

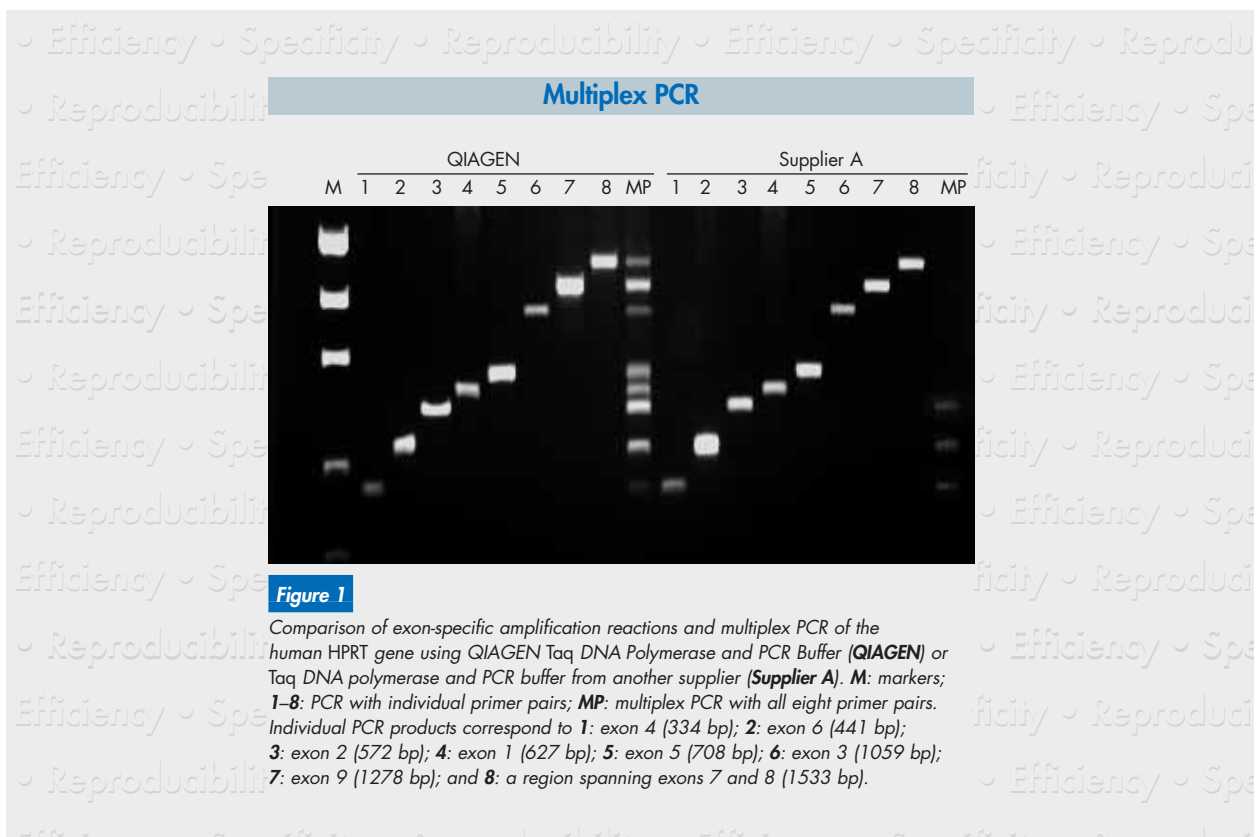
3. Effect of PCR Buffer on multiplex PCR

Multiplex PCR employs different primer pairs in the same amplification reaction. This type of PCR requires extensive optimization of annealing conditions for maximal amplification efficiency of the different primer–template systems. QIAGEN PCR Buffer has been shown to efficiently amplify different primer–template systems with little or no need for optimization of annealing conditions (1). This is due to the balanced combination of NH_4^+ and K^+ , which widens the temperature window for optimal annealing. Therefore, QIAGEN PCR Buffer should be especially appropriate for multiplex PCR.

Hence, we compared QIAGEN PCR Buffer with another commercially available PCR buffer, containing K^+ but not NH_4^+ , in multiplex PCR. As a model system, multiplex PCR of the human hypoxanthine guanine phosphoribosyltransferase (HPRT) gene was examined.

Materials and methods

Amplification reactions contained eight different primer pairs, whose DNA sequences have been published by Rossiter et al. (2). Each amplification reaction contained 250 ng genomic DNA (purified from human blood using the QIAamp® DNA Blood Mini Kit), 2.5 units QIAGEN Taq DNA Polymerase or Taq DNA polymerase of another supplier (Supplier A), 10 pmol of each primer, 200 μM of each dNTP, and 1x QIAGEN PCR Buffer or PCR buffer of Supplier A containing K^+ but not NH_4^+ cations. Final reaction volumes were 50 μl each. The cycling program started with an initial 3-min denaturation step at 94°C, followed by 25 cycles of 30 sec at 94°C, 1 min at 61°C, and 2 min at 68°C. The final extension step was 5 min at 68°C. PCR assays were performed in parallel, and 10 μl of each assay was analyzed on a 1.4% agarose gel in TBE.



Results and discussion

Individual exon-specific sequences from the *HPRT* gene were amplified in separate reactions, using either QIAGEN *Taq* DNA Polymerase and PCR Buffer or *Taq* DNA polymerase and buffer of Supplier A. The PCR should yield eight different products, ranging in size from 334 to 1533 bp. As shown in **Figure 1**, all amplification reactions generated the expected PCR products independent of the *Taq* DNA polymerase and PCR buffer used. In order to determine whether both types of PCR buffer could also be used for simultaneous amplification with all eight primer pairs, the same PCR protocol was repeated but with all 16 primers in one amplification reaction.

Using the PCR buffer from Supplier A, only three PCR products were amplified. These results indicate that even when cycling conditions provide efficient amplification of individual primer–template systems, they may not be optimal for multiplex PCR. This effect is probably due to nonspecific hybridization between the primer molecules themselves and between primers and the PCR products generated in multiplex PCR. In order to overcome these effects, extensive optimization of multiplex-PCR conditions is often necessary when using a PCR buffer containing K^+ but not NH_4^+ .

QIAGEN PCR Buffer, however, allowed efficient amplification of all eight differently sized PCR products without the need for optimization. The unique composition of this buffer reduces nonspecific hybridization (2) and permits efficient amplification of the different primer–template systems under identical conditions. This is essential for successful multiplex PCR.

References

1. *Taq* DNA Polymerase — QIAGEN brings innovation to PCR. (1996) QIAGEN News 1996 No. 5, 2.
2. Rossiter, B.J.F., Grompe, M., and Caskey, C.T. (1991) Detection of deletions and point mutations. In: McPherson, M.J., Quirke, P., and Taylor, G.R., eds. PCR. A Practical Approach. Volume 1. Oxford: University Press. p 67.