



Forensic Biology Section

Operation of 9700 Thermal Cyclers

1. **Scope**

- 1.1. To provide instructions for the operation of the GeneAmp PCR System 9700 thermal cyclers, which are programmable heating and cooling blocks with heated covers that are capable of holding up to 96 samples. The thermal cyclers automate the Polymerase Chain Reaction (PCR) technique for DNA profiling.
- 1.2. The PCR time and temperature profiles are stored in programs, which specify how the instrument should heat and cool samples in a PCR cycle.
- 1.3. The 9700 methods are created using a graphical interface to set temperatures, times, ramp rates and cycle numbers.
- 1.4. Regularly scheduled maintenance and system checks must be performed to verify that the thermal cycler meets temperature accuracy specifications, and to test the temperature uniformity of the sample block. Accuracy in temperature and block uniformity is essential in providing a high-quality amplification product.

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**

- GeneAmp PCR System, model 9700 (Applied Biosystems)
- Temperature Verification System (Digital thermometer with RTD probe).

4. **Quality Assurance**

- 4.1. The thermal cyclers are 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. The sample block calibration temperature indicated by the machine and a NIST-traceable thermometer must be within $\pm 0.75^{\circ}\text{C}$ of the set point. The sample block temperature uniformity range indicated by the NIST-traceable thermometer must be less than 1.0°C .
- 4.4. A performance check consists of a temperature non-uniformity test and should be run at least annually and after a thermal cycler is serviced, repaired, or there is concern as to its proper functioning. Annual is defined as not less than six months and not greater than eighteen months.
- 4.5. The monthly temperature calibration verification test does not need to be run when the quarterly temperature non-uniformity test is run.

5. **Creating a Program**

- 5.1. At the main menu (RUN, CREATE, EDIT, UTIL, and USER), choose CREATE to create a new method from the default method.



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- 5.2. The default method values are 5-minute hold at 94 °C; 25 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds; 7 minute hold at 94 °C; 4 °C hold until the program is manually stopped.
- 5.3. Use the arrow keys to highlight the temperatures and times which need to be changed, deleted, or added.
- 5.4. Cycling and ramp parameters can be modified by highlighting the parameter, pressing the MORE key, then pressing the MODIFY key.
- 5.5. Press STORE to save with new name.
6. **Changing a Program**
 - 6.1. At the main menu (RUN, CREATE, EDIT, UTIL, and USER) choose EDIT to change an existing method.
 - 6.2. Select the method to be edited, press EDIT to view the parameters, and change, delete or add to the parameters of the method by using the arrow keys to highlight the different temperatures and times and entering new values.
 - 6.3. Press STORE to save with new name.
7. **Amplifying Samples in the Thermal Cycler**
 - 7.1. The heated lid of the instrument needs several minutes to reach operating temperature after the instrument is turned on.
 - 7.2. Place a Micro Amp tray of samples in the block with the A1 well in the upper left hand corner.
 - 7.3. Slide the cover forward over the samples and press the lever down to seat the tubes into the block.
 - 7.4. Press RUN and select a program with the arrow keys. Press START to begin running the program. The Reaction Volume and Ramp Speed will be displayed for verification, press START to begin the run.
 - 7.5. After the heated lid reaches operating temperature, the run will begin, and a Run Time screen will display the current step and cycle. Pressing the INFO key will display the User, program name, start time, end time, and reaction volume.
 - 7.6. When the program has finished, press the STOP key, lift the lever on the lid and slide the heated cover back.
 - 7.7. Grasp sample tray with both hands and gently rock it back and forth or gently twist each tube to release tubes from sample wells. Lift the tray out of the instrument and place in a base plate.
 - 7.8. At the end of the run, press the STOP key twice, then press EXIT, and the main menu will appear. Press the power switch to turn the instrument off.



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8. Cleaning the Thermal Cyclers

- 8.1. The sample wells and sample block cover should be cleaned approximately once a month.
- 8.2. If the thermal cycler has been run recently, allow the heated cover time to cool off (at least 30 minutes).
- 8.3. Slide the cover back approximately 1/3 of the way to the rear (where there are notches on the rail) and tilt the cover back to access the underside of the heated cover.
- 8.4. Wipe the bottom of the cover and the top of the sample block wells with a kimwipe soaked in 10% bleach to remove any amplified product.
- 8.5. Clean the wells with a cotton swab soaked in 10% bleach to remove any amplified product.
- 8.6. Clean the wells with a cotton swab soaked in ethanol to remove bleach residue and marker from the inside of the wells.
- 8.7. Soak a kimwipe with ethanol and wipe off any residual bleach from the block and the cover but make certain all of the ethanol has completely evaporated before turning the instrument back on.

9. Temperature Calibration Verification Test (monthly)

- 9.1. Start with the instrument off. Open the thermal cycler cover and clean well A6 with ethanol on a cotton swab.
- 9.2. Place the probe assembly into well A6, position the probe wire out the front end of the instrument, and slide the heated cover forward and close the cover, taking care not to crimp the wires between the cover and the sample block.
- 9.3. Turn on the digital thermometer by pressing the "ON-OFF" key (if the battery is low, the "LO BAT" indicator will appear on the display).
- 9.4. Turn on the thermal cycler and press the UTIL key so the Utility menu appears.
- 9.5. Press the DIAG key so the diagnostic menu appears.
- 9.6. Press the TMP VER key so the temperature verification menu appears.
- 9.7. Press the **TEMP** key to choose the Temperature Verification test and press RUN.
- 9.8. The temperature of the sample block and heated cover will go to 85 °C. The display will show the current temperature of the block cover and the sample block.
- 9.9. When the temperature of the cover is within 10 °C of the sample block temperature, the display will read "Stabilizing at setpoint..." and will count down 3 minutes.
- 9.10. When the clock reaches zero, the display will prompt the user to enter the temperature displayed on the digital thermometer. Enter the digital thermometer's value into the thermal cycler using the keypad and press ENTER.
- 9.11. Next, the temperature of the sample block and heated cover will go to 45 °C. The display will show the current temperature of the heated cover and the sample block.



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- 9.12. When the temperature of the cover is within 30 °C of the sample block temperature, the display will read "Stabilizing at setpoint..." and will count down 3 minutes.
- 9.13. When the clock reaches zero, the display will prompt the user to enter the temperature displayed on the digital thermometer. Enter the digital thermometer's value into the thermal cyclers using the keypad and press ENTER.
- 9.14. The display will show the input temperatures for 85 °C and 45 °C. Press ACCEPT to store these values in the thermal cycler's memory.
- 9.15. The instrument displays "Calibration is Good" if the block average is within 0.75 °C above or below its programmed target temperature. Press EXIT to end.
- 9.16. If the block average is more than 0.75 °C above or below its programmed target temperature, repeat the test. If the thermal cycler fails repeatedly, the instrument needs to be recalibrated by a manufacturer's Service Representative. Label the instrument as "out of order" until serviced.
- 10. Temperature Non-Uniformity Test (quarterly and performance check)**
 - 10.1. Start with the instrument off. Open the thermal cycler cover and clean all the wells in sample block rows A, C, F and H with ethanol on a cotton swab.
 - 10.2. Place the probe assembly into well A1. Position the probe wire out the front end of the instrument. Slide the heated cover forward and close the cover, taking care not to crimp the wires between the cover and the sample block.
 - 10.3. Turn on the digital thermometer by pressing the "ON-OFF" key (if the battery is low, the "LO BAT" indicator will appear on the display).
 - 10.4. Turn on the thermal cycler and press the UTIL key so the Utility menu appears.
 - 10.5. Press the DIAG key so the diagnostic menu appears.
 - 10.6. Press the TMP VER key so the temperature verification menu appears.
 - 10.7. Press the TNU key to choose the Temperature Non-Uniformity test and press RUN.
 - 10.8. The temperature of the sample block will go to 94 °C and the heated cover will remain at 37 °C. The display will show the current temperature of the block cover and the sample block.
 - 10.9. After the temperature of the block reaches 94 °C, the instrument will ramp to 37 °C. When the instrument reaches 37 °C, the display will read "Stabilizing at setpoint..." and will count down 30 seconds.
 - 10.10. When the clock reaches zero, the display will prompt the user to enter the temperature displayed on the digital thermometer. Enter the digital thermometer's value into the thermal cyclers using the keypad and press ENTER.
 - 10.11. The instrument prompts the user to move the probe to the next well. Move the probe to the indicated well and press RUN.



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- 10.12. Repeat the steps for the prompted wells, entering the values into the thermal cycler using the keypad.
- 10.13. After all the wells have been tested at 37 °C, the cover will heat to 94 °C, and all the wells tested at 37 °C will now be tested at 94 °C. At each step the display will prompt the user to enter the temperature displayed on the digital thermometer. Enter the digital thermometer's value into the thermal cycler using the keypad and press ENTER.
- 10.14. At the conclusion of the test, a summary screen appears. Record the temperatures on the Temperature Uniformity Form at this time. The "TNU performance" screen appears, indicating if the instrument is within performance expectations.
- 10.15. Record the +/- value of the temperature non-uniformity test. The instrument passes if the highest and lowest readings for the temperatures differ by 1 °C or less.
- 10.16. If the temperature non-uniformity range is more than 1 °C, repeat the test. If the thermal cycler fails repeatedly, the instrument needs to be recalibrated by a manufacturer's Service Representative. Label the instrument as "out of order" until serviced.