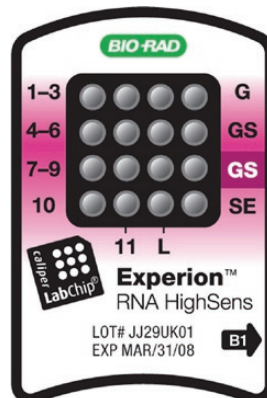
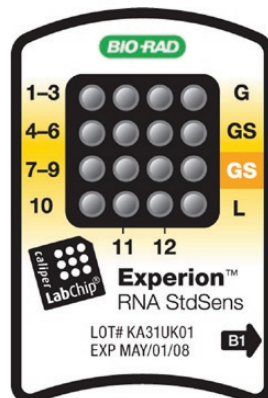


# Experion™ RNA StdSens and HighSens Analysis Kits

## Instruction Manual

Catalog #700-7103  
#700-7105



**BIO-RAD**

## Bio-Rad Technical Support

For help and advice regarding products from the Experion™ automated electrophoresis system, please contact the Bio-Rad Technical Support department, which in the United States is open Monday–Friday, 5:00 AM–5:00 PM, Pacific Time.

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# **1 Experion™ RNA Analysis Kits**

## 1.1 Product Description

The Experion RNA StdSens and HighSens analysis kits are used for RNA analysis with the Experion automated electrophoresis system (Figure 1.1). The Experion system employs LabChip microfluidic technology to automate nucleic acid and protein electrophoresis and analysis, integrating separation, detection, and data analysis within a single platform. Using much smaller sample and reagent quantities than standard analysis methods, the Experion automated electrophoresis system can be used both upstream and downstream of a number of nucleic acid and protein applications.

The Experion RNA analysis kits are used to determine total RNA and mRNA integrity, purity, and concentration. The Experion RNA StdSens analysis kit offers analysis at nanogram levels, and the Experion RNA HighSens analysis kit is used for analysis at picogram levels (Table 1.1). Both kits include the Experion RNA ladder, which has been optimized for automated electrophoresis on the Experion system. They also feature the Experion RNA loading buffer, which contains a 50 bp marker that is used for the proper alignment of samples to the RNA ladder.

RNA analysis with the Experion system compares favorably with other methods for RNA quantitation, such as UV spectroscopy, and provides analysis of both RNA integrity and concentration in a format that is versatile and easy to analyze. In addition, Experion analysis is relatively unaffected by reagents common to RNA preparation, and the electropherogram it generates enables evaluation of the RNA sample for degradation and for the presence of copurifying nucleic acids, such as genomic DNA and tRNA (Woo and Strong 2006). The Experion RNA StdSens and HighSens analysis kits offer single-step total RNA and mRNA analysis in less than 30 min.

For details about how the Experion RNA analysis kits analyze RNA, refer to Appendix A in this manual.

Register your Experion system to ensure you receive important updates on software, tech notes, and manuals. Upon installation, a dialog provides registration instructions.



**Fig. 1.1. The Experion system.** The system includes the following components: 1) automated electrophoresis station, 2) priming station, 3) vortex station used for nucleic acid analysis only, 4) system operation and data analysis tools (software), and 5) analysis kits, which include the (a) chips and (b) reagents for protein (Pro260 kit), standard-sensitivity RNA (StdSens kit), high-sensitivity RNA (HighSens kit), and DNA (DNA 1K and 12K kits) analyses.

**Table 1.1. Experion RNA analysis kit specifications.**

	Experion RNA StdSens	Experion RNA HighSens
Number of samples	12	11
Sample volume	1 µl	1 µl
Quantitation range:		
Total RNA	25–500 ng/µl	200–5,000 pg/µl
mRNA	25–250 ng/µl	—
Limit of detection (total RNA)	5 ng/µl	100 pg/µl
Maximum salt concentration	TE buffer (10 mM Tris, 1 mM EDTA)	DEPC-treated water

## 1.2 Kit Components

**Table 1.2. Components of the Experion RNA analysis kits.**

Item	Description	Volume (per Vial)	10-Chip Kit
RNA StdSens or RNA HighSens chip	Microfluidic chips used for RNA separation		10 chips
Cleaning chip	Chip used for cleaning electrodes		2 chips
RNA gel	Proprietary polymeric sieving matrix	1,250 µl	1 vial
RNA StdSens or RNA HighSens stain	Proprietary fluorescent dye	20 µl	1 vial
RNA StdSens or RNA HighSens loading buffer	Buffer for sample preparation; contains lower marker for alignment of samples to the RNA ladder	900 µl	1 vial
RNA ladder	Standard containing 8 RNA fragments of 200–6,000 nt	20 µl	1 vial
RNA sensitivity enhancer (RNA HighSens kit only)	Proprietary reagent to improve sensitivity	100 µl	1 vial
Spin filters	Used for filtering reagents during sample preparation		3 filters

## 1.3 Storage Conditions

**Table 1.3. Storage conditions.**

Item	Storage	Shelf Life
Experion reagents	4°C	See expiration date on packaging
Experion chips	Ambient	See expiration date on packaging
Experion RNA ladder	–70°C	See expiration date on packaging
Filtered gel (G)	4°C	1 month from filtration; after 1 month, refilter

## 1.4 Additional Requirements

Experion automated electrophoresis station

Experion priming station

Experion vortex station

Microcentrifuge (1,000–10,000 x *g*)

Benchtop vortexer

Aluminum foil

Calibrated pipets and RNase-free, narrow-bore barrier tips (for example, VWR #87001-688 or Rainin #L-10F)

RNase-free microcentrifuge tubes, 0.5 ml or 0.65 ml

RNase-free water (for example, Experion DEPC-treated water, catalog #700-7253)

Benchtop centrifuge (optional, catalog #166-0612)

Extra spin filter for additional filtration steps (as needed, catalog #700-7254)

Experion electrode cleaner (catalog #700-7252)

Foam cleaning swabs (catalog #700-7264)



# 2

## Essential Practices

## 2.1 Storing and Preparing Samples and Reagents

Store all Experion™ reagents except the RNA ladder at 4°C when not in use. Store the RNA ladder at –70°C. Do not store reagents at room temperature for >2 hr, as this will shorten their shelf life.

Before use, allow all kit reagents (except the RNA ladder) to equilibrate to room temperature (15–20 min). Once thawed, gently vortex all kit reagents before use. Before opening the tubes, quickly centrifuge them to collect solution to the bottoms of tubes.

If the RNA gel has frozen, discard it.

Protect the RNA stain and gel-stain solution (GS) from light: store these solutions in a dark place and keep them covered with foil when using them.

The RNA stain contains DMSO, which is hygroscopic. Cap tightly.

Prepare GS on the day of use. Use filtered gel (G) for up to 1 month. After 1 month, refilter G.

Do not use coated or treated pipet tips or microcentrifuge tubes (for example, siliconized polypropylene) for preparation of kit reagents or samples. Use of treated tips or tubes may cause separation artifacts.

Use RNase-free microcentrifuge tubes, pipet tips, and TE buffer for sample and reagent preparation.

## 2.2 Priming and Loading the Chip

To avoid contamination, wear gloves and handle chips by the edges. Never touch the glass portions of the chip.

Load the chip on a benchtop or in the priming station. Never load a chip in the electrophoresis station.

Avoid sources of dust and other contaminants when preparing samples and loading the chip. Foreign particles in reagents, samples, or the wells of the chip interfere with separation. Remove chips from their packaging immediately before use.

It may be easier to load the chip on a white background. Tilt the chip to look for bubbles.

Use narrow-bore filter pipet tips for loading the chip (for example, VWR #87001-688 or Rainin #L-10F).

To avoid introducing air bubbles, do the following (for more help with chip loading, refer to the Experion Training Video in the Experion software Help section under **Contents and Index > Contents > Appendices > Technical Videos**):

- Insert the pipet tip all the way to the bottom of the chip well when dispensing liquids (this reduces the possibility of trapping air)
- Hold the tip vertically, perpendicular to the chip surface. Holding the tip at an angle may trap air bubbles at the bottom of the well
- When expelling liquid, dispense slowly and only to the first stop on the pipet. Using the second stop introduces air and bubbles into the liquid. Reverse pipetting is acceptable

Dislodge bubbles at the bottom of a well with a clean pipet tip, or remove the solution and load it again.

Use a primed and loaded chip within 5 min of loading. When chips are not used within this time, reagents may evaporate, leading to poor results or a chip performance error.

Fill all the chip wells when running an analysis. Use blank samples (prepared with water instead of sample) or replicates if necessary. All 16 electrode pins must be in contact with liquid; otherwise, an IV (current voltage) check failure error will occur.

## 2.3 Running the Analysis

Place the electrophoresis station on a stable surface, where it will not be subjected to vibrations or other movement, and away from direct sunlight and all other potential sources of extreme heat.

Power on the electrophoresis station before launching Experion software.

The first time that the Experion electrophoresis station is used, confirm that communication has been established between the software and electrophoresis station before preparing the reagents.

Do not open the lid of the electrophoresis station during a run. The run will abort if the lid is opened.

## 2.4 General Maintenance

For recommendations on general instrument maintenance, refer to the Experion system manual (bulletin 10001312).

Clean the electrodes after each run (routine cleaning). Cleaning maintains the instrument in optimum condition and prevents buildup and cross-contamination of reagents and samples.

Perform the deep cleaning procedure described in Appendix B to clean the electrodes:

- Prior to first use of the Experion electrophoresis station
- Whenever contamination is suspected or visible (for example, salt deposits or other precipitates) on the electrodes
- Whenever a chip has been left in the electrophoresis station for an extended period of time (for example, overnight)

Never store the cleaning chip inside the electrophoresis station. Store the empty cleaning chip covered to keep the wells clean. Two new cleaning chips are included with every box of chips.

## 2.5 Experion Video Tutorials

For additional information, view the video tutorials available online at [www.bio-rad.com](http://www.bio-rad.com):

North America: Home > Life Science Research > Support > Tutorials > Electrophoresis and Blotting > Experion System Training

Other: Home > Life Science Research > Electrophoresis > Automated Electrophoresis > Experion Training Videos





# **Experion™ RNA Assay Procedure**

For an abbreviated version of this protocol, refer to the Quick Guide provided with the kit.

### 3.1 Set Up the Electrophoresis Station

1. If needed, perform a deep cleaning of the electrodes (see Appendix B for instructions).
2. Power on the computer and then power on the Experion electrophoresis station by pushing the green button in the center of the front panel. The steady green LED above the button indicates the unit is on.
3. Launch Experion software. If the instrument and computer are communicating properly:
  - A green dot and the last 4 digits of the instrument serial number appear at the lower right of the software screen
  - The electrophoresis station icon appears in the upper left corner

When there is no connection, these indicators are absent and a grayed-out instrument icon appears at the upper left of the software screen.

### 3.2 Equilibrate the Kit Reagents

1. Set a heating block or water bath to 70°C. You will use this heating device to denature the samples and the RNA ladder later in the protocol.
2. Thaw the following kit components on ice (15–20 min):
  - RNA samples
  - RNA ladder (red cap)
3. Equilibrate the following kit components to room temperature (15–20 min):
  - RNA stain (blue cap)
  - RNA loading buffer (yellow cap)
  - RNA gel (green cap) (if filtered gel was prepared previously, remove it from storage and equilibrate to room temperature)
  - Sensitivity enhancer (clear cap, RNA HighSens only)
4. Invert each tube several times and then vortex to reincorporate any condensed liquid. Briefly centrifuge the solutions to the bottom of the tubes. Make sure the RNA stain solution (blue cap) is thawed before proceeding.

### 3.3 Filter the Gel and Prepare the Gel-Stain Solution

A gel-stain solution (GS) preparation is sufficient for use with up to three RNA chips. Prepare GS daily and keep it covered with foil until ready for use. If filtered gel (G) is available, skip step 1. Use G within 1 month of preparation. After 1 month, refill it before reuse.

1. Pipet 600 µl RNA gel (green cap) into a spin filter and centrifuge it at 1,500 x g for 10 min. Inspect the tube to ensure all of the gel has passed through the filter and then discard the filter.
2. Prepare the GS. Pipet 65 µl G into an RNase-free 0.65 ml microcentrifuge tube, add 1 µl RNA stain, and vortex for 10 sec. Wrap the tube of GS in aluminum foil to protect the stain from light.
3. **RNA HighSens analysis only:** Centrifuge the GS at 13,000 x g for 10 min.

## 3.4 Prepare the Samples and the RNA Ladder

Once the RNA samples and RNA ladder (red cap) have thawed on ice, vortex them briefly and spin down for a few sec in a microcentrifuge. Both the sample and RNA ladder must be denatured. Use of the RNA HighSens ladder also requires dilution.

### 3.4.1 Experion RNA StdSens Analysis

1. Pipet  $\geq 2$   $\mu$ l RNA sample into separate RNase-free microcentrifuge tubes.
2. Pipet RNA ladder into an RNase-free microcentrifuge tube: use 1  $\mu$ l RNA ladder for each chip plus an extra 1  $\mu$ l RNA ladder to accommodate variations in pipetting (for example, when running 1 chip, use a total of 2  $\mu$ l RNA ladder; for 3 chips, use 4  $\mu$ l RNA ladder).
3. Denature the samples and the RNA ladder by heating the tubes at 70°C for 2 min. Place them on ice for 2–5 min.
4. Vortex briefly and spin down. Keep the tubes on ice.

### 3.4.2 Experion RNA HighSens Analysis

If diluted RNA ladder is available from a previous preparation, skip steps 1 to 5. Do not reheat the diluted ladder.

1. Pipet 5  $\mu$ l RNA ladder into an RNase-free microcentrifuge tube.
2. Transfer 2  $\mu$ l RNA sample into separate RNase-free microcentrifuge tubes.
3. Denature the samples and the RNA ladder by heating the tubes at 70°C for 2 min. Place them on ice for 2–5 min.
4. Spin down the ladder and samples in a microcentrifuge briefly and then place the tube on ice. Add 795  $\mu$ l DEPC-treated water to the denatured ladder. Keep the tubes on ice.
5. Aliquot diluted RNA ladder into RNase-free microcentrifuge tubes, store at –70°C, and avoid exposing it to freeze-thaw cycles.

## 3.5 Prime the Chip

Start the run within 5 min of priming and loading the chip. For help with chip loading, refer to the Experion Training Video: Chip Loading, available in the Experion software Help section under **Contents and Index > Contents > Appendices > Technical Videos**.

1. Pipet 9  $\mu$ l GS into the highlighted well labeled **GS** (gel priming well, Figure 3.1).
2. On the priming station, set the pressure setting to **B** and the time setting to **1**, as specified by the alphanumeric code on the chip (Figure 3.1).
3. Open the Experion priming station and place the chip on the chip platform, matching the arrow on the chip with the alignment arrow on the chip platform. A post on the chip prevents insertion in the wrong position. Do not force the chip into position.
4. Close the priming station by pressing down on the lid. The lid should snap closed.
5. Press **Start**. A “Priming” message appears on the screen of the priming station, and the timer counts down. Priming requires ~30 sec. Do not open the priming station during the countdown.

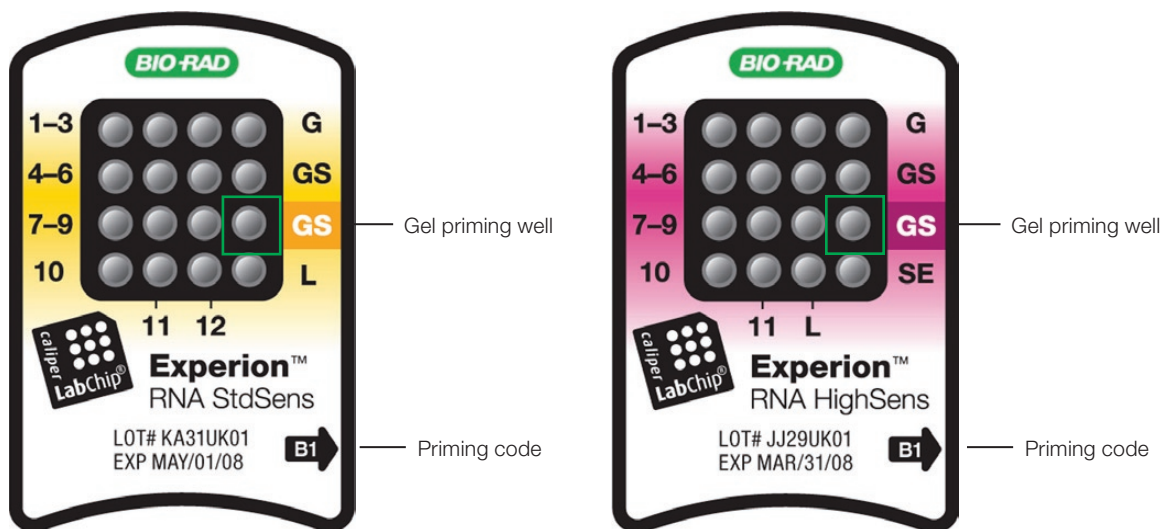


Fig. 3.1. Experion RNA chips. The locations of the gel priming well (GS, highlighted) and alphanumeric priming codes are indicated.

6. An audible signal and “Ready” message indicate that priming is complete. Open the priming station and remove the chip. If the lid sticks, press down on it while pressing down on the release lever.
7. Turn the chip over and inspect the microchannels for bubbles or evidence of incomplete priming. If the chip is primed properly, the microchannels are difficult to see (compare a primed chip to a new, unused chip). If you detect a problem, such as a bubble or incomplete priming, prime a new chip.
8. Place the chip on a clean surface for loading.

Bubbles forced into microchannels during priming take the shape of the microchannel and are elongated, not round.

### 3.6 Load the Chip

1. Pipet 9  $\mu$ l GS into the other well labeled **GS** (Figure 3.2).
2. Pipet 9  $\mu$ l filtered gel (G) into the well labeled **G**.
3. **(HighSens only)** Pipet 6  $\mu$ l sensitivity enhancer (clear cap) into the well labeled **SE**.
4. Pipet 5  $\mu$ l RNA loading buffer (yellow cap) into each sample well (HighSens wells **1–11** or StdSens wells **1–12**, ) and the ladder well, labeled **L**.

Use a new pipet tip for each delivery to prevent contamination of the loading buffer. Alternatively, aliquot 65–70  $\mu$ l loading buffer into an RNase-free microcentrifuge tube and add 5  $\mu$ l to each well from this volume.

5. Pipet 1  $\mu$ l prepared RNA ladder into the well labeled **L**. Every chip must have the RNA ladder loaded into the ladder well labeled **L**.
6. Pipet 1  $\mu$ l sample (or blanks, for example loading buffer, DEPC-treated water, or TE buffer) into the numbered sample wells.



- Inspect all wells for bubbles by holding the chip above a light-colored background and looking through the wells (Figure 3.3). Dislodge any bubbles at the bottom of a well with a clean pipet tip or by removing and reloading the solution.

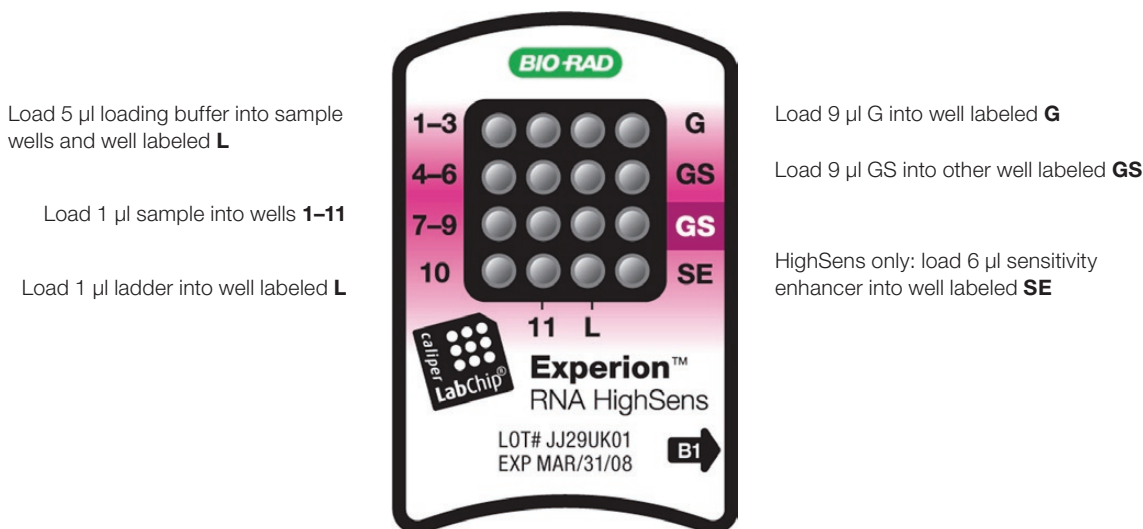


Fig. 3.2. Experion RNA HighSens chip. Wells for loading GS, samples, and ladder are indicated.

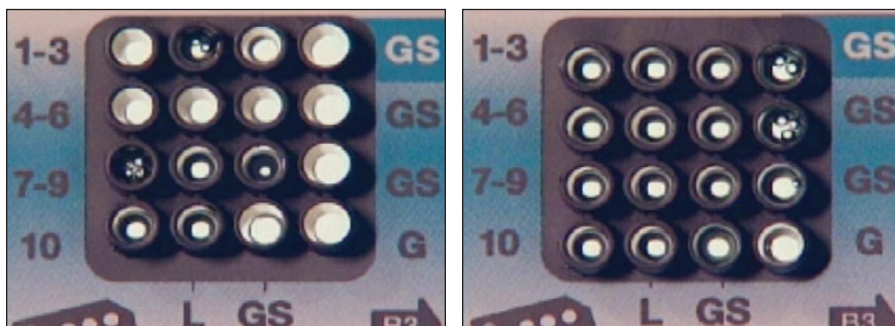

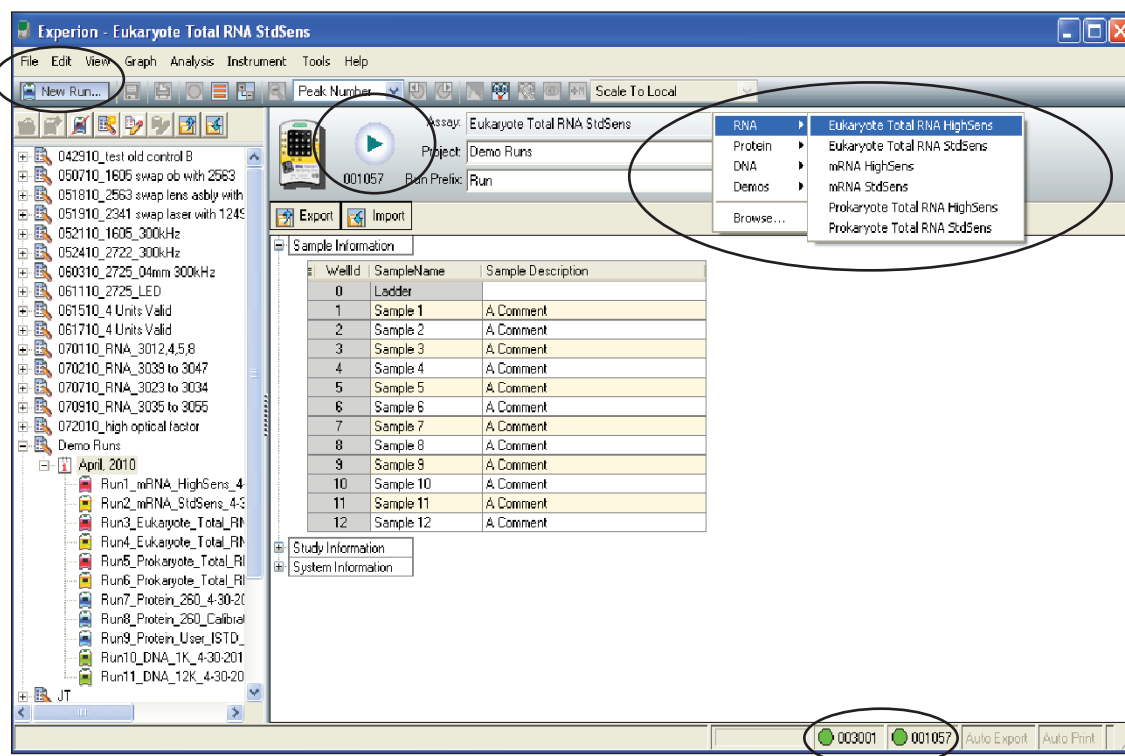


Fig. 3.3. Bubble formation during loading of Experion Pro260 chips. Surface bubbles do not generally cause problems during a run, but bubbles at the bottoms of wells must be removed. **Left**, bubbles trapped at the bottom of wells. The GS and G wells and sample wells 1, 3, and 4-6 contain no solution. Wells 8, 10, and L are filled properly and have no bubbles, but large bubbles have formed at the bottoms of wells 7 and 9 (note the difference in the diameter of the light-colored circles in wells 8 and 9). **Right**, bubbles have formed at the surface of the three GS wells on the right side of the chip; the rest of the wells have no bubbles.

- Slide the chip into the Experion vortex station and turn on the vortex station by pressing **Mix**. Vortexing continues for 60 sec and then automatically shuts off. Remove the chip when the vortex station stops.
- Inspect the wells again to confirm that there is no excessive bubble formation from pipetting and that no liquid has spilled outside the wells during vortexing.
- Place the loaded chip into the Experion electrophoresis station and start the run within 5 min.

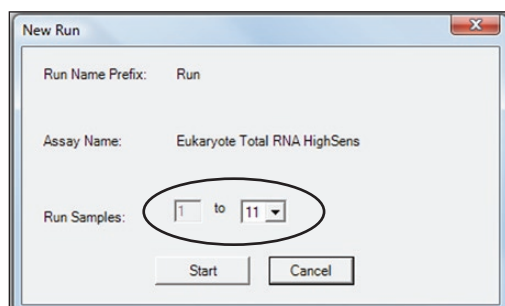
### 3.7 Run the RNA Analysis

1. Open the lid of the electrophoresis station by pulling the release latch. Place the primed, loaded, and vortexed chip on the chip platform and close the lid.
2. In the Experion software toolbar, click **New Run** . In the **New Run** screen (Figure 3.4), from the **Assay** pull-down list, select the type of assay.
3. Either select a project folder from the **Project** pull-down list or create a new project folder by entering a name in the **Project** field or by selecting **File > Project > New**. The project folder appears in the project tree.
4. Enter a name for the run in the **Run Prefix** field and click **Start Run** .



**Fig. 3.4. Details of the New Run screen.** The green dot in the lower right corner indicates that communication between the electrophoresis station and Experion software has been established.

5. In the **New Run** dialog (Figure 3.5), select the number of samples to be analyzed. Though all wells are filled, the Experion system stops the analysis when it reaches the number of samples entered.



**Fig. 3.5. New Run dialog.** The Experion system stops analysis when it reaches the number of samples entered.

- Click **Start**. The green LED in the center of the front panel on the electrophoresis station blinks, and the system performs a number of checks: it confirms that a chip has been inserted, that all wells contain liquid, that electrical connections are made, etc. A calibration counter marks the progress of these calibrations at the upper right of the screen.

Do not open the lid of the Experion electrophoresis station until the run is complete. The lid does not lock. Opening the lid aborts the run.

An “IV Check Error” message indicates the system cannot make electrical contact in one of the wells. This often means there is a bubble at the bottom of the well. Abort the run, and check the chip for bubbles or empty wells. Refill the affected well(s), and start the run again.

- During separation, the sample name is highlighted in the project tree and the electropherogram trace, and virtual gel bands appear in real time:
  - The electropherogram of the sample being separated appears in the electropherogram view
  - The lane corresponding to that sample is outlined in pink and has a dark background

To display the electropherogram of another sample, click on either the sample name in the project tree or on a lane in the virtual gel.
- When analysis is complete (after ~30 min), the instrument beeps and a window opens indicating the end of the run. Select **OK** and remove the chip from the chip platform.
- Clean the electrodes using deionized water within 30 min of each run.

### 3.8 Clean the Electrodes

- Fill a cleaning chip with 800 µl deionized water (0.2 µm-filtered). Gently tap the side of the cleaning chip to remove any trapped bubbles from the wells.
- Place the cleaning chip on the chip platform in the electrophoresis station, close the lid, and leave it closed for 1 min.

Never store the cleaning chip inside the electrophoresis station. Store the empty cleaning chip covered to keep the wells clean. Two cleaning chips are included with each box of chips.

- Open the lid, remove the cleaning chip, and allow the electrodes to dry for 1 min. Close the lid.
- Replace the water in the cleaning chip after use to avoid contamination. For storage, remove the water from the cleaning chip and store the chip in a clean location.

### 3.9 Evaluate the Run

When a run is complete, evaluate the run and the analysis of the data by Experion software.

- Ensure that all lanes (ladder and samples) are visible in the virtual gel. The lower marker (indicated by a pink triangle) should be visible in and aligned across all lanes. If the marker peak is not properly assigned, you may need to manually set the marker (see Section 6.1).

2. Evaluate the separation of the RNA ladder. To display the ladder electropherogram, click the ladder well in the project tree, or click on the lane labeled **L** in the virtual gel. The electropherogram should resemble the one shown in Figure 3.6 and should have the following features (if your ladder does not have these features, see Chapter 7, Troubleshooting for more information):

- Lower marker peak at ~20 sec, at least 5 fluorescence units above the baseline, and distinguishable from the ladder peaks
- Eight resolved ladder peaks
- Flat baseline

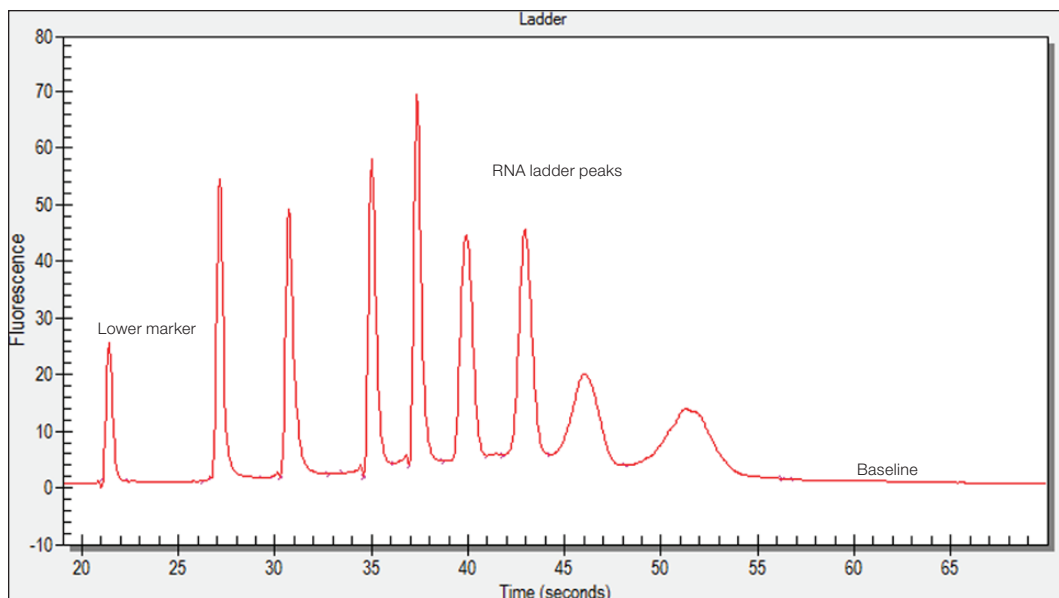


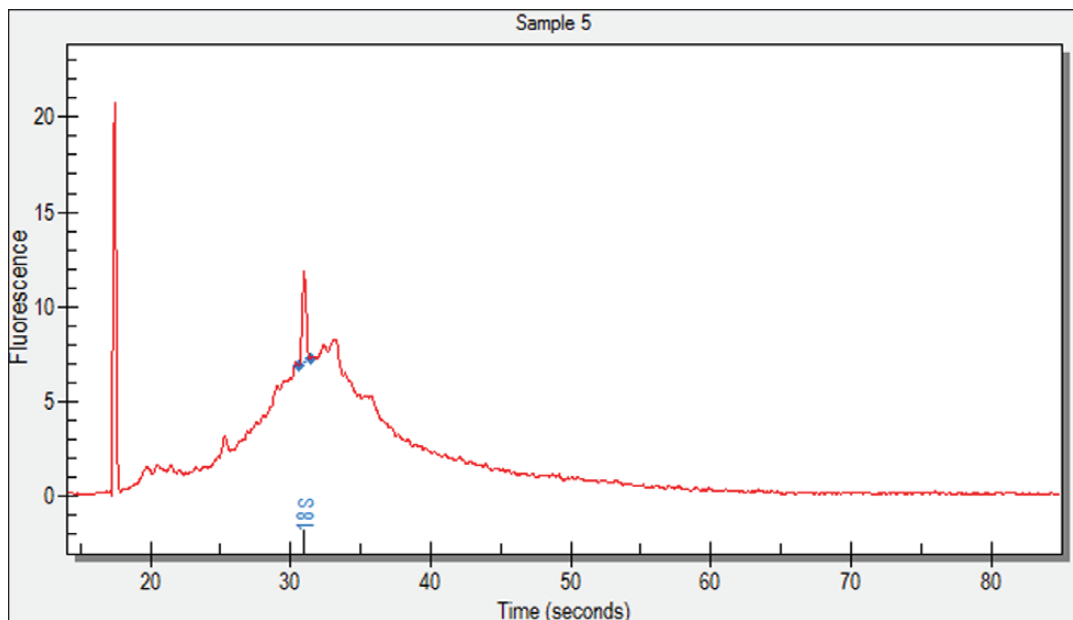
Fig. 3.6. Experion RNA StdSens separation of the RNA ladder in a prokaryotic total RNA assay. Note the flat baseline and resolved peaks.

3. Examine the separation of at least one sample. Click on the sample name in the project tree or on the lane in the virtual gel to view the electropherogram, which should have the following features:

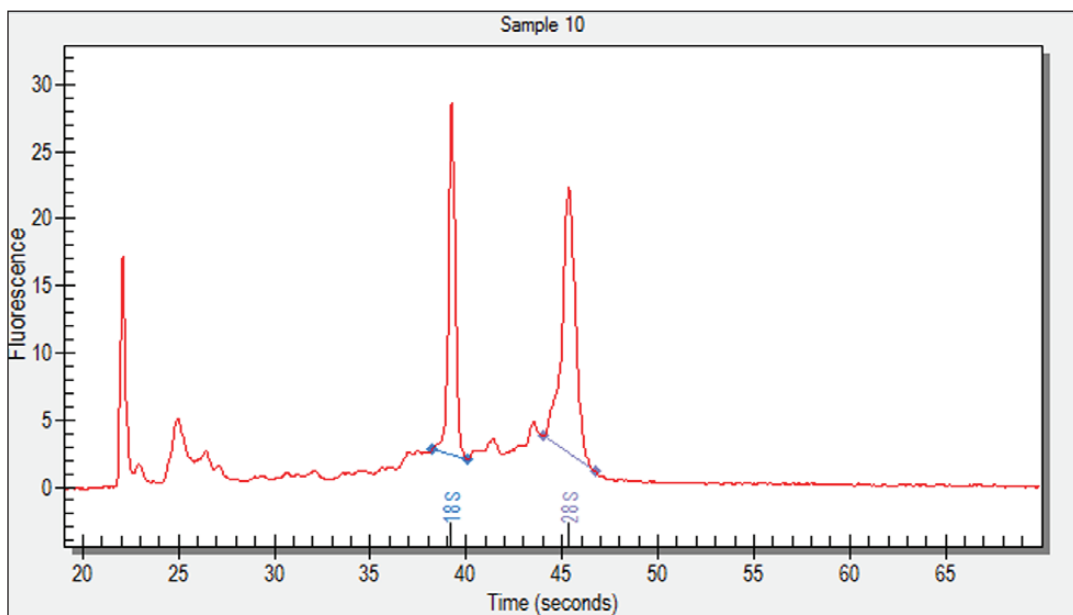
- Lower marker peak at ~20 sec, at least 5 fluorescence units above the baseline, and distinguishable from the sample peaks
- Sample peaks: mRNA analyses often yield broad peaks and, on occasion, contaminating 18S and 28S rRNA peaks (Figure 3.7), while total RNA analyses often yield 2–3 prominent peaks corresponding to rRNA (in eukaryotic sources, 18S and 28S and occasionally 5S; in prokaryotic sources, 16S and 23S) (Figure 3.8)
- Flat baseline

If the lower marker peaks are not properly assigned, manually set the marker (see Section 6.1).

4. Evaluate the data analysis performed by Experion software (see Chapter 4, Data Analysis). If necessary, change the analysis settings and parameters by following the instructions in Chapters 5 and 6.



**Fig. 3.7. Experion RNA HighSens analysis of mRNA.** Note the contaminating 18S rRNA peak identified by Experion software. Analysis time for mRNA samples is longer than for total RNA samples to include the population of longer transcripts and to achieve a smooth baseline.



**Fig. 3.8. Experion RNA HighSens analysis of eukaryotic total RNA.** The 18S and 28S rRNA peaks are identified by Experion software.



# 4

## Data Analysis

This chapter outlines the basic steps and software features used to view and analyze data. For a more detailed description of Experion™ software and its various functions, refer to the software Help menu. For information on how to customize analysis parameters, refer to Chapter 6, Changing Analysis Settings and Parameters.

## 4.1 Viewing Data

The main window of the Experion software user interface contains menus and toolbars, a tree view, and three data views: the electropherogram view, gel view, and results and settings tabs. (Figure 4.1).

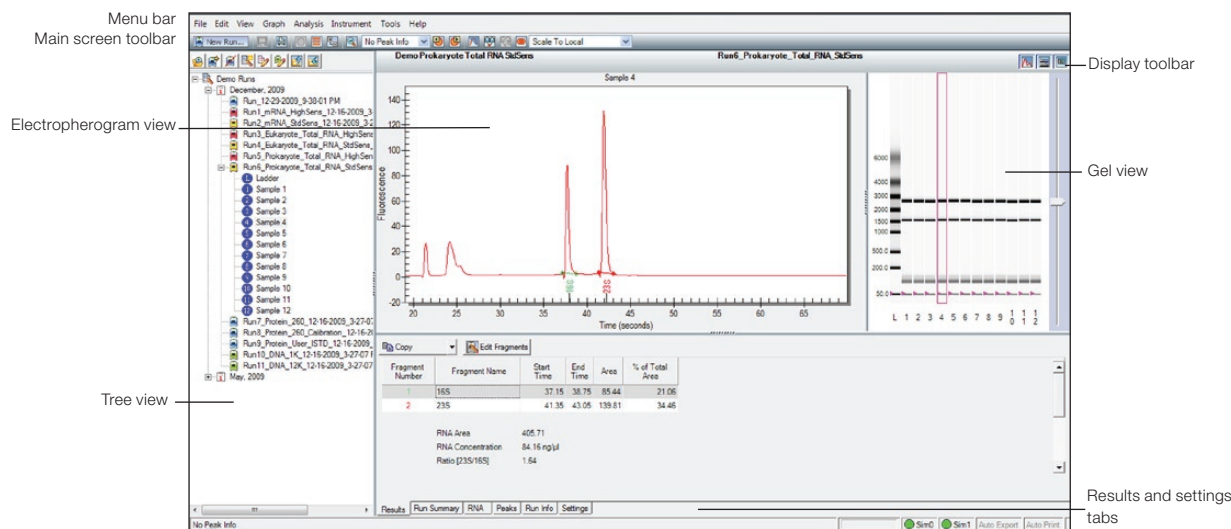


Fig. 4.1. Main window of Experion software. The toolbars, menus, and data views are indicated.

### 4.1.1 Managing Run Files and Project Folders in the Tree View

The tree view (Figure 4.2) displays the project tree, a hierarchical tree of all projects and the data files they contain. Use the project tree and the toolbar above it to:

- Open run files and select samples for viewing
- Create project folders
- Export and import one or more run files
- Edit names of run files and project folders
- Move runs into project folders
- Delete run files and project folders

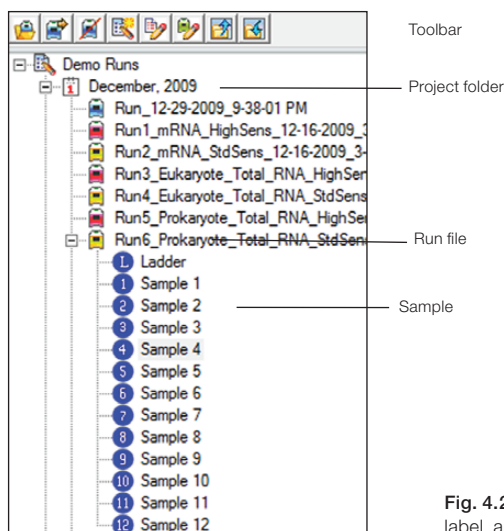


Fig. 4.2. Tree view details. The project folder name, sample well label, and run file prefix can be customized.



To open a run file, double-click on it. The run file opens, displaying the electropherogram and Results tab for the RNA ladder, and the virtual gel for the entire run.

To view the data from a sample, click on the sample name or select the corresponding lane in the gel view.

To edit a run file or project folder name, right-click on it and select **Rename Project** or **Rename Run**. Type in the new name.

To import, export, move, or delete files and folders, click the icons in the toolbar or use the context menu accessed by right-clicking on the items in the tree view. For more details, refer to the Experion software Help file.

#### 4.1.2 General Display Controls

Data in the electropherogram view, gel view, and results and settings tabs are linked. Selecting a sample or peak in one area automatically opens or highlights that sample/peak in the other two areas.

To adjust the relative sizes of the three data views and the tree view, click on and drag the border of the view to its new location.

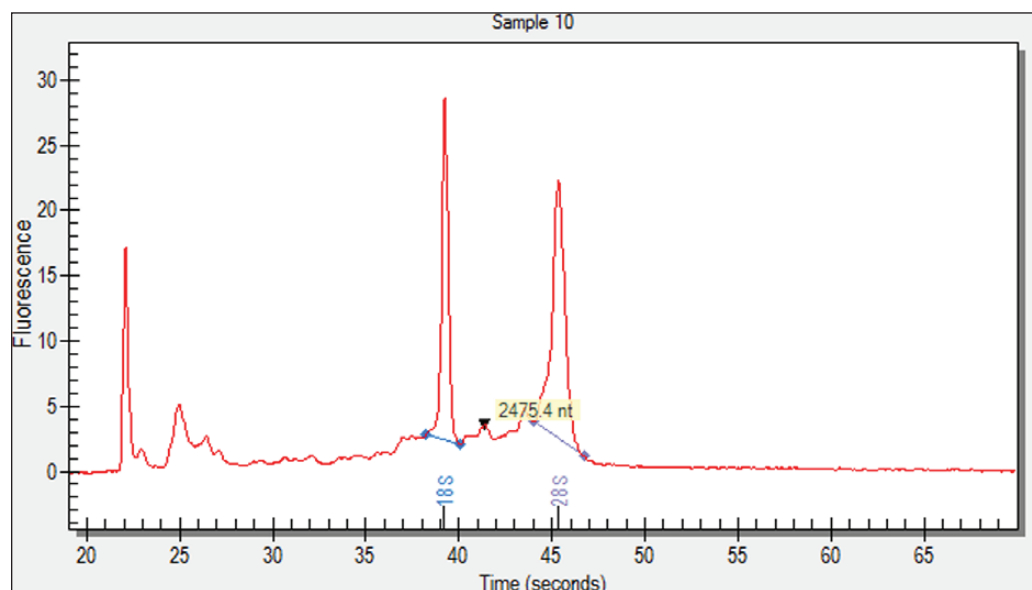
To hide or show one of the three data views, click on the corresponding icon in the display toolbar (located in the upper-right corner of the display, just above the gel view, Figure 4.1).

To zoom in on (expand the view of) a portion of an electropherogram or virtual gel, click on the corner of the area that you would like to enlarge and mouse over to the opposite corner. Double-click anywhere in the electropherogram view to return to the previous view.

#### 4.1.3 Electropherogram View

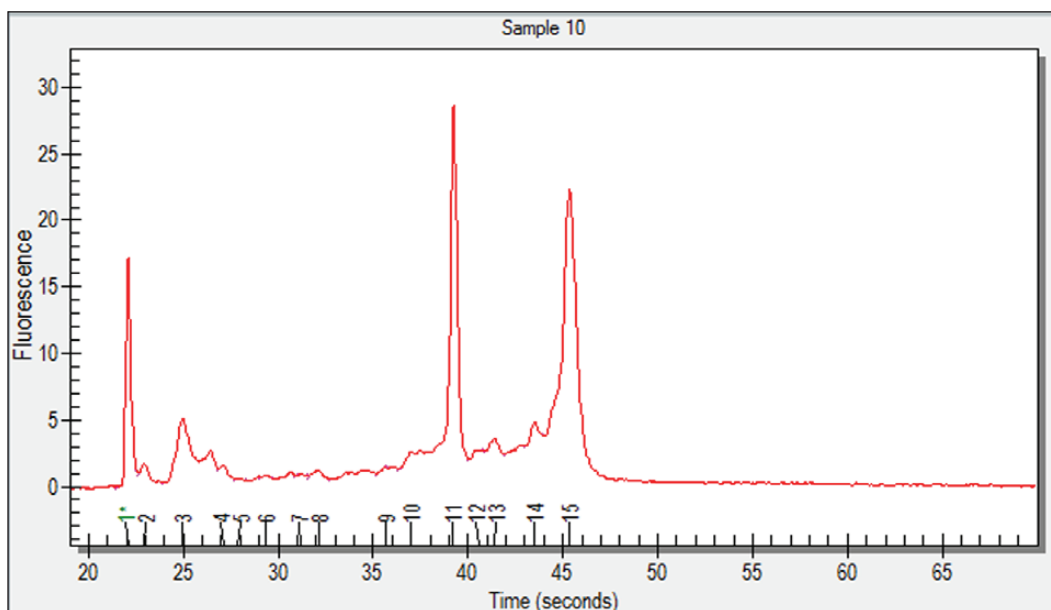
Experion software plots fluorescence intensity vs. migration time to generate an electropherogram for each sample.

Place the cursor over a peak or area of an electropherogram to view its calculated size (Figure 4.3).



**Fig. 4.3. Example of an electropherogram from an RNA StdSens analysis of a total RNA sample.**  
Electropherogram displayed with the Results tab selected. Place the cursor over a peak to reveal its size.

To view the peaks identified by Experion software, click **Peaks** in the results and settings tabs area. In the electropherogram, all peaks identified by Experion software become numbered, and the numbers appear under the peaks (Figure 4.4). Peaks generated by the lower marker used for normalization are numbered and labeled in green with an asterisk (\*).



**Fig. 4.4.** Example of an electropherogram from an RNA StdSens analysis of a total RNA sample. Electropherogram displayed with the **Peaks** tab selected.


Double- or right-click on a peak to select it. An inverted black arrow appears above the peak in the electropherogram, and a pink arrow appears above the corresponding band in the virtual gel. The peak number and corresponding data also appear highlighted in the Peaks tab.

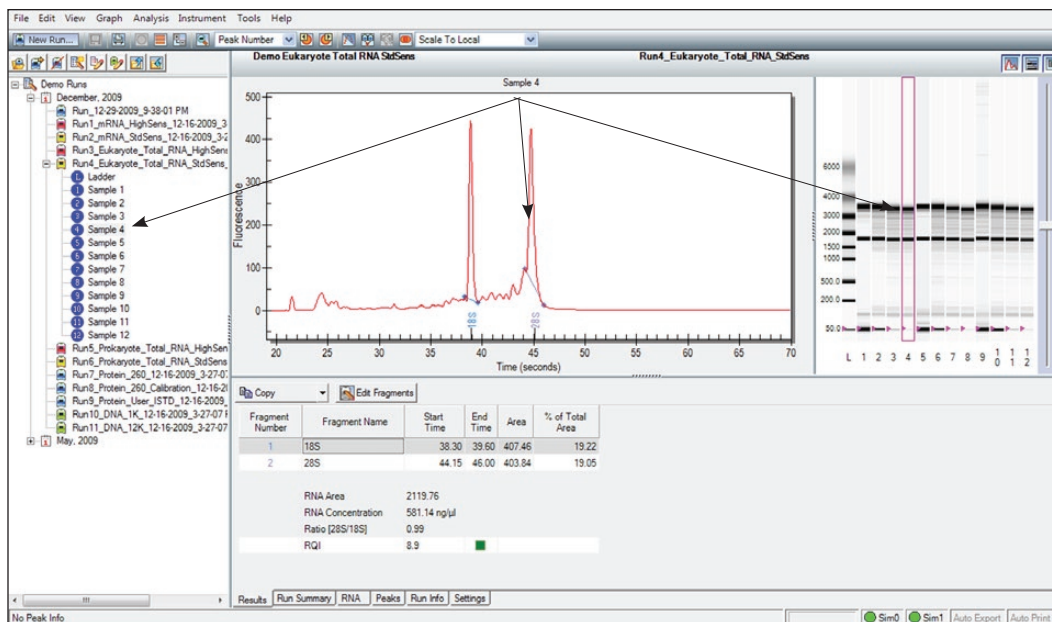
Display electropherograms in three different ways:

- One at a time (single-well view)
- All at once in separate windows (all-wells view)
- As superimposed images (overlays)

#### Single-Well View

This is the default view, which shows the electropherogram data from a single well (Figure 4.5). To return to this display:

1. Click on the sample in the project tree or on the corresponding lane in the virtual gel.
2. In the main screen toolbar, click **View Single Well**  or select **View > Single Well**.
3. The electropherogram appears. The corresponding lane in the virtual gel is outlined in pink.

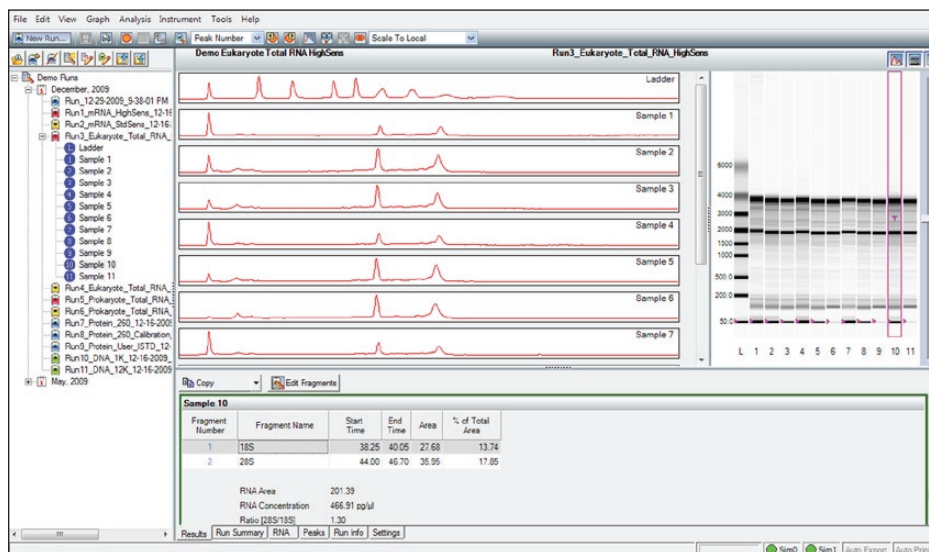


**Fig. 4.5. Single-well view.** As indicated by the arrows, a single electropherogram appears, the name of the sample is highlighted in the project tree, and the corresponding lane in the virtual gel is outlined.

### All-Wells View

To display all electropherograms simultaneously (Figure 4.6):



1. In the main screen toolbar, click **View All Wells** or select **View > All Wells**.
2. Electropherograms for each sample populate the electropherogram view (Figure 4.6). Use the scroll bar to scroll through the electropherograms, or minimize the results and settings table to accommodate the multiple electropherograms.
3. To select and expand a particular electropherogram, double-click on it. The data appear in a single-well view.



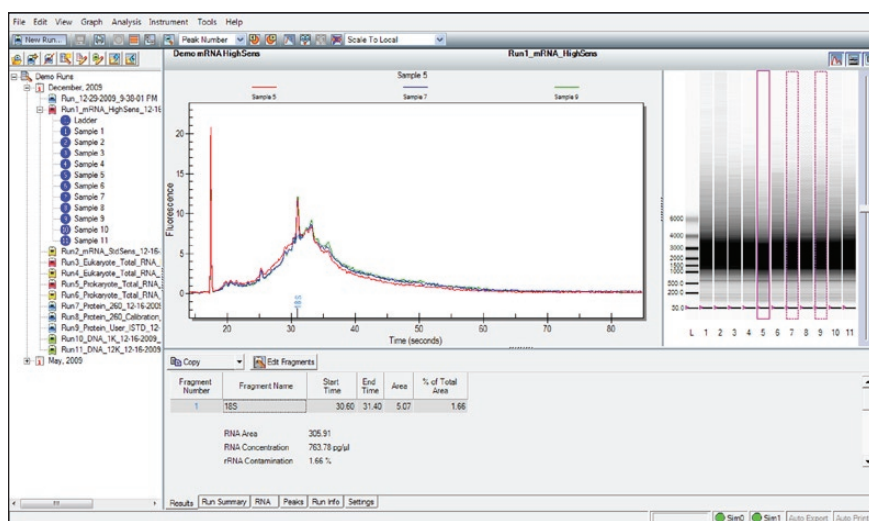
**Fig. 4.6. All-wells view.** All electropherograms are shown in the electropherogram view. Use the scroll bar to view electropherograms.

## Electropherogram Overlays

Superimpose (overlay) multiple electropherograms to facilitate direct comparison among profiles. To overlay a subset of electropherograms (Figure 4.7), use one of the following options:

- Move into the single-well view and, in the main screen toolbar, click **Start Overlay** . Select wells (lanes) in the virtual gel. Each gel lane that is displayed in the electropherogram is outlined. To end the overlay, click **End Overlay** .
- Select the lanes in the virtual gel while holding the **Ctrl** key (for noncontiguous, individual lanes) or the **Shift** key (for lanes next to the original lane selected). To exit, click on a lane in the virtual gel or on a sample name in the project tree
- Select **Analysis > Start Overlay**. Select the lanes to overlay. To end the overlay, select **Analysis > End Overlay**

Electropherograms appear superimposed in a single window, each in a different color. (The colors cannot be changed.) The sample name appears at the top of the electropherogram, in a color that corresponds to the trace. The fluorescence scale of the overlay electropherogram is based on the sample that is selected first; therefore, select the sample with the highest peaks first.



**Fig. 4.7. Electropherogram overlay.** Electropherograms are shown in different colors, and the corresponding lanes in the virtual gel are outlined. In this example, lanes 5, 7, and 9 are superimposed using the overlay feature.

## Peak Labels

To change the type of label displayed, select **Graph > Peak Info** (or use the pull-down list in the main screen toolbar) and select one of the following options:

- **No Peak Info** — displays no peak information
- **Peak Number** — uses sequential numbers for peak identification (default selection)
- **Peak Time** — uses peak migration time (min:sec) for peak labels
- **Peak Height** — uses peak height in units of fluorescence intensity for peak labels
- **Peak Cor. Area** — uses calculated (corrected) peak area for peak labels

## Electropherogram Tags (Annotations)

To annotate a peak in the electropherogram, right-click on the peak and select **Add Tag** (Figure 4.8). A tag is automatically generated with the peak information selected (for example, peak number).

To edit a tag, right-click on it, select **Edit Tag**, and change the information. Click **OK**.

To annotate a region in the electropherogram, right-click in the region and select **Add Tag**. In the **Edit Tag** dialog, enter the annotation and click **OK**.

To move a tag, click and drag the label to the desired place in the electropherogram.

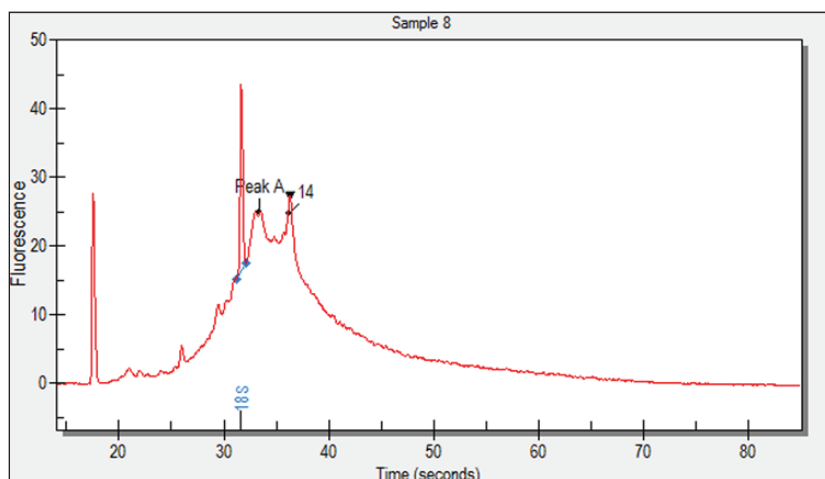


Fig. 4.8. Tagged (annotated) peaks in an electropherogram.

#### 4.1.4 Gel View

Experion software converts electropherogram data into densitometric bands, which appear in the virtual gel (Figure 4.9). Each lane of the virtual gel corresponds to a different sample, and all samples from a chip are shown in a single gel view. When a sample is selected for view as an electropherogram, the corresponding lane in the virtual gel is outlined in pink.

Right-click on a band to select it. A pink arrow appears above the selected band in the virtual gel, and an inverted black arrow appears above the corresponding peak in the electropherogram. The peak number and corresponding data also appear highlighted in the Peaks table. Only one peak can be selected at a time.

To select a different color scheme, choose **View > Gel Color** and select a color from the list. By default, the gel view displays bands as black signals on a white background.

To change the contrast of the bands, use the sliding cursor (Figure 4.9). Changing the contrast does not change the data, but it may improve visualization of faint bands in the virtual gel.

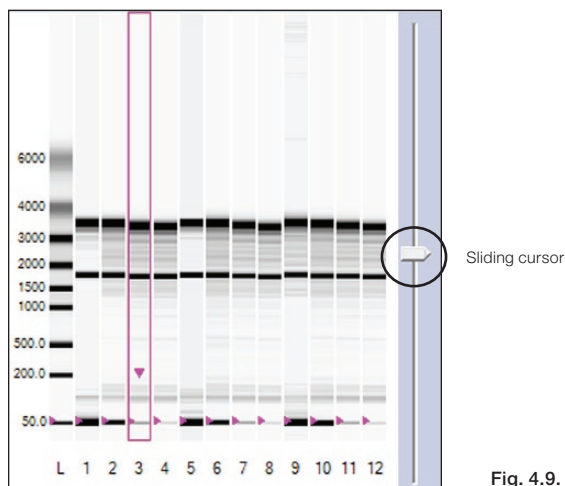
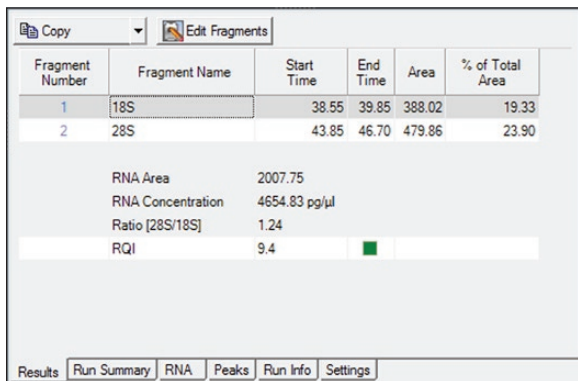


Fig. 4.9. Gel view. Move the sliding cursor to adjust band intensity.

#### 4.1.5 Results and Settings

The six results and settings tabs (Figure 4.10) present options for viewing, customizing, and analyzing separation data (see Section 4.3, Using Results and Settings to View and Annotate Data).

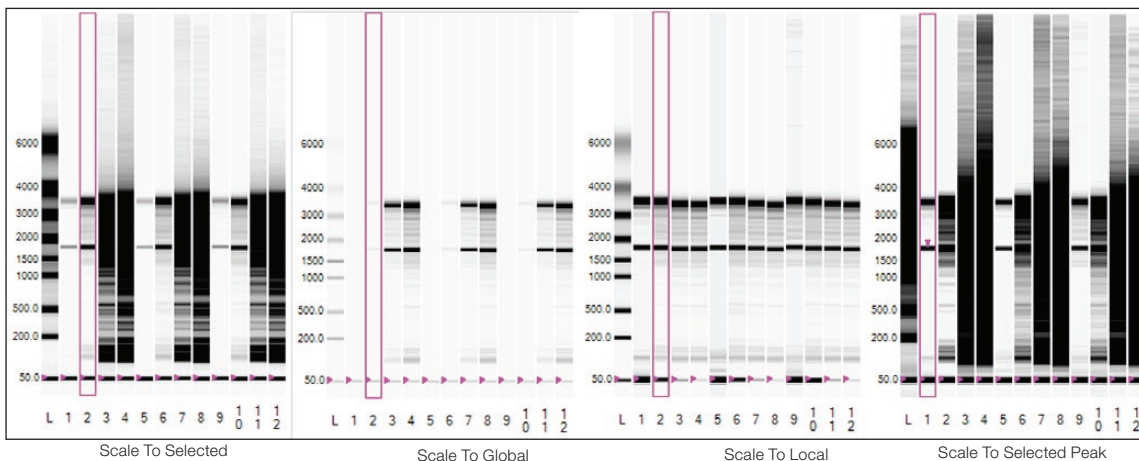


**Fig. 4.10. Results and settings.** Shown is the Results tab for a single sample.

## 4.2 Changing the Fluorescence Intensity Scale

To display more or less detail in the electropherogram traces and lanes in the virtual gel, use the options available under the **Graph** menu. These options determine the y-axis scale (fluorescence intensity) of electropherograms and define the relative scale intensity of the lanes in the virtual gel (Figure 4.11):

- **Scale To Selected** — uses the fluorescence value of the tallest peak in a selected electropherogram to set the scale of the y-axis for all samples. Use this option when comparing (overlying) a well with a high-intensity peak and one with a less intense peak
- **Scale To Global** — uses the fluorescence value of the tallest peak from all the electropherograms to set the scale of the y-axis for all samples. Use this option to create a virtual gel similar to an agarose gel
- **Scale To Local** (default) — uses the fluorescence value of the tallest peak in each electropherogram to set the scale of the y-axis for that electropherogram: each sample is scaled separately. This is the best way to view electropherograms and sample details
- **Scale To Selected Peak** — uses the fluorescence value of the selected peak to set the scale of the y-axis for all samples





**Fig. 4.11. Options for modifying the fluorescence intensity scale.** Examples of how each option affects the display of a virtual gel are shown.




## 4.3 Using Results and Settings to View and Annotate Data

The following options appear under the results and settings tabs:

- **Results** — summarizes analysis data for a single sample. For total RNA, results include peak or fragment name, migration time, area, % of total area, concentration, 28S:18S ratio for the sample, and the RNA quality indicator (RQI). For mRNA, they include an estimation of rRNA contamination and do not include the RQI or 28S:18S ratio. The data in this table can be exported (see Section 4.5.2, Exporting Data Files to Other Applications)  
  
To view the results of a particular sample, click the electropherogram in the all-wells view or lane in the gel view, or select that sample from the project tree. Alternatively, use the arrows in the main screen toolbar   to navigate through the samples
- **Run Summary** — displays a run summary for all samples (wells), including data for each sample name, RNA area and concentration, 28S:18S ratio and RQI information (total RNA) samples, and rRNA contamination (mRNA); right-click to export the data
- **RNA** — summarizes all RNA fragments identified in all samples and categorizes them by their identity (for example, 18S; see below for instructions on how to add fragments for analysis)
- **Peaks** — identifies and provides integration information for peaks in a sample. When you click on the data row for a peak, the row becomes highlighted, an inverted black arrow appears above the corresponding peak in the electropherogram, and a pink arrow appears above the selected band in the virtual gel. To customize the types of data shown, see the instructions below
- **Run Info** — displays sample names and comments; information can be added before, during, or after a run (see below for instructions)
- **Settings** — used mostly for troubleshooting but can be used to edit the parameters used to identify peaks (see Section 6.2, Changing Peak Finding Parameters or the Experion software Help file for more details about these settings)

Experion software automatically detects and names rRNA fragments. To add a fragment to the analysis:

1. Open the **Results** tab and either click **Edit Fragments** or right-click on one of the fragments in the table.
2. In the **Edit Fragments** dialog, click **Add a RNA name to table** . This opens a new row.
3. Enter the information for the RNA fragment and click **OK**.

To customize the types of data shown under the **Peaks** tab:

1. Click the arrow to the left of a column header (or right-click on a column header) to open the **Column Selector** dialog.
2. Use the arrow keys to show, hide, or change the order of display of any of the available headers. For detailed explanations of each setting, refer to the software Help file.
3. Click **OK** to save the changes, or click **Cancel** to close the dialog without saving the changes.

To enter sample names and descriptions:

1. Click the **Run Info** tab and then click on the plus sign (+) next to **Sample Information**.
2. Click on any active cell, enter the sample name and description, and click **Apply**. Click **File/Save** to save the changes. The sample name appears both in the tree view and above the electropherogram.


To enter other information about the experiment:

1. In the **Run Info** tab, click the plus sign (+) next to the **Study Information** and/or **System Information** area(s).
2. Double-click in any blank field, enter the information, and click **Apply**. Click **File/Save**.

## 4.4 Comparing Data from Different Runs


Experion software allows you to overlay runs from different chips (same assay type), enabling direct comparison of electropherograms and virtual gels from multiple chips. You can save and edit this new virtual chip, but you will not be able to make any new calculations. All calculations in the Results tab carry over from the original runs.

In security mode, all run comparisons are saved and cannot be deleted.

1. Click **Create a New Compare Run**  or select **Analysis > New Compare Run**. In the **Compare Runs Setting** dialog, enter a name for the comparison in the **Compare Name** field.
2. Select the project in which you wish to store the comparison from the **Target Project** pull-down list.
3. Select the assay from the **Assay Type** pull-down list and click **Next**. The **Compare Runs** dialog opens, displaying only runs of the same assay type.
4. Double-click to expand the run files you want to compare, or select a run file and click **List Samples**.
5. Click the run file or sample(s), and use the arrows to move them to and from the **Compare** field. To select several lanes at a time, click the sample names while holding the **Ctrl** or **Shift** key. To open a window displaying the selected samples in a gel view, click **Show Gel Lanes**.

Experion software accepts up to 40 samples for comparison (the default is 30). To change the number of wells used, select **Tools > Options**. Under the **Advanced** tab, change the number under **Visible Compare Runs Gel** lanes.

Only one ladder can be used per comparison. If a ladder is not selected, the ladder used in the first run file is used as the ladder for the entire virtual chip. If an additional ladder is inserted in the virtual chip, it is treated as a sample.

6. Select **Realign Data** to align the results of all the runs and click **OK**. The separations appear together in one window as a single experiment. In the Results table, the sizes and concentrations for fragments in each sample are unchanged; the original sizes and concentrations are shown.
7. To change the lanes used in the run comparison:
  - a. Open a run comparison by double-clicking the file name in the tree view and then clicking **Edit Compare Run**  or selecting **Analysis > Edit Compare Run**.



- b. Select the samples you want to add or delete, and use the arrows to add or remove them from the comparison.
  - c. To view the samples within a run, double-click the run file or select it and click **List Samples**.
8. Click **OK** to process the changes and view the results.

## 4.5 Saving, Exporting, and Printing Data

### 4.5.1 Saving Data Files

Data are automatically saved to a run file. Names for run files are given according to the selection made in the **Run Files** tab of the **Options** dialog.

To designate a new name for a file, select **File > Save As** and enter the desired name.

To view the selections or make changes in the automatic naming of files, select **Tools > Options > Run Files**. The following options appear:

- **Create Run name by combining** — select the options to be added to the file name. Options include the prefix (enter the desired characters), assay class (adds the assay type to the file name), instrument name, date, and time
- **Run file folder** — choose between default (saves files to the directory C:\Program Files\BioRad Laboratories\Experion Software\Data) and custom (enables navigation and selection of another folder)
- **Create daily folders** — select to enable creation of daily subfolders by date. When this option is selected, the software stores all run files from the same date in a single folder

### 4.5.2 Exporting Data Files to Other Applications

To export data to another program (for example, Excel software), select **File > Export Data**. In the **Export** dialog, select the desired options. For a complete description of all export options, refer to the Experion software Help file.

To export files automatically after a run is completed:

1. Select **Tools > Options** and click the **Advanced** tab.
2. Select **Auto Export** and then click **Settings**.
3. Select the options for **Auto Export** and click **Apply**.

To quickly copy and paste electropherograms, virtual gels or lanes in the gels, or data tables into another application, use the options under the **Edit** pull-down list.

### 4.5.3 Printing Data Files

You can print both graphic and tabulated data from a run.

To choose the print options, select **File > Print** and select the desired options in the **Print** dialog. For a complete description of all printing options, refer to the Experion software Help file (search term “printing data”).

To print files automatically after a run is completed:

1. Select **Tools > Options** and click the **Advanced** tab.
2. Select **Auto Print** and then click **Settings**.
3. Choose the desired options in the **Experion Print** dialog and click **Apply**.

To save or print the data as a PDF file, select **File > Print** and select **Print PDF**.



5

# Assessing RNA Quantity and Quality

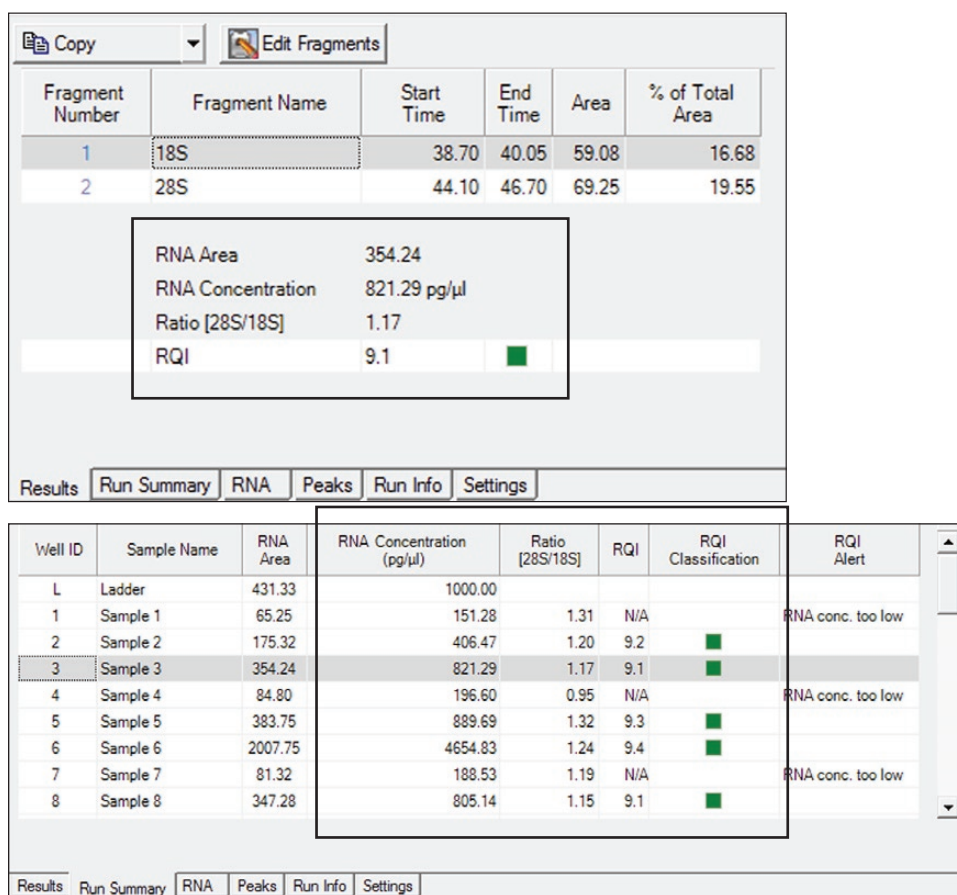
## 5.1 RNA Quantitation

Experion™ software begins RNA quantitation by comparing the time-corrected area (corrected area) under the electropherogram of a sample (excluding the lower marker) with the corrected area under the electropherogram of the RNA ladder. It then uses this value, the ladder concentration (160 ng/μl for the RNA StdSens assay and 1,000 pg/μl for the RNA HighSens assay), and a correction factor to determine the RNA concentration of the sample. Results are displayed under the Results and Run Summary tabs (Figure 5.1).

## 5.2 RNA Quality Indicator (RQI)

The RNA quality indicator (RQI) feature of Experion software allows you to estimate the level of degradation in a eukaryotic total RNA sample. The RQI algorithm compares the electropherogram of RNA samples to data from a series of standardized, degraded RNA samples and automatically returns a number between 10 (intact RNA) and 1 (degraded RNA) for each sample (Table 5.1). RQI analysis is performed for all samples with an Experion StdSens or HighSens analysis kit.

To view RQI data, select either the **Results** (selected sample) or **Run Summary** (all samples) tab (Figure 5.1).



**Fig. 5.1. Experion quantitation results.** RQI results appear with quantitation results under the Results (top) and Results Summary (bottom) tabs. Examples shown are for a total RNA Experion RNA HighSens analysis.

**Table 5.1. RQI values, indicators, and their interpretation.** These values can be customized.

RQI Value	Color Indicator	Meaning
7–10	Green	Acceptable quality
4–6	Yellow	Possibly acceptable quality
1–3	Red	Unacceptable quality

Experion software also issues RQI alerts when RQI calculations may not be reliable. When one of the following limitations or anomalies is detected, an alert is displayed in the Run Summary tab:

- RNA conc. too low — concentration of RNA is too low (<10 ng for the RNA StdSens assay and <200 pg for HighSens assay)
- Critical anomaly — RQI calculations cannot be performed, sample should be assayed again (if this error occurs in >1% of samples, contact Bio-Rad Technical Support)

To ensure the best results from RQI analysis, make sure that:

- Ladder peaks are identified correctly
- Lower marker is identified correctly
- 18S and 28S fragments are within normal time windows (this may require that you edit a fragment)

For more information about how the RQI is calculated and its comparison to the RNA integrity number (RIN), see bulletin 5761.

For information about RNA degradation and its effect on data quality, see bulletins 5893, 5452, and 5315.

RQI color indicators and value assignments can be customized. The default values are approximations. Acceptable RNA quality varies depending on the application. Validate your RQI values for your application and set the ranges accordingly.





# Changing Analysis Settings and Parameters

Once an Experion™ analysis is complete and you have examined the quality of the run and of the data, you can reanalyze the data by changing some of the settings. Most of the steps outlined in this chapter are used for troubleshooting purposes or are optional; they are not required in most analyses.

## 6.1 Manually Setting a Marker

To designate a peak as a marker (and change the alignment of a sample), right-click on the peak in either the electropherogram (single-well view) or the **Peaks** tab and select **Manually Set Lower Marker** to define the peak as the new lower marker. Do this if no or incorrect peaks have been selected as the lower marker.

Pink triangles in the virtual gel indicate markers. The markers should be aligned across all lanes.

## 6.2 Changing Peak Finding Parameters

Peak find parameters tell the peak-find algorithm whether a peak is significant.

To edit the parameters used by Experion software for finding peaks in a selected sample/well:

1. Select the sample by clicking on the lane in the virtual gel (or on the sample name in the tree view).
2. Under the **Settings** tab, edit the parameters under **Peak Find Settings**:
  - **Min. Peak Height** — minimum height required for a peak to be integrated (accepted values are 0.1–1,000). Determine the appropriate value for this parameter by zooming in on a small peak in an electropherogram and reading its fluorescence value on the y-axis
  - **Min. Peak Width** — minimum amount of time that must elapse before a peak is recognized (accepted values are 0.1–10, with lower values yielding more peaks)
  - **Slope Threshold** — represents the variation in fluorescence units over time required to detect a peak (accepted values are 0.01–10, with lower values yielding more peaks)

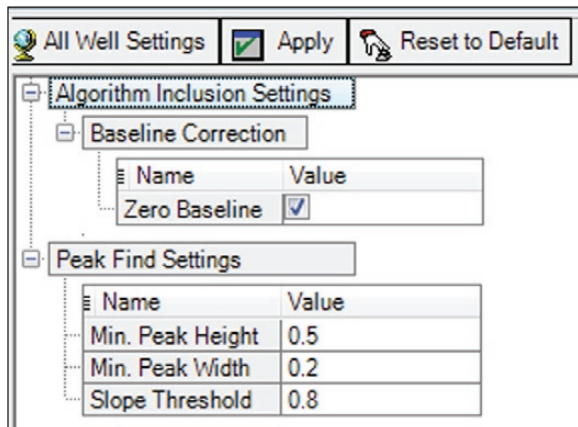


Fig. 6.1. Peak Find Settings options.

3. Click **Apply** to apply the changes and reanalyze the data. Click **Save** in the main screen toolbar to save the new conditions or click **Reset to Default** and **Apply** to recover the default settings.

To modify the peak-find settings for all sample wells:

1. Under the **Settings** tab, click **All Well Settings** or select **Analysis > All Wells Settings**.
2. In the **All Wells Settings** dialog, expand **Peak Find Settings** and select the type of modification you would like to apply (see above for descriptions).
3. Click **OK** to apply changes and **Yes** to overwrite the settings. Click **Save** in the main screen toolbar to save the new conditions. To reset to default settings, click **Reset to Default**.

Do not adjust the Peak Find Settings values so that the markers are eliminated from analysis.



## 6.3 Changing General Settings

These settings appear in the **Settings** tab, in the **All Wells Settings** dialog under **General Settings** (Figure 6.2). They apply to all samples in a run.

- **Data Frequency** — displays the rate of data collection in Hz; cannot be edited
- **Filter Width** — determines the width of the polynomial (in sec) to be applied to the data for filtering (noise reduction). Set this variable to less than twice the width of peaks (0.3–10)
- **Polynomial Order** — defines the power series applied to fit the raw data. Valid values are 1–10, with lower values yielding smoother curves

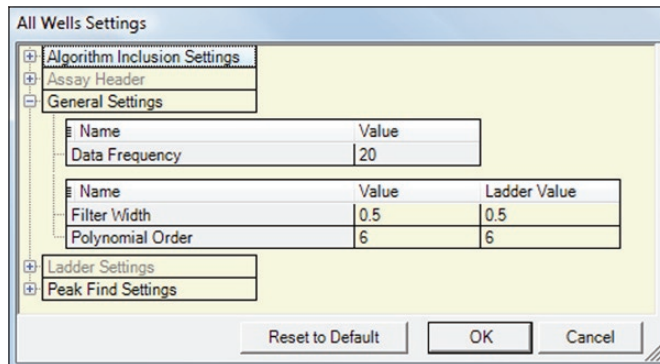


Fig. 6.2. General Settings options.

## 6.4 Baseline Modification

Experion software subtracts the average baseline value from the signal. This does not affect the shape of the curve; it shifts the baseline down to zero. The default settings for baseline modification are appropriate for most samples.

To shut off this baseline modification method for a selected sample:

1. Under the **Settings** tab, deselect the **Zero Baseline** option under **Baseline Correction**.
2. Click **Apply** to apply the changes and reanalyze the data, and click **Save** in the main screen toolbar to save the new conditions or choose **Reset to Default** and **Apply** to recover the default settings.

To edit the parameters used by Experion software for adjusting the baseline for all samples:

1. Either open the **Settings** tab or select **Analysis > All Wells Settings**.
2. In the **All Well Settings** dialog, expand **Algorithm Inclusion Settings** and deselect the **Zero Baseline** modification. Click **OK**.
3. In the **Experion Application** dialog, click **Yes** to save changes and reanalyze the data. Click **Save** in the main screen toolbar to save the new conditions.

To reset to default settings, click **Reset to Default** in the **Settings** tab under **All Well Settings** and then click **Save Run** in the main screen toolbar.

## 6.5 Turning Analysis Off

To turn analysis off, select **Analysis > Turn Analysis Off**. Analysis remains off for all runs until it is turned back on.

Turning analysis off displays the raw data from a run; it deactivates alignment, shifting the positions of the lower marker in each sample. In addition, the pink arrows (in the virtual gel) indicating the positions of the markers appear only in the ladder lane. With analysis off, the migration of the markers may not be perfectly aligned (Figure 6.3). Turning analysis off can help reveal if a problem is isolated (one or a few wells have the marker out of alignment) or is general (alignment problems across the entire chip).

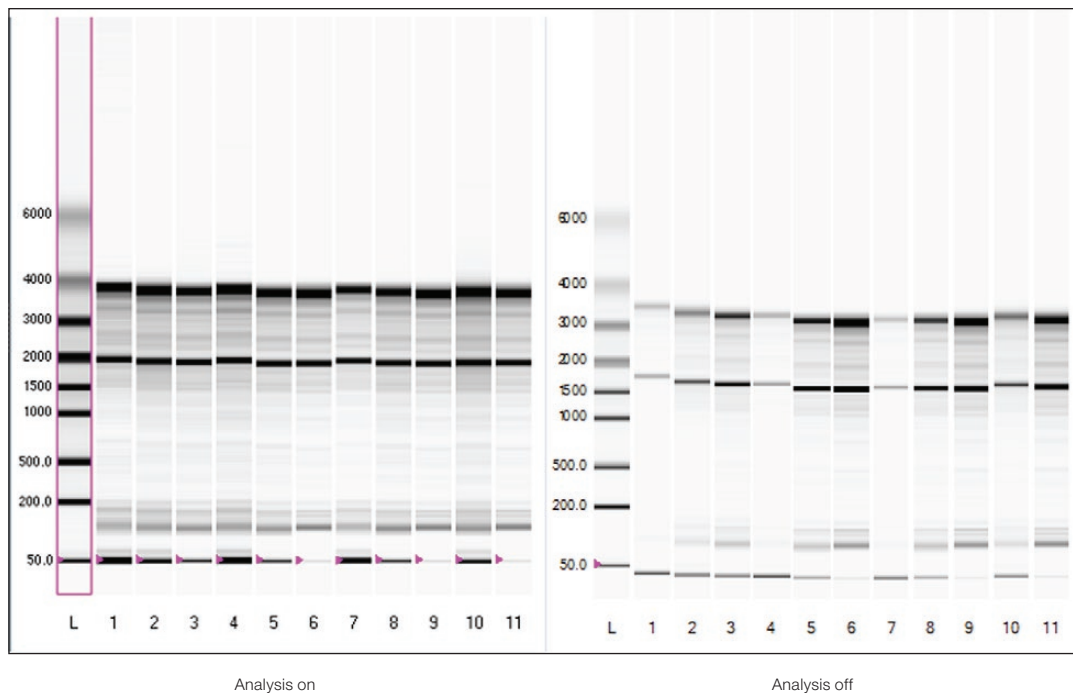




Fig. 6.3. Effects of turning analysis off.

## 6.6 Manual Peak Integration

This function enables definition or refinement of ladder peak areas.

1. Select a sample and click  or select **Analysis > Manual Integration** to start manual integration.
2. Adjust the baseline by clicking and dragging the dots at the ends of the peaks (Figure 6.4).  
If a peak in the electropherogram is not detected by the software, right-click on that peak and select **Add Peak**. Zoom in on the peak for a better view of the baseline.
3. Save any changes by selecting **File > Save Run As** (use a new file name), or revert to the original baseline and integration by clicking .

Manual integration applies to the ladder well (L). Moving back to automated integration removes changes made during manual integration unless those changes are saved. The baselines of sample rRNA peaks can be modified by repositioning the end points of the peak baseline, but this affects only rRNA ratios; concentrations will not be affected.

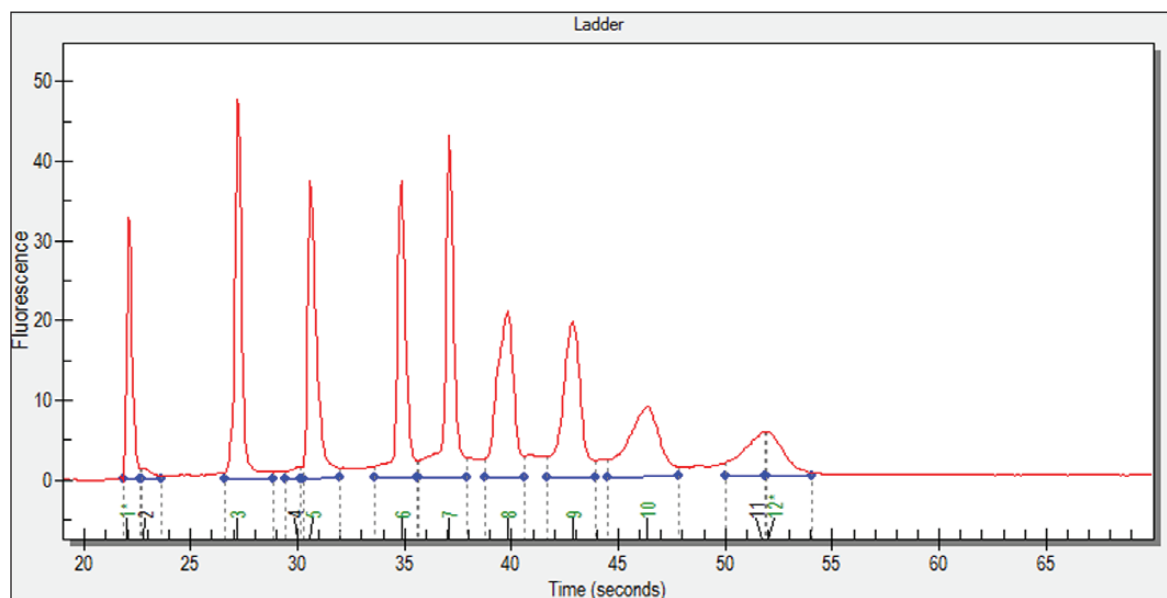


Fig. 6.4. Manual integration mode indicates the beginning and end of peak baselines with blue dots.



# 7 Troubleshooting

## 7.1 Electrophoresis and Priming Stations

Error	Probable Cause	Recommended Action
<b>IV Check Failure</b> (Experion™ software)	One or more wells are not filled correctly, and the electrodes are not immersed	Fill all sample wells of the chip with blank or replicate samples Ensure pipets are properly calibrated
	Air bubbles are interfering with the electrical contact in one or more of the wells	Stop the run, remove the chip, and use a clean pipet tip to remove the sample and apply a new aliquot  When pipetting, insert the tip vertically and to the bottom of the well. Dispense the liquid slowly. Do not expel air at the end of the pipetting step. Dispense only to the first stop on the pipet. Positive (reverse) pipetting may also be used
	An electrode is loose or disconnected	Perform a conductivity test with the cleaning chip or conductivity chip from the Experion validation kit (catalog #700-7051). Add 850 µl water to the chip, place the chip in the electrophoresis station, and close the lid. In Experion software, select <b>Tools &gt; Diagnose Instrument</b> and select <b>Conductivity</b> in the <b>Instrument Diagnostics</b> dialog. If the test passes, focus on the sources of error above. If it fails, contact Bio-Rad Technical Support with the results
	Lid is not closed	Close the lid
	Chip is not primed properly	Repeat the analysis using a new chip
<b>Check Seal</b> (Priming station)	Priming seal is worn, torn, or clogged	Replace the priming seal (catalog #700-7031)
	Chip is not positioned correctly in the priming station	Align the arrow on the chip with the arrow on the platform
	Gel-stain solution (GS) was added to the wrong well for priming	The priming well is highlighted on the chip. See Section 3.5, Prime the Chip
No chip detected	Chip not positioned properly, empty wells, or incorrect reagents	Make sure chip is positioned properly and correct reagents used
Run aborts before all wells are analyzed	Computer power saver options are causing runs to abort (this is a problem only with Experion software prior to version 3.0)	Upgrade to the newest software version  Confirm all power saver and/or power options are turned off before starting the assay. Do not let the computer “sleep” during a run. If the computer goes to sleep, the run will abort

## 7.2 Experion RNA Analysis

Error	Probable Cause	Recommended Action
Concentrations are not reproducible (CV > 20%)	Sample preparation procedures are inconsistent	Use the same reagents and follow the same procedures for each sample and each run. Do not modify the protocol outlined in Chapter 3 to compensate for RNA concentration or sample variability
Peaks were not detected in the RNA ladder	An air bubble is at the bottom of the ladder well or microchannels due to poor priming or sample loading; air bubbles can interfere with the contact between the electrode and the liquid	Check the chip for air bubbles after priming and sample loading: <ul style="list-style-type: none"> <li>■ After priming: turn the chip over and make sure the microchannels are filled with gel-stain solution (GS). If the chip is primed correctly, the wells should be hard to see. Compare the primed chip to an unprimed chip if necessary. Do not touch the glass</li> <li>■ After sample loading: tilt the chip and view it from different angles against a light background</li> </ul>

Error	Probable Cause	Recommended Action
No peaks detected in some samples	There is not enough sample in the wells	Ensure pipets are calibrated and that correct volumes have been added to the wells
	Samples were not prepared properly	Review the sample preparation procedure detailed in Chapter 3
	RNA stain was photobleached	Protect RNA stain and gel-stain solution (GS) from light Prepare new GS; use a new tube of RNA stain if necessary
	Particulates are clogging the microchannels	Use only high-quality 0.2 µm-filtered water (such as Experion DEPC-treated water, not autoclaved water) Verify that gel (G) and GS were properly filtered If using samples that contain particulates, perform a quick microcentrifuge spin of the prepared sample to pellet particulates before loading Remove solutions from the top of the liquid to prevent introducing particulates pelleted during centrifugation
No peaks detected in any samples	Gel-stain solution (GS) is old or photobleached	Prepare fresh GS
	Chip was primed with incorrect pressure and time settings	Check settings on the priming station and repeat analysis with a new chip
	Particulates are clogging microchannels	Use only high-quality, 0.2 µm-filtered water (such as Experion DEPC-treated water, not autoclaved water) Verify that gel (G) and GS were filtered properly If using samples that contain particulates, perform a quick microcentrifuge spin of the prepared sample to pellet particulates before loading Remove solutions from top of liquid to prevent introducing particulates pelleted during centrifugation
Lower marker is missing	Samples do not contain loading buffer or were not prepared properly	Review the sample preparation procedure in Chapter 3
Electropherogram peaks are much smaller than expected (peaks are <50% of what is expected)	Not enough sample was added	Ensure pipets are calibrated to accurately deliver ≤10 µl
	Too much salt in sample; salt ions compete with sample ions during injection	Ensure RNA is in buffer of low ionic strength (for RNA StdSens use TE or water; for RNA HighSens use water) Analyze a dilution series of sample in water on another chip. If peak heights and concentrations of diluted sample increase, salt is too high in the original sample. Determine proper dilution to minimize salt effect
	Gel-stain solution (GS) is old or photobleached	Prepare fresh GS
	Reagents, specifically RNA stain, were not brought to room temperature prior to use; if stain is not equilibrated to room temperature, it will be more concentrated when it is added to gel, which may result in lower peaks and areas	Repeat analysis with a new chip, new tube of stain, and properly equilibrated reagents
	Filtered gel (G) or gel-stain solution (GS) were mislabeled or used improperly. RNA chips contain wells for GS that carry sample through microchannels and supply dye for analysis	Ensure chip was primed with GS Ensure G was applied to well labeled G and GS was used in all wells marked GS

Error	Probable Cause	Recommended Action
Peaks are small, broad, or missing in some samples but present in other samples on the same chip	Contaminants are present	Prepare samples in a different buffer or dilute in water Do not use autoclaved water for diluting samples or ladder. Use only high-quality 0.2 µm-filtered water (such as Experion DEPC-treated water)
	RNA ladder and samples were not denatured completely	Heat samples and RNA ladder for 2 min at 70°C before loading Do not use RNA ladder that has been repeatedly heated and frozen
Late migration (peaks broaden and are delayed over the course of the run)	Air bubbles are interfering with electrical contact in one or more of the wells	Turn analysis off (see Section 6.5, Turning Analysis Off, for more information). Late migration is indicated if there is a drift in the migration of lower marker across the chip. Refer to the troubleshooting tips that follow Stop run, remove chip, and use a clean pipet tip to remove or dislodge the bubbles, or remove and replace the solution in affected wells When pipetting, insert the tip vertically and to bottom of the well. Dispense liquids slowly. Do not expel air at the end of the pipetting step. Dispense only to the first stop
Peaks migrating faster than usual; electropherogram appears compressed	Electrophoresis station temperature is inappropriate or fluctuating during run (should be 30–35°C); there is no cooling unit in the electrophoresis station, so if the temperature changes during the run, samples exhibit different separation characteristics	Ensure the temperature of the room is appropriate and stable, and place the electrophoresis station away from all heat sources, such as windows or ovens
Sizing is incorrect	RNA ladder peaks and lower markers were improperly assigned by the software	Manually assign lower marker (follow instructions in Section 6.1, Manually Setting a Marker), if necessary
Quantitation is incorrect	Lower marker was not properly assigned by the software	Check that lower marker was properly assigned. Manually select the marker, if necessary (follow instructions in Section 6.1, Manually Setting a Marker)
	Pipetting and/or dilution errors occurred during sample preparation or chip loading	Ensure calculations and dilutions are correct Ensure pipets are calibrated, and use pipets that accurately deliver volumes of ≤10 µl Do not modify the sample preparation and loading protocols described in this manual Ensure the total RNA concentration of samples is within the linear dynamic range; if total RNA concentration is unknown, analyze a sample dilution series Review the essential practices described in Chapter 2 before initiating another analysis with a new chip
	Samples are old or have not been stored properly and RNA degraded	Prepare fresh samples and try the analysis again
Ghost peaks or contaminants appear in electropherograms	Electrodes are contaminated	Perform the deep cleaning procedure described in Appendix B and software Help section (search term “electrodes”) Use only 0.2 µm-filtered water (such as Experion DEPC-treated water) for diluting samples and the RNA ladder; do not use autoclaved water
	RNA ladder degraded	Aliquot the RNA ladder for single use and store at –70°C



Error	Probable Cause	Recommended Action
Lower marker is identified incorrectly	Experion software selected incorrect peak to correspond to the marker; such an error affects alignment and sizing	Manually select the markers by following the instructions in Section 6.1
Virtual gel does not resemble an agarose gel separation of same samples	Gel view is a direct interpretation of electropherogram data. By default, fluorescence intensity of bands in each lane is scaled to intensity/peak height of highest peak in that sample (scale to local). Consequently, each gel lane may have a different scale of fluorescence intensity	Adjust scaling of the virtual gel to <b>Scale To Global</b> (see Section 4.2). This adjusts the scale on all electropherograms Adjust the intensity of the virtual gel using the sliding cursor
Baseline is fluctuating (for example, into very negative and positive values)	Air bubbles are interfering with electrical contact in one or more of the wells  Sample is in inappropriate sample buffer  Chip is not completely primed  Samples are too concentrated; RNA levels are out of range of assay	Stop the run, remove the chip, and remove and replace solution in affected wells  Ensure RNA is in buffer of low ionic strength (for Experion RNA StdSens use TE or water, for Experion RNA HighSens use water)  Prime and load a new chip  Dilute the samples and repeat the analysis
RNA ladder or samples appear degraded	RNase contamination of electrodes, reagents, or consumables    Improper storage	Perform the deep cleaning procedure described in Appendix B and software Help section (search term "electrodes")  Use only 0.2 µm-filtered water (such as Experion DEPC-treated water) for diluting samples and the RNA ladder  Use RNase-free consumables and RNase-free, filter pipet tips; change pipet tips with each use  Store RNA ladder and samples appropriately (for example, at -70°C)

## 7.3 Contacting Technical Support

If you are having problems with a run and would like the Bio-Rad Technical Support team to review your data, please submit the following files to **LSG\_TechServ\_US@bio-rad.com** for analysis:

- Run file (C:\Program Files\Bio-Rad Laboratories\Experion Software\Data\<daily folder name>\<run name>.bdf)
- Run log (C:\Program Files\Bio-Rad Laboratories\Experion Software\Data\<daily folder name>\<run name>.log)
- Packet file (C:\Program Files\Bio-Rad Laboratories\Experion Software\Data\<daily folder name>\Packet\<run name>.txt)
- System log (C:\Program Files\Bio-Rad Laboratories\Experion Software\Backup\Version X\Experion\_System.log or .txt)
- Lot numbers of the RNA chips and the Experion RNA analysis kit

To export the run file, log file, and packet file, select the run(s) in the tree view and click **Export Selected Runs**. The software exports the files into folders with the corresponding project name(s).





# Appendices

## Appendix A: How the Experion™ System Works

The Experion automated electrophoresis system performs electrophoresis of samples within a microfluidic chip. Within each chip, a series of microchannels connects the sample wells to a separation channel and buffer wells. A set of electrodes in the electrophoresis station applies a voltage across the microchannels, causing charged molecules in the samples to migrate into and through the separation channel. Samples are run sequentially, with a sufficient lag between them to prevent cross-contamination. For separation, the microchannels are filled with a proprietary gel-stain solution (GS) that acts as a sieving matrix; therefore, the sample RNA fragments migrate through the separation channel at a rate based on their size and charge<sup>1</sup>. Finally, the fragments interact with a fluorescent dye during separation and are detected as they pass a laser and photodiode detector (laser-induced fluorescence).

RNA analysis is accomplished with the Experion RNA StdSens and HighSens analysis kits, which perform analyses based on the following sample types:

- mRNA
- Total RNA — eukaryotic
- Total RNA — prokaryotic

Overall, RNA analysis with the Experion system involves the following steps:

- Preparing the chip (priming and loading) — Priming fills the microchannels of the microfluidic chip with GS, which contains both the sieving matrix and fluorescent dye. RNA samples and loading buffer are then added
- Running the chip — The chip is inserted into the electrophoresis station, and as the instrument lid is closed, electrodes come into contact with the solution in the wells. Voltage is applied to the sample wells of the chip, causing the charged RNA fragments to migrate into the separation channel. In the separation channel, the different fragments separate as they move at different rates through the gel matrix, depending on their size. During separation, the fluorescent dye intercalates between the bases of the fragments
- Detecting the fragments — As the fragments migrate toward the end of the separation channel, a laser excites the dye, causing it to fluoresce if it is bound to RNA. A photodiode detects the fluorescence, and Experion software plots the fluorescence intensity vs. time to produce an electropherogram and a virtual gel image
- Analyzing the data — Following separation, Experion software subtracts background noise, removes spikes, identifies and integrates peaks, and assigns their sizes, concentrations, and quality (RNA quality indicator, RQI, value for eukaryotic total RNA). Following analysis, parameters may be changed and the data reanalyzed

### How Experion Software Analyzes RNA Fragments

#### Data Presentation

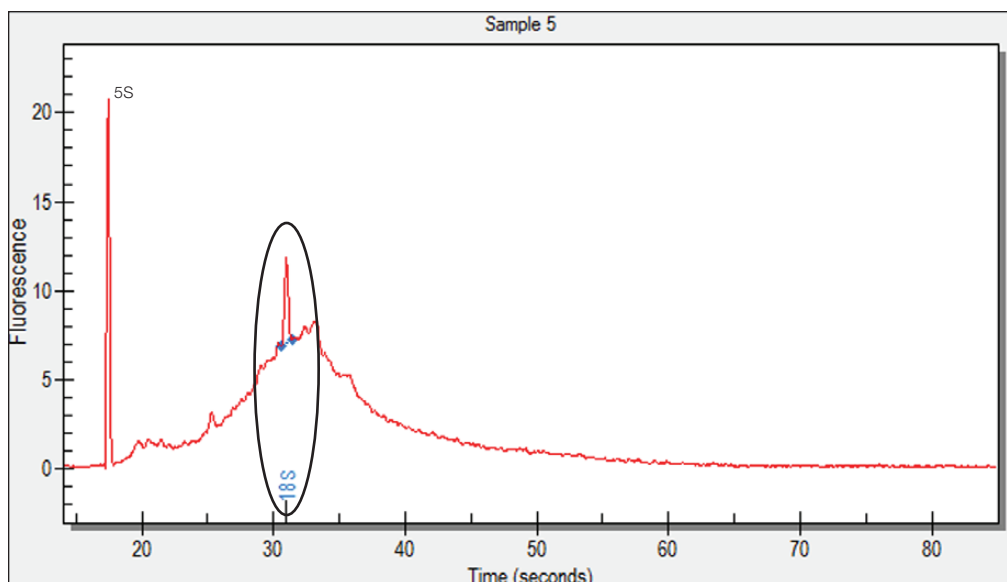
As the photodiode detects fluorescent signals from the dye-RNA complexes, Experion software converts the signal into an electropherogram (a plot of fluorescence vs. time). Experion electropherograms generally have the following features (Figure A.1):

- Sample peaks — signals generated by the sample fragments
- Lower marker — signal generated by the lower marker, which is included in the RNA loading buffer to normalize the separation of fragments across all wells in a chip

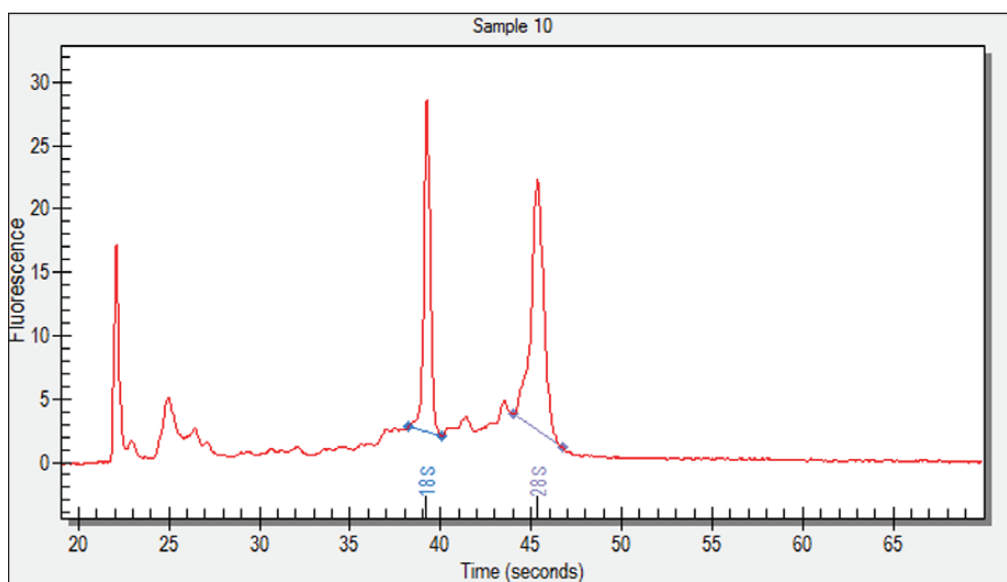
<sup>1</sup> RNA samples are heat-denatured to minimize the effects of shape or structure on migration.

The appearance of the electropherogram depends on the type of RNA assay performed:

- mRNA analyses often yield broad peaks or a smear and, on occasion, contaminating 18S and 28S rRNA peaks (Figure A.1). In addition, mRNA samples require more time for analysis than total RNA samples to ensure inclusion of the population of longer transcripts and to achieve a smooth baseline
- Total RNA analyses yield 2–3 prominent peaks corresponding to rRNA (18S and 28S and occasionally 5S in eukaryotic sources, 16S and 23S in prokaryotic sources, Figure A.2)

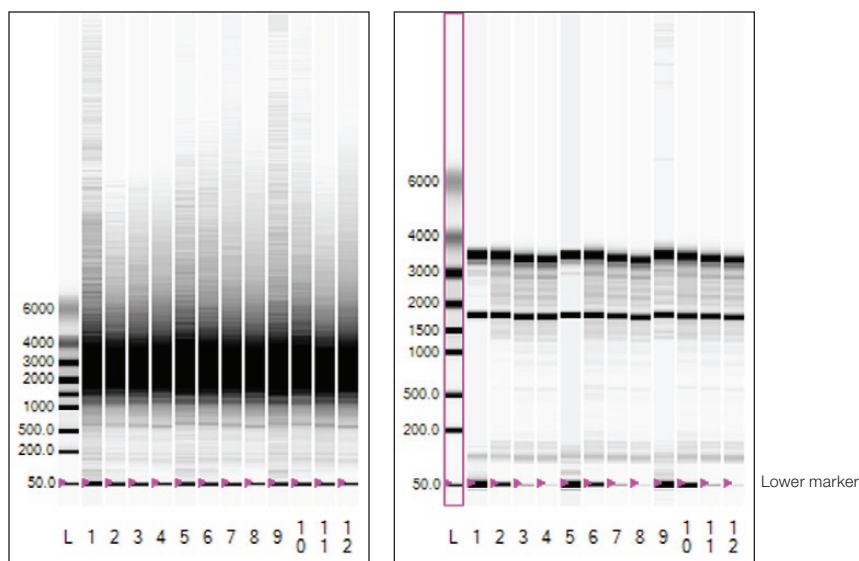


**Fig. A.1.** Electropherogram generated by an Experion RNA HighSens analysis of mRNA. The 18S rRNA peak is identified by Experion software.



**Fig. A.2.** Electropherogram generated by an Experion RNA HighSens analysis of eukaryotic total RNA. The 18S and 28S rRNA peaks are identified by Experion software.

Once separation occurs, the data are converted into a densitometric, gel-like image, or virtual gel (Figure A.3). Each lane in the virtual gel corresponds to a different sample. The sample peaks and lower marker seen in the electropherogram also appear in the virtual gel.



**Fig. A.3. Virtual gel generated by Experion RNA StdSens analysis of mRNA (left) and eukaryotic total RNA (right).** Shown are separations of the RNA ladder (L) and 12 samples on a single chip. Pink triangles mark the position of the lower marker.

Once separation is complete, the software subtracts background noise, removes spikes, identifies and integrates peaks, and assigns their sizes and concentrations. The results of data analysis are tabulated and presented in a Results table at the end of analysis (Figures A.4 and A.5). The types of data available include the following:

- Concentration of mRNA or total RNA (in ng/μl or pg/μl, depending on the assay)
- rRNA contamination (mRNA assays only)
- 28S/18S ratio (eukaryotic total RNA assays) or 23S/16S ratio (prokaryotic total RNA assays)
- RNA quality indicator (RQI, eukaryotic total RNA assays)

Copy		Edit Fragments			
Fragment Number	Fragment Name	Start Time	End Time	Area	% of Total Area
1	18S	38.70	40.05	59.08	16.68
2	28S	44.10	46.70	69.25	19.55
RNA Area		354.24			
RNA Concentration		821.29 pg/μl			
Ratio [28S/18S]		1.17			
RQI		9.1			
Results Run Summary RNA Peaks Run Info Settings					

**Fig. A.4. Results tab generated by Experion RNA StdSens analysis of eukaryotic total RNA.** Shown is the Results tab for a single sample.

## Data Analysis: Normalization, Sizing, Quantitation, and Quality Determination (RQI)

Following separation, Experion software analyzes RNA fragments using one or more of the following:

- Internal 50 bp lower marker for normalizing the migration times of samples in different wells
- RNA ladder for determining fragment size (sizing) and concentration (quantitation)
- RNA quality indicator (RQI, eukaryotic total RNA analysis only) to quantitate the state of degradation of a sample

### Normalization: Aligning Peak Migration Times

To compensate for small variations in each separation, Experion RNA analysis uses an internal 50 bp marker to normalize the migration times among samples. The marker is included in the Experion RNA loading buffer and so is added to each sample and the RNA ladder (Figures A.1–A.3). Inclusion of the marker and the normalization process ensures that the system software properly identifies peaks.

### RNA Sizing: Determining Peak Sizes

The first sample to be analyzed is the RNA ladder, which contains 8 RNA fragments of 200–6,000 nt. Experion software constructs a standard curve of migration time as a function of fragment size from the RNA ladder separation. It then calculates the size of the RNA fragments from the sample wells by comparing their migration times to the standard curve.

### Quantitation: Determining RNA Amount

Experion software begins RNA quantitation by comparing the time-corrected area (corrected area) under the electropherogram of a sample (excluding the lower marker) with the corrected area under the electropherogram of the RNA ladder. It then uses the corrected area, the known ladder concentration (160 ng/μl for the Experion RNA StdSens assay and 1,000 pg/μl for the Experion RNA HighSens assay), and a correction factor to determine the RNA concentration of the sample. Results are displayed under the Results and Run Summary tabs (Figures A.4 and A.5).

### Quality Determination (RQI)

The RQI algorithm compares the electropherogram of RNA samples to data from a series of standardized, degraded RNA samples and automatically returns a number between 10 (intact RNA) and 1 (degraded RNA) for each sample (Figures A.4 and A.5 and Table 5.1). RQI analysis is performed for samples with an Experion StdSens or HighSens analysis kit. The RQI feature of Experion software allows you to quantitate the level of degradation in a eukaryotic total RNA sample. Refer to bulletin 5761 for more details about RQI.

Well ID	Sample Name	RNA Area	RNA Concentration (pg/μl)	Ratio [28S/18S]	RQI	RQI Classification	RQI Alert
L	Ladder	431.33	1000.00				
1	Sample 1	65.25	151.28	1.31	N/A		RNA conc. too low
2	Sample 2	175.32	406.47	1.20	9.2	■	
3	Sample 3	354.24	821.29	1.17	9.1	■	
4	Sample 4	84.80	196.60	0.95	N/A		RNA conc. too low
5	Sample 5	383.75	889.69	1.32	9.3	■	
6	Sample 6	2007.75	4654.83	1.24	9.4	■	
7	Sample 7	81.32	188.53	1.19	N/A		RNA conc. too low
8	Sample 8	347.28	805.14	1.15	9.1	■	

Results
Run Summary
RNA
Peaks
Run Info
Settings

**Fig. A.5. Run Summary tab.** RQI results appear with quantitation results under the Results (Figure A.4) and Results Summary tabs. Example shown is for a total RNA Experion RNA HighSens analysis.

### How Experion Analysis Differs from Gel-Based Methods

Though the Experion RNA analysis kits and other gel-based electrophoresis methods both use a gel matrix and voltage differences to separate RNA fragments according to their charge:mass ratio, there are a number of fundamental differences between the two methods that may affect how specific fragments are separated and how data are interpreted (Table A.1).

**Table A.1. Comparison of Experion RNA analysis and agarose gel electrophoresis.**

Aspect	Agarose Gel Electrophoresis	Experion RNA Analysis
Sieving polymer (matrix)	Formulation can be changed to affect resolution	Fixed formulation
	Agarose	Polymer (not crosslinked)
RNA stain	Multiple available	Single, proprietary stain
	Intercalates RNA fragments	Intercalates RNA fragments
Detection	Separate staining step; fragments stained after separation	Integrated separation step; fragments stained during separation
	Separate destaining step	Destaining not required
	Requires photography or other imaging equipment for documentation, analysis	Laser excites the stain, detection using a photodiode, both incorporated
Types of separation	Native and partially to completely denaturing	Partially denaturing
Downstream applications	Separated fragments available for excision, electroelution, blotting applications	Fragments not available for further analysis
Reagent and sample usage	Moderate: ng to µg sample, ml to L reagents	Minimal: pg to µg sample, µl reagents
Waste generation	Moderate to extensive (ml to L)	Minimal (µl)
Overall analysis time	Hours (depends on gel size, staining method, and methods of documentation and analysis)	30 min/chip (11 or 12 samples per chip)
Data analysis	Manual; requires additional equipment and software	Automated (with flexibility of manual adjustments)



## Appendix B: Deep Cleaning Procedure

This manual procedure applies Experion™ electrode cleaner directly to the 16 platinum pins in the electrode manifold. It is the most stringent method of cleaning the Experion electrode pins and helps ensure RNase-free operation of the system.

The need for this deep cleaning will vary from lab to lab and depends on the frequency of use of the Experion system and on the types of assays (protein, RNA, or DNA analysis) that are performed.

Perform deep cleaning:

- Prior to first use of the Experion electrophoresis station
- Between a protein and DNA or RNA analysis (an Experion electrophoresis station used to analyze protein samples will become contaminated with RNases and should be cleaned prior to any RNA analysis), or between a DNA and RNA analysis
- Whenever RNase contamination is suspected or other contamination (for example, salt deposits or other precipitates) is visible on the electrodes
- Whenever a chip has been left in the electrophoresis station for an extended period of time (for example, overnight)

Perform this procedure with the electrode manifold in place (installed in the electrophoresis station).

It requires the following supplies:

- Experion electrode cleaner (catalog #700-7252)
- Experion DEPC-treated water (catalog #700-7253)
- Foam cleaning swabs (catalog #700-7264)

**Warning:** Keep the chip platform dry during this procedure. Either cover the chip platform using plastic wrap and a paper towel or foil, or place a cleaning chip on the chip platform.

1. Shut off power to the electrophoresis station.
2. Add 0.5–1 ml Experion electrode cleaner to a microcentrifuge tube. Insert a swab into the solution until it is saturated with the electrode cleaner.
3. Use the swab to gently scrub each electrode pin one at a time, on all four sides. Do not press too hard on the pins, as they may bend. Finally, clean the tip of each pin. Add more electrode cleaner to the swab as necessary.

-Or-

Move the swab up and down, and side to side, 2–3 times along the columns and rows of pins. Finally, clean the tip of each of pin. Add more electrode cleaner to the swab as necessary.

4. Repeat steps 2 and 3 twice, each time with a fresh swab soaked in DEPC-treated water.
5. Fill a cleaning chip with 800 µl DEPC-treated water, place it in the electrophoresis station, and close the lid for 5 min.
6. Remove the chip and allow the pins to dry completely by leaving the lid open for 5–10 min.

Refer to the Experion system manual for more details and images.

## Appendix C: Glossary

### All-wells view

Mode of display within the electropherogram view wherein electropherograms of all wells are shown

### Baseline

Line of initial data that serves as the control, or signal representing no fluorescence

### Chip

Microfluidic device used to perform separation using the Experion system

### Electropherogram

Trace recording of signal (fluorescence intensity) as a function of time; displayed in the electropherogram view

### Electropherogram view

Portion of the Experion software user interface dedicated to display of electropherogram data

### Electrophoresis station

Instrument that houses all the electrical, optical, and hardware components necessary to perform separation, staining, destaining, band detection, and imaging of samples; equipped with a universal power supply to operate at 100–240 V, 50–60 Hz

### Gel priming well

Well of the microfluidics chip used to fill the microchannels of the chip with gel-stain solution (GS) during priming

### Gel-stain solution (GS)

A mixture of gel matrix and stain; used for sample separation and detection

### Gel view

Portion of the Experion software user interface dedicated to display of virtual gel data

### LabChip

Microfluidic “lab-on-a-chip” technology developed by Caliper Life Sciences; the Experion automated electrophoresis system was built on LabChip technology

### Lower marker

RNA fragment (50 bp) included in the Experion RNA sample buffer and used to normalize the separation of fragments across all wells in the chip

### Microchannels

Fine channels approximately the diameter of a human hair are used for samples separation in Experion microfluidic chips

### Microfluidic technology (microfluidics)

Multidisciplinary field comprising physics, chemistry, engineering, and biotechnology that deals with the behavior, precise control, and manipulation of microliter and nanoliter volumes

### Normalization

Alignment of peak migration times across different sample wells of the same chip; the lower marker is used by Experion software for this purpose

### Overlay

Function of Experion software that allows different electropherograms to be superimposed for easier comparison

### Peak

Signal in an electropherogram generated and identified by the peak-find settings to be significantly different from the baseline

### Priming

Filling the microchannels of the microfluidic chip with gel-stain solution in preparation for analysis

### Priming station

Instrument used to prepare chips for sample analysis; applies pressure to the priming well of the microfluidic chip and fills the network of microchannels with the gel-stain solution

### Project folder

User-defined folder containing multiple run files comprising a particular project

### Project tree

Hierarchical tree of all saved projects and the data files they contain

### Quantitation

Determination of the concentration of an RNA sample

### Results and settings

Portion of the Experion software user interface dedicated to display of tabulated analysis results and analysis settings and parameters

**RNA ladder**

RNA standard containing 8 RNA fragments of 200–6,000 nt; separation data from the ladder are used by Experion software for size determination of sample fragments and quantitation of RNA samples

**RNA quality indicator (RQI)**

Quantitative measure of integrity of eukaryotic total RNA, generated by Experion software and ranging from 1 (completely degraded) to 10 (intact)

**Run file**

File containing data for a single run

**Single-well view**

Function of Experion software that allows an electropherogram from a single well to be viewed

**Sizing**

Determination of the size, in nt, of an RNA fragment

**Spin filter**

Polypropylene spin tubes with filter membranes used to remove particulate matter from the gel-stain (GS) and gel (G) by centrifugation

**Tree view**

Portion of the Experion software user interface dedicated to display of the project tree; used to locate and manipulate data files and organize runs into projects

**Virtual gel**

Densitometric, gel-like image generated from electropherogram data and displaying peaks as bands; displayed in the gel view

**Vortex station**

Instrument used to mix RNA or DNA samples and loading buffers (supplied in the kits) in the chip wells; used for nucleic acid applications only

## Appendix D: Ordering Information

<b>Catalog #</b>	<b>Description</b>
700-7001	Experion™ System, 100–240 V, for RNA and DNA analysis, includes electrophoresis station, priming station, vortex station, software, USB2 cable
701-7001	Experion System, 100–240 V, for RNA and DNA analysis, includes electrophoresis station, priming station, vortex station, software, USB2 cable, Experion RNA StdSens starter kit
700-7111	Experion RNA StdSens Starter Kit, includes 3 RNA StdSens chips, 2 cleaning chips, Experion reagents, 2 spin filters, total RNA standard, cleaning swabs, electrode cleaner, tips, and tubes to run 3 chips
700-7103	Experion RNA StdSens Analysis Kit for 10 Chips, includes 10 RNA StdSens chips, 2 cleaning chips, 1,250 µl RNA gel, 20 µl RNA StdSens stain, 20 µl RNA ladder, 900 µl RNA StdSens loading buffer, 2 spin filters
700-7105	Experion RNA HighSens Analysis Kit for 10 Chips, includes 10 RNA HighSens chips, 2 cleaning chips, 1,250 µl RNA gel, 20 µl RNA HighSens stain, 20 µl RNA ladder, 900 µl RNA HighSens loading buffer, 100 µl RNA sensitivity enhancer, 2 spin filters
700-7153	Experion RNA StdSens Chips, 10, plus 2 cleaning chips
700-7154	Experion RNA StdSens Reagents and Supplies for 10 Chips, includes 1,250 µl RNA gel, 20 µl RNA StdSens stain, 20 µl RNA ladder, 900 µl RNA StdSens loading buffer, 2 spin filters
700-7155	Experion RNA HighSens Chips, 10, plus 2 cleaning chips
700-7156	Experion RNA HighSens Reagents and Supplies for 10 Chips, includes 1,250 µl RNA gel, 20 µl RNA HighSens stain, 20 µl RNA ladder, 900 µl RNA HighSens loading buffer, 100 µl RNA sensitivity enhancer, 2 spin filters
700-7251	Experion Cleaning Chips, 10
700-7254	Experion Spin Filters, 10
700-7252	Experion Electrode Cleaner, 250 ml
700-7253	Experion DEPC-Treated Water, 100 ml
700-7051	Experion Validation Kit, 3 test chips, qualification procedures, dongle
700-7264	Cleaning Swabs, lint free, for electrode deep cleaning, 25
700-7031	Experion Priming Seals, 2









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