APPENDIX IV

THE THERMO-CYCLER USED IN AMPLIFICATION IS GENE AMP PCR SYSTEM 9600

To use this machine for amplification, create the Method and the program for the experiment.

Creating a Method:

A method is a series of up to 17 programs linked together.
For example, you can link a HOLD program to a CYCL program and conclude you experiment. Access the main menu.

> Select option   9600
> RUN-CREATE-EDIT-UTIL

1.   Press the **OPTION** key to select CREATE, then press **ENTER**, the following display appear

> Create program
> HOLD-CYCL-AUTO-METH

2. Press the **OPTION** key to select METH, then press **ENTER.** The following display appears.

> Link progs: _ _
> _ _ _ _ _ _ _

3. Type the program numbers of the programs you want linked. After you type each number, press
    ENTER. For example, to link programs 1,3,and 5, your display would look like this:

> Link progs: 1–
> 2- 3- 5- – –

4. Press **STEP** to move to the next display. If you are linking seven or more programs in a method, the following display appears:

```
  _  _  _  _
_  _  _  _  _
```

   This display let you link up to ten additional programs in the method.
   NOTE: You cannot link a method.

5. Type the program numbers of the programs you want linked. After you type each number, press **ENTER**.

6. Press **STEP** to move to the next display.

```
Meth # ???          25.6 °C
RUN-STORE-PRINT-HOME
```

   This display allows you to run, store, or print the method.

7. Press **OPTION** key to move the cursor beneath **STORE**, then press **ENTER**. The following display appears:

```
Store
Enter program #  x
```

   X is the next available number.

8. Press **ENTER** to store the method under the displayed number (1-150), the following display appears:

```
Store
Protect program?  NO
```

   Now your method stored, so you need to program your programs which linked in your method To do this follow the steps as following:

Creating a program:

1. Access the main menu.

> Select option   9600
> RUN-CREATE-EDIT-UTIL

2. Press the OPTION key to select CREATES, then press **ENTER.**
    The following display appears:

> Create program
> HOLD-CYCL-AUTO-METH

> Press the **OPTION** key to select the type of program – HOLD, CYCL or AUTO
> to create program, then press **ENTER.**

3. Specify the thermal cycling parameters for the program.
    The last display to appear allows you to run, store, or print the program. For
    example, this Display appears at the end of a CYCL program
    Program type CYCL   Program Number (not yet assigned)

> CYCL # ???   xx. XC
> RUN-STORE-PRINTHOME

4. In the most cases, you will want to store the program and assign it a number
    so that it can be run at any time. Use the following procedure to store the program

Storing the program:

1. Press the **OPTION** key to move the cursor beneath **STORE**, then press **ENTER.**
    The following display appears:

> Store
> Enter program # x

> The number ''x'' is the next available number. If this is the first file being stored,
> "x" would be a "1" on the display. Up to 150 programs can be stored.

2. Press ENTER to store the method under the displayed number, OR
   To use a different number, type the number (1-150), then press ENTER.
   The following display appears:

> Store
> Protect program? N<u>O</u>

3. The above display gives you the option of protecting the file under a user I.D.
   By doing so, the file cannot be overwritten or deleted unless the user number is
   entered. Select YES or NO by pressing the **OPTION** key, then press **ENTER**.
   [Always selects NO unless if you want to keep it forever and will be under your I.D.
   number].

CYCLE: The Cycle Program

A CYCL program contains the thermal ramp and hold segment patterns for PCR cycling.
It contains up to nine set-points. Each set-point comprises a ramp segment and a hold
segment for the specific target temperature. The pattern can be repeated up to 99 times.
Typically there are two or three set-points in a CYCL program. A complete cycle
typically required less than two to four minutes.
The Displaying in a CYCL Program: example for displays CYCL program

> <u>3</u> Temperature
> PCR

> Setpt #1 Ramp 0:0<u>0</u>
> 94.0C   Hold 0:30

> Setpt #2 Ramp   0:0<u>0</u>
> 55.0C   Hold 0:30

> Setpt #3 Ramp 0:0<u>0</u>
> 72.0C   Hold   0:30

> Total cycles = 35
> Pause during run? NO

Adjust your program temperature and number of cycles according your experiment protocol by following the table above. Store your programs as usual under the same number you create.