



FOOD SAFETY LAB / MILK QUALITY IMPROVEMENT PROGRAM
Standard Operating Procedure

Title: **Thermal Cycler Use**

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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to explain how to sign up for and correctly use the six Applied Biosystems 2720 Thermal Cyclers in Room 356D of the Food Safety Laboratory.

1.2 Scope

This SOP applies to members of the Food Safety Laboratory, the Milk Quality Improvement Program Laboratory, the Laboratory for Molecular Typing, and the Worobo Laboratory, as well as members of other laboratories using the thermal cyclers and visitors.

1.3 Definitions

Thermal cycler (also known as a thermocycler, PCR machine or DNA amplifier): a laboratory apparatus most commonly used to amplify segments of DNA via the polymerase chain reaction (PCR). Thermal cyclers may also be used in laboratories to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. The device has a thermal block with holes where tubes holding the reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps.

Sample block: The wells on the instrument that hold the reaction tubes during thermal cycling. The block houses an internal Peltier heating/cooling unit. The sample block is made of aluminum to provide optimal thermal transfer rate.

96-well tray: Distinct from a PCR rack, this tray fits onto the thermal cycler block and positions individual or strip 0.2 mL reaction tubes to provide the thermal contact necessary for thermal cycling.

Film compression pad: Foam rubber pads that are placed on top of a 96-well reaction plate sealed with adhesive film during thermal cycling. The pads support a proper thermal seal between the thermal cycler and reaction plates when using adhesive films. Without the pads, the volume of the reaction may decrease due to evaporation.

1.4 Safety



Both the heated cover and the sample block of the thermal cycler get hot enough to cause severe burns. Avoid opening the cover when the sample block is dangerously hot, and be careful not to touch the heated lid plate while the thermocycler lid is open.

Full details on instrument safety can be found in the instrument user guide, pp x-xxii. The user guide is located on the Food Safety wiki as an addendum to this SOP.

Centrifuges must be properly balanced to operate safely. If you have not been trained on the laboratory centrifuges, refer to the Lab Responsibilities list to learn who will train you.

SECTION 2 MATERIALS

- Reaction to be thermocycled, in one of the following formats (all supplies are available in room 358B):
 - Individual 0.2 mL reaction tubes with caps
 - 0.2 mL reaction strip tubes with caps
 - 96-well reaction plate
- 96-well tray, if using individual or strip reaction tubes (room 356D)
- Film compression pads, if using 96-well reaction plates (room 356D)
- Mini centrifuge, if using individual or strip reaction tubes (room 356D, and around the lab)
- Bucket centrifuge, if using 96-well reaction plates (room 350C)



SECTION 3 PROCEDURES

3. Thermal Cycler Use

a) Preparation

i) Six ABI 2720 thermal cyclers are available for use, and are referred to by nickname: Belle, Bubba, Chocolate Thunder, Thelma, Thing One, and Totoro.

ii) It is recommended that you sign up in advance to use a thermal cycler, particularly if you wish to use a specific instrument. Many lab members regularly use the thermal cyclers, and failure to sign up may result in no available instruments when you wish to run your reaction.

(a) Sign up for the time you need on the instrument you plan to use by writing your name and the start and end time of your use on the white board in room 356D.

iii) It is important to centrifuge all reactions that will run in the thermal cycler before placing them in the instrument. This concentrates all liquid in the bottom of the tube, and eliminates air bubbles. Both of these factors could compromise the success of the reaction.

(a) For reactions in 0.2 mL individual or strip reaction tubes, spin briefly in the mini centrifuge fitted with the 0.2 mL tube adapter, located next to the thermal cyclers.

(b) For reactions in 96-well reaction plates, spin for 3 minutes at 4,000 rpm and at 4° C in the bucket centrifuge.

(c) For both centrifuges, be sure to balance the load to avoid accidents and failure.

b) Starting the Instrument

i) The power switch on the thermal cycler is located on the rear of the instrument, above the power cord receptacle. Switch the instrument on. You should hear the whirring of the fan.

ii) The startup screen will appear. After several seconds, this will be replaced by the Main Menu.



c) Selecting a User

- i) From the Main Menu press F5 (User). The Select User Name screen appears.
- ii) Use the arrow keys to highlight the desired user. Press F1 (Accept) to return to the main menu.

d) Adding Yourself as a User (optional)

- i) In many cases, the protocol you need to run will already be programmed onto the instrument under an existing user. In this case, you do not need to add yourself as a user. If you will be adding many new protocols and wish to add yourself as a user, and the instrument has available user name slots, you may do so. If there are no name slots available, check with Sherry or Martin before removing an existing user to ensure that the protocols stored there are no longer needed.
- ii) From the Main Menu press F5 (User). The Select User Name screen appears.
- iii) Press F2 (New). The User Name screen appears.
- iv) Spell out your name by using the arrow keys to move the highlighter at the right of the screen to the first letter of your name, then press Enter, then highlight the second letter and press Enter, etc. When you have finished entering your name (up to six characters), press F1 (Accept) to save it.
- v) The Security Code screen appears. Press F5 (Cancel).
- vi) The Select User Name screen appears. With your name highlighted, press F1 (Accept) to return to the Main Menu.

e) Loading Samples into the Instrument

- i) Raise the lever to disengage and raise the heated cover.
- ii) Loading the samples depends on the format of your reactions:
 - (1) For 0.2 mL individual or strip reaction tubes, make sure that the 96-well tray is in place. Place the tubes in any location on the sample block, pressing down on the caps to make sure the tubes are firmly seated.
 - (2) For 96-well reaction plates, remove the 96-well tray from the sample block. Insert the plate into the block so the well marked A1 is in the upper left corner of the block, and the notched corner of the plate is in the upper right corner of the block.



Press down on the plate to make sure it is firmly seated. **Place 2 film compression pads on top of the plate.**

iii) Pull the lever down to engage the heated cover.

f) Running an Existing Method

i) From the Main Menu press F1 (Run). The Stored Methods screen appears.

ii) Select a method by highlighting one of the displayed methods using the arrow keys.

iii) Press F1 (Start). The Select Method Options screen appears.

iv) Enter the reaction volume using the number keys, then press F1 (Start).

(1) The maximum programmable volume for the thermal cycler is 100 μ L.

v) Wait for the heated cover to reach 103 °C. The Run Time screen displays and the method you selected starts running.

vi) To find out when the run will end, press F4 (Info). The Method Information screen appears. When you are through viewing this screen, press F4 (Return) to redisplay the Run Time screen.

g) Creating and Storing a New Method

i) From the Main Menu press F2 (Create). The Create/Edit screen appears with a default method displayed.

ii) Make a change to the top line to alter the number of holds, cycles, and temperature steps.

(1) For example, use the arrow keys to move the highlighter to the 1 in the upper left corner of the screen. Press 2, then Enter. Notice that you've added a hold (another time and temperature segment) to the graph.

iii) Make changes to the values below the top line to alter the temperatures and times of the program. Use the arrow keys to move the highlighter to a temperature or time on the graph and change the parameter.

(1) For example, move the highlighter to the 5:00 time at the left of the screen in the pre-PCR portion. Press 5 3 0 then press Enter, for 5:30.

iv) To create an indefinite hold at the end of your program, enter the time 99:59 for the final hold.



- v) Auto-incrementing or decrementing temperature control parameters is useful for touchdown PCR and other applications.
 - (1) Use the arrow keys from the Create/Edit screen to select a time or temperature parameter in the PCR segment.
 - (2) Press F1 (Modify). The Select Modification screen appears.
 - (3) Press F1 (AutoX). The AutoX screen appears.
 - (4) Highlight the PCR time or temperature parameter that you want modified when you run your method.
 - (5) Use the numeric keypad to change numeric values. Press F2 (+) or F3 (-) to change the plus or minus signs. The selected sign displays in the current field. After you press Enter or an arrow key, an asterisk (*) appears for parameters that have been modified.
 - (6) Press F1 (Accept) to accept all entries on the AutoX screen and return to the Modify screen.
 - (7) Press F4 (More) to return to the Create/Edit screen.
 - vi) Other advanced features that can be programmed into methods can be found on page 4-9 of the user manual.
 - vii) Press F2 (Store) to save the method. The Store Method on Instrument screen appears.
 - viii) Press F3 (Method). The Method Name screen appears.
 - ix) Press the CE key to clear the field. Then spell out the desired method name by highlighting each letter and pressing Enter after each one.
 - x) Press F1 (Accept). The Store Method on Instrument screen appears again with your method name. On the 2720 Thermal Cycler, a method has a method name, and it is associated with a particular user.
 - xi) Press F1 (Accept). The system saves your method and returns to the Main Menu.
- h) Ending a Run and Powering off the Instrument**
- i) Typical protocols end with an indefinite hold at 4° C, meaning that the run will not end until you instruct it to.



- ii) Press the Stop key. The Confirm Stop screen appears. Press the Stop key again to stop the run. The run stops and the End of Run screen appears. Press F5 (Exit) to return to the Main Menu.
- iii) Power the instrument off using the switch on the back.
- iv) Raise the lever to disengage and raise the heated cover.
- v) Carefully remove your tubes or plate.

SECTION 4 TROUBLESHOOTING

My reaction(s) failed

All laboratory thermal cyclers are verified and tested on a biannual basis. The record of the last test is taped to the top of each instrument, and detailed reports are available in the verification and testing binder on the shelf in room 356D. Before concluding that the thermal cycler is at fault, check your reaction controls. If you still suspect a malfunction with one of the instruments, inform the individual responsible for thermal cycler maintenance (see Lab Responsibilities list). They can use SOP 5.18 on the Food Safety Wiki ([Thermal Cycler Calibration Verification and Temperature Non-Uniformity Testing](#)) to determine whether or not an issue exists.

There was a power failure during my protocol

The thermal cyclers are not normally connected to the building's uninterruptible power supply. If you are concerned about a power outage during your run, there are two red outlets above the thermal cyclers Thing One and Chocolate Thunder that may be utilized. In the event of an unexpected power outage, these outlets may be used for any of the thermal cyclers to resume functioning.

An automated restart function on the thermal cyclers allows for power outages and safe continuation of a PCR run after resumption of power. The instrument does the following in a power failure:

- Restarts or continues the PCR experiment.
- The instrument determines what temperature was being approached, or was holding. Upon resumption of power, it will go to that temperature and count down the time remaining in the hold as soon as the temperature is within the specified clock start limit.

The instrument will not power on

- Check that the GFCI breaker on the power outlet that the thermal cycler is plugged into has not been tripped. If it has been tripped, reset it by pressing the RESET button.
- Check that the power cord is plugged firmly into both the instrument and the wall outlet.



- Inform the individual responsible for thermal cycler maintenance (see Lab Responsibilities list). They can check the instrument fuses, and if this does not resolve the issue they can arrange for professional repair.

The instrument displays an error message, or I'm having a different problem

The troubleshooting section of the ABI 2720 manual, beginning on page 7-1, describes all possible error messages and how to resolve them. You may also inform the individual responsible for thermal cycler maintenance (see Lab Responsibilities list). If they are unable to resolve the issue, they can arrange for technical support or professional repair.

SECTION 5 REFERENCES

[Applied Biosystems 2720 Thermal Cycler User Guide](#)

https://en.wikipedia.org/wiki/Thermal_cycler