Torch Melting Curve Analysis with FilmArray 2.0 Software

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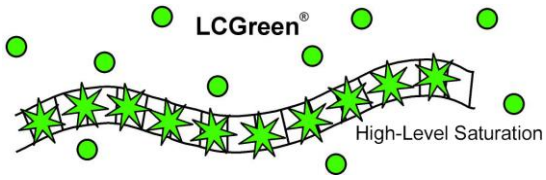
1. Introduction

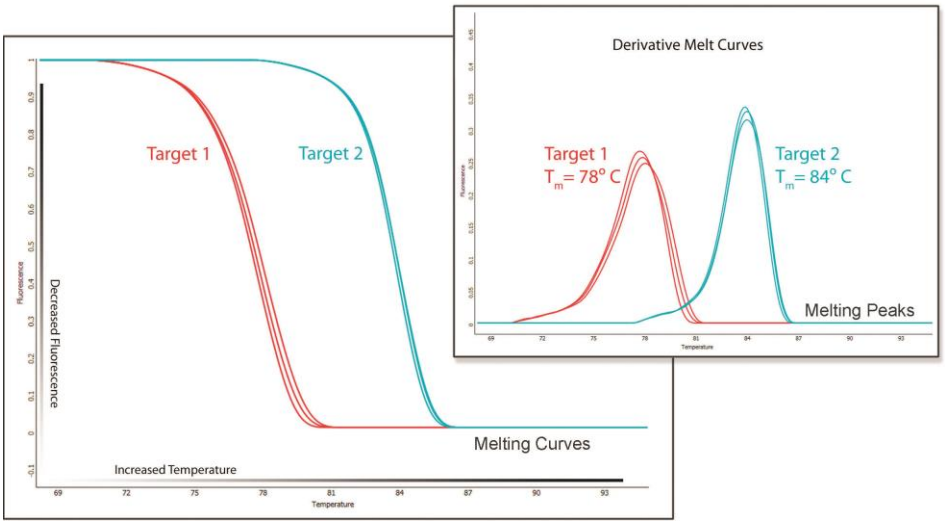
FilmArray PCR reactions contain the DNA binding dye LCGreen® Plus. LCGreen Plus is incorporated into the copies of DNA as they are made during each PCR cycle. When bound to double-stranded DNA, the dye fluoresces and the fluorescence is detected by the FilmArray instrument. As the temperature is increased and the copies of double-stranded DNA melt, the LCGreen Plus dye is released and a reduction in fluorescence is detected.



Copies of double-stranded DNA generated during PCR (called PCR products or amplicon) will have unique sequences based on the template that was amplified. Amplicon length and sequence determines the temperature at which the double-stranded DNA will melt apart, which is known as the melting temperature (Tm) of the amplicon. PCR products made from different targets will have different sequences and, therefore, different Tms.

After the last cycle of PCR, the FilmArray instrument gradually raises the temperature of the reaction from approximately 60°C to 94°C. As the temperature reaches the Tm of an amplicon, the amplicon denatures and fluorescence drops, releasing LCGreen Plus. This produces a melting curve, seen in the graph below, which shows the rapid decline in fluorescence. A melting peak with a specific Tm is generated for each amplicon by plotting the negative derivative of the melting curve.

Figure 1. Melting Curves for Two Different Targets with Unique Amplicon Sequences



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The FilmArray uses melting curve analysis to identify pathogen specific PCR product. Since the sequence and Tm of an amplicon from a specific target is known and consistent, pathogen-specific PCR product can be identified as being copied from that target. Non-specific PCR products with different Tms are excluded.

2. Install FilmArray 2.0 Software

FilmArray 2.0 software is required to import and view run data, including melt curve data from the FilmArray Torch system. This section provides instructions for installing the software on a computer running the Windows 7 operating system (32-bit or 64-bit).

Before installing:

- Download the required software or order the installation CD:
    - Obtain the FilmArray 2.0 installation software:
        - Visit the BioFire E-Document Center (https://www.online-ifu.com/ITIFA20) to download the installer.

          NOTE: A high-speed internet connection is recommended for downloading these files.

        - Contact Customer Support to Order the installation CD.
    - If the designated computer uses a Windows 32-bit OS, install the update:
        - Visit the BioFire E-Document Center (https://www.online-ifu.com/ITIFA202A) to download the update.
        - Contact Customer Support to order the Update CD.
- Save all open files in the event the setup installer instructs a reboot for the computer.

To install the FilmArray 2.0 software:

1. If using the FilmArray 2.0 installation CD, insert the disc into the CD drive. If downloaded from the BioFire E-Document Center, begin at step 2.
2. Navigate to the installation file and double-click the file **FilmArray Setup.exe**.
3. On the setup assistant window (see the following image), select the check box to agree to the license terms and then select **INSTALL** to install the software.
4. When the setup assistant confirms the software has been successfully installed, select **FINISH** to close the installer.

To install the update for the Windows 32-bit OS:

1. If using the Update CD, insert the disc into the CD drive. If downloaded from the BioFire E-Document Center, begin at step 2.
2. Navigate to the update and double-click the file **FA2 32-bit Update.exe**.
3. On the setup assistant window, select **INSTALL** to install the update.
4. If the software detects an existing installation of the update on the designated computer and the version of that software matches the update being installed, select **REMOVE** to uninstall the existing update.
5. When the setup assistant confirms the update has been successfully installed, select **FINISH** to close the installer.

3. Export Data from Torch

When exporting runs from Torch, database files are saved with the extension .db.

To export runs to a file:

1. Insert a removable drive into an available USB port on the front of the System Base.

NOTE: One or more removable drives, including USB flash drives, external CD/DVD drives, and external hard drives, can be connected to the USB ports on the FilmArray Torch System Base.

2. Select **Browse Runs** on the toolbar to view a list of runs in the database. For specific results, filter the list by selecting the **Search** icon and using the search form that appears (see the "Search Run Data" section in the *FilmArray Torch Operator's Manual*).

3. Select **Options** and then select **Export Runs** on the Options menu that appears.
4. Select the run or runs desired to export and then select **OK**. If multiple removable drives are connected to the USB ports on the System Base, select a destination device when the display prompts you to "Select a removable drive for the save destination." A number of helpful features will help to complete this task:
    - To move through the list of runs, select the arrow buttons (<<, <, >, >>).
    - To select all the runs, select the **Select All** button.
    - To deselect the entire selection of runs, select the **Deselect All** button.
5. Enter a name for the export file by using the keyboard on the display and then select **Save**. The message "Exporting run…" appears while the system is exporting runs to the removable drive.

NOTE: The operator can cancel the export process before it completes by selecting **Cancel** on the export process dialog. Any runs that were exported before selecting Cancel will be saved to the chosen location.

6. It is safe to remove the removable drive from the USB port on the System Base once the message "Successfully exported [n] runs to file" appears.

4. Import Data into FilmArray 2.0 from Torch

This feature enables the operator to import FilmArray runs from another FilmArray database onto the computer being used.

To import runs from a file:

1. Insert the removable drive into the computer with the FilmArray 2.0 software installed.
2. Open the FilmArray 2.0 software.

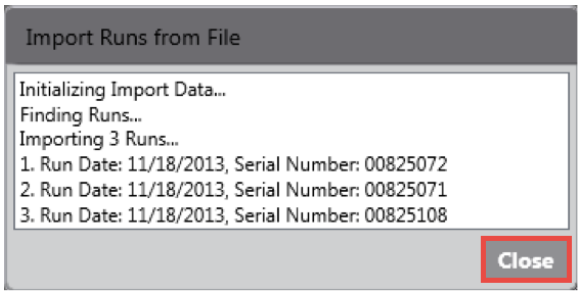
3. In the **Browse Runs** tab, right-click anywhere in the Results area and then select **Import Runs from File** on the menu that appears. The Select Database dialog will display.

NOTE: Import Runs from File is one of two Browse Runs context menu options that are independent of the selected runs: The operator can select a run or runs to access this option, but the particular run(s) selected are immaterial.

4. Navigate to the desired .db file location and then select **Open** to start the import process.
5. During the import process, the Import Runs from File dialog will display the run date and serial number of the runs being imported.

NOTE: The operator can cancel the import process before it completes by selecting the **Cancel** button in the bottom-right corner of the dialog. Any runs that were imported before selecting Cancel will remain in the database, but any runs not yet imported are not processed.

6. Whether the operator allows the process to complete or selects **Cancel**, it is necessary to select the **Close** button, when it appears, to close the dialog.



5. View Melting Curve Analysis Results

The operator can view melting curve analysis results for each control and pathogen assay in a run through the Evaluator tab. The Evaluator tab is only used to evaluate the test results and cannot be used to operate the instrument or perform a run.

The Evaluator is accessed from the Browse Runs tab. When an individual run has been selected and opened from Browse Runs, a new, run-specific tab appears and is identified by the Pouch Serial Number.

To access run-specific Evaluator tabs:

1. In the **Browse Runs** tab, double-click the desired run in the Results table. The tab for that run will open.

   Multiple run-specific windows can be open at the same time. Each window contains the following features. The figure that follows shows where each feature is located on the screen.

Feature	Description	Number in Figure Below
Information Bar	This section of the screen summarizes information about the run in the following fields: Pouch Serial Number, Pouch Lot Number, Pouch Type, Instrument Name, Date, Operator, Protocol, Sample ID, and Status.	1
Summary Tab	This tab shows the pouch status, organisms that were positive in the run, and whether the controls passed or failed. If there is a positive interpretation or a	2

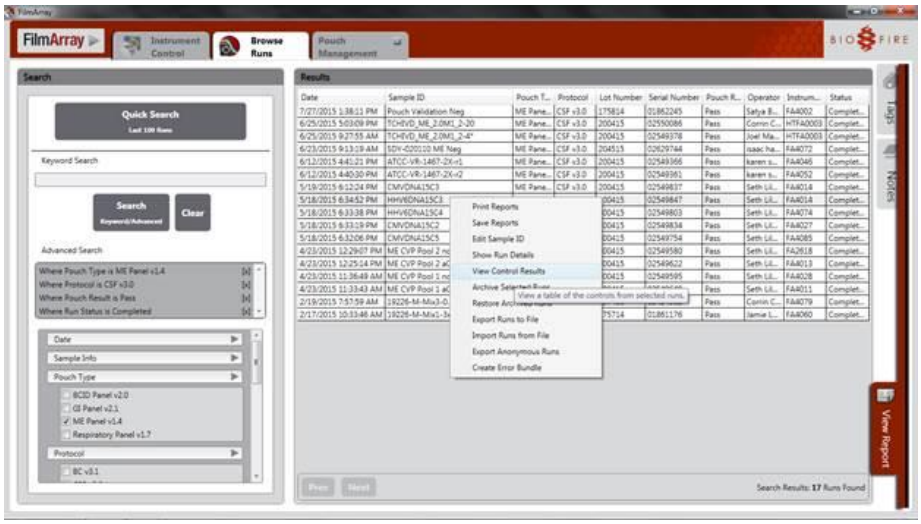
	failed control, the operator can double-click on the text to display the curves.	
Control Tab	This tab shows each control used in the run and whether it passed. To view melt curve data for a control, select the control name from the list in the Control section. The selected control's curve will be displayed in the graph, and information regarding the control will display in the Assays box.	3
Interpretation Tab	To view information and the curves for each interpretation, select the individual organism. The Interpretation tab displays each of the organisms tested in the FilmArray pouch. To view melt curve data in the Melt Peaks Chart box for an organism: 1. Select the organism interpretation from the list in the Interpretation tab. The selected curves will be displayed in the Melt Peaks Chart box, and information for the assays used for the interpretation will be displayed in the Assays box. 2. View additional information by placing the mouse cursor over a melt curve and reading the tooltip that appears.	4

6. View Control Results

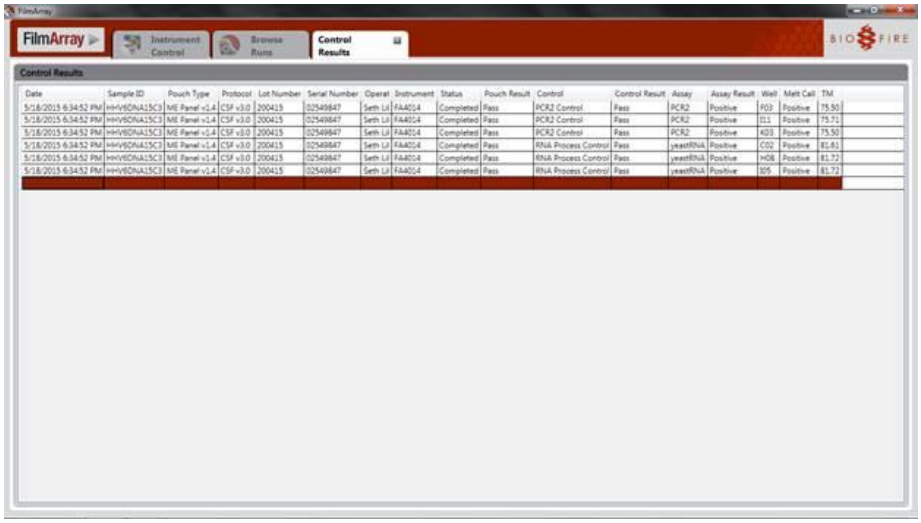
The FilmArray 2.0 View Control Results feature enables an operator to view the control results for a set of runs. The control data that is displayed on the Control Results tab can be copied to Notepad as a tab-delimited table, and then exported in a .txt file. An operator can import the .txt files into other applications (such as Microsoft Excel) for analysis.

To view and export control run data:

1. On the FilmArray 2.0 Browse Runs tab, select the runs to be viewed.
2. Right-click the selected runs and choose **View Control Results** on the pop-up menu (see the following image).



3. Copy the data that is displayed on the Control Results tab by pressing **Ctrl+A** and then **Ctrl+C**.



4. Open a new document in Notepad (Start > All Programs > Accessories > Notepad).

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5. Paste the table into the blank document by pressing **Ctrl+V**.
6. Save the document as a .txt file on a removable drive.
7. Import the .txt file with the program that will be used to analyze the control results. For example, Microsoft Excel.

Technical Support Contact Information

BioFire Diagnostics is dedicated to providing the best customer support available. For any questions or concerns about this process, please contact the FilmArray Technical Support team for assistance.

BioFire Technical Support
Email: support@biofiredx.com
Phone: +1-801-736-6354, select Option 5 and then Option 1

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