



FOOD SAFETY LAB / MILK QUALITY
IMPROVEMENT PROGRAM

Standard Operating Procedure



Title: **Thermal Cycler Calibration Verification and Temperature Non-Uniformity Testing**

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***Thermal Cycler Calibration Verification and
Temperature Non-Uniformity Testing***

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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this SOP is to detail the protocols for performing two temperature diagnostic tests on the lab's 6 Applied Biosystems 2720 Thermal Cyclers: the Calibration Verification Test and the Temperature Non-Uniformity test. Passing of these tests indicates that the instruments' internal thermometers are correctly calibrated and that the instruments' Peltier elements are in good working order.

1.2 Scope

This SOP may be used by the lab member(s) assigned to thermocycler maintenance.

1.3 Definitions

Calibration Verification Test: This test verifies the temperature calibration of the thermal cycler by measuring the stabilized temperature of well A6 at 45°C and 85°C.

Temperature Non-Uniformity Test: This test verifies the temperature uniformity of the sample block by measuring the stabilized temperatures of 8 specific wells at 55°C and 95°C.

1.4 Safety

Both the heated cover and the sample block of the thermal cycler get hot enough to cause severe burns. Follow the on-screen instructions on the thermal cycler to avoid opening the cover when the sample block is dangerously hot, and be careful not to come into contact with the heated lid plate while the thermocycler lid is open.



SECTION 2 MATERIALS

- An ABI 2720 thermal cycler
- Cotton swabs
- Mineral oil
- Perkin-Elmer 0.2-mL Sample Block Module Temperature Verification Kit:
 - Temperature verification frame
 - 0.2-mL probe assembly
 - Omega 869C thermometer
- Record sheets



SECTION 3 PROCEDURES

1. Obtaining the Temperature Verification Kit

1.1. The BRC is in possession of this kit. To borrow it from them, send a request in an email to the genomics lab manager, at BRC_genomics@cornell.edu. Alternatively, you may ask for it in person at the DNA sequencing lab in 147 Biotech.

1.1.1. The temperature probe has the appearance of two 0.2 mL tubes. The tube with the black tip contains the sensor, and this is the part of the probe that must be inserted in the well specified later on.

2. Performing the Calibration Verification Test

2.1. Power on the thermal cycler to be tested. Open the cover and remove the plastic rack, if present.

2.2. Coat well A6 of the sample block with mineral oil using a cotton swab.

2.3. Place the sensor in well A6 of the sample block, with the second tube in well B6.

2.4. Place the temperature verification frame into the sample block over the thermometer probe and wire, guiding the wire beneath the sample frame and through the channel cut in the front edge of the sample frame. It is crucial that the wires leading to the probe are beneath the sample frame and exit through the channel to prevent their damage.

2.5. Press down firmly on both the sensor and on the temperature verification frame to ensure that they are seated securely in the sample block.

2.6. Close the thermal cycler lid and pull the lever down.

2.7. Turn the switch on the left side of the digital thermometer from “OFF” to “200.” The switch should be in the center position, and the readout should now display the current temperature of the sample block.

2.8. From the thermal cycler Main Menu, press F4 to open the Utilities submenu.

2.9. Press the F1 key to open the Diagnostics submenu.

2.10. Press the F3 key to open the Temperature Verification submenu.

2.11. Press the F1 key to open the Calibration Verification routine.

2.12. Press the F1 key to begin the test.



- 2.13. Both the block and the cover will heat to 85°C. Once both have reached their setpoints, an on-screen countdown of 3:00 will begin.
- 2.14. When the time is up, the countdown will be replaced by a prompt to “Enter actual block temperature.” Read the thermometer at this point, enter the value in the correct field on the paper record sheet, and use the numeric keys to enter the value into the thermal cycler. Once you have entered the value, press the Enter key.
 - 2.14.1. It is not necessary to enter the decimal point. To enter a value of 45.1, one would key in “451” followed by the Enter key.
- 2.15. The system automatically begins the second temperature reading at the 45°C setpoint. Repeat step 2.14 for the second temperature reading.
- 2.16. Power off the digital thermometer.
- 2.17. At the conclusion of the test, a summary screen will appear displaying the entered values. If these values match those written on the record sheet, press F1 to accept.
- 2.18. The results will be displayed on the screen:
 - 2.18.1. If the thermal cycler is properly calibrated, the message will read “Calibration is good.” This means the instrument passed.
 - 2.18.2. If the thermal cycler is not properly calibrated, the message will read “Instrument may require service. Contact Applied Biosystems Technical Support.” This means the instrument failed.
- 2.19. Write whether the instrument passed or failed in the proper field on the record sheet.
- 2.20. Press the F5 key to exit the Calibration Verification test.

3. Performing the Temperature Non-Uniformity Test

- 3.1. Open the lid of the thermal cycler, remove the temperature verification frame, and then remove the temperature probe.
- 3.2. Use a cotton swab to coat wells A1, A12, C4, C9, F4, F9, H1, and H12 with mineral oil.
- 3.3. Place the sensor in well A1 of the sample block, with the second tube in well A2.
- 3.4. Repeat steps 2.4-2.7.
- 3.5. From the Temperature Verification submenu, press the F2 key to start the Temperature Non-Uniformity Test.



- 3.6. Press the F1 key to begin the test.
- 3.7. The sample block will heat to 95°C, then cool and stabilize at a setpoint of 55°C. Once it has, a 0:30 countdown will begin.
- 3.8. Repeat step 2.14.
- 3.9. The instrument screen will prompt you to move the probe to well A12. To do so, first repeat step 3.1. Place the sensor in well A12 and the second tube in well A11. Repeat steps 2.4-2.6.
- 3.10. Press the F1 key to test well A12. Once again, the sample block will heat to 95°C, then cool and stabilize at a setpoint of 55°C. Once it has, a 0:30 countdown will begin.
- 3.11. Repeat step 2.14.
- 3.12. Repeat steps 3.9-3.10 for wells C4, C9, F4, F9, H1, and H12, as prompted by the instrument screen.
- 3.13. The thermocycler will now begin testing the 95°C setpoint. You will be prompted to move the probe back to well A1. Do so by repeating step 3.9.
- 3.14. Repeat steps 3.6 to 3.12, following the prompts on the instrument screen, for the 95°C setpoint.
- 3.15. Once the final temperature value has been entered into the thermal cycler, power off the digital thermometer.
- 3.16. At the conclusion of the test, a summary screen appears displaying all entered temperatures. Verify that they are correct from the written record sheet. Press the F1 key to accept these values.
- 3.17. The results will be displayed on the screen:
 - 3.17.1. If “Pass” appears after both setpoint temperatures, then the instrument passed.
 - 3.17.2. If “Fail” appears after one or both of the setpoint temperatures, then the instrument failed.
- 3.18. Write the TNU values at both setpoints and whether that setpoint passed or failed on the record sheet.
- 3.19. Press the F5 key to exit the Temperature Non-Uniformity test.
- 3.20. Repeat step 3.1



4. Repetition

- 4.1. If any thermal cycler fails either test, repeat that test to ensure repeatability and a lack of operator error in its performance.
- 4.2. Confirmed failures should be brought to the attention of the lab manager, who will schedule the affected thermal cycler(s) for maintenance.
- 4.3. Repeat steps 2-3 for all other thermal cyclers.

5. Cleanup

- 5.1. Use one or more cotton swabs to remove the mineral oil from all tested sample wells.
Replace the plastic frame and close the thermal cycler lid.
- 5.2. Carefully remove the mineral oil from the temperature probe.
- 5.3. Return the digital thermometer, temperature probe, and temperature verification frame to their carrying case. Make sure the digital thermometer is turned off.
- 5.4. Return the Temperature Verification Kit to the BRC Sequencing Lab.



SECTION 4

TROUBLESHOOTING

- Any issues with the Temperature Verification Kit should be brought to the attention of the BRC staff.
- Failure of the two tests described above cannot be remedied by user service. Do not attempt to perform any repairs on the thermal cyclers.



SECTION 5

REFERENCES

- https://tools.thermofisher.com/content/sfs/manuals/cms_041483.pdf
- http://tools.thermofisher.com/content/sfs/manuals/cms_041173.pdf
- In-person consultation with Thomas Stelick, the BRC genomics laboratory manager



SECTION 6

METHOD VERSION & CHANGES

VERSION	DATE	EDITOR	COMMENTS
Version 1	01/13/2016	Sam Reichler	Original SOP
Version 2	04/21/2020	Jordan Skeens	Updated to 04/2020 SOP template Title page: updated the SOP# to 5.4.2 Section 1.1: Removed reference to specific lab manager and updated contact email