VeriFlex Blocks technology enhances PCR assay optimization

Introduction

Before the mid-1980s, having a method to easily and specifically amplify any DNA sequence seemed unthinkable. However, the introduction of the polymerase chain reaction (PCR) quickly changed that. From the time of its inception, amplification of nucleic acids using PCR has been an indispensable tool in the biologist's toolbox and has revolutionized all aspects of biological research.

The key to the specificity of a PCR assay is in the sequences of the oligonucleotide primers. The forward and reverse primers in a PCR assay are designed to hybridize to specific sequences in the target DNA within a narrow range close to the melting temperature (T_m). In turn, the melting temperature depends on a variety of factors, including the concentrations of nucleotides, salts, and other components. Each of these factors may have different effects on the forward and reverse primers and, in an Applied Biosystems™ TaqMan™ Assay, also influence the binding of the oligonucleotide probe. Although the effect of many of these factors can be predicted, in practice, it is often easier to empirically determine the effective melting temperatures in an assay. To do this, researchers will perform the PCR reaction across a range of temperatures, above and below the predicted T_m, to see which option gives the best results. This can involve dedicating a single-temperature PCR instrument to multiple runs, each at a slightly different temperature.

PCR instrument manufacturers recognized that there was a need for instruments that could vary the temperature across the heat block. They therefore designed PCR instruments that generated a temperature gradient across the reaction heat block. These blocks had a user-defined minimum temperature at one end of the block and a maximum temperature at the other end of the block, with a gradient between these temperatures forming passively across the block (Figure 1, left). In this system, though, a true linear gradient is not possible, since interactions with non-heating elements at the edges of the block affect the end temperature. Usually, the temperature gradient observed in such systems follows a sigmoidal curve [1]. Although temperature gradients indeed allowed variable temperatures in a single run, it was difficult to know exactly what the temperature was in individual wells.

The innovators working with the Applied Biosystems™ products portfolio took a different approach. They designed a platform with up to six individual heating/cooling elements that divided the block into up to six zones (Figure 1, right). A user could control each zone independently, and define up to six different heating profiles for a PCR reaction (Figure 2). Testing has shown that the temperature control within the Applied Biosystems™ VeriFlex™ Blocks enables a truly linear and precise response when compared to gradient-block thermal cyclers (**see application note**). VeriFlex technology has been incorporated into all of the latest Applied Biosystems™ PCR instruments, including endpoint instruments such as the ProFlex™ system and flagship real-time instruments such as the QuantStudio™ 7 Pro system.

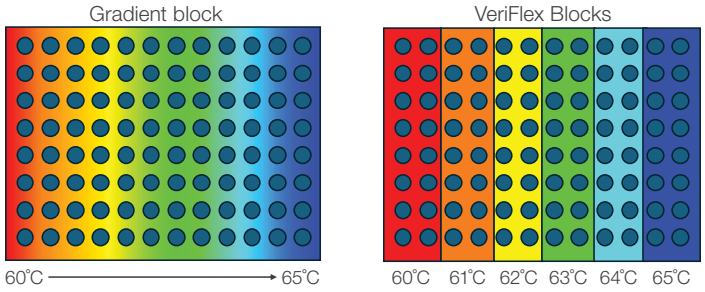

Figure 1. Temperature distribution in a gradient thermal cycler block (left) and in a VeriFlex thermal cycler block (right). The temperatures are precisely known and controllable in the VeriFlex Blocks.

Figure 2. Changing temperatures for the VeriFlex Blocks is easy and intuitive.

One of the most obvious benefits of the VeriFlex system is that it facilitates setting up optimization reactions for PCR assays. Because the temperatures are precisely defined and controlled, the temperature enabling the best performance for an assay can be assessed with confidence. For example, a user testing primers for the first time can set up a PCR run with annealing temperatures of 56°, 57°, 58°, 59°, 60°, and 61°C. This can be particularly useful when optimizing a multiplex PCR assay, when there are up to 18 different oligos (2 primers and 1 probe for 6 different targets) in a reaction. Based on the results of this experiment, the temperature that gave the best results for all subsequent assays can be used.

The VeriFlex system also brings other less obvious benefits. For example, if it is found that different PCR primers have different optimal temperatures, a user can run subsequent reactions with those different conditions for different primers on one instrument. To maximize efficiency, the VeriFlex zones can be set at different temperatures—for example, at 57°C for gene A and 63°C for gene B. This would allow for simultaneous running of 48 samples under each condition, enabling optimal use of the instrument.

VeriFlex technology also supports Sanger sequencing workflows. In the Applied Biosystems™ BigDye™ sequencing workflow, the cycle sequencing step depends on a sequencing primer annealing to one end of the target to be sequenced. As in a PCR reaction, the annealing of the sequencing primer is temperature dependent, and therefore can be optimized using the VeriFlex system. Figure 3 shows a template that was sequenced with the same primer at six different annealing temperatures and one extension temperature (60°C). Notice that the best results were obtained when the annealing was at 50°C—there were fewer mixed peaks after about base 30, and the overall read was long.

Finally, when a single temperature is needed for non-PCR incubations, thermal cyclers with VeriFlex Blocks can be used as programmable non-cycling heat blocks. For example, different zones could be dedicated to restriction digestion (37°C), cDNA synthesis (45°C), and proteinase K digestion (65°C). This provides an extra level of flexibility in a single instrument, saving valuable bench space.

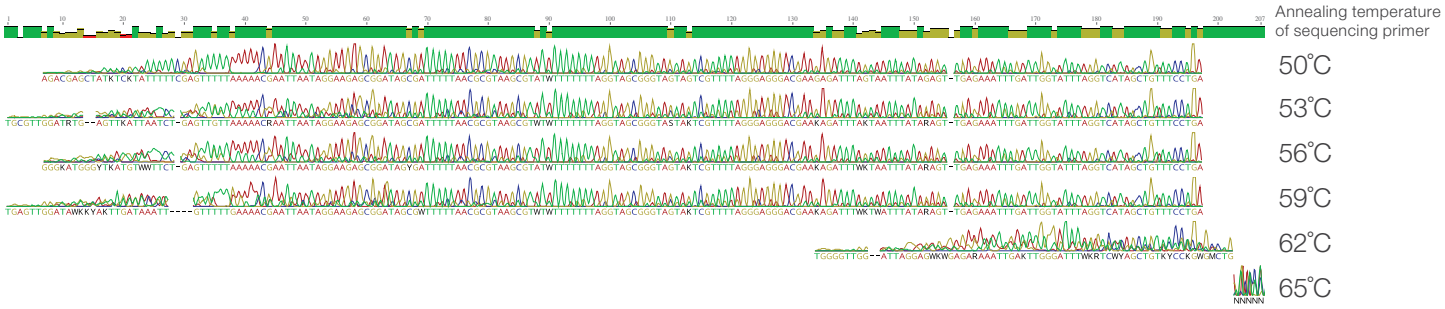

Figure 3. Illustration of VeriFlex Blocks usage to optimize a Sanger sequencing reaction. In this case, the best results were seen when the annealing temperature was 50°C.

Conclusion

The current line of Applied Biosystems PCR instruments builds on innovations developed by Thermo Fisher Scientific chemists and engineers. The VeriFlex system, incorporated into our most modern instruments, is one innovation that helps bring increased control over your PCR reactions, giving you confidence that the science is the best it can be.

For more information, check out our **application note**.

To learn more about how VeriFlex Blocks technology can help you with your research, contact a sales representative for a demo or a quote.

Learn more at **thermofisher.com/quantstudio**