



Forensic Biology Section

Operation of ProFlex Thermal Cycler

1. Scope

- 1.1. The ProFlex thermal cycler is a programmable instrument that heats and cools up to 96 samples. The thermal cycler automates the Polymerase Chain Reaction (PCR) for DNA profiling.
- 1.2. The PCR time and temperature profiles are stored in methods which specify how the instrument amplifies Short Tandem Repeat (STR) DNA profiles that are then run on a Capillary Electrophoresis (CE) instrument. The methods are created using a graphical interface to set temperatures, times, ramp rates and cycle numbers.

2. Safety

- 2.1. The thermal cycler sample block and cover become very hot. Do not touch these surfaces while the machine is in operation or severe burns could result.

3. Equipment

- ProFlex PCR System (Life Technologies)
- VeriFlex 9 channel Temperature Verification Kit “TVK” (Life Technologies)

4. Quality Assurance

- 4.1. The thermal cycler is calibrated at the factory.
- 4.2. An internal validation or performance check must be performed before an instrument can be used in casework or database work.
- 4.3. Tests are run on each thermal cycler monthly, quarterly, annually, and after service or repairs.

5. Amplifying Samples in the ProFlex

- 5.1. Place the tray of samples in the block with the A1 well in the upper left-hand corner.
- 5.2. Close the cover over the samples, pressing the lever to seat the tubes into the block.
- 5.3. Touch the **Open Method** icon at the bottom of the Home screen.
- 5.4. Three folders will be displayed: Public, Casework, and Database:
 - 5.4.1. The ‘Fusion Direct-Amp 25 cycles’ is in the Database folder.
 - 5.4.2. The ‘Fusion Casework 30 cycles’ and ‘Y23 Casework 30 cycles’ are in the Casework folder.
 - 5.4.3. The ‘95 Degree Denaturation’ is in the Casework and Database folders.
 - 5.4.4. There are no methods in the Public folder.
- 5.5. Touch the **folder** that contains the method needed, then touch the **method’s name**.
- 5.6. Touch **Verify Block**.
- 5.7. The Run Parameters screen will be displayed. Verify the correct method is selected.
- 5.8. You can choose to retain the default Run ID or touch the **Run ID** to edit it. The default Run ID is YYYY_MM_DD_HHMMSS, where “YYYY_MM_DD” is the date and “HHMMSS” is the time.
- 5.9. Touch **Add Comments** to enter comments about the run. Type comments and touch **Enter** to save.
- 5.10. Touch **Start Run**. The cover will heat to the required temperature and the run will begin. While the run is in progress, the Home screen displays the Temperature and Time remaining.



Forensic Biology Section

Operation of ProFlex Thermal Cycler

5.11. Touch the Time/Temperature **Status Dial** to see the run parameters and additional data during the run (Stages, Start Time, End Time, Time Remaining, Cycle #, etc.).

5.12. When the run is complete, the Status Dial on the Home screen displays “Done, Remove Samples”. Touch **Done** once you have removed the samples.

6. Creating a Method

6.1. Touch **New Method** from the Home screen.

6.1.1. Touch **Open Template** to create a new method using an existing template and select from the categories listed in the Select Method Template screen.

6.1.2. Touch **Open Method** to create a new method using an existing method and select from the methods listed in the Select Method screen.

6.2. The Method Edit screen will open, showing a preview of the run. Touch **Edit** (or anywhere on the graphical profile) to edit the parameters.

6.2.1. To add or delete a step, touch **Manage Steps**. Buttons to **Add** or **Remove** steps will appear. Press **Done** to finish managing the steps.

6.2.2. Touch **Advanced Options** to edit the block, ramp rate, or simulation mode. Press **Done** to save any edits.

6.2.3. Touch **Save As** to save an edited template as a new method.

7. Monthly Cleaning

7.1. The sample wells and sample block cover should be cleaned approximately once a month.

7.2. If the thermal cycler has been run recently, allow the instrument time to cool off (at least 30 minutes) so the heated cover and wells can cool off.

7.3. Wipe the cover and the top of the sample block wells with ethanol on a kimwipe.

7.4. Clean inside the wells with ethanol on cotton swabs to remove any ink from the wells.

7.5. Make certain all the ethanol has evaporated before turning the instrument back on.

7.6. If the instrument becomes contaminated with PCR product or biological material, decontaminate with 10% bleach solution on cotton swabs or kimwipes. To prevent corrosion, rinse any metal with deionized water after using bleach on the block or cover.

8. Monthly Tests

8.1. The monthly **Self Verification Test** checks the instrument hardware (block, heated cover, etc.). The test takes ~ 3 minutes.

8.1.1. Touch **Settings**, then **Maintenance and Services**, then **Self Verification Test**, and then **Start Test**.

8.1.2. The test results are displayed as a report. Insert a USB drive into the port on the front and touch **Export to USB** to copy the report, and then touch **Cancel** to return to the menu.

8.1.3. The Self Verification Test can also be used after service or when instrument errors occur.



Forensic Biology Section

Operation of ProFlex Thermal Cycler

- 8.2. The monthly **Heated Cover Test** checks the cover's heating element. Takes ~ 5 minutes.
 - 8.2.1. Touch **Block Verification Test**, then **Verify Block temperature**, then **Heated Cover**, and then **Next**.
 - 8.2.2. Turn on the TVK, insert the probe into the indicated wells (middle zone), insert the TVK USB cord, and touch **Start Test**.
 - 8.2.3. The test results are displayed as a report. Unplug the TVK USB cord from the instrument, insert a USB drive into the instrument, and touch **Export to USB** to copy the report.
 - 8.2.4. Touch **Cancel** to return to the Verify Block Temperature menu.
9. **Quarterly Test**
 - 9.1. The quarterly **Temperature Non-Uniformity Test** checks the calibration of the three temperature zones in the block. The test takes ~ 30 minutes (three 10-minute tests).
 - 9.1.1. Touch **Temperature Non-Uniformity**, and then **Next**.
 - 9.1.2. Turn on the TVK, insert the probe into the indicated wells, insert the TVK USB plug into the port by the screen, and touch **Start Test**.
 - 9.1.3. The instrument will prompt you to move the TVK probe to the next two zones.
 - 9.1.4. The test results are displayed as a report. Unplug the TVK USB cord from the instrument, insert a USB drive into the instrument, and touch **Export to USB** to copy the report. Touch **Cancel** to return to the Verify Block Temperature menu.
 - 9.2. Open the test reports with Microsoft Word to print the reports or save them as PDFs.
 - 9.3. If an instrument fails any test, repeat the test. If an instrument fails a test repeatedly, contact the Life Technologies service department. Label the instrument as "out of order" until serviced.
10. **Annual Test**
 - 10.1. The annual **Temperature Verification Test** checks the calibration of the three temperature zones in the block. The test takes ~ 75 minutes (three 25-minute tests).
 - 10.1.1. Touch **Settings**, then **Maintenance and Services**, then **Block Verification Test**, then **Verify Block temperature**, then **Temperature Verification**, and then **Next**.
 - 10.1.2. Turn on the TVK, insert the probe into the indicated wells, insert the TVK USB plug into the port by the screen, and touch **Start Test**.
 - 10.1.3. The test results will be displayed as a report. Unplug the TVK USB cord from the instrument, insert a USB drive into the instrument, and touch **Export to USB** to copy the report. Touch **Cancel** to return to the menu.
 - 10.2. Open the test report with Microsoft Word to print the report or save it as a PDF.
 - 10.3. If an instrument fails the test, repeat the test. If the test fails repeatedly, contact the Life Technologies service department. Label the instrument as "out of order" until serviced.



Forensic Biology Section

Operation of ProFlex Thermal Cycler

11. Performance Check

- 11.1. Performance checks are done on instruments that have already had an internal validation.
- 11.2. Performance checks are done when an instrument has had service performed on it, when an instrument is reallocated from one function to another (e.g., moved from database work to casework), or a new instrument is purchased (for a make and model of instrument that has been previously validated).
- 11.3. For a new instrument, the monthly, quarterly, and annual QC checks should be run on the instrument before it is approved for use. All the QC checks for the instrument should “pass”. This establishes the baseline performance of the new instrument. A Positive Control, Negative Control, and a sample of known DNA profile can also be run on the new instrument (see below).
- 11.4. For an instrument that has had service or is being reallocated from one function to another, a Positive Control, Negative Control, and a sample of known DNA profile should be amplified (preferably with the same samples run in duplicate on another qualified instrument) and then run on a genetic analyzer. The expected profiles should be obtained from each sample. This confirms the instrument is still performing as expected.