BIO·RAD

iCycler Thermal Cycler Gradient Feature

The iCycler Thermal Cycler now offers a temperature gradient option for optimization of assay conditions. The gradient feature is available when using the 96 well, 200 µl sample block. Gradients may be programmed at any step of any cycle of the protocol. The gradient option allows input of a temperature gradient of 1–25 degrees in total temperature span at temperatures as low as 40 °C and as high as 99 °C.

Thermal Gradients in the iCycler are formed from the front to the rear of the sample block resulting in eight specific temperatures for assay optimization. This innovative design allows for evaluation of the maximum assay components at each specific temperature. Each row includes twelve wells for evaluation of assay components such as $MgCl^{++}$ or primer concentrations.

The temperature gradients formed by the iCycler take a non-linear yet highly reproducible shape that allows for excellent prediction of actual sample temperatures at each sample row. Extensive validation of the gradient temperatures on multiple sample reaction blocks indicate that the non linear gradient is both predictable and reproducible.

Precision temperature ramping rates ensure that each sample well achieves set temperatures, including gradient and uniform set temperatures, at the same time. This is critical to the objective evaluation of the variety of temperature and assay conditions.

Programming a Temperature Gradient

Temperature Gradients may be specified when editing a sample protocol. **Temp Gradient** is an option available when a temperature field is highlighted and the **F3-Option** is selected. (Figure 1.0)
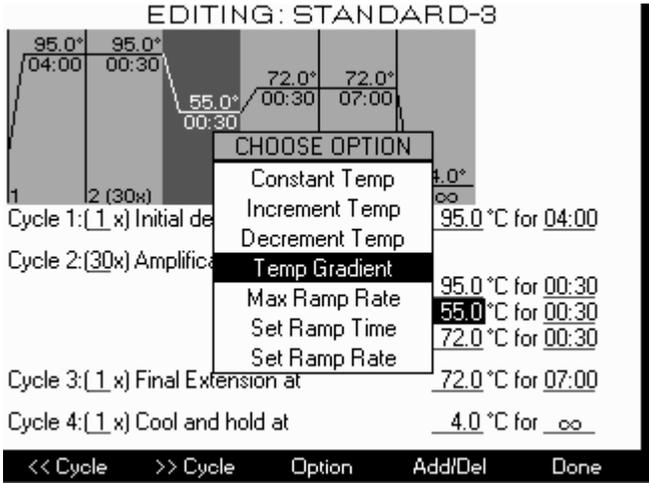

Fig. 1.0. Edit Options: Temp Gradient.

Once **Temp Gradient** is selected, the default minimum gradient of 1° from first to second temperature is displayed. Edit the temperature fields for the minimum and maximum temperatures required. The display will update with the expected temperatures for each row of samples. Thermal Gradients in the iCycler thermal cycler are formed from the front to the rear of the sample block resulting in eight specific temperatures. Row A is the hottest temperature specified and Row H is the coolest temperature specified. Values for each sample row are displayed in the editing screen and will be stored in the run and validation reports.

Note that if the first temperature is a higher value than the second temperature an error message is displayed and the second temperature is corrected to the nearest acceptable value. Any input temperature out of the range of acceptable values for gradient will be corrected to the nearest acceptable value. The minimum temperature value for any gradient is 40 °C and the maximum temperature value for any gradient is 99 °C. While input of as little as a 1° temperature gradient is possible, the minimum recommended gradient is 5°. This selection reflects the best achievable accuracy at each row of specific temperatures. The accuracy of the iCycler thermal cycler is +/- 0.3° allowing for a maximum deviation of 0.6° from row to row. (Although extensive validation would indicate the actual variation to be much smaller.) Since there are eight rows of temperatures, 0.6 x 8 rows = 4.8 ° » 5.0° minimum accurate gradient span.
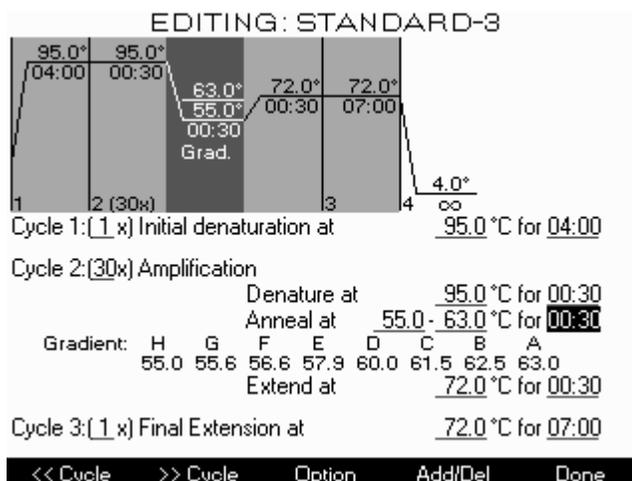

Fig. 1.1. Editing Protocol: Display of gradient temperatures.

Running Gradient Protocols

When a temperature gradient has been indicated, the temperature measurement mode must either be block mode or algorithm mode. Due to the nature of a temperature gradient, sample mode of temperature control is not possible. The following message will flash on the screen and the temperature measurement mode options will again be available for selection.

The temperature gradient will also appear in the graphical presentation of the protocol with the minimum and maximum temperatures and the indication "Grad." This graphical presentation of the protocol appears in the run screen while the protocol is running (fig1.2).
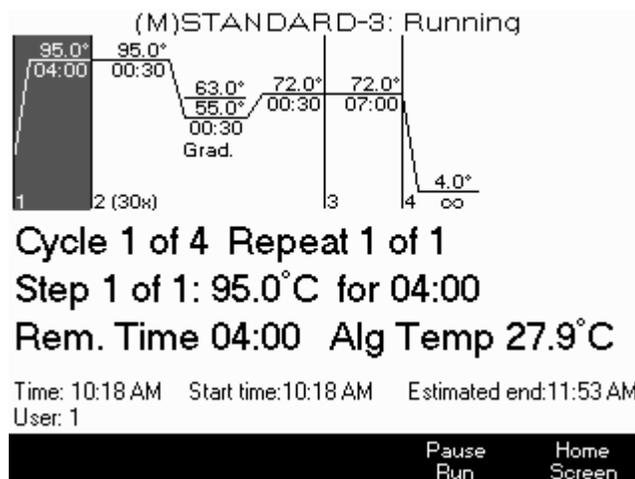

Fig. 1.2. Running Protocol: Temperature Gradient Indicated.

Run and Validation Reports

The temperature gradient values are stored with the run and validation reports in the same format as shown in the editing screen. In this way temperature values may be recalled and reviewed after the assay results are complete (fig 1.4).
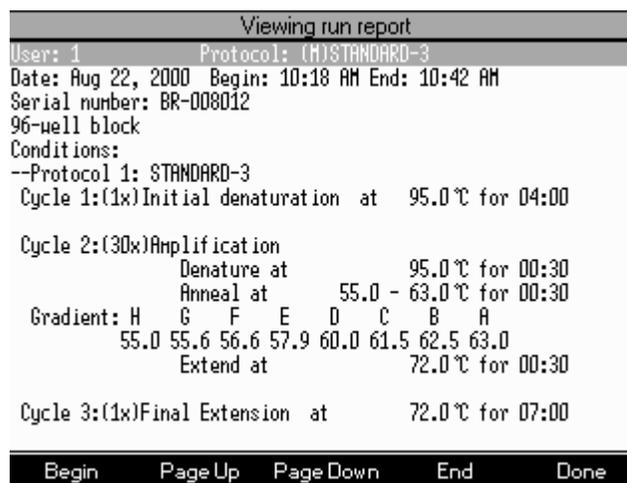

Fig. 1.3. Reports: Gradient Temperatures Stored.

Gradient Specifications

Input Gradient Range:	**1–25 °C**
Functional Gradient Range:	**5–25°C**
Minimum Temperature of Gradient:	**40 °C**
Maximum Temperature of Gradient:	**99 °C**
Gradient Accuracy:	**+/- 0.4 °C**
Gradient Uniformity:	**+/-0.4 °C**