

OPERATION OF THE MODEL T100 THERMOCYCLER

The following procedure is intended to accompany the BIOL 3702L laboratory exercises entitled *PCR Characterization of Escherichia coli* and *PCR Characterization of Enterococcus*. The protocol below is specific for the Model T100 Thermocycler (Fig. 1; Bio-Rad Laboratories, Inc.). For additional details regarding the Model T100, the manual can be downloaded from the following URL: <http://www.bio-rad.com/webroot/web/pdf/lsr/literature/10020523.pdf>.

Two thermocyclers are available for these exercises.

- The thermocycler labeled “COOPER_1” should be used by those groups performing the PCR with *Escherichia coli* isolates.
- The thermocycler labeled “COOPER_2” should be used by those groups performing the PCR with *Enterococcus* isolates.

Procedure

- 1) Turn on the thermocycler using the switch in the rear of the instrument. Allow the thermocycler to go through its self-testing protocol. When this has been completed, a screen with four different icons should appear.
- 2) Gently lift the lid of the thermocycler to expose the reaction plate.
- 3) Arrange the PCR tubes evenly across the reaction plate.
- 4) Be sure the green plastic ring surrounding the reaction plate is in the proper orientation. One side is intended for the use of flat-top PCR tubes, whereas the other side is intended for use with dome-cap PCR tubes.
- 5) Gently close the lid of the thermocycler.
- 6) On the screen, gently press the “Saved Protocols” icon. A new screen will appear.
- 7) On the left side of the screen is a list of folders. If the button labeled “Main” is not highlighted in green, gently press it.
- 8) To the immediate right of the green-highlighted “Main” folder button should be a list of pre-entered programs.
 - If using the thermocycler labeled “COOPER_1” to assess *E. coli*, gently press the button labeled “EC-TYPE”.
 - If using the thermocycler labeled “COOPER_2” to assess *Enterococcus*, gently press the button labeled “ENTID&VR”.
- 9) At the bottom of the screen is another series of icons. Gently press the one labeled “RUN”.

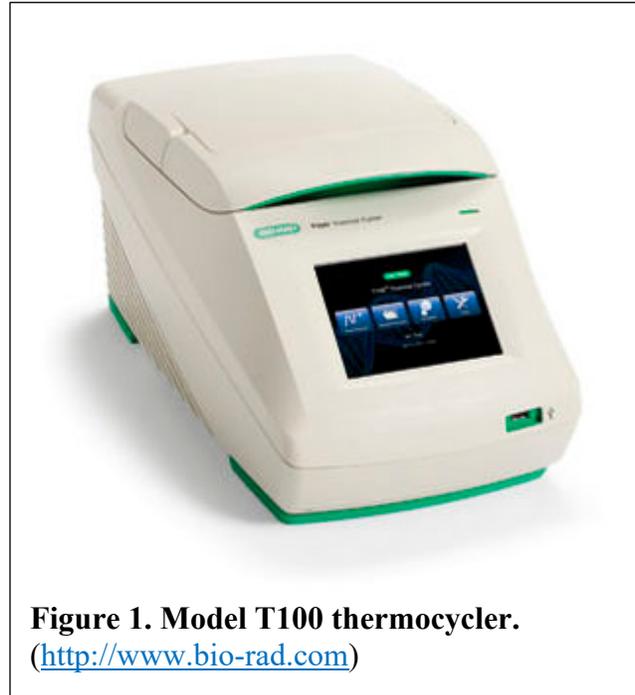


Figure 1. Model T100 thermocycler.
(<http://www.bio-rad.com>)

- 10) A small screen insert will appear. A button indicating the volume of the PCR will be highlighted. The volume for the PCR involving *E. coli* is 20 μ l, whereas the volume for the PCR involving *Enterococcus* is 25 μ l.
 - If the volume is correct, press the button labeled “OK”. This will start the PCR.
 - If the volume is incorrect, press the highlighted volume button. Another keypad-like screen will appear. Enter the correct volume using the keypad numbers. Then press the button labeled “OK”. This will start the PCR.
- 11) The time remaining to complete the PCR is shown at the top of the screen. Typically, program “EC-TYPE” will be completed in 3 hours, whereas program “ENTID&VR” will be completed in 2 hours. Both reactions shall end with the PCR tubes being held at 4°C until retrieved.

Note: The holding time at 4°C is indefinite. However, it is never wise to allow the thermocycler to operate at this temperature longer than necessary. Overnight holds should be strictly limited. Always consult with your laboratory instructor when anticipating an overnight hold at 4°C.

- 12) When the PCR is complete, gently press the icon labeled “CANCEL” at the bottom of the screen. This will terminate the program.
- 13) Gently lift the lid of the thermocycler to expose the reaction plate and the PCR tubes. Remove the PCR tubes. Gently close the lid of the thermocycler.
- 14) These tubes can be placed in the refrigerator (4°C) for short-term (1-2 days) storage or frozen at -20°C for long-term (> 2 days) storage. Alternatively, the PCR products can be processed immediately (e.g., electrophoresis).
- 15) Turn off the thermocycler using the switch in the rear of the instrument.