BERGEN COUNTY | Technical Schools

Stem Cell Lab

Applied Biosystems

9800 Fast Thermal Cycler

Bergen County Technical Schools

Stem Cell Lab

Room 213

Applied Biosystems 9800 Fast Thermal Cycler Information Sheet

The Applied Biosystems 9800 Fast Thermal Cycler is an instrument used in the lab to amplify segments of DNA, using the principles of the Polymerase Chain Reaction (PCR). The instrument consists of two main components, a sample block and a heating cover. Up to 96 0.2mL PCR tubes can be placed into the sample block and be amplified. Once the samples are placed into the appropriate locations, the instrument uses the heating cover and sample block to raise and lower the temperature based on a pre-programmed time and temperature cycle.

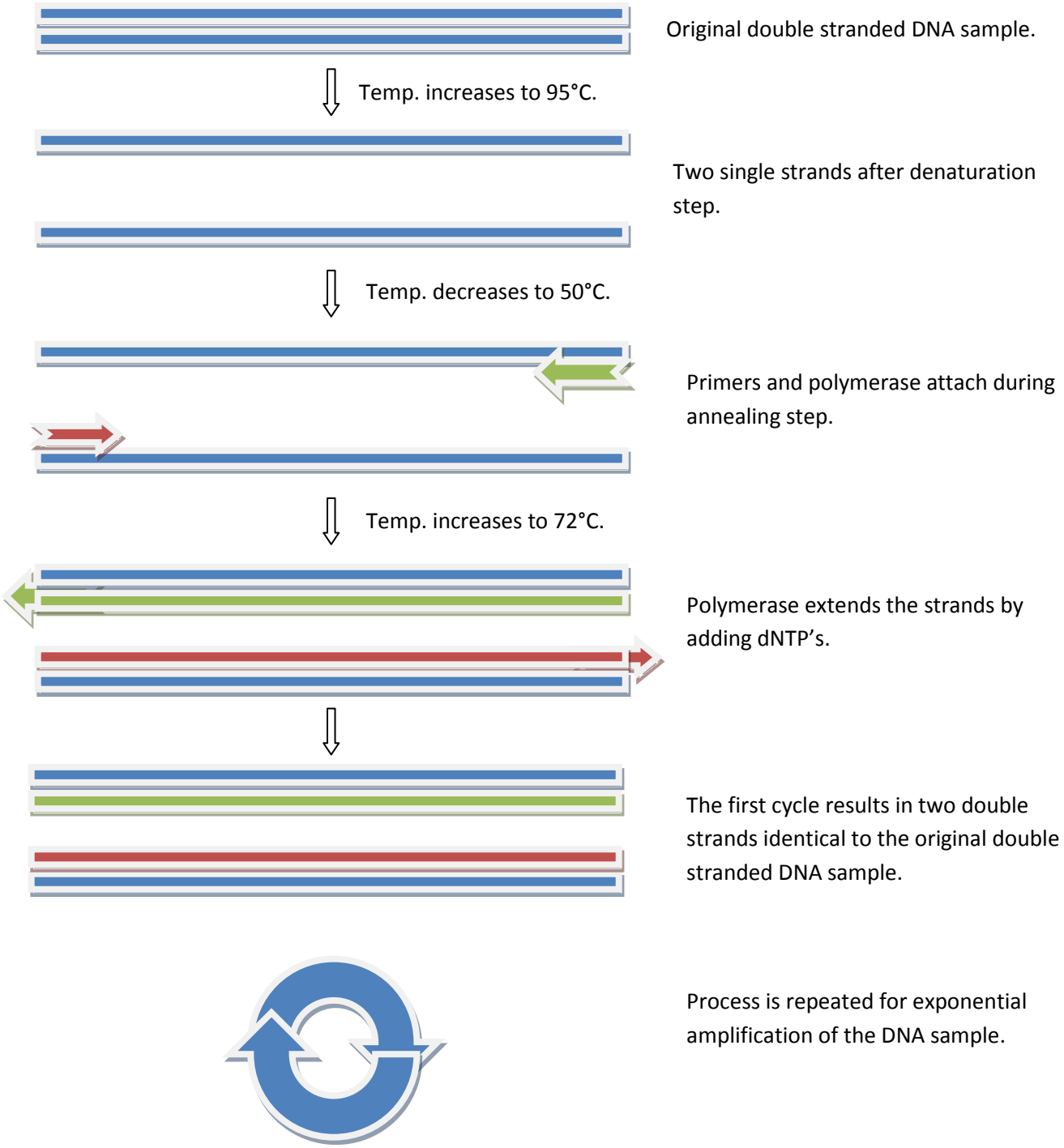
The instrument does not use a computer to program in a PCR cycle, so the instrument and its LCD display are all that is needed to program all time and temperature settings for a user created cycle.

Principles of the Polymerase Chain Reaction

PCR is a process of DNA amplification. By starting with even a single strand of DNA, after 30 cycles, there will be over 1 billion copies of that DNA strand. The reason for this is that during each cycle, the DNA strand will denature, primers will attach, and will then elongate, resulting in a doubling of the initial number of strands. This exponential growth allows for rapid amplification of the DNA.

For a PCR reaction to work, there must be a template strand of DNA, two primers complimentary to the sense and anti-sense strands of the DNA, a DNA polymerase (such as Taq), deoxynucleoside triphosphates (dNTP's), and a buffer. During each cycle, the mixture first goes through the denaturation step, where the temperature is raised to approximately 95°C, and the DNA strands separate from their compliment. During the annealing step, the primers and polymerase bind to the single stranded DNA once the temperature is dropped to approximately 50°C. In the elongation step, the polymerase extends the DNA by adding the dNTP's into the DNA strand. This occurs

when the temperature is raised to approximately 72°C. The cycle is normally then repeated at least 30 times to amplify the DNA.



Original double stranded DNA sample.

Two single strands after denaturation step.

Primers and polymerase attach during annealing step.

Polymerase extends the strands by adding dNTP's.

The first cycle results in two double strands identical to the original double stranded DNA sample.

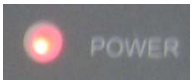
Process is repeated for exponential amplification of the DNA sample.

Applied Biosystems 9800 Fast Thermal Cycler Quick Start Guide

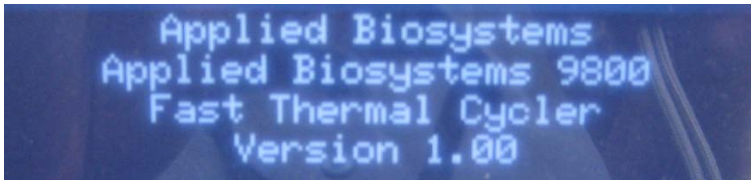
Starting Up the Instrument

1. Turn on the Applied Biosystems 9800 Fast Thermal Cycler by pressing the **Power Button**, located on the front of the instrument.

   a. A **Red Light** will appear under the LCD display when the instrument is powered on.



   b. The instrument will go through a start-up procedure when powered on. The message on the LCD display will read the instruments name and the software version installed.



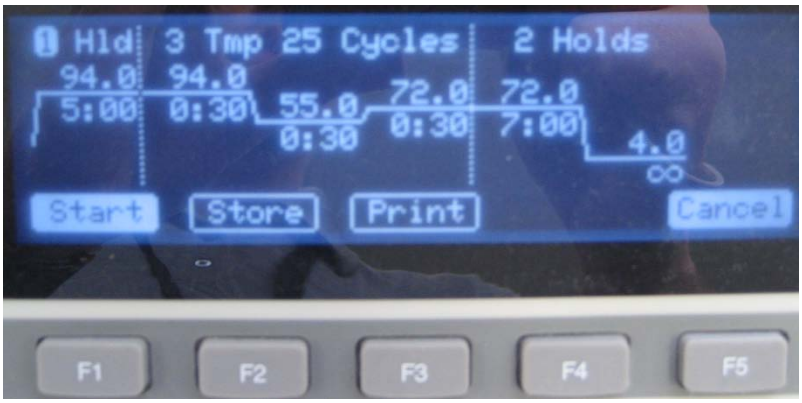
Note: There is not a computer or external software associated with this instrument. Once the initial start-up and self-test are complete, all operations are controlled via the **Keypad** and **LCD screen** of the instrument.

2. Once the instrument is ready to use, the **Main Menu Screen** will appear on the **LCD Screen**.

Creating a PCR Protocol

1. On the *Main Menu Screen*, press the **F1 Button** for **Create**.

2. The next screen that appears has all of the times and temperatures for the run in the form of a chart.



   a. In the first hold section:

      i. Enter the **Number of Holds** that should be performed prior to the run. Press the **Down Arrow**.

      ii. Enter the **Temperature** for each hold that needs to be performed. Press the **Down Arrow**.

      iii. Enter the **Time** for each hold that needs to be performed. Press the **Right Arrow** to proceed to the next section.

   b. In the PCR cycling screen:

      i. Enter the **Number of Temperature Settings** to be used in the cycle. Press the **Right Arrow**.

      ii. Enter the **Number of Cycles** to be performed. Press the **Down Arrow**.

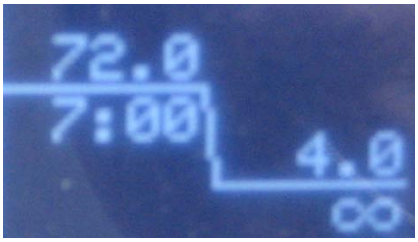
iii. Enter the **Temperature** that will be run for each stage of the cycle, and move to the next by pressing the **Right Arrow**. Press the **Down Arrow**.

iv. Enter the **Time** that each temperature will be run for in each stage of the cycle and move to the next by pressing the **Right Arrow**. Press the **Right Arrow** to proceed to the last section.

c. In the final hold screen:

i. Select the **Number of Holds** to use after the sample has completed the PCR cycle. Press the **Down Arrow**.

ii. Enter the **Temperature** for the final hold, and then press the **Down Arrow**.

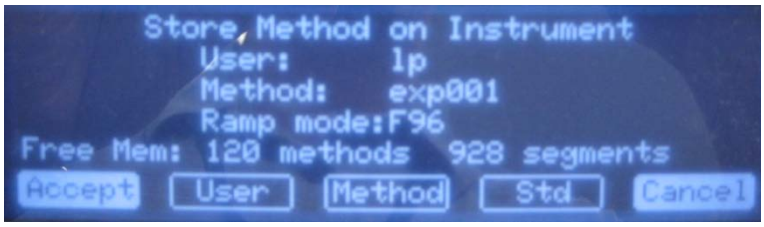


1. For the final hold, if the sample should be kept cool until use, the temperature should be set to 4°C.

iii. Enter the **Time** for the final hold.

1. If the temperature should be kept cold indefinitely until the user removes the samples from the instrument, the time entered should be 99:59, which will show up as ∞.

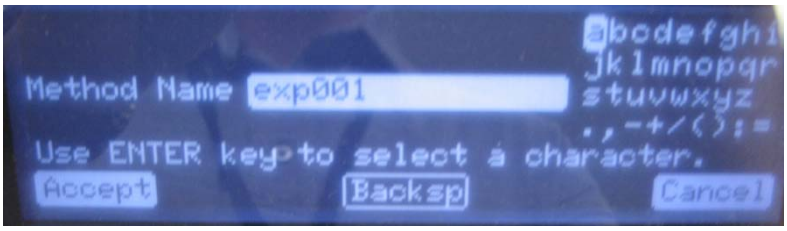
d. Once the entire cycle is established, press the **F2 Button** to **Store** the information in the instrument's memory.



e. Press the **F3 Button** to change the name of the **Method**.

Note: The method name should be something that represents the type of protocol or sample that is being amplified, and possibly the user's initials.

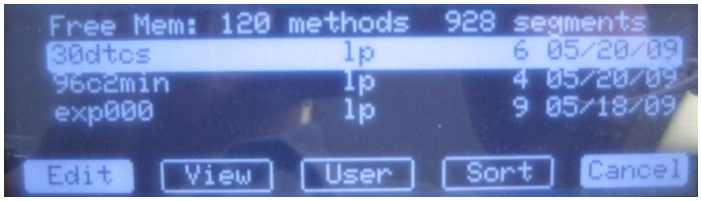
f. Enter the method or protocol name by using the arrows to select letters on the on-screen keypad.



g. Once the method has been defined, press **F1** to **Accept** the information.

Editing a PCR Protocol

1. On the *Main Menu Screen*, press the **F3 Button** to **Edit**.



2. Select the protocol to edit by using the **Up** and **Down Arrows**, and select the correct protocol by pressing the **F1 Button** to **Edit**.

3. Repeat the steps for defining a PCR protocol from the previous section.

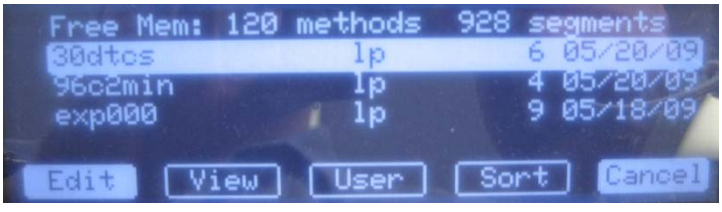
Running a PCR Protocol

1. Place the samples in the **Heating Block** by lifting the lever on the **Sample Heating Lid**, and pushing it back.

   a. The samples can be placed into any of the 96 openings in the heating block.

2. Once samples are placed in the instrument, pull the **Sample Heating Lid** back over the samples, and pull the lever back down.

3. On the *Main Menu Screen*, press the **F1 Button** to **Run**.



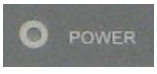
4. Select the protocol to run by using the **Up** and **Down Arrows**, and select the correct protocol by pressing the **F1 Button** to **Start.**

5. Enter the **Volume of the Sample** in the tubes using the **Keypad**, and press **F1** to **Start** the established protocol.
6. The instrument will follow the protocol that was entered. The time it will take the instrument to complete the run will depend on the number of cycles, temperatures, times for each temperature, and length of the holds.
7. When completed, the samples can be removed from the Sample Heating Block by repeating Steps 1 and 2.

Shutting Down the Instrument

1. When the instrument is done being used:
    a. Make sure all samples were removed from the **Heating Block**.
    b. Shut the **Power Off** by pressing the **Power Button** on the front of the instrument.

        i. The **Red Light** under the LCD display will turn off when the instrument is powered off.



        ii. The **LCD Screen** will also turn **Black**.
    c. **Make sure the station is neat before leaving the instrument!**

Applied Biosystems 9800 Fast Thermal Cycler Safety Sheet

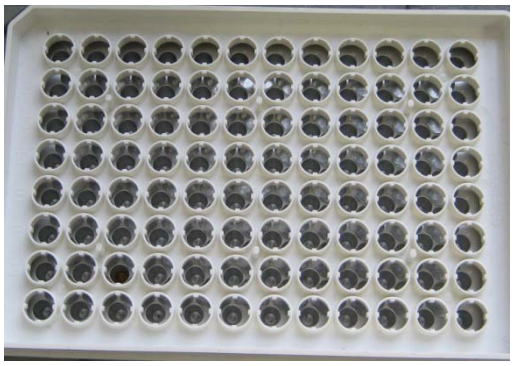
1. Samples should be handled according to good laboratory procedures and methods in order to prevent accidents.
2. When handling or dealing with chemicals or biological samples, be sure to wear appropriate protection (goggles, gloves, lab coat).
    a. Check chemical or sample MSDS sheets to determine the appropriate safety precautions.
3. Dispose of all waste solutions according to waste disposal procedures.
4. Do not remove any panels or cords from the instrument to avoid electrical shock.
5. If any liquid should fall near the instrument, do not operate the instrument. Fluid seepage into internal components creates a potential shock hazard, and can cause the instrument to not work properly.
6. During the instrument's operation, the temperature of the heated cover can be as high as 108°C, and the temperature of the sample block can be as high as 100°C. Before performing the procedure, keep hands away until the heated cover and sample block reach room temperature.
7. To protect yourself against burns, do not open the heated cover or touch the sample block when the word "Hot" is displayed on the screen. This indicates the block temperature is over 50°C.
8. Do not operate the instrument in a Cold Room or refrigerated area. The instrument is intended to be used at temperatures ranging from 15°C to 30°C.
9. Paper or other flammable objects should be kept clear of the heating cover and sample block while the instrument is running to avoid possible fires.
10. Cleaning the workstation around the instrument is necessary. Never attempt to clean any internal spaces of the instrument.
11. Food and drinks should not be placed on or near the instrument.

Applied Biosystems 9800 Fast Thermal Cycler Instrument Information Sheet

9800 Fast Thermal Cycler Instrument Overview

1. Sample Heating Cover
2. 96-well Sample Heating Block
3. Instrument Power Button
4. Instrument LCD Display
5. Instrument Keypad

Sample Block Information



- Sample block capable of running 96 0.2µL PCR sample tubes.

Instrument Keypad Information

1. Function Keys
2. Stop Thermal Cycling Button
3. Power On/Off LED Light
4. Numeric Keypad
5. Directional Movement Buttons