Application Forum

New Developments in PCR

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Introduction

Using the unique DNA polymerases based on fusion protein technology as a starting point, Finnzymes has re-engineered all aspects of PCR technology, revolutionizing traditional PCR and enabling new applications.

DNA Polymerases: The Fusion Protein Technology

PCR employs an in vitro DNA replication system that mimics processes found in nature. Because the replication machinery is not truly reconstituted, but rather patched together from a few important components, PCR is slower, less robust, and more prone to errors than DNA replication in vivo. The fusion protein technology developed by Wang et al. (1) sought to more closely recreate in vivo replication systems. To this end, a processivity factor (a thermophilic, non-specific, double-stranded DNA binding protein) was fused directly to a DNA polymerase, such that it acted in cis, thereby reducing the complexity of the replication system as a whole (1).

Finnzymes' Phusion® and Phire® DNA polymerases represent the fruits of this rational design. In addition to the expected increase in processivity, these PCR enzymes exhibit a number of desirable features, including resistance to various PCR inhibitors and faster overall performance. In conventional PCR, the hold steps for denaturation, annealing, and extension are typically 15–60 s, and 35 cycles of PCR could take several hours, even for short PCR products. Using Phusion or Phire DNA polymerases, PCR protocols are significantly shorter due to time savings at every PCR step:

1. Shorter denaturation step (1–5 s), as higher temperatures can be used due to increased temperature stability of these DNA polymerases.
2. Shorter annealing step (5–20 s) due to primer-template complex stabilization effect and because two-step PCR protocols are more often applicable due to the effective 5–8°C increase in primer Tm.
3. Shorter extension step (15–30 s/kb) due to high processivity of these polymerases.

PCR Instruments and Consumables: New Technological Solutions

The novel polymerases based on fusion technology have subsequently given rise to a new generation of instruments, consumables, and kits that take full advantage of

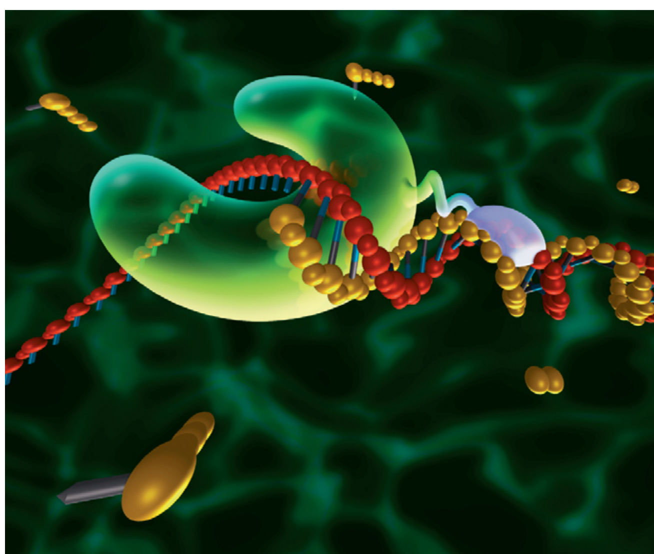

Figure 1. Structure of Phusion® High-Fidelity DNA Polymerase showing the double stranded DNA binding protein fused to the N terminus of the enzyme. Features of this polymerase include increased processivity, extremely high fidelity, and exceptional resistance to various PCR inhibitors.

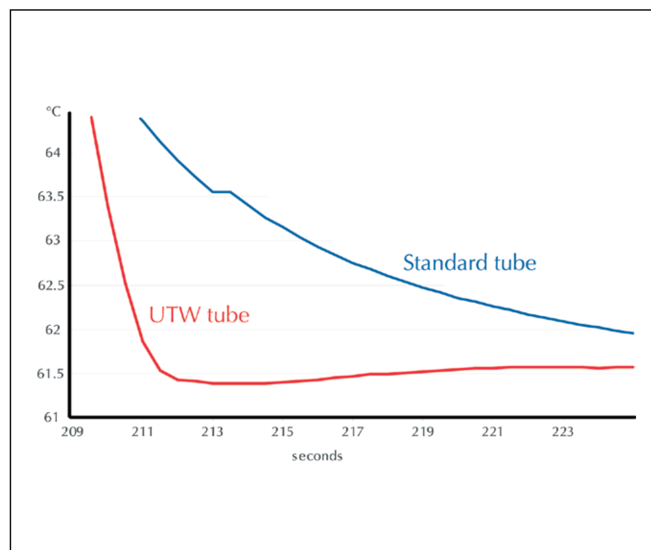

Figure 2. UTW® vessels allow faster thermal transfer to PCR sample. Thermal profile of a 30-μl sample during block temperature change from 98°C to 61.5°C. Standard thin-wall tubes cause the sample to lag by more than 2.5°C and 10 s as compared to UTW vessels.

the enhanced properties bestowed by the fusion protein technology. The rate-limiting step in PCR is no longer the reaction itself, but rather other factors such as the PCR instrument and the plastic consumables used in it.

It is important to note that when hold times are decreased to <15 s, it becomes critical that the instrument and consumables are engineered such that the sample reaches the specified temperature and remains at that temperature for the programmed time. In a typical PCR system, it may require ≥15 s for the sample to actually reach the set temperature, and thus fast protocols often exhibit unacceptable variability across the sample block, or from run to run. Studies using in-sample temperature probes have confirmed wide variation in actual sample temperatures for many PCR systems (2).

Development of the Finnzymes Piko® Thermal Cycler was based around the small Piko PCR Plate, which is one-fourth the size of a conventional microplate. This smaller plate format enabled the design of a PCR instrument with significant technological improvements: high thermal uniformity and thermal accuracy, fast ramp rates and extremely short block settling times (<1 s). These features ensure that PCR samples will achieve the programmed temperatures quickly with minimal variation from well to well and instrument to instrument.

The final rate-limiting step in the PCR protocol is the heat transfer between the block and the reaction mixture: The thinner the tube walls the faster the heat transfer and, thus, the protocol. Ultra-thin walled UTW® reaction vessels are manufactured with a novel method of injection molding polymers. UTW vessels have walls that are <150 microns thick—half the thickness of conventional thin-wall PCR tubes. This allows thermal transfer from block to sample to occur with minimal delay.

Finnzymes' High Performance PCR takes advantage of the benefits of fusion polymerases to increase the speed and yield of PCR reactions. Phusion, a polymerase consisting of a novel Pyrococcus-like enzyme fused to a processivity enhancer, features high processivity, extremely high fidelity and yield as inherent properties of the engineered polymerase. Combining this enzyme with Piko Thermal Cyclers and UTW reaction vessels delivering additional speed and uniformity to the reactions results in synergistic benefits: high fidelity PCR performance with increased yields in just a fraction of the time when compared to conventional PCR.

Direct PCR: Taking Advantage of Resistance to Inhibitors

Phusion and Phire DNA polymerases exhibit surprising resistance to several common PCR inhibitors. This feature has enabled a wide range of Direct PCR protocols that eliminate the need for DNA purification before PCR. Typically, two approaches have been developed for each starting material. If a small piece or volume of the sample is placed directly into a PCR reaction, a 5-min initial denaturation step releases enough DNA for PCR. In cases where multiple PCR reactions are to be run from a single sample, a tissue punch may be incubated briefly in incubation buffer, and a small quantity of this extract is used for the PCR reaction. Based on this strategy, optimized kits and protocols for blood, plants, and for other tissues have been developed.

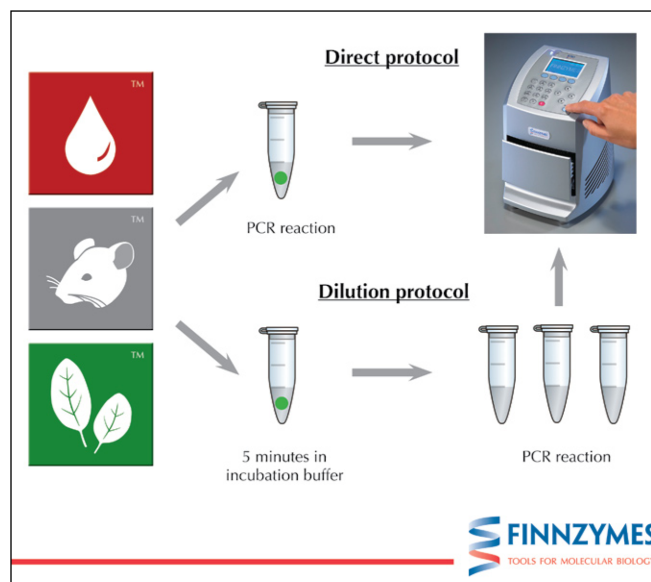

Figure 3. Direct PCR workflow. Direct and Dilution PCR protocols are illustrated. In both cases, no DNA isolation is required. Using Piko Thermal Cycler and UTW vessels, the total time from sample to completed PCR reaction can be as little as 30 min.

References

1. **Wang, Y., D.E. Prosen, Mei, L., J.C. Sullivan, M. Finney, and P.B. Vander Horn.** 2004. A novel strategy to engineer DNA polymerases for enhanced processivity and improved performance in vitro. Nuc. Acids Res. *32*:1197-1207.
2. **Kim, Y.H., I. Yang, Y.-S. Young-Seuk Bae, and S.-R. Park.** 2008. Performance evaluation of thermal cyclers for PCR in a rapid cycling condition. BioTechniques *44*:495-505.