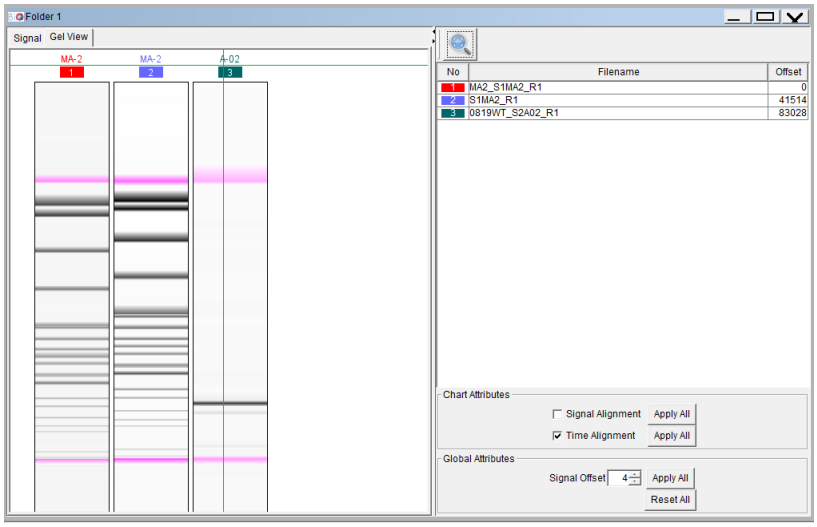


Figure 5-10 Use ”Main Panel→View→Gel view setting→Invert” for Inverting the Gel View

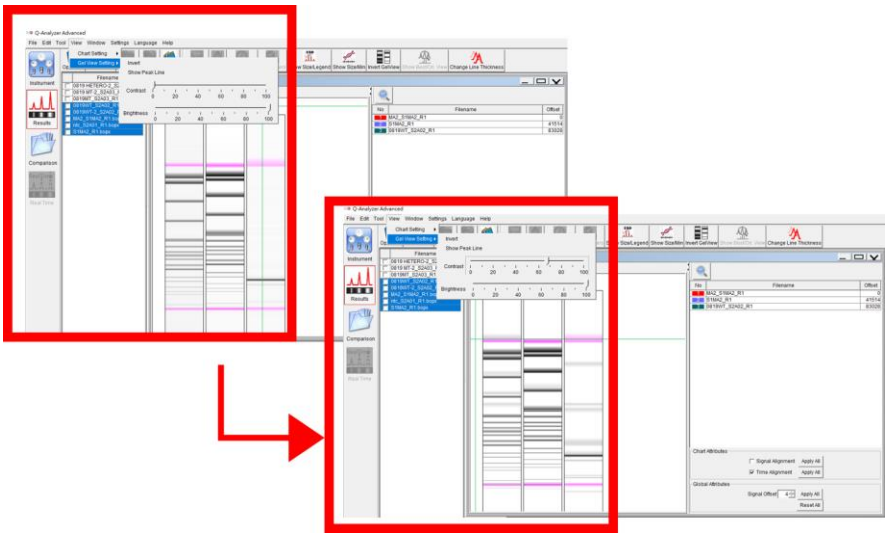


Figure 5-11 Use “Main Panel→View→Gel view setting→Contrast” for Contrast Adjustment of Gel View

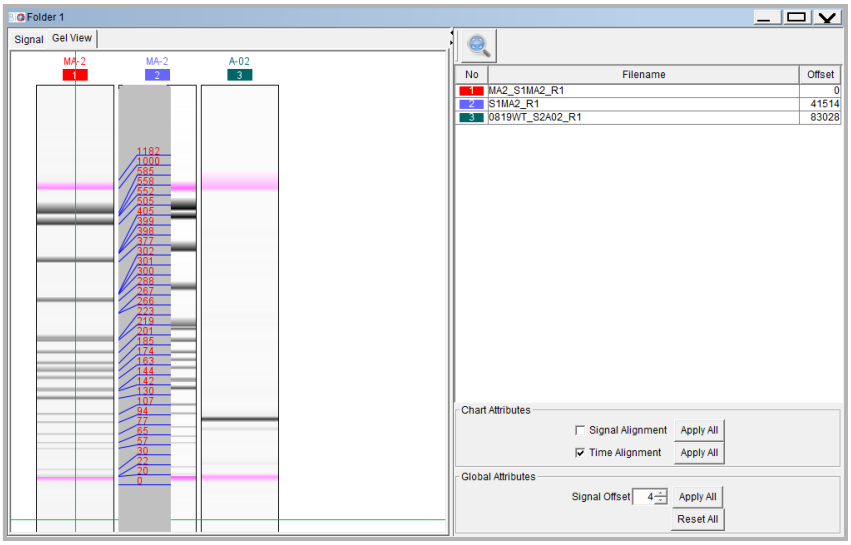


Figure 5-12 The Base Pairs of Peaks Appearance when You Move Cursor on Each Line

5.2.1 Export the Report of Compare Result

To save the results of data comparison, user can follow the steps below to export the report of the **Folder**. After entering the result files into **New folder** and completing the comparison, right click and select **Export report** (Figure 5-13). *Q-Analyzer*™ will automatically generate the report file and select the format (Preview/PDF/DOC/XLS), then enter the file name and save the file to the designated folder (Figure 5-14).
User can save the comparison results by using the function **Save Folder**, too.
You can find the **Save Folder** option under **File** at the Main Frame. (Figure 5-15)

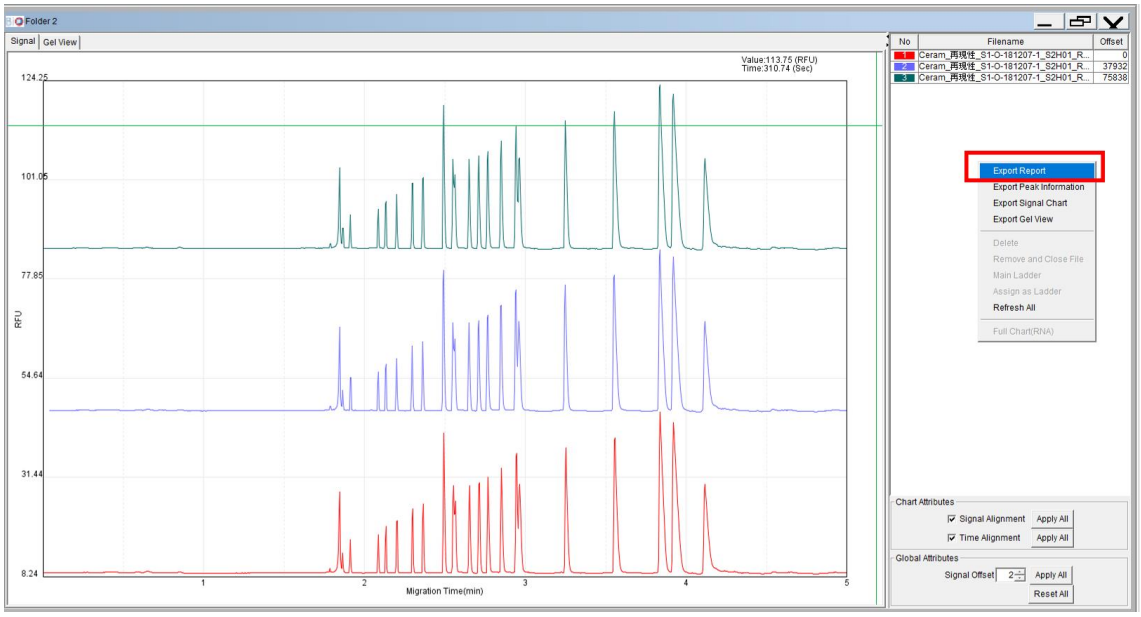


Figure 5-13 Right click and select **Export report**

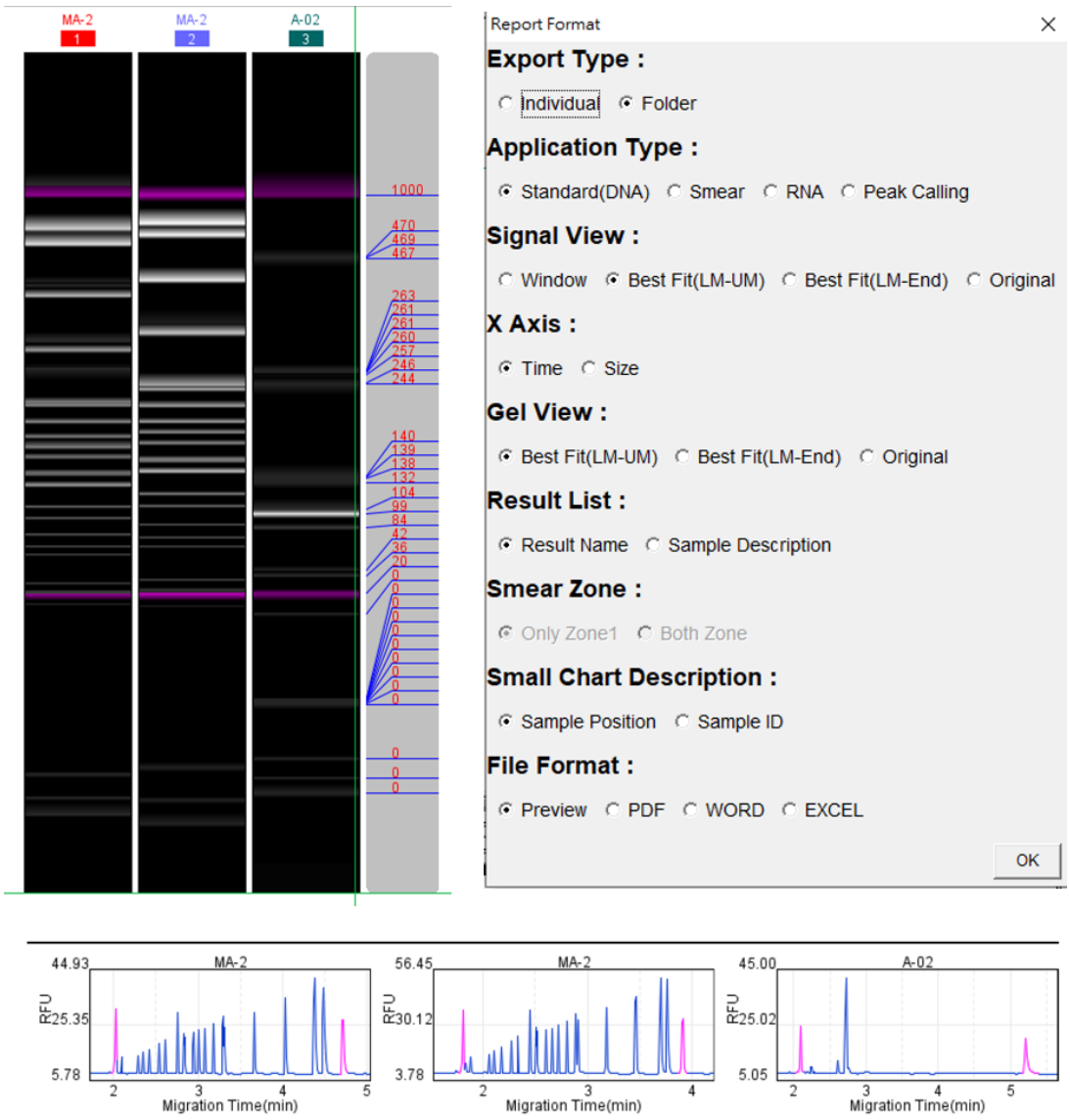


Figure 5-14 Select the file format, enter the file name, and save the file to the designated folder

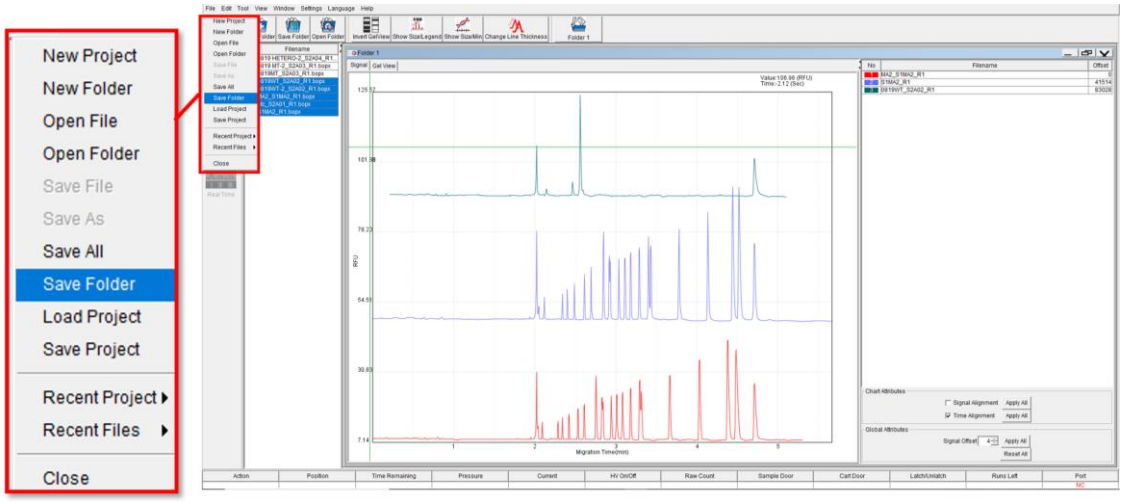


Figure 5-15 Function of **Save Folder**

5.3 Total RNA Quality Analysis

To understand the quality of RNA sample, user can assign 18S and 28S in order to obtain the RNA quality number (RQN) by conducting the steps below.

The RNA tab will be enabled, unless the result file is generated by RNA application when you use Method Selector in choosing the method (Figure 5-16).

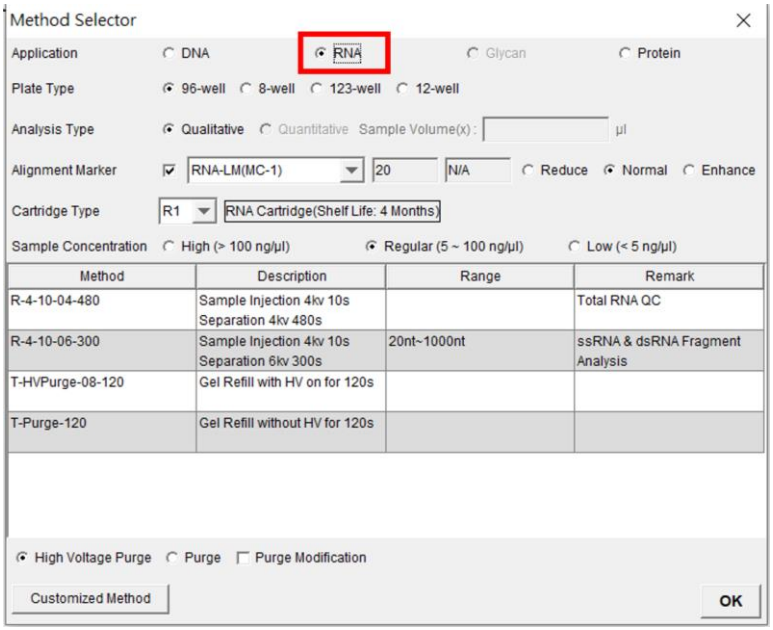


Figure 5-16 Choosing RNA in Method Selector

Step 1. Select the **RNA** tab to calculate the RQN (Figure 5-17).

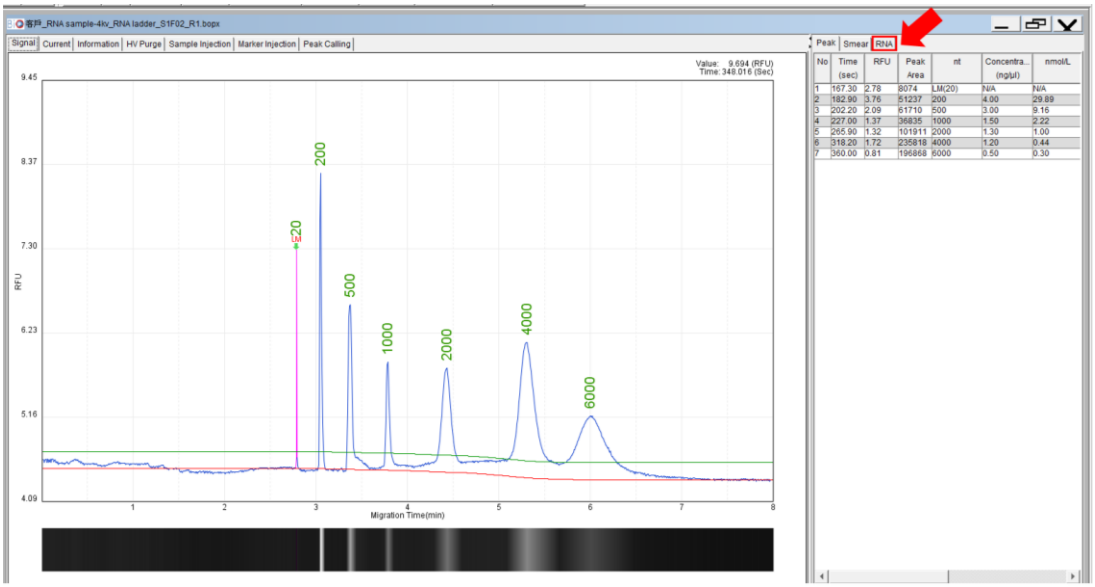


Figure 5-17 RNA Sample Result

Step 2. Select **Eukaryotic RNA** or **Prokaryotic RNA** (Figure 5-18 A)

Step 3. Click **Auto assign** to assign the orange and green lines for “18S”, “28S” RNA (Eukaryotic) or “16S”, “23S” RNA (Prokaryotic) (Figure 5-18 B).

Step 4. If the lines are not properly cover the RNA area, drag the orange and green lines to cover the designated areas.

Step 5. If you want to save the settings, click **RNA factor** button to edit and save the RNA factor table (Figure 5-18 C).

Step 6. Click **Calculate**, the 28S/18S or 23S/16S ratio and the RQN (RNA Quality Number) will appear on the screen (Figure 5-18 D).

Step 7. Click the right button in the gray region to export the report

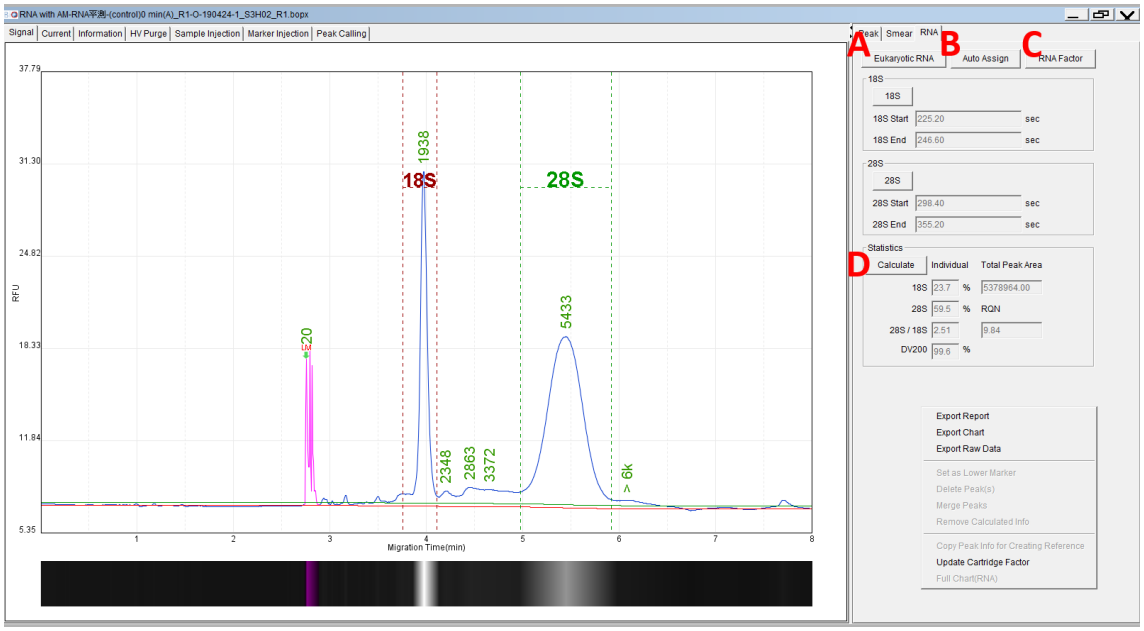


Figure 5-18 RNA Quality Check Panel

5.4 Smear DNA Analysis (gDNA QC for NGS)

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

To conduct the analysis, do the following steps:

Step 1. Click the Smear tab (Figure 5-19 A) (Alternatively, you can select Smear from the Toolbar).

Step 2. Enter the percentage of the target distribution (e.g. 50%) or the bp range of the target region (Figure 5-19 B-C). Then, click Apply. Figure 5-19 D shows the detail information of the assigned region, including the Average bp and concentration of the region (nmol/L).

Step 3. Modify the Interval of the size range if necessary, and then click Apply to apply the setting. Doing so will help you get the more detail of the distribution (Figure 5-19 E).

Step 4. In addition, you can edit the other zone by selecting Zone2 and the editing rules are the same as mentioned above. This function helps user get the ratios between Zone1 and Zone2 which provided more information about these two Zones (Figure 5-19 F).

Step 5. All information about the distribution will show up in the below table.

Step 6. Click the right button on the right panel to export the report (Figure 5-20). User can export a report with two zones information, select “both zone” at Smear zone

***Note**: To use the Smear function, the result data must be calculated.

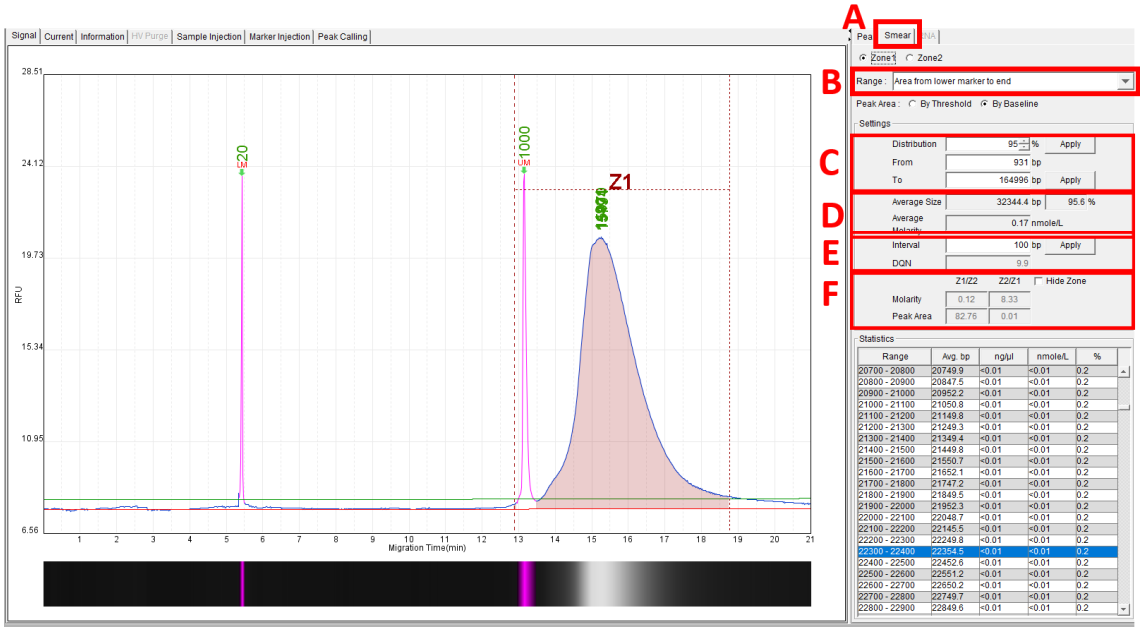
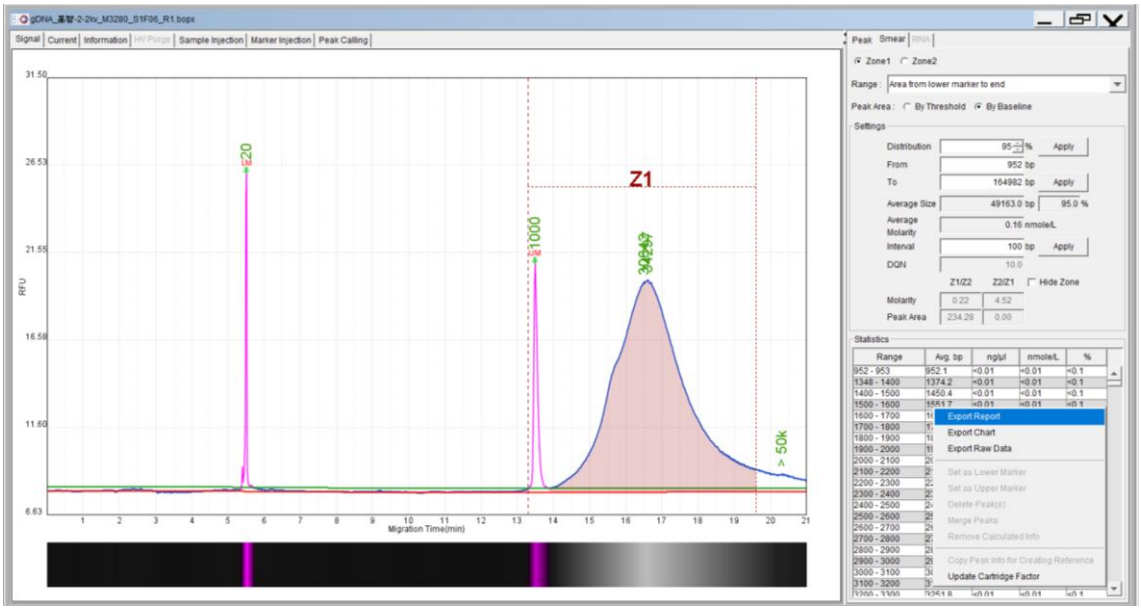


Figure 5-19 Fragmented Genomic DNA Sample Result



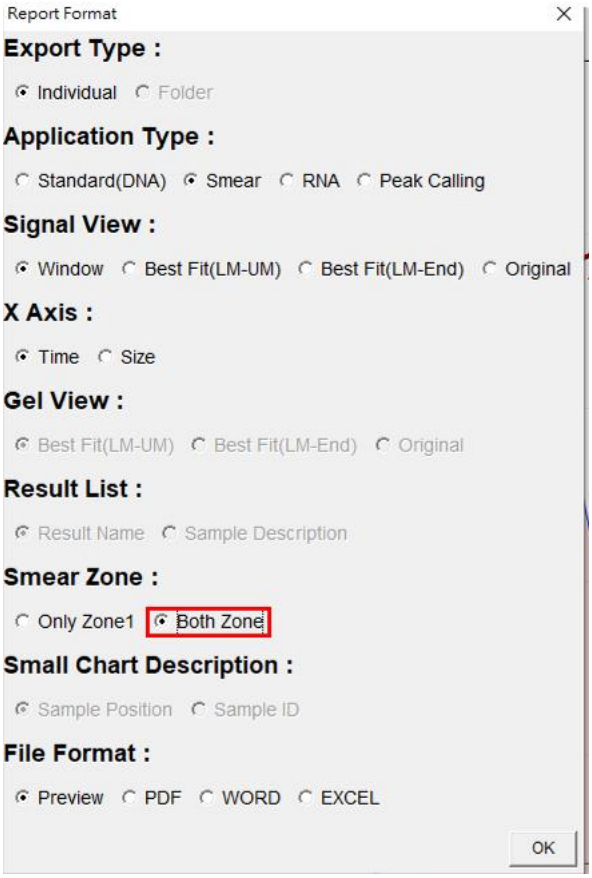


Figure 5-20 Export the Smear Report

5.5 Peak Calling Function for Clinical Testing

Peak Calling function allows user to quickly distinguish the target peaks. *Q-Analyzer*™ will follow the rules in the **Peak Calling table** and report "**+**" when the peaks are found and show "**-**" when the peaks are not found.

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

Step 1. Click **Edit → Peak Calling Table** to load the Peak Calling table (Figure 5-21 A).

Step 2. Click the right button and select **Add Row** to add a new panel (Figure 5-21 B).

Step 3. Set up the new panel. Enter the criteria in the columns for the peak (Figure 5-21 C) and save as a new file (Figure 5-21 D) to the default folder.

- Target: The center value of the peak.
- Upper/Lower Margin (size): The upper/lower margin of the peak target 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 Legend name.

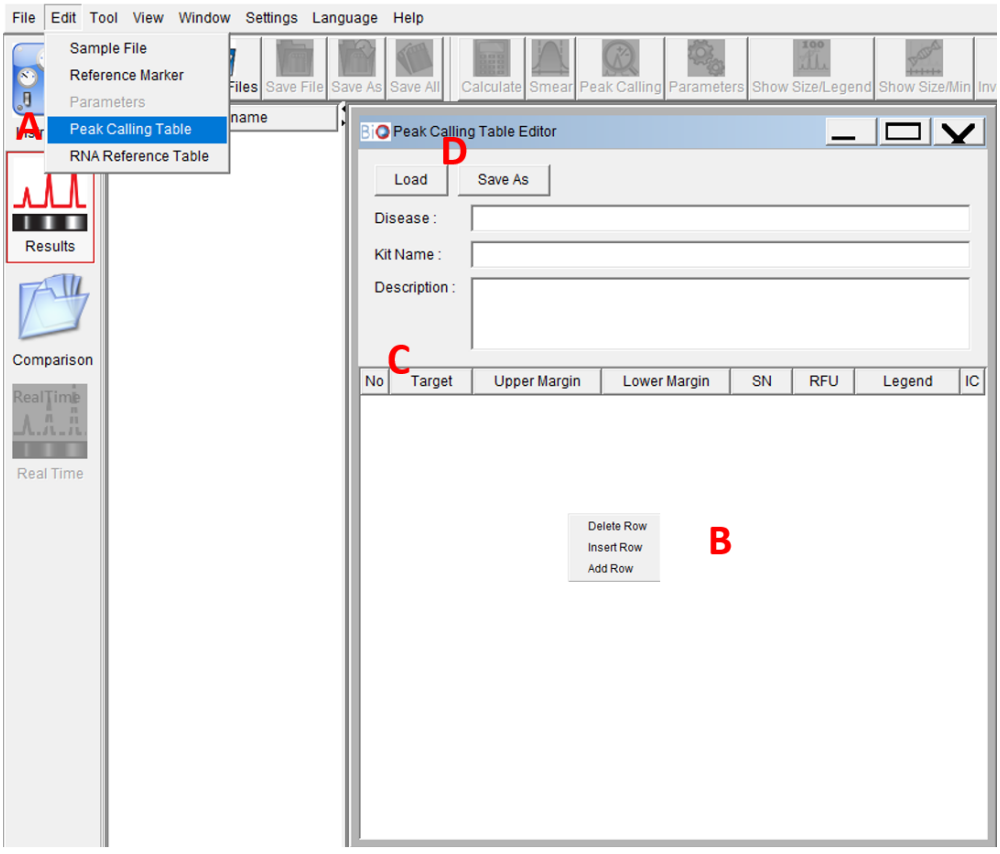


Figure 5-21 Peak Calling Table

Step4. Return to the page of your result files, calculate the fragment size and select the **Peak calling** tab. (Alternatively, you can select **Peak Calling** from the Toolbar or **Tool→Peak Calling**. Figure 5-22 A-B).

Step5. Browse the desired file and load your Peak Calling Table (Figure 5-22 C).

Step6. Click **Signal** tab and return to the chart, the software will automatically scan the peak signal. Click **Show Size/Legend** button on the Toolbar, the legend of target peak will be displayed on the window. You can also click the right button to export the report. (Figure 5-23)

***Note**: Incorrect fragment size might affect the result of Peak Calling. Please ensure your experiment is properly calibrated and the peak has the correct bp value.

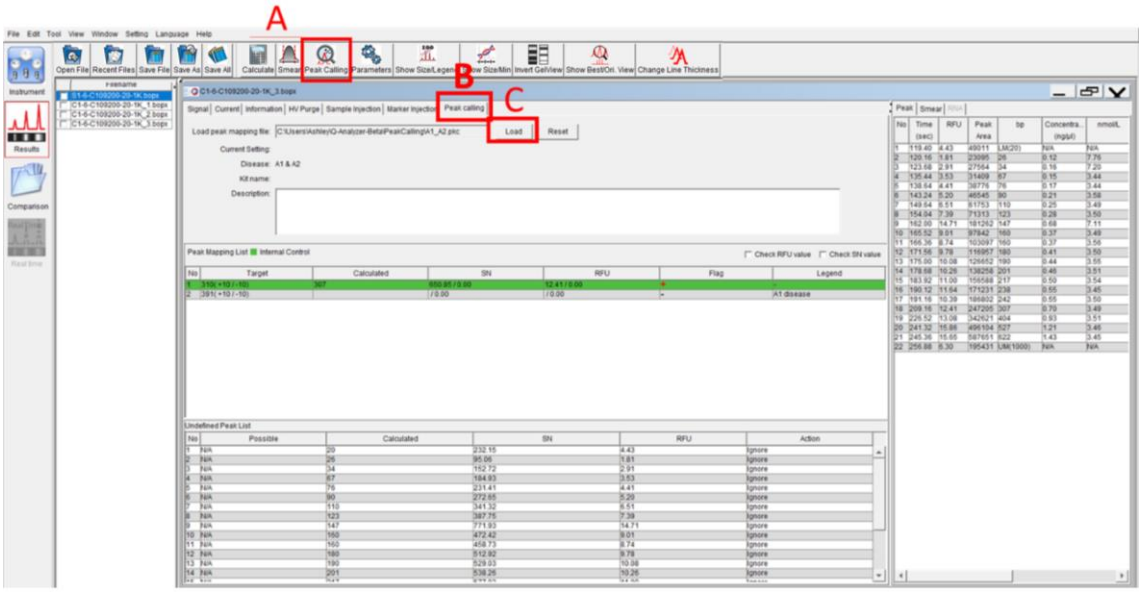


Figure 5-22 Fragment Size Calculation and Peak Calling Selection

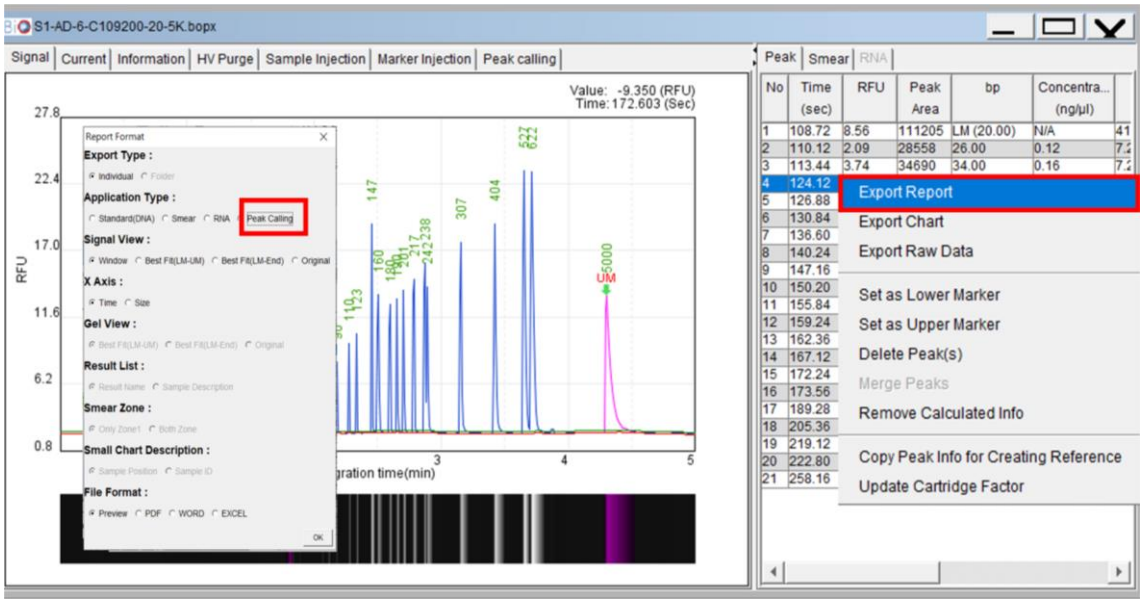


Figure 5-23 Loading Panel and Exporting the Report

Appendix A-Troubleshooting

✧ **Please exclude these issues first …**

- Insert the pin into the small hole of cartridge cap and press it all the way in.
- Every sample and marker should be over 20 µl (using 0.2ml tube). Buffer tray should be loaded with proper volume.
- The tube setting at the MA1~MD3 area should be secure tightly **(Hold the plate by your hand and press down the tube tightly into the well by thumb)**.
- Alignment marker and Size marker should be placed at the correct position
- Instrument (include pump) power need to be turned on. USB cord and air tube should be connected well.
- *Q-Analyzer*™ should be updated to the latest version.

1. Unpacking, packing process and Installation

Q1：How to unpack the cargo and what should we notice when moving the instrument?

A：

- About the unpacking process, please follow the unpacking guide we provided in the box or download from the website.
- Please keep the box and cushioning materials. Because you will need all these pieces of stuff when you are going to transport the instrument.

Q2：What are the minimum computer requirements and recommendations?

A：

- Please refer to Section 1.2 System Installation.

Parameter	Minimum requirements	Recommendations
Operation system	Win 7, 8, 10	Win 10
Processor (CPU)	Intel i3 up	Intel i7
Memory (RAM)	4G	8G up
Storage	1 TB	2T up
Monitor	1440* 900 up	1920* 1080 up
Others		Internet

- *Q-Analyzer*™ use about 30-40 MB of RAM

Q3：What is the size of the result file?

A：Each file is 120 KB; 1 GB can store 8,333 files; 8 GB can store 66,664 results.

Q4：Do we need to install the software first or connect the cable first?

A：*Follow the Quick start of instrument installation and software installation.

Step1. Install the instrument & Pump

- Connect the Instrument with pump

* The pump might need to be placed on the floor

Step2. Install *Q-Analyzer*™ in PC

- the set-up program is stored in the software key with the latest version. Please insert the software key to install *Q-Analyzer*™.

Step3. Connect the USB with instrument & PC and open *Q-Analyzer*™

- to avoid restarting the PC again

Q5：Can power adaptors be used alternately?

A：No.

Different devices need different voltage (power) to trigger. Not enough power may influence the normal function of the instrument. Please follow the label on the adaptor wire.

2. Instrument not working or working abnormally

2.1 Before use

Q1：Pressing the machine power, but have no response?

A：

Step1. Please check the socket is useful and the power adaptor is correct.

Step2. Visual check the connector (loose or worn-out) and appearance of the power cord.

Step3. Try to enter *Q-Analyzer*™.

Step4. If the instrument is offline and indicator does not light up, the power switch

is out of order.

Step5. If the instrument is online but indicator does not light up, the indicator light is malfunction.

Step6. Open the service panel and check the J1 and J6 connector. For the details, please contact your local distributor or BiOptic.

Q2：How should we do when PC cannot detect the connection of $Qsep_{100}$?

A：

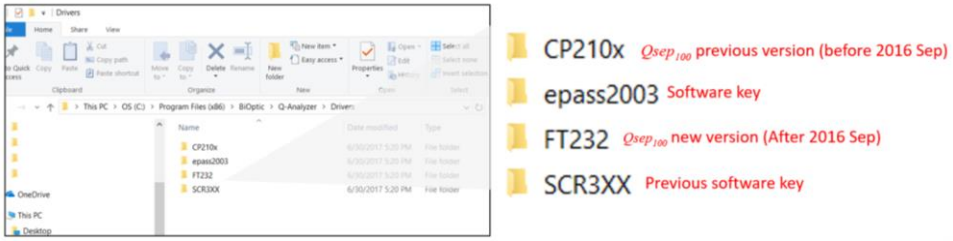
First, you can use another PC to try again. If it still cannot detect, please follow the following steps.

Step1. Please check the power light of the instrument.

Step2. Restart the PC again.

Step3. Check the USB connection. (Check the USB port & USB wire)

Step4. Go to Device Manager and check the USB Serial Converter. If show "!", please install the driver. The driver also included in the $Q\text{-}Analyzer^{TM}$ setup file.



Q3：How should we do when PC cannot connect with $Qsep_1$ WiFi mode?

A：

Step1. You must confirm that is no other PC or laptop connecting to this $Qsep_1$, because it may block the new connection.

Step2. Check the LED light after power turns on

Step3. Confirm whether you can see $Qsep_1$ in the WiFi connection list or another instrument in the list.

- See other instruments in WiFi list, but no $Qsep_1$ display in the list after restart $Qsep_1$ => Report to BiOptic
- Check if $Qsep_1$ is listed on your cell phone WiFi list，but not display on PC/laptop. Reboot the network card or change to another PC/laptop

For details, please follow the "$Qsep_1$ Operation Quick Start".

Q4：What does the light of $Qair_{box}$ mean?

A：

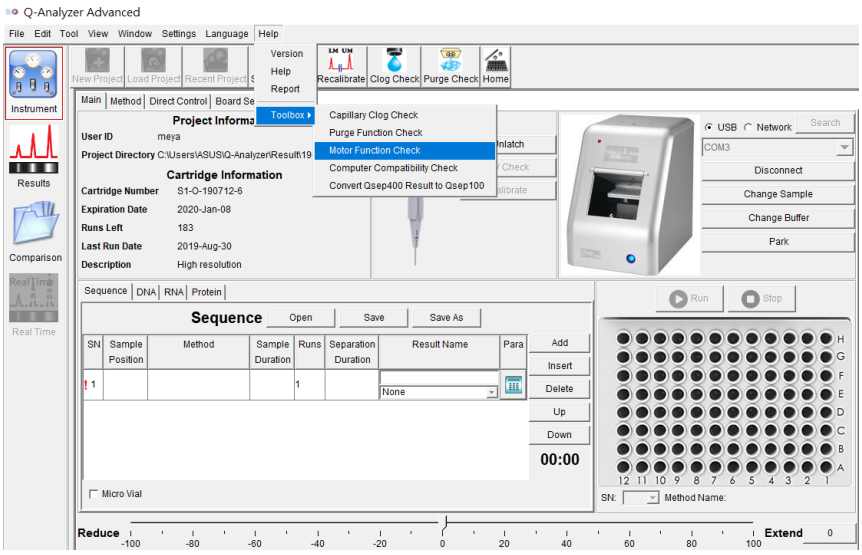
Blue light: Power on
Green light: Pressure OK
Red light: Alert

2.2 During use

Q5：If the buffer tray can't move smoothly, how should we do?

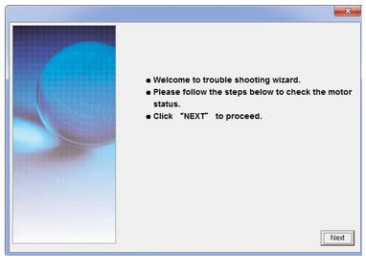
A：Please refer to section 4.3.8 Help: Motor Function Check and use motor function check to check each action and report to us.

Step1. To the Main Page>> Help>> Motor Function Check

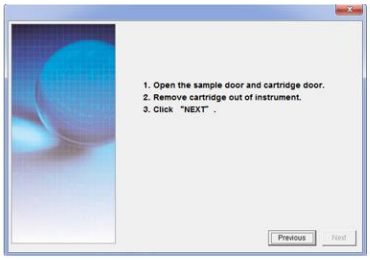


Step2. Please follow the instructions in the window popping up.

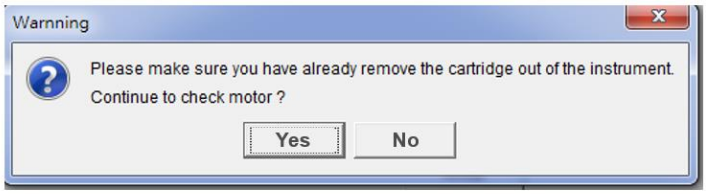
1


● Welcome to trouble shooting wizard.
● Please follow the steps below to check the motor status.
● Click "NEXT" to proceed.
Next

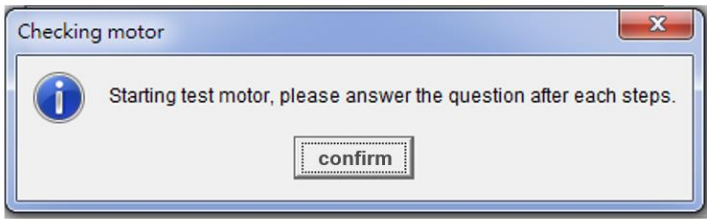
2


1. Open the sample door and cartridge door.
2. Remove cartridge out of instrument.
3. Click "NEXT" .
Previous
Next

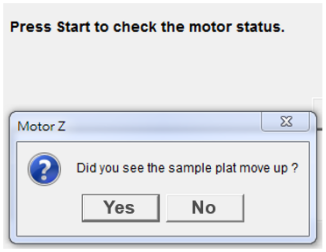
3


Warnning
Please make sure you have already remove the cartridge out of the instrument.
Continue to check motor ?
Yes
No

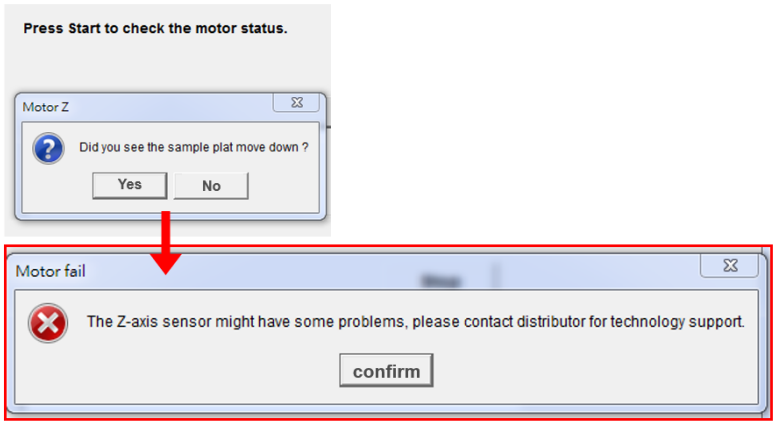
4


Checking motor
Starting test motor, please answer the question after each steps.
confirm

5


Press Start to check the motor status.
Motor Z
Did you see the sample plat move up ?
Yes
No

6


Press Start to check the motor status.
Motor Z
Did you see the sample plat move down ?
Yes
No
Motor fail
The Z-axis sensor might have some problems, please contact distributor for technology support.
confirm

Q6：The $Qair_{box}$ works frequently, over 3 times/min

A：

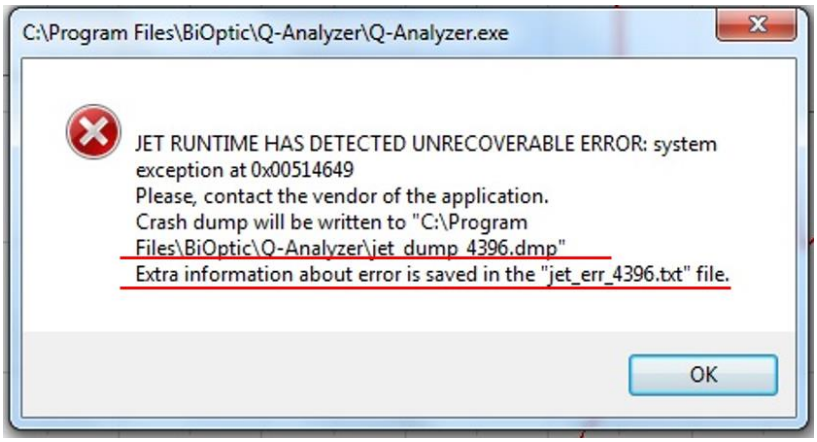
- Check the air leaking of the system airway. You can also use the bubbling water to check it.
- We have used three kinds of filters. The right one is the strongest, which can be used longer than the other two. And the middle white one is stronger than the left blue one.



- the cartridge cap locks too tight or too loose may also cause air leaks.
  1. Too tight: The distance between the cartridge cap and the purge piston will be enlarged.
  2. Too loose: The distance between the cartridge cap and gel reservoir will be enlarged.

Q7：Jet error window pop-out.

A：Please send all the files mentioned in the warning window back to the BiOptic.



3. Cartridge issues

3.1 Before use

Q1：HV check failed or Calibration failed.

A：

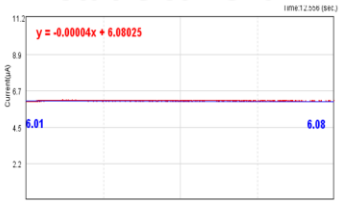
HV check

- Make sure follow the “unpacking guide” to unpack the new cartridge
- New cartridge has an inner cap to prevent gel stock in the cartridge cap, please use a needle to punch a small hole at the inner cap.

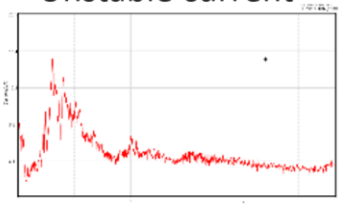
Push the pin down to the end then move up and down for 2-3 times

- During the HV check, to check the current is stable or not in the final 10 seconds (2μA). After HV check, if the current is still unstable, please repeat this step 2-3 times.

Stable current



Unstable current



- **Stable Current:**
  If the current is stable, then please change to fresh alignment marker, and the volume of alignment should be above 20ul. Then you can do the following steps.
- **Unstable Current:**
  Please use purge function to purge this new cartridge for 15-30min and put in hot water if needed.
  If the cartridge still can’t pass the calibration, please send the report (zip file) back to us.

Calibration

- Use C109100 20bp& 1kb (MA-1/ M/AM-01) alignment marker to do calibration.
- The criteria:

1. The result needs to have two peaks (more than two or less than two will also failed) and the S/N must be 60 times of noise.
2. Peak time must be within the specified range, cannot be shift too far.

- If the calibration failed, user can do it again, and it will extend 10s of the HV purge (10n, n= calibration number) and 30s of separation in next calibration.
- The cartridge will be locked after 10 times failed.

*Note: make sure user place above 20µl of 20bp& 1kb in MA1 (M) position properly.

Hold the plate by your hand and press down the tube of alignment marker tightly into the well by thumb.

Q2：What's wrong with the pop-out error window?

A：

Error Code		Checkpoint	Preliminary treatment
01	RFID module is out of order	RFID Reader	• Re-latch
02	Can't find the Tag	RFID Reader or RFID tag	• Re-latch • Use the other cartridge to confirm
05	Incorrect format of RFID Tag	RFID tag	• Re-latch
06	Error from RFID decoding	RFID tag	• Re-latch

3.2 During use

Q3：How to check if the cartridge is clogged or not and how to rescue the dry out cartridge?

A：

- Using the purge station to see whether the gel drop comes out. If not, soak the cartridge tip in the beaker with hot water for 10-15mins and then purge again.
- It will be even better to soak and purge at the same time

Table 1: The recommend settings

Cartridge	S1	S2	F3	U1	R1	P1	G1
			For New Cartridge				
Duration (Sec.)	120	90	240	240	120	90	120
Pressure (Mpa)	0.35	0.35	0.35	0.35	0.35	0.35	0.35
			For Clogged Cartridge				
Duration (Min.)	30	20	60	60	30	20	30
Pressure (Mpa)	0.5	0.5	0.5	0.5	0.5	0.5	0.5

- For the details, please refer to the section 3.5 Capillary Clog Check.

Q4：What if the cartridge has expired, but still has runs left?

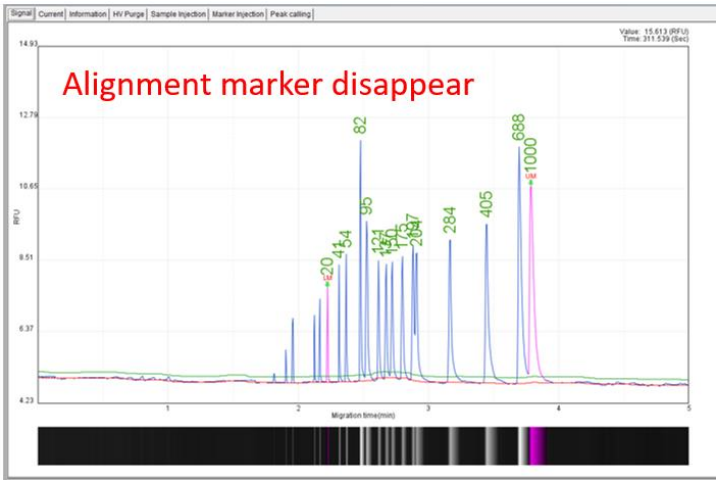
A：

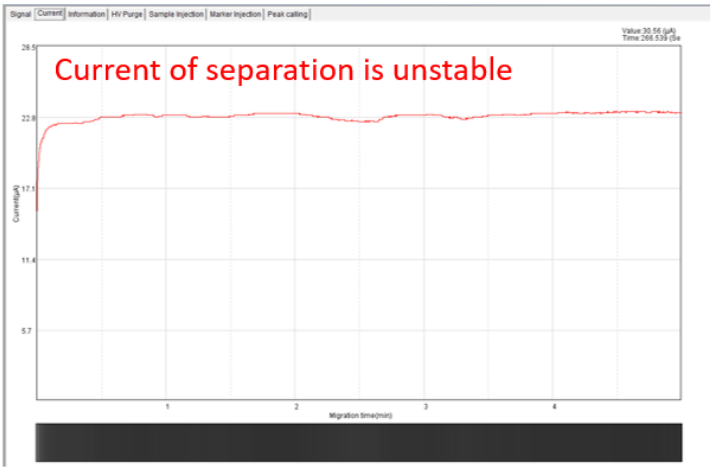
- We are not solving the issues from expired cartridge.
- About the expired cartridge, user can use them to do the “pre-analysis” or use to test the sample with unknown buffer condition, because sometimes unknown buffer may damage the capillary.

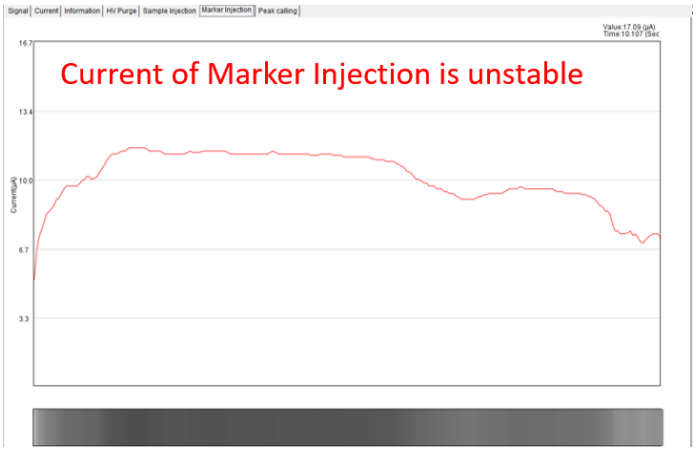
Q5：What if the cartridge has sucked the mineral oil? How to recover it?

A：The condition is that Alignment marker volume <10ul and cover oil.

- When sucked the mineral oil, it will lead to the Alignment Marker disappear, current of separation and marker injection are unstable.







- The solution is that try to "purge 15-30 minutes to recover it". Sometimes the cartridge will recover.

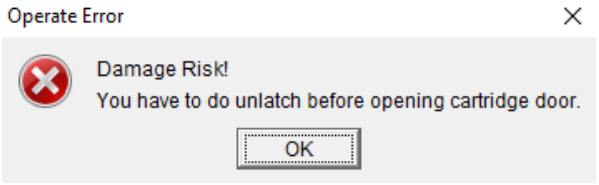
Q6：Anything needs to notice during insert or take out the cartridge?

A：

- Please stand up and insert the cartridge vertically.
- Be careful not to hit the tip of the cartridge.

- When finishing the analysis, click "unlatch" before you open the cartridge door.
- If you do not press unlatch before opening the card door, the system will pop up

a warning window. It might damage the anode pin.



3.3 Storage

Q7：How to store the cartridge? And relative buffer?

A：

- For the details, please refer to the document of “Storage Condition of BiOptic’s Products”.
- Cartridge cannot be stored in the $Qsep_1$!
- Cartridge can be stored in the instrument for 2~3 days, but not in $Qsep_1$!
- If the cartridge will be idle for more than 3 days, please put the cartridge in the cartridge shell and avoid the light.
- Store the cartridge in the instrument, then user will need to add few drops of mineral oil on the top of park & Separation position of buffer tray to prevent the solution evaporation.
- Place the cartridge back to the package, user might need to gently insert the cartridge tip into the soft gel in the box.

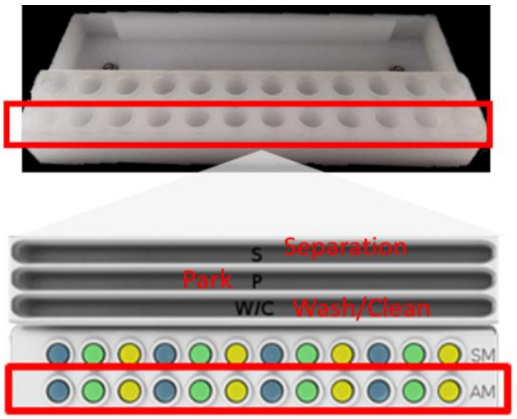
4. Questions of operation

Q1：How do I place the $Qsep_{400}$ Alignment marker and size marker?

A：

- Please refer to section 3.1.1 Figure 3-5 or $Qsep_{400}$ operation Quick Start.

AM-01 (position 1, 4, 7, 10)	20-1k (C109100)
	20-5k (C109102)
	20-1.5k (C109109)
	20-15k (C109110)
AM-02 (position 2, 5, 8, 11)	RNA-LM (C109120)
	Protein-LM (C104605)
AM-03 (position 3, 6, 9, 12)	User Define AM



※ Make sure tubes with alignment marker are at the assigned position

Q2：How do I prepare the unknown sample before $Qsep$ analysis?

A：

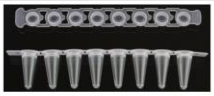
- About the sample with unknown buffer condition, please dilute the sample with dilution buffer. Because some unknown chemicals and organic solvents may influence the coating of capillary and damage the cartridge, hence, dilute the sample may reduce the affection of cartridge.

Sample type	
Samples with high salt buffer (PCR reagent, RFLP…etc)	**dilute with 0.1X Dilution buffer**
Samples with low salt buffer (or H_2O)	**dilute with 1X Dilution buffer**
Samples with unknown buffer (FFPE, cfDNA…etc)	**dilute with 0.1X Dilution buffer**

Q3：The minimum volume of sample?

A：

- When using a 0.2 ml PCR tube, the minimum volume of sample is 20 µl.
- When using a 0.1 ml tube, the minimum volume of sample is 10 µl.
- The patented micro-vial can be used for the rare sample. Only needs 2 µl to finish one run. *Micro-vial is NOT allowed to be used in N1 cartridge.

		Volume
0.2 ml PCR tube		≥ 20 µl
0.1 ml tube		≥ 10 µl
0.1 ml tube		≥ 10 µl
microvial (C104250)		≥ 2 µl

Q4：The sample consumption (per run)?

A：

- For different cartridges, all the sample consumption is < 0.1 µl per run.

Q5：What is the air pressure range of each instrument?

A：Please refer to Operation manual- Hardware.

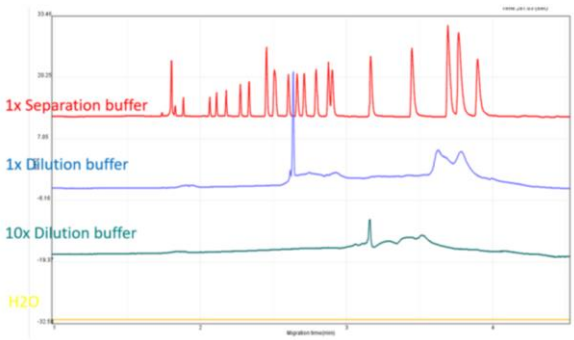
Instrument	Pressure range
$Qsep_{100}$	**50 psi ~ 75 psi**
$Qsep_{1}$	**50 psi ~ 65 psi**
$Qsep_{400}$	**50 psi ~ 75 psi**

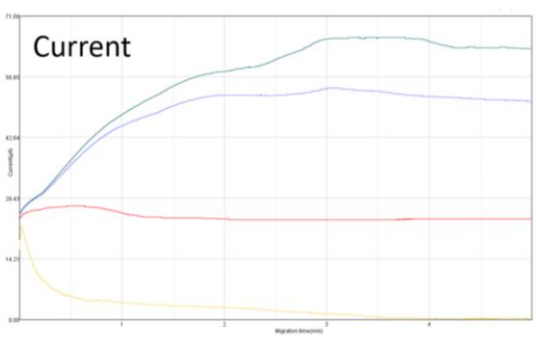
*$Qsep_{100}$, which was produced before 2016 Sep., does not have this function because it uses the old motherboard.

Q6：What if we use the wrong buffer?

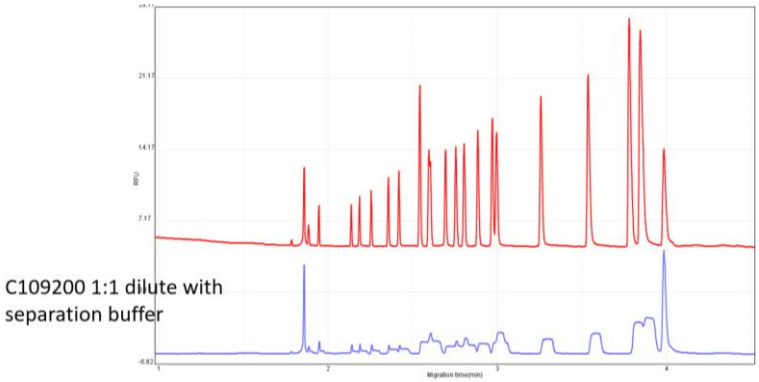
A：

- Using dilution buffer to do the separation, the peaks pattern becomes weird and the current raise.



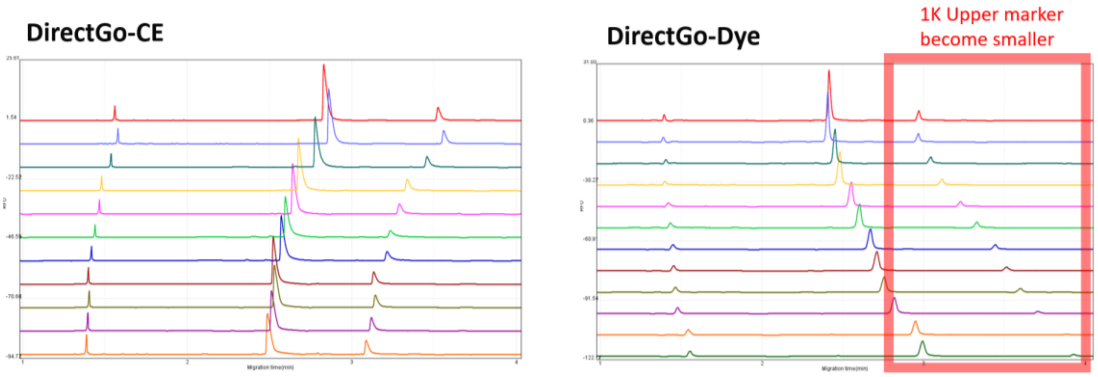


- Use separation buffer to dilute sample, current is OK, but pattern becomes weird



Q7：Will dye or organic solvent influence cartridge? How to pretreat the sample?

A：

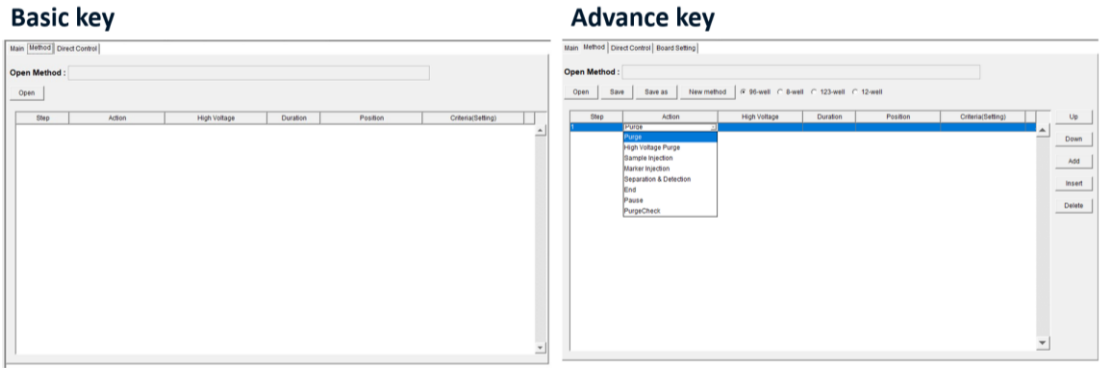


- Please try the sample dilution or even cleanup will be better.
- If the sample is very rare and precious, dilute the sample and use N1 cartridge. And add a 5-10mins purge to every 10 runs.

Q8：What is the difference between Basic key and Advance software key?

A：

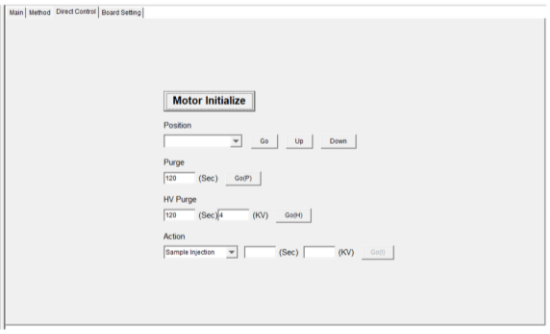
- Create Method



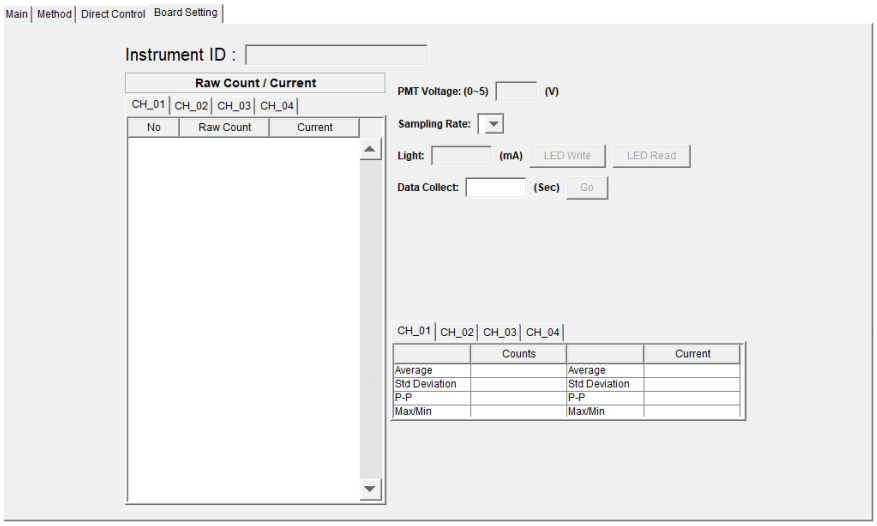
- More function

Basic key

Advance key



- Board Setting. (Advance key only!)

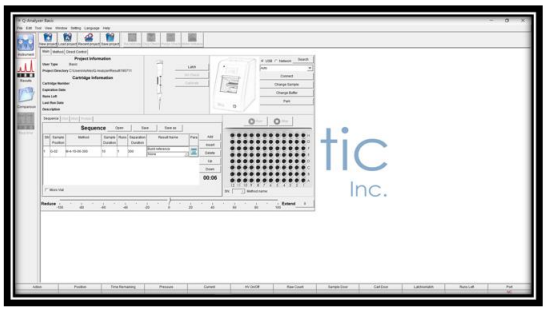


Q9：What is the difference between *Q-Analyzer*™, *Q-Editor*™ and *Q-viewer*™?

A：

- Please refer to software operation manual section 2.2 Software Mode.

Q-Analyzer



Q-Editor

Q-Viewer

Software Level table

Condition / Mode	Software Key (Advance) detected	Software key (Basic) detected	Instrument connected	Q-Editor License	None
Password	✓	✓			
Q-Analyzer (Advance)	✓				
Q-Analyzer (Basic)		✓	✓		
Q-Editor				✓	
Q-Viewer					✓

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

Q10：Why should I do recalibration?

A：

The benefits of Recalibration are:

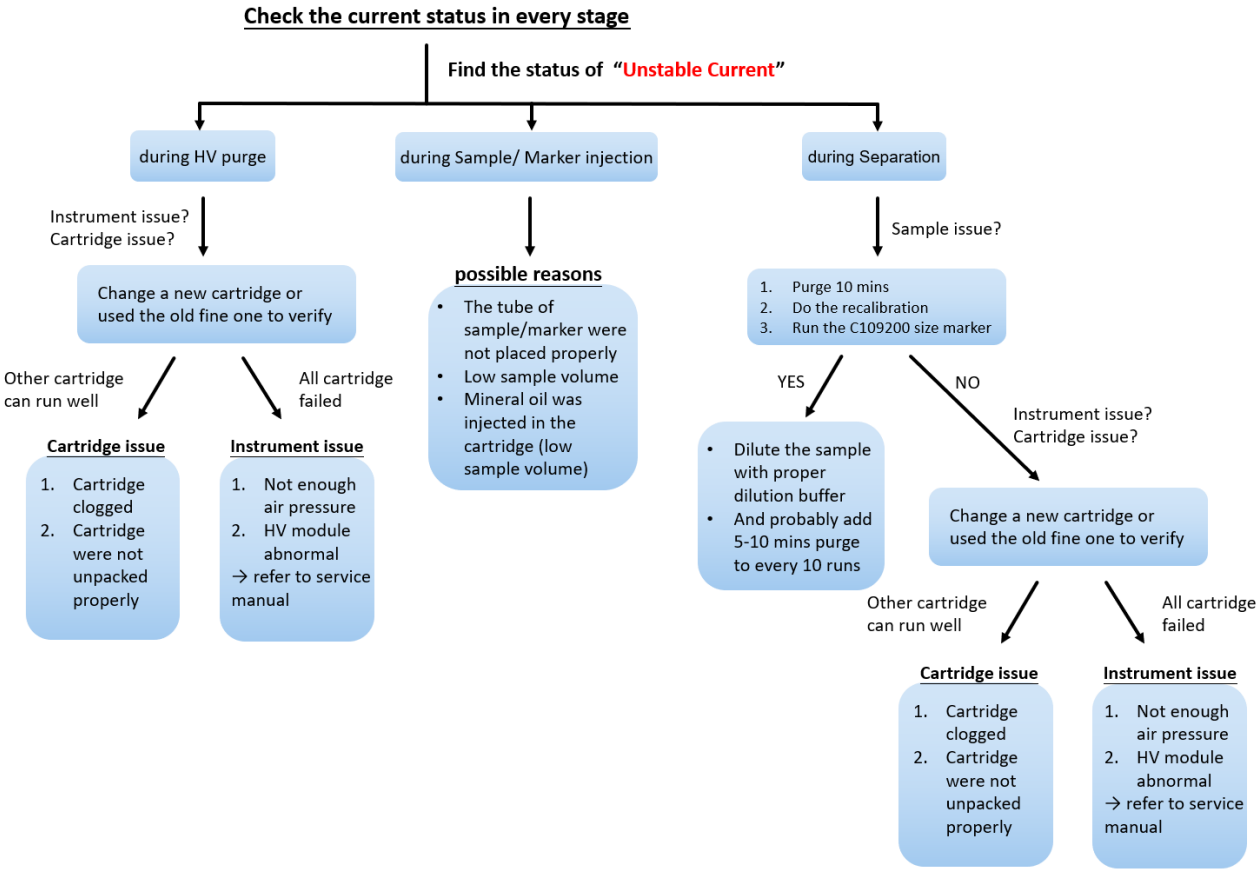
- Check the cartridge is good or not
- Recalibrate the lower and upper marker

5. Unexpected results

Q1：What kind of conditions will cause unstable current?

A：

- Most of the unstable current issues come from the sample and cartridge. When you encounter some result issues, please check sample and cartridge first.
- Please check the current status in every stage and follow the following steps to verify the possible reasons.



Q2：What to do if alignment marker and samples are migrating too slowly and the upper alignment marker does not appear on the result?

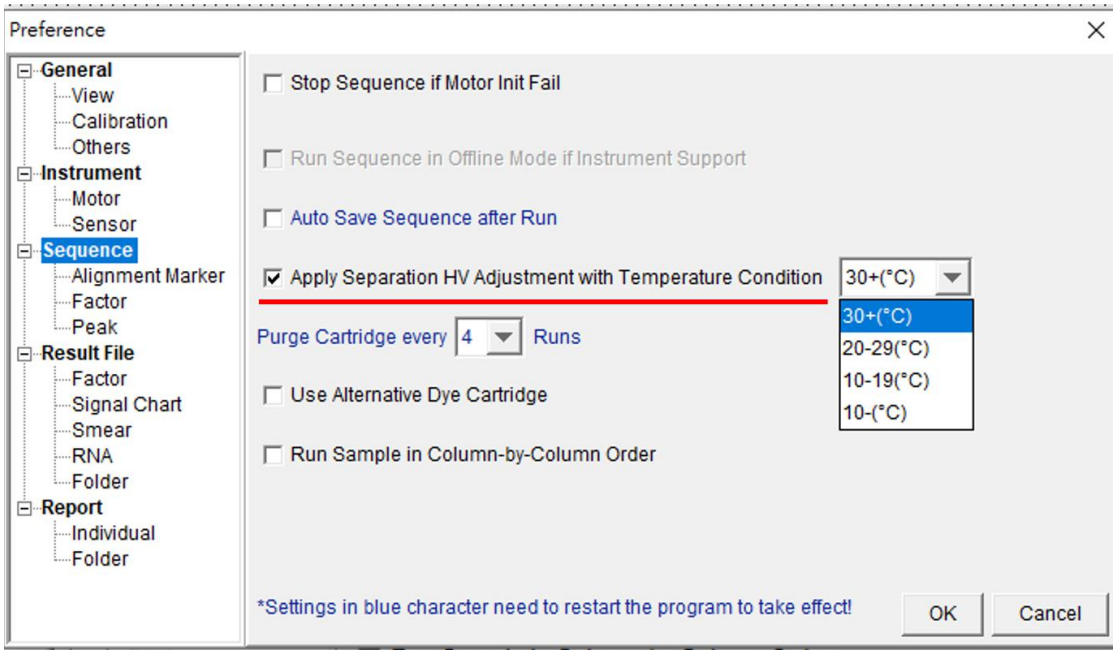
A：

Step1. Check the method is selected properly or not.

Step2. Check the alignment marker is fresh or not.

Step3. Check the current in each step is okay or not.

- If the current is unstable during one of the steps, please refer to 5-Q1 to resolve the issue.
- If the current is stable, it might be the environmental issue, such as temperature.
  - 10 ℃: Speed up the separation current (1.2x)
  - 10-19 ℃: Speed up the separation current (1.1x)
  - 20-29 ℃: Room temperature
  - 30 ℃+: Slow down the separation current (x0.9)



Q3：Identify wrong alignment marker or size marker

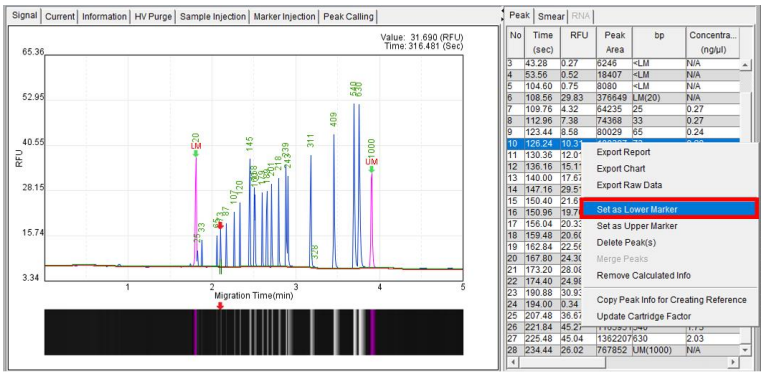
A：Alignment marker is to calibrate of migration time for each run, and it is very important and affect to the sizing result.

- **Recalibrate (please refer to section 3.4)**
  To run alignment marker first and the software will remember the factors of lower/ upper marker and recognize it in the following run.

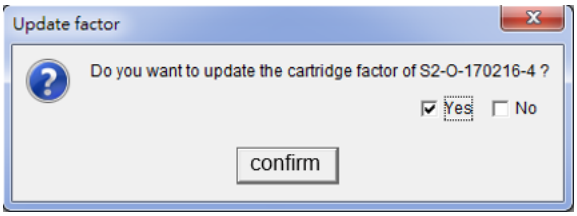
- **Manually update the lower and upper marker**

  Update the alignment in first run, and the following results will be updated to the factors

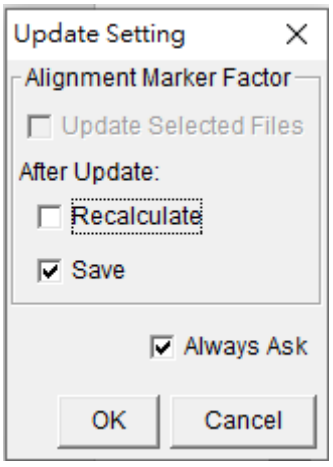
  Step 1. Set as lower marker.



  Step 2. Update the cartridge factor.



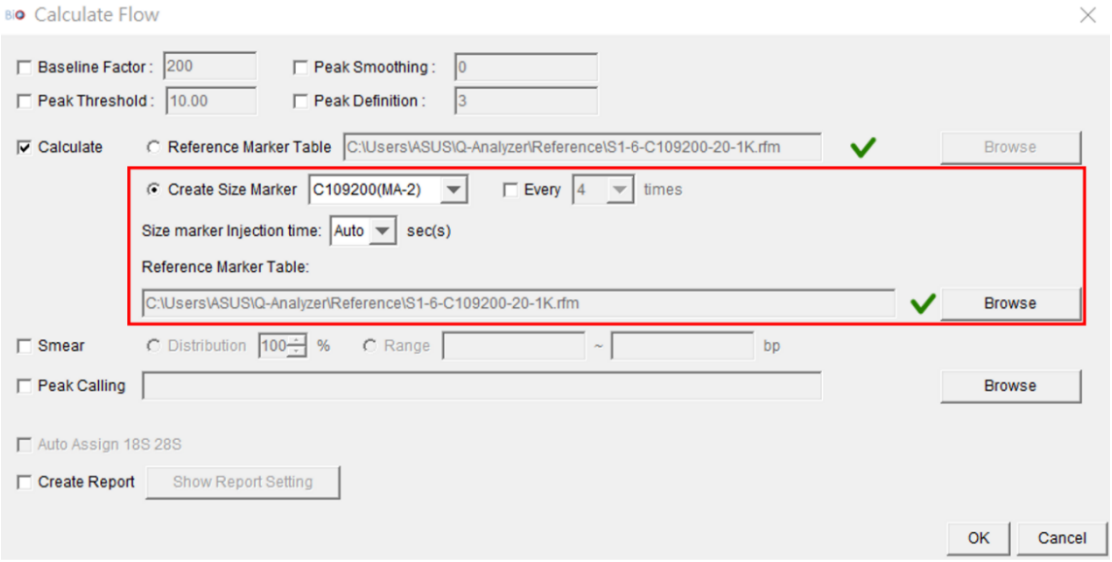
  Step 3. recalculate and save the result.



Q4：The calculated size is wrong

A：

- Environment may influence the migration time, so use the “create size marker” function (Remember to place the C109200 size marker on MA2). Please refer to 3.1.2



- The specifications of all the same types of instrument on the market are also about 10% deviation.
- The platform we provide for DNA analysis is native condition, so the conformation of double strand DNA may also affect the migration.

Q5：Is the concentration display in result table reliable?

A：

- The software calculates the concentration of Peak, which is according to “Peak area” and some parameters to correspond to Size marker (SM) which is near the target Peak to estimate.
- If you just want to compare the samples which are similar buffer condition. You can refer to this data.
- The **salt ion concentration** in the sample affects the injection of charged molecules. Therefore, if the salt ion concentration of the sample is different from that of size marker, the concentration estimated by its Peak area will different with exact concentration.

Q6：The current is stable, but there is no signal at all, even the alignment marker does not appear. RFU near zero

A：

- If there are other cartridges, please use it to confirm the situation. Even the

cartridge that has been zeroed can be used to confirm that the machine is OK or not.

- RFU near zero indicate there is no signal been detected. The major issue may come from the light pathway (Ex/Em). Please refer to the service manual and check if the J2 (LED) and J3 (PMT) connectors are loose. For further information, please contact local distributor or BiOptic.

Q7：The current is stable, but there is no signal except the alignment marker

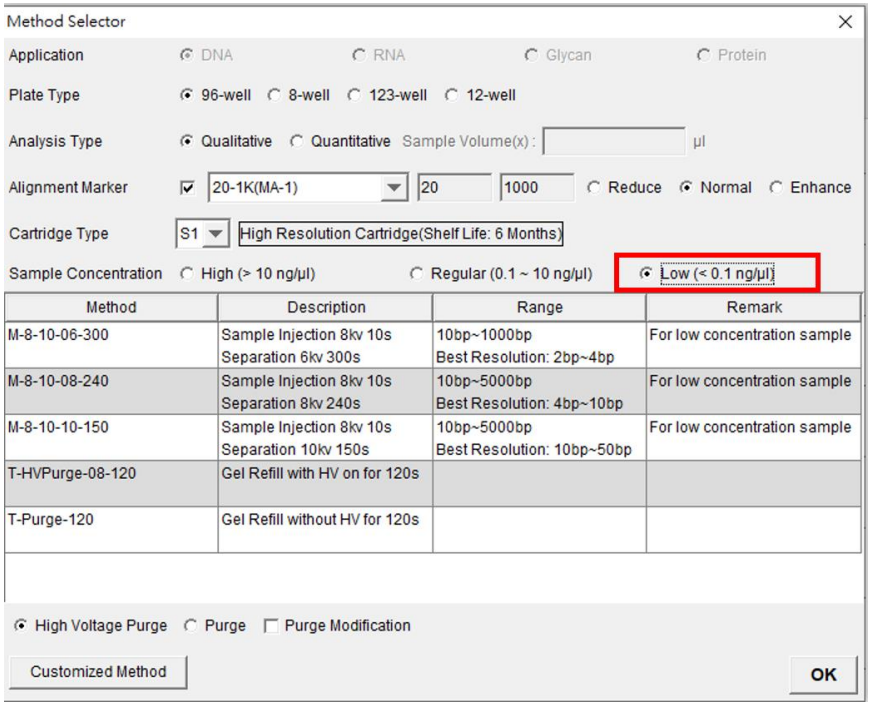
A：

- The issue properly caused from the sample. You can run the size marker to confirm).

Step1. You have to check the sample injection, the current is stable or not.

Step2. Check the sample concentration.

If the sample concentration is too low, you can select the “Low (<0.1 ng/μl)” in method. Besides, you can also use our N1 High sensitivity cartridge to analyze these kind of samples.



Step3. Check the salt concentration of the samples.

If the salt concentration is too high, please follow the 4-Q2 to dilute the sample with 0.1X dilution buffer or Clean up the sample will be better.

Q8：One of the 4 Channel shows different result with others.

A：

Step1. Check the samples which are belong to that failed channel. Make sure all

the sample volumes are over 20 µl.

Step2. Please check the current.
Take four 0.2 ml PCR tube and add 100ul separation buffer in each. Place them at A-01, A-04, A-07 and A-10. Extend the sample injection to 60 seconds and do the analysis.

Step3. If the current is unstable, please do the same process as the clogging single channel cartridge.

Step4. If it still unstable, this channel might be damaged, just ignore this channel. Place dilution buffer at the corresponding wells.

6. Specific application issues (RNA, Protein …)

6.1 R1 RNA cartridge

Q1：When running RNA samples, are there any special things to consider?

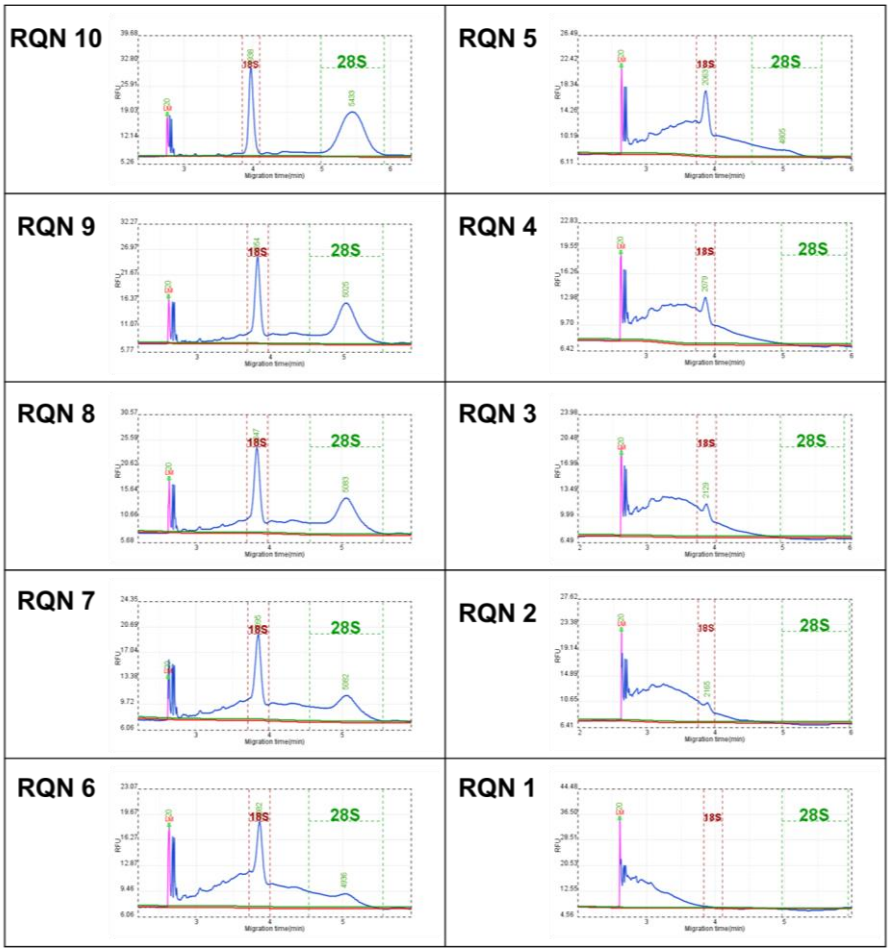
A：

- Clean up everything to avoid the contamination of RNase.
- Use tissue with ethanol to wipe the cartridge tip
- The separation buffer and dilution buffer we provided are 10x stock and need to dilute to 1x via DEPC-water.
- Alignment Marker: 5x lower Marker dilute to 1X via dilution buffer, and place in MC1.
- Sample Treatment: heat the RNA sample at 95/ 70℃ for 2-5 minutes, and place on ice until analysis.

Q2：What is the criteria of RQN?

A：

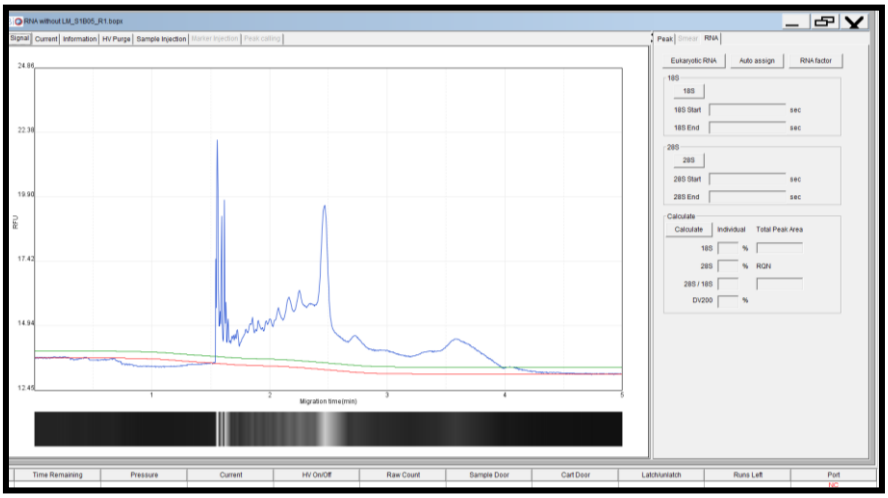
- The larger the RQN value, the higher the integrity of the RNA, but what kind of RNA sample is beneficial for the subsequent experiments
- According to the experimental design, you should create your own criteria because maybe this RQN value is good in A laboratory but is not good enough in B lab.



Q3：Why the result shows "N/A" in RQN?

A：

- Did you use lower marker?
  No lower marker, no information
- In the analysis results, the system has been unable to interpret 18S and 28S.
  The integrity of RNA is very bad.

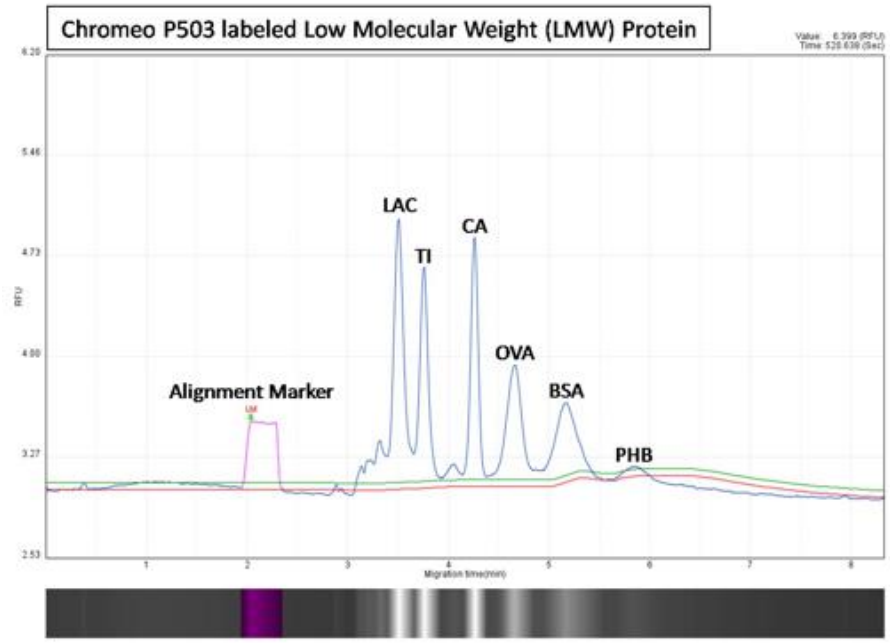


6.2 P2 Protein cartridge

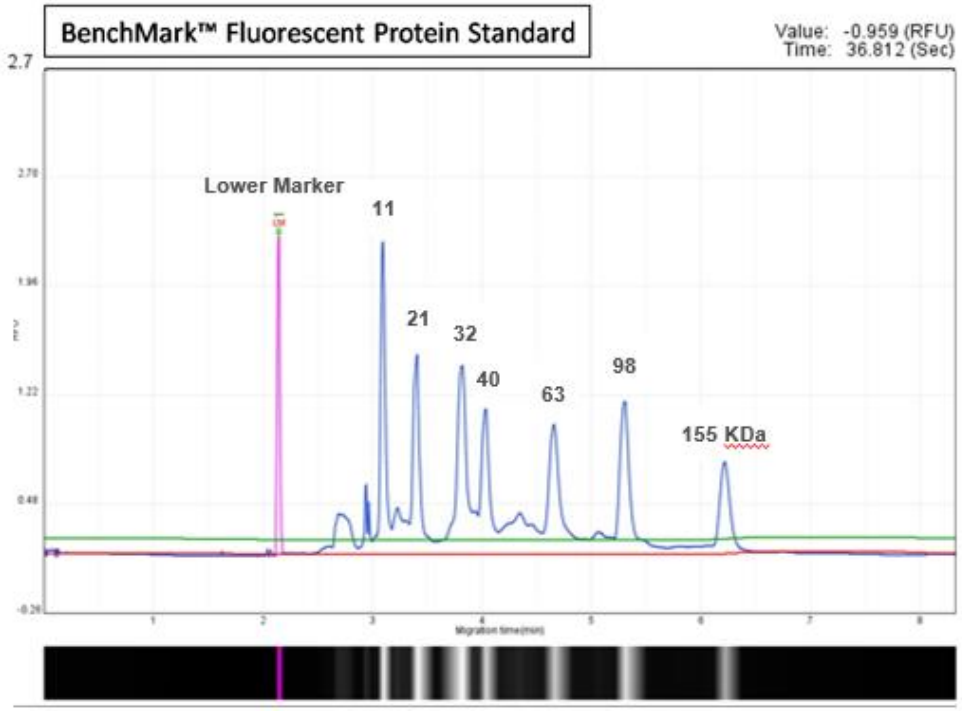
Q4：What Protein Alignment marker should I use?

A：

- It depends on what labeling dye you choose.
- For Chromeo P503, you can use the lower marker we provide in cartridge box.



- For Alexa488, just define the “Free dye” as lower marker



Q5：Which instrument can I use to analyze protein sample? Or what labeling dye should I choose?

A：

- Basically, we will choose the protein labeling dye according to the Ex/Em range of the machine.
- We suggest user using Alexa488 labeling dye and $Qsep_{100}$ Advance, $Qsep_{100}$ Advance has better sensitivity and there is commercialize pre-stained ladder from Thermo Fisher, which can provide better size accuracy.

Q6：How to do the labeling process?

A：

- For the details, please refer to the website to download the latest labeling protocol.

7. Maintenance

Q1：How often should I change the Marker and Buffer?

A：

For $Qsep_{100}$ and $Qsep_{400}$

- Every 96 samples or every 3-5 days
- User need to add few drops of mineral oil to cover the Separation buffer and 10ul oil on Marker
- The buffer tray can be washed and reused

For $Qsep_1$

- Separation buffer need to be changed every 20 runs
- System will also pop out a window to remind user to change the separation buffer.

Q2：What happens if I don't change the filter for a long time??

A：

- There may be insignificant air leaks.
- Not enough pressure may reduce the replacement efficiency of gel in the cartridge. Old gel may influence the current and even the analysis result may have some unpredictable affect.

Q3：How to clean the instrument regularly?

A：

- The outside of $Qsep$ could wipe with dry cloth. No detergent or ethanol, it might

destroy the color.

- Inside of instrument, you can use 70% ethanol to clean it.

8. Others

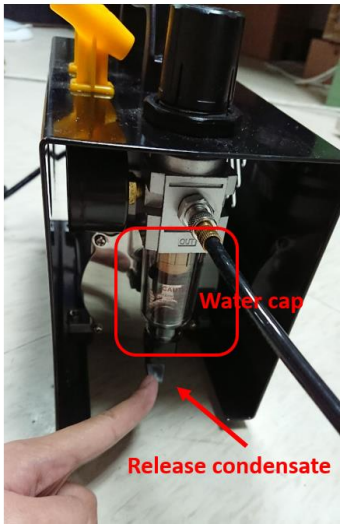
Q1：Should we turn off the instrument and pump every day?

A：

No need.

For the Pump:

User might need to remove the condensate in the water cup before running the sample.



Q2：How should we do before discard the cartridge?

A：

Step1. Bend the cartridge tip to prevent the tip jab to someone.

Step2. Wear the gloves

Step3. Open the cap and remove the inner cap.

Step4. Pour the gel into the container of waste liquid and wash the reservoir.

Step5. The cartridge can throw into the trash can.

Report to BiOptic

- Fill the “Troubleshooting Form”
- Clearly identify the time when issue occurs.
- Sample information, include sample type, buffer condition, bp or MW
- Describe your problem in detail, including words, figures, video, result files and report (.rar).
- Try to exclude the dummy issue first.