Center for Homogeneous DNA Analysis

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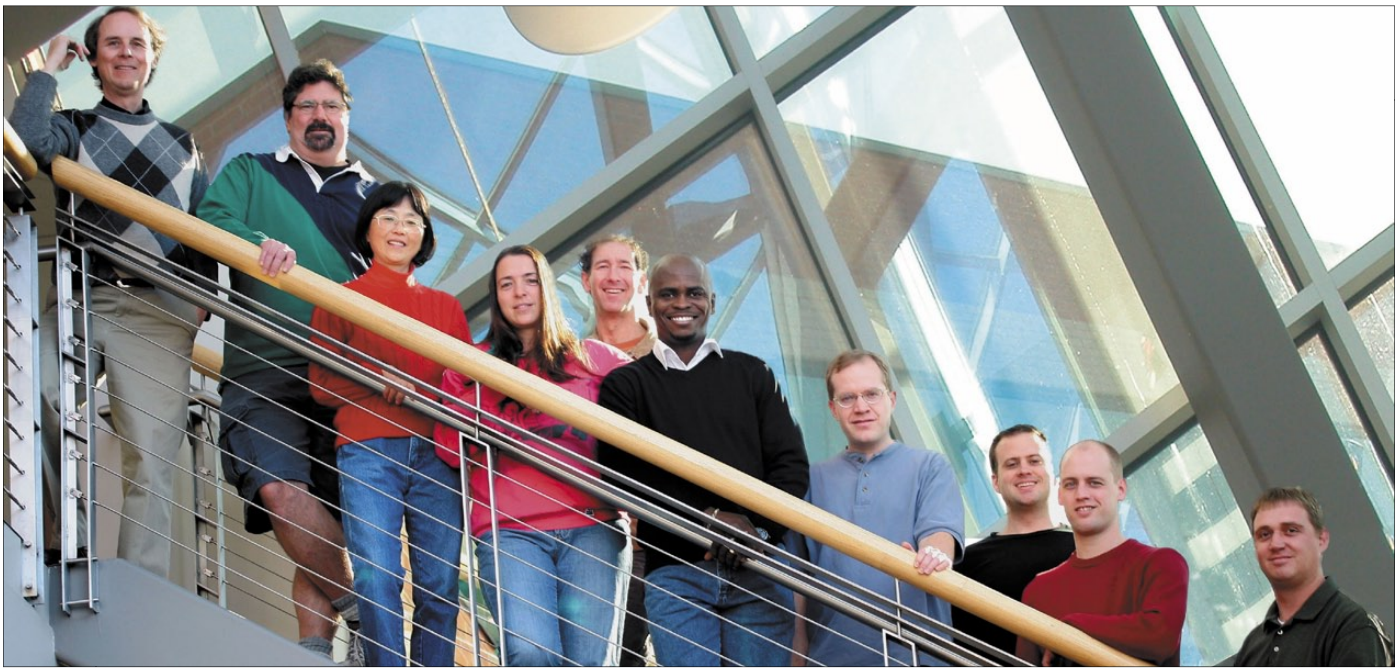

The People: From left to right: Carl Wittwer (Principal Investigator), Robert Pryor (Senior Research Specialist), Ying Wang (Lab Technician), Gudrun Reed (Senior Research Specialist), Robert Palais (Research Associate Professor), Oluwole Elenitoba-Johnson (Engineer), Matthew Poulson (Lab Assistant), Scott Sundberg (Graduate Student), Jesse Montgomery (Graduate Student), and Roscoe Errigo (Undergraduate Student). Not pictured: Luming Zhou (Research Associate) and Jana Kent (Research Associate).

The Research

Our laboratory develops simple methods for analyzing DNA. In the early 1990s we introduced rapid-cycle PCR techniques for achieving DNA amplification in 10–15 min. In the mid-1990s, we adapted flow cytometry optics to thermal cycling for real-time monitoring of PCR, resulting in the commercial "LightCycler." As part of this development, SYBR Green I, hybridization probes, and melting analysis were first applied to real-time PCR. Since 2000, we have focused on melting analysis as a method that does not require any separations, electrophoresis, or covalently labeled oligonucleotide probes. Only two PCR primers and a dye are needed. DNA melting is a fundamental property of DNA, and if followed closely, can provide much more information than previously imagined. We recently developed high-resolution melting methods with saturation dyes to provide rapid, inexpensive methods for mutation scanning (identification of heterozygotes), genotyping, and sequence matching. The combination of rapid-cycle PCR and high-resolution melting analysis enables meaningful results in less than 15 min.

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The Technique

The yield and specificity of PCR depends on accurate temperature cycling. Glass capillaries have become popular because they allow rapid and accurate thermal cycling. However, one concern about glass capillaries is that they can break. Recently, plastic capillaries have been introduced as an alternative to glass capillaries. However, the viability of plastic capillaries as opposed to glass, especially for rapid cycling applications, was not clear. Rapid cycling typically uses 0 s denaturation and annealing times. No holding periods are used because denaturation and annealing occur very rapidly (<1 s) after the sample reaches the target temperatures. The plastic capillary walls were thicker than the glass capillary walls, and the thermal conductivity of the plastic was much lower than glass, both predicting altered thermal effects on the sample. Indeed, while samples in glass capillaries amplified well by rapid cycling, samples in plastic capillaries failed to amplify. One out of three targets could be amplified in plastic if 5–20 s denaturation and annealing times were used. Although plastic capillaries may prove adequate in some slow cycling protocols, the sample temperature/time course is very different in glass capillaries. For rapid cycling, glass capillaries are clearly the better option.

See "Plastic versus glass capillaries for rapid-cycle PCR" on page 487.