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Notes & Tips

Lab assembly of a low-cost, robust SYBR green buffer system for quantitative real-time polymerase chain reaction

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The SYBR green approach for real-time PCR does not require the synthesis of expensive probes and is very sensitive. We sought to develop a SYBR-based system assembled from off-the-shelf reagents, which would rival or surpass commercial kits at a fraction of the cost. Others have reported lab-made mixtures but issues of stability and efficiency were noted [1,2]. Our amplifications were done on an iCycler iQ Bio-Rad station, with quantitation, melt curve, and PCR efficiency analysis derived by the station's software. Of the factors that influence PCR efficiency and specificity, we tested $MgCl_2$ concentration and combinations of additives (Triton X-100, betaine, formamide, trehalose, and PEG400). The melting temperature profiles and agarose gel electrophoresis migrations show a single amplicon of the expected size, witness to the reaction's specificity (Fig. 1A). Best results were obtained with 2 mM $MgCl_2$ in presence of 2.5% formamide and 0.6 M trehalose (Fig. 1A). Note that formamide (f) and trehalose (t) lower the melting temperature of the amplicons (Fig. 1A). A typical reaction is assembled as follows: 2 µl of 10× core buffer (100 mM Tris–HCl, pH 8.5, 500 mM KCl, 1.5% Triton X-100, 20 mM $MgCl_2$, 2 mM dNTPs, and 100 nM fluorescein for well-factor correction), 6 µl of 2 M trehalose (Sigma Chemicals), 0.5 µl formamide, 0.1 µl of a 1/100 SYBR green dilution in dimethyl sulfoxide (Molecular Probes/Invitrogen), 1 µl (1 U) of *Taq* polymerase, the primers (250 nM each, final concentration, primer sequences are listed in Supplemental Methods), and water to 20 µl. We then assessed the dynamic range and sensitivity of our buffer system. As Figs. 1B and C show, we were able to quantify target DNA over 7 orders of magnitude and down to 10^2 copies per reaction. We have also successfully amplified 70% GC-rich templates (Supplemental Methods) using our buffer and a standard temperature schedule. Finally, we compared the sensitivity of our buffer system with the sensitivity of the commercial mix recommended for the Bio-Rad platform (iQ SYBR Green Supermix; used according to the manufacturer's protocol) using 10-fold serial dilutions of a DNA template. Despite the fact that our buffer system yields much higher absolute fluorescence levels, both buffer systems have similar sensitivities (Fig. 2) and maximal PCR efficiencies (102.6 and 103.5%). The consistently high absolute fluorescence and sensitivity of our buffer system were confirmed with various primer pairs (Table 1) and we ruled out a fluke deficiency in the response range of the reference commercial kit by testing four different lots that yielded very similar results (data not shown). DNA-binding dyes alternative to SYBR green are now available. We thus tested whether one such dye, EvaGreen (Biotium), also works in our buffer system and possibly improves its sensitivity. EvaGreen and SYBR green yield essentially identical results (Supplemental Methods), but SYBR is currently 10,000 times cheaper per reaction.

We describe here a stable buffer system that routinely amplifies target DNA over 7 orders of magnitude and detects as little as 100 molecules, performances that are in line with those of the best commercial kits. There are several differences between our and the commercial buffer systems. We use wild-type *Taq* polymerase produced in our lab (see Supplemental Methods), whereas the thermostable polymerases of commercial kits often require an activating heating step (hot-start DNA poly-

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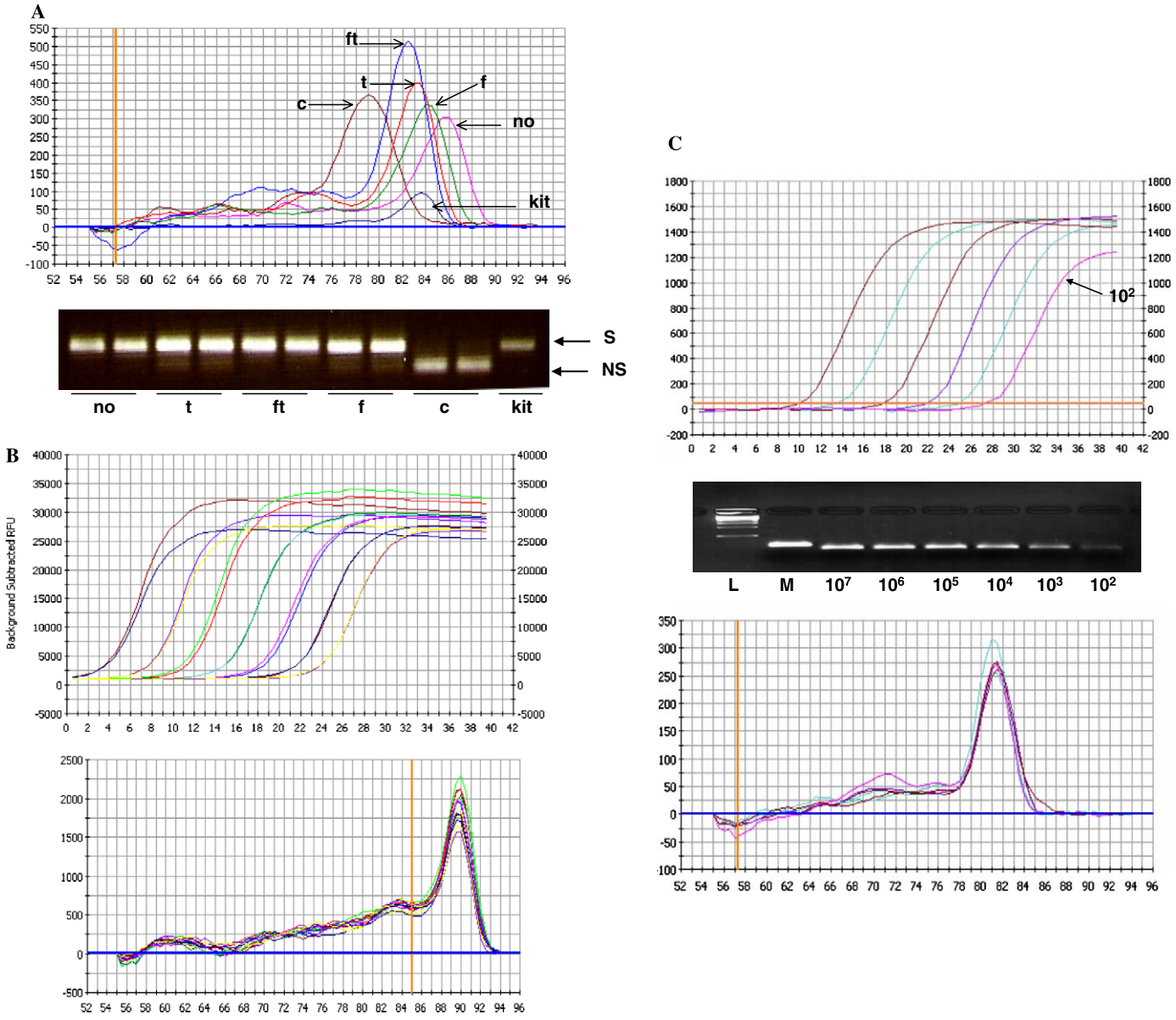

Fig. 1. (A) Effect on specificity of 2.5% formamide (f), 0.6 M trehalose (t), 2.5% formamide + 0.6 M trehalose (ft), or no additives (no). The control reaction (c) has both 2.5% trehalose and 0.6 M formamide but no cDNA was added. A reaction with a commercial SYBR green kit was performed for comparison (kit). Input GAPDH (Accession No. M17701) cDNA was 8700 copies in 0.5 μg rat brain cDNA prepared as previously described [3]. (Top) Melt curve analysis of amplifications. *y* axis, first derivative of the base-line-subtracted fluorescence; *x* axis, temperature. (Bottom) 3% Agarose gel displaying the specific amplicons (S) and a nonspecific amplification artifact (NS). (B) Amplification range of the lab-made system measured with seven 10-fold serial dilutions of a rat CaM-KIIN (Accession No. AF271156) gene fragment, starting at 9×10^9 copies. (Top) Base-line-subtracted fluorescence as a function of cycle number. (Bottom) Melt curve analyses of the real-time PCR amplifications. (C) Amplification sensitivity of the lab-made system. Detection of 10-fold serial dilutions of template DNA bearing a mouse TSPY-like1 (Accession No. BC017540) gene fragment, starting with 10^7 copies down to 10^2 copies. (Top) Fluorescence as a function of cycle number. (Middle) 3% Agarose gel display of the 88-bp amplicons as primed with the indicated copy numbers ("L" molecular weight markers, "M" a 117-bp marker). (Bottom) Melt curve analyses. Primer sequences are detailed in Supplemental Methods.

merase), which purportedly improves specificity. Here, we show that in our system wild-type *Taq* polymerase is at least as specific as the modified *Taq* used in the commercial kit that we assessed (Fig. 1A). Our buffer system contains 0.6 M trehalose, a compatible solute known to confer heat stability to proteins. This probably explains in part the higher fluorescence signal obtained with our preparation. A typical reaction using our mix comes to approximately 2 cents (USD) with lab-made *Taq* polymerase (less than 10 cents with commercial *Taq* polymerase), whereas a reaction using a commercial kit costs roughly 1 USD. In summary, we detail here conditions permitting highly sensitive real-time PCR and run-to-run consistency at a fraction of the cost of commercial

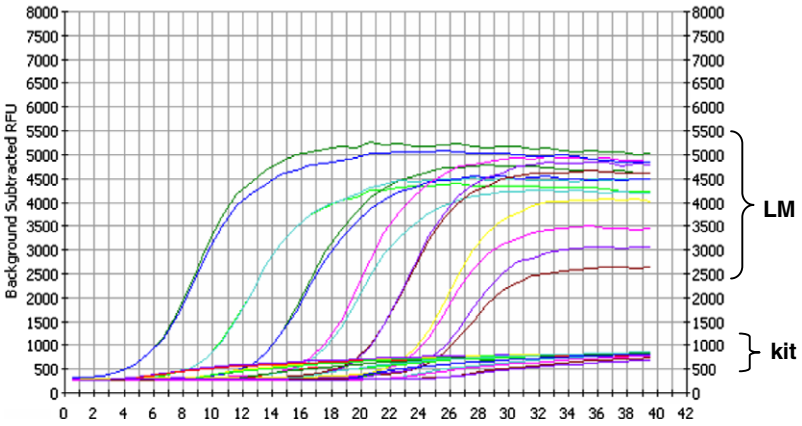

Fig. 2. Comparison between our buffer system and a commercial mix. Sensitivity of the lab-made (LM) and commercial kit (kit) systems were measured with seven 10-fold serial dilutions of a rat CaM-KIIN gene fragment, starting at 9×10^9 copies.

Table 1
Comparison between the lab-made and the commercial kit for the amplification of Arc (Accession No. 019361) and GAPDH (Accession No. M17701) from 0.5 µg rat brain cDNA

Threshold cycle C_t	Arc
27.7	Lab-made buffer
28.5	Lab-made buffer
28.6	Lab-made buffer
28.9	Commercial kit
28.9	Commercial kit
29.7	Commercial kit
Threshold cycle C_t	GAPDH
21.0	Lab-made buffer
21.1	Lab-made buffer
20.9	Lab-made buffer
21.3	Commercial kit
21.1	Commercial kit
20.9	Commercial kit

Triplicates were done and the C_ts are listed. Primer sequences are listed in Supplemental Methods.

alternatives. The method should be valuable for institutions performing high-throughput real-time PCRs and for laboratories with limited financial resources.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ab.2005.12.002.

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