## Experion<sup>™</sup> DNA 1K and DNA 12K Analysis Kits

## **Instruction Manual**

Catalog #700-7107 #700-7108





#### **Bio-Rad Technical Support**

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### **1.1 Product Description**

The Experion DNA 1K and 12K analysis kits are used for DNA analysis with the Experion automated electrophoresis system (Figure 1.1). The Experion system employs LabChip microfluidic technology to automate electrophoresis and analysis by 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 DNA 1K and DNA 12K analysis kits allow analysis of DNA fragments of 15–1,500 bp and 50–17,000 bp, respectively. These DNA assays provide high sensitivity and excellent resolution (down to 5 bp) over a broad dynamic range (Table 1.1). Consuming only 1  $\mu$ l of sample for each analysis, the Experion automated system can analyze 1–11 samples in ~40 min. These assays are recommended for rapid, efficient analysis of restriction digests, amplified DNA, microsatellites, and amplified fragment length polymorphisms (AFLPs).

For details about how the Experion DNA analysis kits analyze DNA, 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.

	Experion DNA 1K	Experion DNA 12K
Number of samples	11	11
Sample volume	1 µl	1 µl
Total run and analysis time	40 min	30 min
Sizing range	15–1,500 bp	50–17,000 bp
Resolution	25–100 bp: 5 bp 100–700 bp: 5% 700–1,000: 10%	100–1,500 bp: 10% 1,500–12,000 bp: 20%
Quantitation range (≥100 bp)	0.5–50 ng/µl	0.5–50 ng/µl
Limit of detection	0.1 ng/µl	0.1 ng/µl
Sizing accuracy	±10%	±15%
Sizing reproducibility	CV ≤5%	CV ≤5%
Quantitation accuracy (in TE)	±25%	±20%
Quantitation reproducibility <sup>1</sup> (in TE)	CV ≤20%	CV ≤20%
Maximum salt concentration:		
KCl or NaCl MgCl <sub>2</sub>	200 mM 15 mM	250 mM 15 mM

#### Table 1.1. Experion DNA analysis kit specifications.

<sup>1</sup> Applies to inter- and intrachip variation.

### **1.2 Kit Components**

### Table 1.2. Components of the Experion DNA analysis kits.

Item	Description	Volume (per Vial)	10-Chip Kit
DNA chip	Microfluidic chips used for DNA separation		10 chips
Cleaning chip	Chip used for cleaning electrodes		1 chip
DNA gel	Proprietary polymeric sieving matrix	250 μl (DNA 1K) 650 μl (DNA 12K)	3 vials 1 vial
DNA stain	Proprietary fluorescent dye	40 µl	1 vial
DNA loading buffer	Buffer for sample preparation; contains lower and upper markers for alignment of samples to the DNA ladder	750 µl	1 vial
DNA ladder	DNA standard containing 11 DNA fragments of 25–1,000 bp (DNA 1K) or 100–10,380 bp (DNA 12K)	20 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
Gel-stain solution (GS, prepared)	4°C	1 month from filtration; do not 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 DNase-free narrow-bore tips (for example, VWR #87001-688 or Rainin #L-10F) DNase-free microcentrifuge tubes, 0.65 ml DNase-free water (for example, Experion DEPC-treated water, catalog #700-7253) Extra spin filter for additional filtration steps (as needed, catalog #700-7254) Experion electrode cleaner (catalog #700-7252)



### 2.1 Storing and Preparing Samples and Reagents

Store all Experion<sup>™</sup> reagents at 4°C when not in use. Do not store reagents at room temperature for >2 hr, as this will shorten their shelf life.

Before use, allow all kit reagents 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 DNA gel has frozen, discard it.

Protect the DNA 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 DNA stain contains DMSO, which is hygroscopic. Cap tightly.

Use prepared GS for up to 1 month. Discard after 1 month; do not refilter.

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 DNase-free microcentrifuge tubes, pipet tips, and TE buffer as needed. Do not use autoclaved water for sample or 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 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. A new cleaning chip is 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:

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 Automated Electrophoresis System

# B Experion<sup>™</sup> DNA 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. Equilibrate the following kit reagents to room temperature for 15-20 min:
  - DNA stain (blue cap)
  - DNA loading buffer (yellow cap)
  - DNA gel (green cap)
- 2. 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 DNA stain solution (blue cap) is thawed before proceeding.

### 3.3 Prepare the Gel-Stain Solution

A gel-stain solution (GS) preparation is sufficient for use with at least four DNA chips. Use the GS within 1 month. Keep prepared GS at room temperature and covered with foil until ready for use. If the GS was already prepared, equilibrate it as detailed above.

### 3.3.1 Experion DNA 1K Analysis Kit

- 1. Prepare the GS by adding 12.5 µl DNA stain (blue cap) to a tube of 250 µl DNA 1K gel (green cap). Vortex the GS for 10 sec, and then spin it down briefly in a microcentrifuge.
- 2. Transfer the GS to a spin filter, and label and date the tube.
- 3. Centrifuge the spin filter for 15 min at 2,400  $\times$  *g*. Inspect the tubes to ensure all of the gel has passed through the filters, and then discard the filters. Blue staining of the filter membrane is normal.
- 4. Wrap the tube of GS in aluminum foil to protect the stain from light.

### 3.3.2 Experion DNA 12K Analysis Kit

- 1. Prepare the GS by combining 10 µl DNA stain (blue cap) and 200 µl DNA 12K gel (green cap) in a DNase-free 0.65 ml microcentrifuge tube. Vortex the GS for 10 sec and then centrifuge briefly.
- 2. Transfer the GS to a spin filter, and label and date the tube.
- 3. Centrifuge the spin filter for 10 min at  $1,500 \times g$ . Inspect the tubes to ensure all of the gel has passed through the filters and then discard the filters. Blue staining of the filter membrane is normal.
- 4. Wrap the tube of GS in aluminum foil to protect the stain from light.

### 3.4 Prepare the Samples and the DNA Ladder

Each chip can analyze up to 11 DNA samples. All wells of a chip must be filled for the electrophoresis station to operate properly.

- 1. Remove the DNA ladder from storage, vortex to mix, then centrifuge briefly. Place the ladder on ice.
- 2. Prepare the samples as necessary to meet the following requirements for analysis:
  - DNA concentration Samples should have a DNA concentration within the linear range of the assay (0.1–50 ng/µl). Make an appropriate dilution of the sample, for example in TE buffer (10 mM Tris, 1 mM EDTA, pH 8), as needed
  - Salt concentration The concentration of salt in the DNA sample must be <250 mM KCl or NaCl, 15 mM MgCl<sub>2</sub>. High salt concentrations increase conductivity, reducing the amount of sample injected onto the chip and affecting assay sensitivity and potentially reducing quantitation accuracy and precision. Make an appropriate dilution of the sample in TE buffer or water, as needed
  - Restriction enzyme deactivation Restriction enzyme digests may require the addition
    of EDTA to chelate metal ions and/or heat inactivation of the enzyme prior to running the
    chip. Failure to inactivate the restriction enzyme may result in degradation of the internal
    DNA markers, inaccurate sizing, or peak broadening

### 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 µI GS into the highlighted well labeled GS, gel priming well (Figure 3.1). Insert the pipet tip vertically and to the bottom of the well when dispensing. Dispense slowly to the first stop on the pipet, and do not expel air at the end of the pipetting step.
- 2. On the priming station, set the pressure setting to **C** and the time setting to **3** or **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 or 60 sec. Do not open the priming station during countdown.
- 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.

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

8. Place the chip on a clean surface for loading.

### 3.6 Load the Chip

- 1. Pipet 9 µl GS into the 3 other wells labeled **GS** (Figure 3.2).
- Pipet 5 µl DNA loading buffer (yellow cap) into each sample well and the ladder well (wells 1–11 and L, Figure 3.2).

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.

- Pipet 1 µI DNA ladder into the well labeled L. Every chip must have the DNA ladder loaded into the ladder well labeled L.
- 4. Pipet 1 µl sample (or blanks, for example water or TE buffer) into sample wells 1–11.
- 5. 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.
- 6. 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.
- 7. 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.
- 8. Place the loaded chip into the Experion electrophoresis station and start the run within 5 min.



Fig. 3.2. Experion DNA chip. Wells for loading buffer, samples, GS, 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.

### 3.7 Run the DNA 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 Mew Run**. In the **New Run** screen (Figure 3.4), from the **Assay** pull-down list, select either **DNA** > **DNA** 1K or **DNA** > **DNA** 12K.
- 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

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+ R 052410 2722 300kHz	Sample Inform	ation					
🕣 🗟 060310_2725_04mm 300kHz	Welld	SampleName	Sample Description	Restriction	Digest		
B 061110_2725_LED	0	Ladder					
is 061510_4 Units Valid     is	1	Sample 1	A Comment				
E 061710_4 Units Valid	2	Sample 2	A Comment				
⊕ ₩ 070110_RNA_3012,4.5,8	3	Sample 3	A Comment				
	4	Sample 4	A Comment				
	5	Sample 5	A Comment				
0/0910_HNA_3035 to 3055	6	Sample 6	A Comment				
Drzulo_nign optical ractor		Sample /	A Comment				
Demo Huns	0	Sample 0	A Comment				
Punt mPNA HighSans 4	10	Sample a	A Comment				
Bun2 mBNA StdSens 4-3	10	Sample 11	A Comment				
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- Run9_Protein_User_ISTD_							
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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.

New Run	X
Run Name Prefix:	Run
Assay Name:	DNA 1K
Run Samples:	
[	Start Cancel



6. 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.

- 7. 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.

- 8. When analysis is complete, the instrument beeps and a window opens indicating the end of the run. Select **OK** and remove the chip from the chip platform.
- 9. Clean the electrodes using deionized water within 30 min of each run.

### 3.8 Clean the Electrodes

- 1. 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.
- 2. 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. A cleaning chip is included with each box of chips.

- 3. Open the lid, remove the cleaning chip, and allow the electrodes to dry for 1 min. Close the lid.
- 4. 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.

- 1. Ensure that all lanes (ladder and samples) are visible in the virtual gel. The markers (indicated by pink triangles) should be visible in and aligned across all lanes. If the marker peaks are not properly assigned, you may need to include or exclude peaks, or manually set the markers (see Sections 6.2 and 6.3).
- Evaluate the separation of the DNA 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):
  - Two marker peaks
  - Eleven resolved ladder peaks between the lower and upper marker
  - Flat baseline





Fig. 3.6. Separation of the DNA ladder. Top, DNA 1K ladder. Bottom, DNA 12K ladder. Note the flat baseline and well-resolved peaks. All identified peaks are numbered, and the lower and upper markers are indicated by green asterisks (\*).

- 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 (for example, Figure 4.3):
  - Two resolved marker peaks
  - Sample peaks located between the upper and lower markers
  - Flat baseline

If the upper and lower marker peaks are not properly assigned, you may need to include or exclude peaks, or manually set the marker (see Sections 6.2 and 6.3).

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



This chapter outlines the basic steps and software features used to view and analyze data. For a more detailed description of Experion<sup>™</sup> 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



To open a run file, double-click on it. The run file opens, displaying the electropherogram and **Results** tab for the DNA 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 versus migration time to generate an electropherogram for each sample. In the electropherogram, all peaks identified by Experion software are numbered, and the numbers appear under the peaks (Figure 4.3). Peaks generated by the markers and used for normalization (for example, the lower and upper markers) are numbered in green and labeled with an asterisk (\*).

Place the cursor over a peak or area of an electropherogram to view its calculated size (Figure 4.3). To display the sizes of all the peaks in the electropherogram, select **Peak Size** in the **Select Peak Information** pull-down list (main screen toolbar).



Fig. 4.3. Example of an electropherogram. All identified peaks are numbered, and the lower and upper markers are indicated by a green asterisk (\*). Place the cursor over a peak to reveal its size.

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 Results 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 4.4). 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 O or select View > Single Well.
- 3. The electropherogram appears. The corresponding lane in the virtual gel is outlined in pink.



Fig. 4.4. 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 4.5):

- 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 4.5). 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.

Demo DNA	1K		_		F	lun10_DN	IA_1K					1
2009 IRNA HighSens 12:16:2009 3:1	mll				Mr			Ladder	ì			
IRNA_StdSens_12-16-2009_3-2: Likasyote_Total_RNA_HighSens_ Likasyote_Total_RNA_StdSens_	1 mar	mu		MIL				Sample 1				
tokaryote_Total_RNA_HighSens tokaryote_Total_RNA_StdSens_ totein_260_12-16-2009_3-27-07	1 mair	mu		MM	٨			Sample 2				
totein_260_Calibration_12-16-20 totein_User_ISTD_12-16-2009_1 DNA_1K_12-16-2009_3-27-07 P	munt	mil		MIL	m			Sample 3	1500 L			1
der ple 1 ple 2	MA			M.	M		1	Sample 4	E 500.0	===		==
ple 3 ple 4 ple 5	M			M	M			Sample 5	300.0			-
6 7 8	ma			mn	Mh			Sample 6	200.0		===	-
xe 9 xe 10 xe 11 xe 11 xe 11 xe 12 xe 2006 2 27 071					Mh			Sample 7	100.0		_	
			۸	٨	Mh			Sample 8	15.0			
		····		M	Mh			Sample 9		1234	5678	9 10
				18				Of alome?	1			
Elmple 3												
Peak State	Peak Number	Mig. Time (secs)	Size (bp)	Corrected Area	Area Ratio	Conc (nglul)	Molarity (nmol/l)	% Total	Observation	Comments		
<b>X</b>	1	37.80	15	270.18					Lower Marker			



#### **Electropherogram Overlays**

Superimpose (overlay) multiple electropherograms to facilitate direct comparison among profiles. To overlay a subset of electropherograms (Figure 4.6), 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. 4.6. Electropherogram overlay. Electropherograms are shown in different colors, and the corresponding lanes in the virtual gel are outlined. In this example, lanes 3, 6, and 7 are superimposed using the overlay feature.

#### Peak Labels

Experion software automatically labels detected peaks with numbers for easy identification, and the upper and lower markers used for normalization are indicated in green and with an asterisk (\*).

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 (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
- Peak Size uses calculated size (bp) for peak labels
- Peak Molarity uses calculated molarity (nmol/L) for peak labels

#### **Electropherogram Tags (Annotations)**

To annotate a peak in the electropherogram, right-click on the peak and select **Add Tag** (Figure 4.7). 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.





Fig. 4.7. 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 4.8). 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.

Place the cursor over an area in the virtual gel to view its estimated size in base pairs (bp). If a band corresponds to a peak identified by Experion software, view the sample name, peak number and calculated size, concentration, and percentage of total DNA by placing the cursor over the band (Figure 4.8).

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 Results 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 4.8). Changing the contrast does not change the data, but it may improve visualization of faint bands in the virtual gel.





#### 4.1.5 Results and Settings

The six results and settings tabs (Figure 4.9) present options for viewing, customizing, and analyzing separation data (see Section 4.3, Using Results and Settings to View and Annotate Data).

Peak State	Peak Number	Mig. Time (secs)	Size (bp)	Corrected Area	Area Ratio	Conc (ng/µl)	Molarity (nmol/l)	% Total	Observation	Comments
	1	37.80	15	259.13					Lower Marker	
	2	39.51	23	104.16	0.6964	2.22	144.14	12.74		
	3	40.48	32	120.19	0.8036	2.47	115.34	14.17		
	4	47.71	104	111.25	0.7438	1.97	28.80	11.31		
	5	48.14	108	134.84	0.9015	2.38	33.29	13.66		
	6	74.06	410	132.35	0.8849	2.12	7.84	12.16		
	7	75.40	435	157.24	1.0513	2.49	8.67	14.26		
	8	90.61	1027	114.25	0.7639	1.86	2.75	10.67		
	9	91.73	1173	117.16	0.7833	1.92	2.49	11.03		
<b>T</b>	10	94.25	1500	149.57					Upper Marker	
	Sample Peaks Found	8								
	Total Concentration	17.4 ng/µl								
	Total Molarity	343.3 nmol/l								

Fig. 4.9. 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 4.10):

- 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 (overlaying) a sample 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 samples 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. 4.10. 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 all detected peaks in a single sample. 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 <sup>(1)</sup>/<sub>(2)</sub> to navigate through the samples. See below for instructions on how to customize the types of data shown under this tab
- Run Summary displays a run summary for all samples; right-click to export the data
- DNA Search Result summarizes user-defined known DNA fragments: lists the samples that contain a peak that fits with the search of size, as defined under the DNA Search Criteria tab and performs statistical calculations for the size, concentration, and percentage of total DNA for each peak identified
- DNA Search Criteria used to compare statistics for specific DNA fragments in the samples (see Section 6.1, Designating and Searching for Specific DNA Fragments for information on how to designate and then search for known fragments)
- 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 also be used to edit the parameters used to identify peaks (see Section 6.4, Changing Peak Finding Parameters, or the Experion software Help file for more details about these settings)

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

- 1. Right-click on any of the column headers in the Results tab (or click the arrow to the left of the column header). The **Column Selector** dialog opens.
- 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.

Under the Results tab, total concentration data appear at the bottom of the table. This calculation represents the total DNA concentration of the original sample.

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 white (active) cell, enter the sample names and descriptions, and click **Apply**. Click **File/Save** to save the changes. The sample name appears above its 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.
- 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 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.

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### 5.1 DNA Quantitation Methods

Experion<sup>™</sup> software enables DNA quantitation by several approaches: percentage determination and concentration determination (yielding either ng/ul or molarity) (Table 5.1). These calculations are performed automatically for each peak identified by Experion software and presented in the Results tab (Figure 5.1).

uantitati	on Method			Outp	ut	Description						
Percentag Concentra	e Determinati ation Determin		% Tot ng/ nmol	tal ⁄µl /L	Percentage of total DNA Relative concentration (against upper marker)							
Parala Stata	Peak Number	Mig. Time (secs)	Size (bp)	Corrected Area	Area Ratio	Conc (na/ul)	Molarity (nmol/l)	% Total	Observation	Comments	•	
	1	37.80	15	250.65				1	ower Marker			
	2	47.54	102	159.46	1.0166	2.70	40.11	10.63				
	3	48.36	111	248.82	1.5863	4.18	57.18	16.45				
	4	78.35	489	332.57	2.1203	4.88	15.12	19.20				
	5	79.39	512	444.31	2.8327	6.51	19.29	25.61				
	6	81.78	575	476.79	3.0397	7.15	18.84	28.11				
<b>T</b>	7	94.25	1500	156.85				l	Upper Marker			
-	Sample Peaks Found Fotal Concentration	5 25.4 na/ul									•	

Table 5.1. DNA quantitation methods used by Experion software.

Fig. 5.1. Results table showing quantitation data for peaks in a sample. Experion software automatically performs % total and relative concentration determinations for each peak identified in every sample.

### 5.2 Performing Percentage Determination

Experion software automatically calculates a percentage of total DNA (% total) value for each detected peak and lists the results in the **% Total** column of the **Results** tab (Figure 5.1). Double-click on a peak in the electropherogram (or right-click on a band in the virtual gel) to highlight the data for that peak in the Results tab.

To exclude a peak from the % total calculation, right-click on the peak (in either the single-well electropherogram view or Results table) and select **Exclude Peak** in the context menu. The new calculations for the remaining peaks appear in the Results tab.

This change affects a selected sample. To exclude multiple peaks from multiple samples, use the **Peak Find Settings** options in the **Settings** tab (see Section 6.3, Excluding a Peak From Analysis and 6.4, Changing Peak Finding Parameters) and adjust the peak detection criteria.

### 5.3 Performing Concentration Determination

Experion software also automatically performs quantitation of all peaks in a sample by a single-point calibration to the upper marker. Results are presented in the following columns of the Results tab:

- Conc. (ng/µl)
- Molarity (nmol/l)

To view these default relative quantitation results, double-click on a peak in the electropherogram or right-click on a band in the virtual gel to highlight the data for that peak in the Results tab.

The algorithm used to calculate DNA concentration of a sample peak involves the following steps:

1. The DNA concentration per peak area is first estimated using the concentration and peak area of the internal upper marker.

[DNA]<sub>est</sub> of sample peak = Sample peak area • [upper marker]/marker area

2. Because the ratio of DNA concentration to peak area depends on peak size, a peak injection correction factor is applied. The [ladder peak]/ladder peak area ratios for the DNA ladder peaks that encompass the sample peak are used to calculate the correction factor.

Conc.  $(ng/\mu I) = [DNA]_{est}$  of sample peak • correction factor

The amount of dye incorporated into the DNA fragment generally increases linearly with size. The actual composition of the DNA fragment can affect the amount of dye that is incorporated; this is particularly evident with smaller DNA fragments.

3. The concentration is then converted to molarity using the following equation (660 is an average atomic mass per bp):

Molarity (mol/L) = Conc.(ng/ $\mu$ l) / (660 • peak size in Kbp) • 10<sup>6</sup>

Experion Automated Electrophoresis System

# 6 Changing Analysis Settings and Parameters

Once an Experion<sup>™</sup> 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 Designating and Searching for Specific DNA Fragments

- 1. Designate and search for specific DNA fragments in your samples:
  - a. In the DNA Search Criteria tab, click Add DNA Name M, or in the electropherogram, rightclick on a peak and select Add to Search List. The DNA Search List Criteria tab opens and contains the size and tolerance range for that entry.
  - b. Click in the field under DNA Name and enter the fragment name.
  - c. Click in the field under Size (bp) and enter the size of the fragment in bp.
  - d. Click in the field under the **±bp mass** column to enter the new value for the tolerance in bp.
- 2. A list of all the peaks that fit the criteria appears in the **DNA Search Result** tab. Separate tables appear for each fragment designated in the search criteria list.
- 3. To delete designated fragments ("DNA names") from a search, click **Delete DNA Name** in the toolbar of the **DNA Search Criteria** tab.

### 6.2 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 Results table and select **Manually Set Lower Marker** or **Manually Set Upper Marker** to define the peak as the new lower or upper marker. Do this if no or incorrect peaks have been selected as the lower or upper marker.

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

### 6.3 Excluding a Peak from Analysis

To exclude one or more peaks from analysis of a single sample, right-click on the peak in either the electropherogram (single-well view) or Results table and select **Exclude Peak** from the context menu. This change affects only a selected sample.

To exclude multiple peaks from multiple samples, open the **Settings** tab and use the **Peak Find Settings** options in the **All Wells Settings** dialog (see Section 6.4, Changing Peak Finding Parameters) to adjust the criteria by which peaks are detected.

Excluded peaks are not included in total DNA calculations. Therefore, excluding a peak from analysis affects percentage determination (% total calculations) for the other peaks in a sample.

### 6.4 Changing Peak Finding Parameters

The parameters entered 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:
  - Slope Threshold represents the variation in fluorescence units over time required to detect a peak (accepted values are 0.2–1,000, with lower values yielding more peaks)
  - 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)
- 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.

### 6.5 Changing General Settings

These settings appear in the **Settings** tab, in the **All Wells Settings** dialog under **General Settings** (Figure 6.1). They apply to all samples in a run.

- Data Frequency displays the rate of data collection in Hz
- Upper Marker Concentration displays the concentration of the upper marker
- Lower Marker Concentration displays the concentration of the lower marker
- Time Window Ratio defines the window in which the software searches for a marker
- Display Start Time displays the time Experion software starts looking for peaks
- Display End Time displays the time Experion software stops looking for peaks
- Ladder Concentration concentration of each fragment in the ladder
- Use Time Corrected Areas corrects for peaks of different sizes that transition the detector at different rates
- 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

All Wells Settings		
Algorithm Inclusion Settings     Assay Header     General Settings		
i Name	Value	
Data Frequency	20	
Upper Marker Concentration (ug/ml)	2.5	
- Lower Marker Concentration (ug/ml)	5	
Use Upper Marker	✓	
Time Window Ratio	0.1	
<ul> <li>Display Start Time</li> </ul>	20	
Display End Time	130	
Ladder Concentration	4	
Use Time Corrected Areas		
E Name	Value	
Filter Width	0.5	
Polynomial Order	6	
Eadder Settings     Peak Find Settings		
Reset to Default OK	Cancel	Fig. 6.1. General Settings options

### 6.6 Baseline Modification

To change the method used by Experion software for adjusting the baseline in a single sample:

- 1. Under the **Settings** tab, select one of the following two options under **Algorithm Inclusion Settings** (Figure 6.2):
  - Baseline Correction (default) subtracts the baseline from the signal. The baseline represents a slow, rolling component in the signal. Generally, this method affects the original shape of the curve and subsequent calculations. The more complex the baseline, the greater impact subtraction might have on the signal. For well-resolved peaks with a slow rolling component; this method, however, can normalize calculations across the chip. If peaks are poorly resolved, baseline correction can cause considerable artifacts
  - Zero Baseline subtracts the average baseline value from the signal. This method does not affect the shape of the curve; it shifts the signal down to zero. If the Baseline Correction option results in undesirable artifacts (small peaks do not appear, or too many peaks appear), change this setting to see if it remedies the problem
- 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** to recover the default settings.

Deselecting both options makes the original signal(s) appear. Sometimes it is important to see the difference between the signals across the chip if no correction is applied.

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 select the type of modification you would like to apply (see above for descriptions or Figure 6.2). Click **OK**.
- In the Experion Application dialog, click OK and Yes to to overwrite the sample settings. 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.

All Wells Settings	
Algorithm Inclusion Settings	٦
Baseline Correction	
Name Value	
Baseline Correction	
Assay Header	
General Settings     Jadder Settings	
Peak Find Settings	
Reset to Default OK Cancel	

Fig. 6.2. Algorithm Inclusion Settings options.

### 6.7 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 upper and lower markers 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 and the position of the upper marker may drift (Figure 6.3). Turning analysis off can help reveal if a problem is isolated (one or a few wells have markers out of alignment) or is general (alignment problems across the entire chip).



Fig. 6.3. Effects of turning analysis off.

### 6.8 Manual Peak Integration

This function enables definition or refinement of peak areas for the ladder and all sample lanes.

- 1. Select a sample and click or select **Analysis > Manual Integration** to start manual integration.
- 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 A.

Manual integration applies to individual sample wells. Moving back to automated integration loses changes made during manual integration unless those changes are saved.



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



Error	Probable Cause	Recommended Action		
IV Check Failure	One or more wells are not filled correctly, and	Fill all sample wells of the chip with blank or replicate samples		
(Experion <sup>™</sup> software)	the electrodes are not immersed	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		
Check Seal	Priming seal is worn, torn, or clogged	Replace the priming seal (catalog #700-7031)		
(Priming station)	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 the chip is positioned properly and correct reagents are used		
Run aborts before all wells	Computer power saver options are causing	Upgrade to the newest software version		
are analyzed	runs to abort (this is a problem only with Experion software prior to version 3.0)	Confirm that 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 aborts		

### 7.1 Electrophoresis and Priming Stations

### 7.2 Experion DNA 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 DNA concentration or sample variability			
Peaks were not detected in A the DNA ladder w sc tr lice	An air bubble is at the bottom of the ladder well or microchannels due to poor priming or	Check the chip for air bubbles after priming and sample loading:			
	sample loading; air bubbles can interfere with the contact between the electrode and the liquid	<ul> <li>After priming: turn the chip over to make sure the microchannels are filled with gel-stain solution (GS).</li> <li>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> </ul>			
		<ul> <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	Samples were not prepared properly	Review the sample preparation procedure detailed in Chapter 3				
	DNA stain was photobleached	Protect the DNA stain and gel-stain solution (GS) from light				
		Prepare new GS; use a new tube of DNA 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 the 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				
No peaks detected in any samples	Gel-stain solution (GS) is old or has been photobleached	Prepare fresh GS				
	Chip was not primed with the correct pressure and time settings	Check settings on the priming station and repeat the analysis with a new chip				
	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 the GS was filtered properly				
		If using samples that contain particulates, perform a quick microcentrifuge spin of the prepared sample to pellet particulates before loading				
Lower and/or upper marker is missing	Samples do not contain sample buffer or were not prepared properly (if both are missing)	Review the sample preparation procedure in Chapter 3				
	Late migration	If only the upper marker is missing, refer to the troubleshooting tips for late migration (below)				
Electropherogram peaks are much smaller than expected (peaks are <50% of what is expected)	Too much stain in the gel-stain solution (GS); sample cannot destain completely, and the background is high; too much stain can contribute to high background and low sample and ladder peaks	Prepare new GS using a new tube of stain. Ensure stain is thawed completely before preparation				
	Not enough sample was added	Ensure pipets are calibrated				
		Use pipets that accurately deliver volumes of ${\leq}10~\mu\text{I}$				
	Too much salt in the sample; salt ions compete with the sample ions during injection	Analyze a dilution series of the sample in water on another chip. If the peak heights and calculated DNA concentrations of the diluted sample increase, the salt is too high in the original sample. Determine the proper dilution to minimize the salt effect				
	Gel-stain solution (GS) is old or has been photobleached	Prepare fresh GS				
	Reagents, specifically the DNA 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 the gel, which may result in significantly lower peaks and areas	Repeat the analysis with a new chip, new tube of stain, and properly equilibrated reagents				
	Gel-stain solution (GS) was mislabeled or	Ensure chip was primed with GS				
	used improperly. DNA chips contain specific wells for GS that carry the sample through microchannels and supply dye for analysis	Ensure GS was used in all wells marked GS				

Error	Probable Cause	Recommended Action			
Peaks are small, broad, or missing in some samples,	Contaminants are present	Prepare samples in a different buffer or dilute them in water, if necessary			
but present in other samples on the chip		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)			
Late migration (peaks broaden and are delayed over the course of the run)		Turn analysis off (see Section 6.7, Turning Analysis Off, for more information). Late migration is indicated if there is a drift in the migration of the lower marker across the chip and the upper marker migrates later and runs off the top of the gel view. Refer to the troubleshooting tips that follow			
	Air bubbles are interfering with electrical contact in one or more of the wells	Stop the run, remove the 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 the 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 the run (should be $30-35^{\circ}$ C); there is no cooling unit in the electrophoresis station, so if the temperature changes during the course of a run, the 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	DNA ladder peaks and upper and lower markers were improperly assigned by the software	Exclude peaks (follow the instructions in Section 6.3, Excluding a Peak From Analysis) or manually assign markers (follow the instructions in Section 6.2, Manually Setting a Marker), if necessary			
Quantitation is incorrect	Upper and lower markers were not properly assigned by the software	Check that both markers were properly assigned. Manually select the marker(s), if necessary (follow the instructions in Section 6.2, Manually Setting a Marker)			
	Pipetting and/or dilution errors occurred during	Ensure calculations and dilutions are correct			
	sample preparation or chip loading	Ensure pipets are calibrated, and use pipets that accurately deliver volumes of ${\leq}10~\mu\text{I}$			
		Do not modify the sample preparation and loading protocols described in this manual			
		Ensure that the total DNA concentration of samples is within the linear dynamic range before proceeding with the sample preparation instructions described in Section 3.4; if total DNA concentration is unknown, run a DNA analysis of a dilution series of the sample			
		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 the DNA degraded	Prepare fresh samples and try the analysis again			
	DNA is <100 bp	Small DNA fragments may bind dye differently, depending on base composition			
Ghost peaks or contaminants appear in	Electrodes are contaminated	Follow the deep cleaning procedure described in Appendix B and the software Help section (search term "electrodes")			
electropherograms		Use only 0.2 µm-filtered water (such as Experion DEPC- treated water) for diluting DNA samples and the DNA ladder; do not use autoclaved water			

Error	Probable Cause	Recommended Action		
Upper or lower marker is identified incorrectly	Sometimes Experion software selects incorrect peak to correspond to the upper or lower marker; such an error affects alignment and sizing	Manually select the upper and lower markers by following the instructions in Section 6.2		
Virtual gel does not resemble an agarose gel	The gel view is a direct interpretation of the electropherogram data. By default, the	Adjust scaling of the virtual gel to <b>Scale To Global</b> ; this also adjusts the scale on all the electropherograms.		
separation of the same samples	fluorescence intensity of the bands in each lane is scaled to the intensity/peak height of the highest peak in that sample (scale to local). Consequently, each gel lane may have a different scale of fluorescence intensity	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	Stop the run, remove the chip, and remove and replace solution in affected wells		
	Chip is not completely primed	Prime and load a new chip		
	Samples are too concentrated; DNA levels are out of the range of the assay	Dilute the samples and repeat the analysis		

### 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 DNA chips and the Experion DNA 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). Experion Automated Electrophoresis System



### Appendix A: How the Experion<sup>™</sup> 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 DNA fragments migrate through the separation channel at a rate based on their size and charge. Finally, the fragments interact with a fluorescent dye during separation and are detected as they pass a laser and photodiode detector (laser-induced fluorescence).

DNA analysis is accomplished with the Experion DNA 1K and 12K analysis kits and 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. DNA 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 DNA 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 the DNA. 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 and concentrations.
   Following analysis, parameters may be changed and the data reanalyzed

#### How Experion Software Analyzes DNA Fragments Data Presentation

As the photodiode detects fluorescent signals from the dye-DNA 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 peak(s) signal(s) generated by the sample fragment(s)
- Upper and lower markers signals generated by the upper and lower markers, which are
  included in the DNA loading buffer to normalize the separation of fragments across all wells
  in a chip. The upper marker is also used as an internal standard for relative quantitation
  (see below). For each lane, comparison of the corrected area of this peak with the
  corrected area of every detected peak allows estimation of relative concentration

Once separation occurs, the data are converted into a densitometric, gel-like image, or virtual gel (Figure A.2). Each lane in the virtual gel corresponds to a different sample. The sample peaks and upper and lower markers seen in the electropherogram also appear in the virtual gel.



Fig. A.1. Electropherogram generated by an Experion DNA 1K analysis. Peaks identified by Experion software are numbered, and the relative positions of the lower and upper markers are indicated by asterisks (\*).



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 arealso tabulated and presented in a Results tab (Figure A.3). The types of data available include the following:

- Fragment size (in bp)
- Concentration: relative concentration under Conc. (ng/µl) and Molarity (nmol/l)
- % Total sample (percentage determination)

Peak State	Peak Number	Mig. Time (secs)	Size (bp)	Corrected Area	Area Ratio	Conc (ng/µl)	Molarity (nmol/l)	% Total	Observation	Comments	
_	1	37.80	15	250.65					Lower Marker		Ĩ
	2	47.54	102	159.46	1.0166	2.70	40.11	10.63			
	3	48.36	111	248.82	1.5863	4.18	57.18	16.45			
	4	78.35	489	332.57	2.1203	4.88	15.12	19.20			
	5	79.39	512	444.31	2.8327	6.51	19.29	25.61			
	6	81.78	575	476.79	3.0397	7.15	18.84	28.11			
<b>T</b>	7	94.25	1500	156.85					Upper Marker		
	Sample Peaks Found Total Concentration	5 25.4 na/ul									
Results Ru	n Summary DNA Searc	h Result DNA Se	arch Criteria	Run Info	Settings						

Fig. A.3. Results tab generated by an Experion DNA 1K analysis. Shown is the Results tab for a single sample.

#### Data Analysis: Normalization, Sizing, and Quantitation

Following separation, Experion software analyses DNA fragments using one or more of the following:

- Internal markers (upper and lower) for normalizing migration times of samples in different wells
- DNA ladder for determining fragment size (sizing)
- Internal upper marker for determining concentration (quantitation)

#### Normalization: Aligning Peak Migration Times

To compensate for small variations in each separation, Experion DNA analysis uses two internal markers to normalize the migration times among samples. The two internal markers, an upper marker (1,500 bp/17,000 bp) and lower marker (15 bp/50 bp), are included in the Experion DNA loading buffer. Therefore, both of these markers are added to each sample and the DNA ladder (Figure A.1). Inclusion of these markers and the normalization process ensure that the software properly identifies peaks.

#### **DNA Sizing: Determining Peak Sizes**

The first sample to be analyzed is the DNA ladder, which contains 13 DNA fragments (including two markers) of 15–1,500 bp (DNA 1K) or 50–17,000 bp (DNA 12K). Experion software constructs a standard curve of migration time as a function of fragment size from the ladder separation. It then calculates sizes of the DNA fragments from the sample wells by comparing their migration times to the standard curve.

#### **Quantitation: Determining DNA Amount**

Experion software offers two different types of DNA quantitation methods: percentage determination and concentration determination. All quantitation measurements are based on the time-corrected peak area (corrected area) of each peak identified in an electropherogram. The corrected area of a peak is proportional to the amount of the DNA it represents in a mixture.

- Percentage determination measures the percentage of each fragment in a mixture (% total). This method is commonly used for determining DNA content, purity, and stability. The accuracy of this method depends on the dye-binding efficiency of each component in the DNA mixture
- Concentration determination provides the amount of the fragment(s) in a mixture rather than just a percentage of the total. Experion software quantitates a DNA sample peak by comparing the sample peak area relative to the concentration:peak area ratio of the upper marker and applying a correction factor to account for the size dependence of fluorescence

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

Though the Experion DNA analysis kits and other gel-based electrophoresis methods both use a gel matrix and voltage differences to separate DNA 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).

Aspect	Agarose Gel Electrophoresis	Experion DNA Analysis
Sieving polymer (matrix)	Formulation can be changed to affect resolution	Fixed formulation
	Agarose	Polymer (not crosslinked)
DNA stain	Multiple available	Single, proprietary stain
	Binds DNA fragments	Intercalates DNA fragments
Detection	Separate staining step; fragments sometimes stained after separation	Integrated separation step; fragments stained during separation
	Requires photography or other imaging equipment for documentation, analysis	Laser excites the stain, detection using a photodiode, both incorporated
Types of separation	Denaturing and native	Native
Downstream applications	Separated fragments available for excision, electroelution, blotting applications	Fragments not available for further analysis
Reagent and sample usage	Moderate to extensive (ml to L)	Minimal (µI)
Waste generation	Moderate to extensive (ml to L)	Minimal (µI)
Overall analysis time	Hours (depends on gel size, staining method, and methods of documentation and analysis)	30-40 min/chip (11 samples per chip)
Data analysis	Manual; requires additional equipment and software	Automated (with flexibility of manual adjustments)

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

### **Appendix B: Deep Cleaning Procedure**

This manual procedure applies Experion<sup>™</sup> electrode cleaner directly to the 16 platinum pins in the electrode manifold. It is the most stringent method of cleaning the Experion electrode pins.

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**

### % Total

Calculation performed by Experion<sup>™</sup> software to show the percent of the total DNA comprised by a particular peak; used in purity assessments

### 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

#### **DNA** ladder

DNA standard containing 11 DNA fragments of 100–10,380 bp (DNA 12K) or 25-1,000 bp (DNA 1K); separation data from the ladder are used by Experion software for size determination of sample fragments

#### 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

DNA fragment included in the Experion DNA sample buffer and used to normalize the separation of fragments across all wells in the chips; for the DNA 1K kit, the lower marker is 15 bp and for the 12K kit it is 50 bp

#### 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 and upper markers are 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 a DNA fragment in a sample

#### **Relative quantitation**

Method of quantitation in which concentrations are determined against a single-point internal standard, such as the upper marker

#### **Results and settings**

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

#### 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 bp, of DNA fragments

#### 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

#### Upper marker

DNA fragment included in the Experion DNA sample buffer and used to normalize separation of fragments across all wells in the chips; also used as an internal standard for relative quantitation by default. For the DNA 1K kit, the upper marker is 1,500 bp, and for the 12K kit it is 17,000 bp

#### 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**

#### Catalog # Description

- 700-7001 Experion<sup>™</sup> System, 100–240 V, for RNA and DNA analysis, includes electrophoresis station, priming station, vortex station, software, USB2 cable 700-7107 Experion DNA 1K Analysis Kit for 10 Chips, includes 10 DNA chips, 1 cleaning chip, 3 x 250 µl DNA 1K gel, 40 µl DNA 1K stain, 20 µl DNA 1K ladder, 750 µl DNA 1K loading buffer, 3 spin filters 700-7108 Experion DNA 12K Analysis Kit for 10 Chips, includes 10 DNA chips, 1 cleaning chip, 650 µl DNA 12K gel, 40 µl DNA 12K stain, 20 µl DNA 12K ladder, 750 µl DNA 12K loading buffer, 3 spin filters 700-7163 Experion DNA Chips, 10, plus 1 cleaning chip 700-7164 Experion DNA 1K Reagents and Supplies, for 10 chips, includes 3 x 250 µl DNA 1K gel, 40 µl DNA 1K stain, 20 µl DNA 1K ladder, 750 µl DNA 1K loading buffer, 3 spin filters 700-7165 Experion DNA 12K Reagents and Supplies, for 10 chips, includes 650 µl DNA 12K gel, 40 µl DNA 12K stain, 20 µl DNA 12K ladder, 750 µl DNA 12K loading buffer, 3 spin filters 700-7261 Experion DNA 1K Ladder, 20 µl 700-7262 Experion DNA 12K Ladder, 20 µl 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

Experion Automated Electrophoresis System



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 Belgium 09 385 55 11
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 Czech Republic 420 241 430 532
 Denmark 44 52 10 00
 Finland 09 804 22 00
 France 01 47 95 69 65

 Germany 089 31 884 0
 Greece 30 210 777 4396
 Hong Kong 852 2789 3300
 Hungary 36 1 459 6100
 India 91 124 4029300
 Israel 03 963 6050

 Italy 39 02 216091
 Japan 03 6361 7000
 Korea 82 2 3473 4460
 Mexico 52 555 488 7670
 The Netherlands 0318 540666
 New Zealand 0508 805 500

 Norway 23 38 41 30
 Poland 48 22 331 99 99
 Portugal 351 21 472 7700
 Russia 7 495 721 14 04
 Singapore 65 6415 3188
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