Application Note A01-009A

Gradient profiles and adaptive ramping

Prime

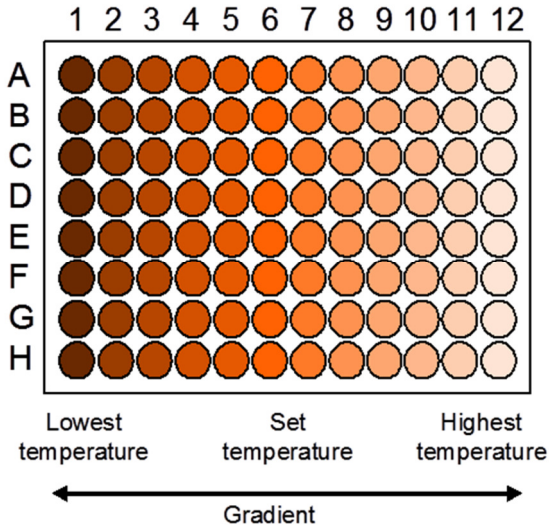
Introduction

The gradient feature of a thermal cycler block is most often used to optimise the annealing temperature of a PCR. Although PCR primers are usually supplied with theoretical melting temperatures (T_m), these can be calculated in different ways which may give widely varying values and therefore it is recommended that the annealing temperature is determined experimentally. Using a gradient can identify the optimal temperature for the PCR on a specific instrument and is especially important when changing a sensitive assay from one thermal cycler to another.

The hold time for the annealing step is also important because this is when the primers bind specifically to the target to allow extension to occur. The length of time required can depend on the reagents and thickness of the plastic consumables used and should also be determined experimentally. As with all experimentation, when optimising a PCR it is important to change only one variable at a time. In this application note we physically measure the gradient across the thermal cycler block and demonstrate that whenever a gradient is applied to the block, the hold time for the subsequent step remains constant. This is known as adaptive ramping.

Block gradient

When programming a gradient on the 3PrimeG, PrimeG or Prime Elite, the programmed temperature is the temperature in the centre of the block and the gradient is the variation at the two extremes, with the left hand column (column 1) being the coolest and the right hand column (column 12 in a 96-well block) the hottest (Fig. 1).



The PrimeG is capable of producing a gradient of temperatures across its block by using the four independent heating channels that divide the block into quarters from left to right. The gradient function controls each of these channels at a different temperature, producing a near linear gradient across the block.

The maximum temperature gradient which can be set is dependent on the model and block type with up to 30°C on a 96-well block for the Prime Elite or 14°C on a 3PrimeG.

***Fig. 1**: Gradient spread in a 96-well block.*

Measuring the block gradient

Experiments were performed using a Techne PrimeG thermal cycler. Twelve temperature probes were placed across the thermal cycler block, one in each column as shown in Fig. 2. The probes were connected to a TAS unit connected in turn to a PC running QTAS 2 Temperature Acquisition Software for Thermal Cycler Analysis (Hain Lifescience UK Ltd).

The thermal cycler was programmed to run four separate 3-step programs of 95°C for 1 min, 55°C for 1 min and 72°C for 1 min (5 cycles) with a gradient on the 55°C step of 29°C (maximum), 20°C, 10°C or 5°C. Temperature data for the twelve columns across the block was measured by the temperature probes and recorded by the PC software. Data for each gradient profile was plotted against time.

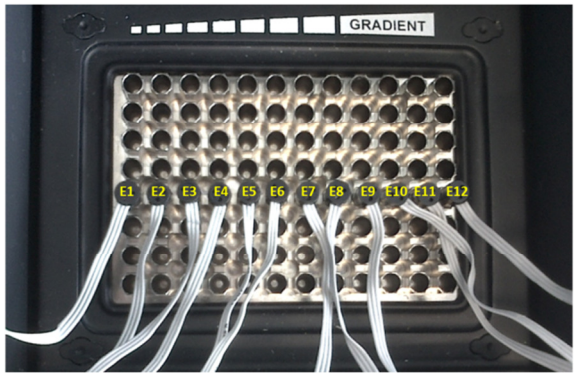


***Fig. 2**: Temperature probes placed across the block wells*

Results

Fig.3 shows the temperature profiles measured for each gradient program and Fig. 4 the same on an expanded scale for the gradient step of cycle 3.

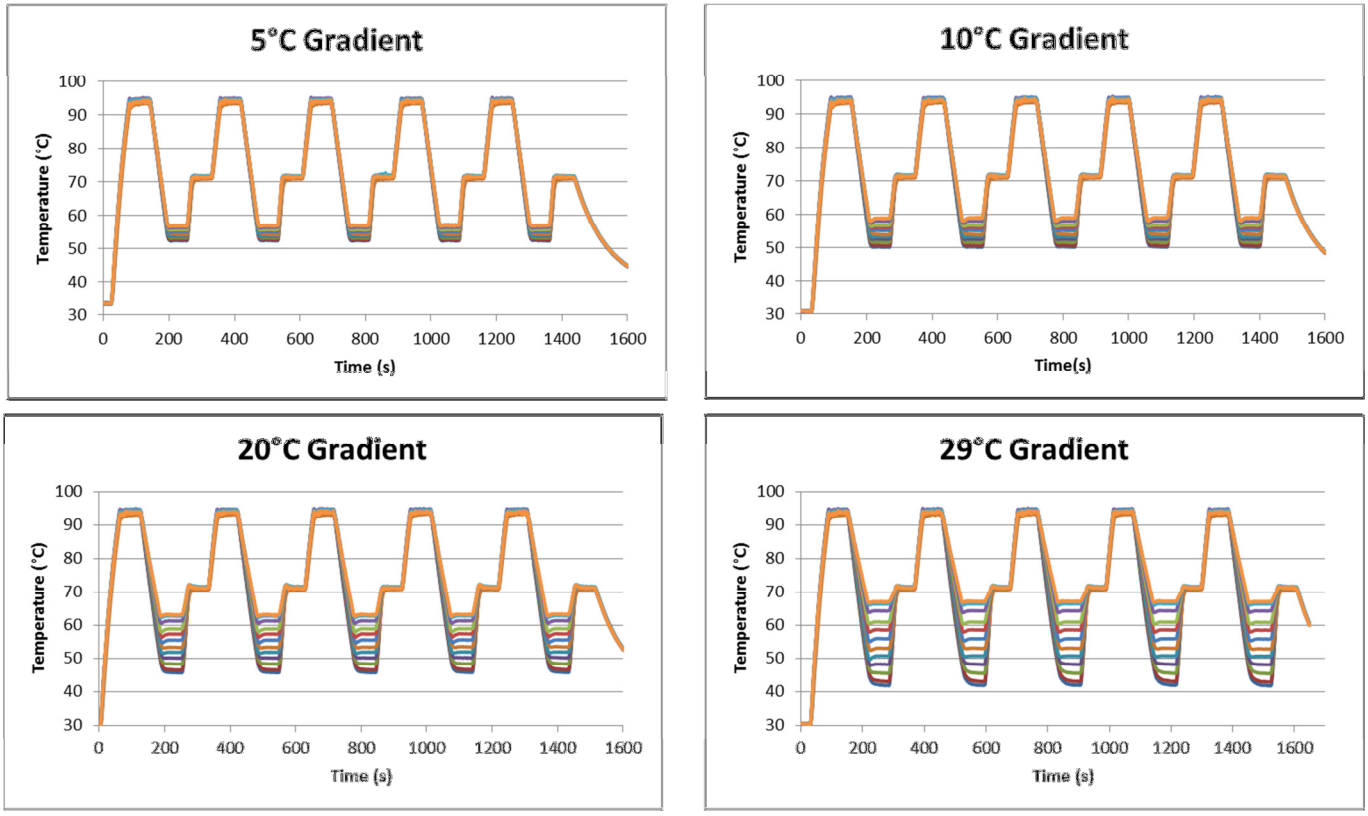


***Fig. 3:** Temperature profile plots measured using probes placed across row D of the thermal cycler block.*

The data obtained using the temperature probes clearly shows how the block adapts the ramping rate in each column in order to achieve the required well temperatures at the same time. Similarly, at the end of the gradient step, the heating ramp rates vary so that the next temperature point is reached simultaneously in each well. For example on cooling to the annealing temperature with a 29°C gradient, E1 ramped down at a rate of 0.73°C/s while the rate in E12 was only 0.41°C/s. Heating rates to the extension step were 1.41°C/s for E1 but only 0.18°C/s for E12. The overall result is that on the gradient step, the hold time is the same for each well regardless of temperature.

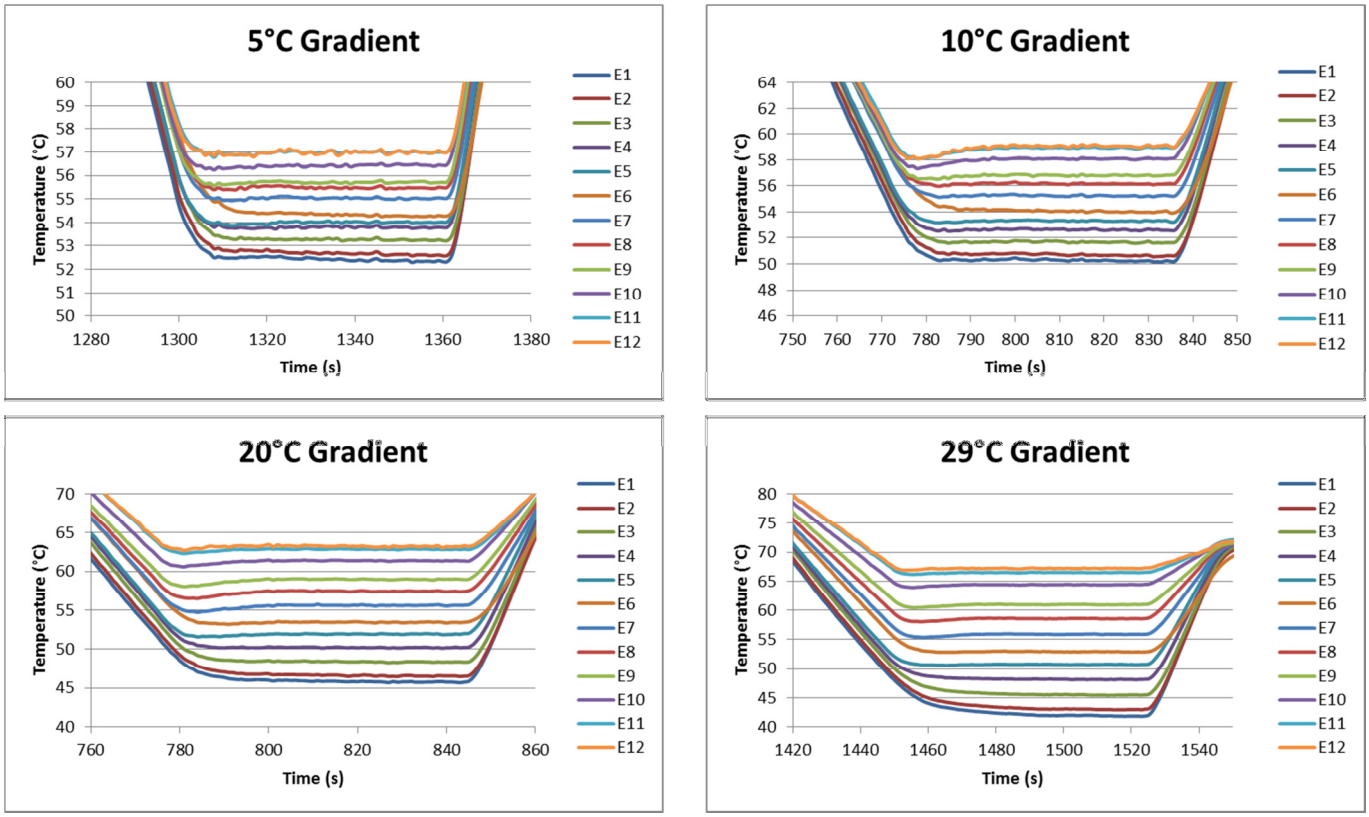


Fig. 4: *Gradient step profile for cycle 3 of each of the programs.*

A gradient calculator is included in the Settings menu of all gradient-enabled Prime units. The calculator is used to display the actual temperatures of the block for any given gradient. While the gradient is mainly linear there are small variations at the extremes of the gradient due to thermal losses from the edges of the block and a gain of heat from the adjacent columns. This is the reason why the first column does not reach the lowest ideal temperature of the gradient and the last column cannot reach the highest temperature. Fig. 5 shows how the measured gradients compare to the values given in the gradient calculator for each of the four programs. The results demonstrate that the actual values are very similar and that the gradient calculator presents a close representation of actual block temperatures.

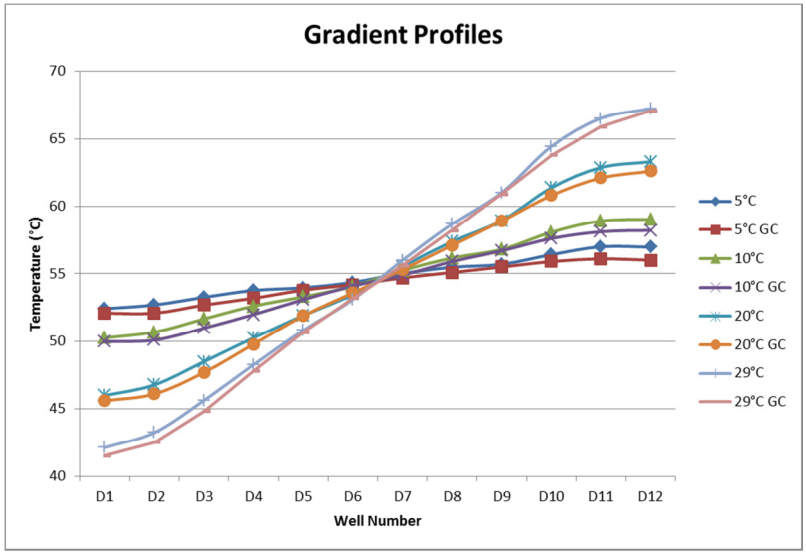


Fig 5*: Gradient profiles for each of the annealing steps. The measured temperatures are shown compared to the temperatures given in the gradient calculator (GC) feature located in the Settings menu.*

Conclusions

The gradient function of the Prime thermal cyclers is a useful feature which can be used to improve the results of a PCR by allowing a simple optimisation step to be performed in a single run.

When using a gradient, Techne thermal cycler blocks are designed to give accurate hold times for each thermal cycling step using automatic adaptive ramping. This ensures that whatever gradient range is used, the step hold time is the same for each well.

Could the differences in ramp rate in transferring from one temperature to another have an effect on the reaction? This could be the case, especially when large gradients are applied across the block. However for thorough optimisation, an initial broad gradient test is generally followed by a much narrower gradient to refine the annealing temperature; in this case the ramp rates will vary much less between wells and will not have as much influence.

In summary, automatic adaptive ramping ensures that the hold times across each of the twelve columns during a gradient step are identical, thus minimising the variables during PCR optimisation.