Fast Blocks and Fast qPCR Reagents: A Comparison of a Next-Generation Real-Time PCR System and Reagent to Their Predecessors

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Introduction

Bio-Rad Laboratories, Inc. is about to celebrate its tenth year in the field of quantitative PCR (qPCR). We have developed various generations of instruments: from the original iQ™ and MyiQ™ systems, to the Opticon™ series and Chromo4™ and MiniOpticon™ platforms, to our latest CFX96™ and CFX384™ real-time instruments. We have also developed a steady stream of PCR reagents: from iTaq™ DNA polymerase and iQ™ SYBR® Green supermix, to iProof™ high-fidelity DNA polymerase and various iTaq fast supermixes with ROX, to our most recent addition, SsoFast™ EvaGreen® supermix.

Performance is also expected to evolve over time. In this spirit, we thought it would be interesting to perform assays under suboptimal conditions to test how technological improvements in both instrumentation and reagents enhance (or adversely affect) results. In this study, we compared the performance of our own reagents on our own instruments.

It is well known that under properly optimized conditions, qPCR systems and quality reagents are expected to generate robust results across a large dynamic range. To test range limits, assays to test qPCR instrumentation and reagents were run under optimal and slightly suboptimal annealing conditions in conjunction with reduced annealing/extension times. Assays were run on the MyiQ single-color real-time PCR detection system and on the CFX96 real-time PCR detection system with either iQ™ SYBR® Green supermix or SsoFast EvaGreen supermix.

Methods

PCR Primers/Template DNA

- Forward primer: available on request
- Reverse primer: available on request
- The template used GAPDH phagemid vector (clone 522094, I.M.A.G.E. Consortium)
- Amplicon size: 169 bp

qPCR Assay Annealing Optimization

A previous gradient real-time qPCR assay spanning 55–70ºC and using 200 nM of each primer, 1000 copies of template DNA per well, and iQ™ SYBR® Green supermix was run on the MyiQ system. The optimal amplification temperature range for this assay was 55–64ºC.

Reaction Conditions

The DNA template was mixed in a 10-fold series from 10^8 to 10^2 copies per well with forward and reverse primers (200 nM final each), either iQ™ SYBR® Green supermix or SsoFast EvaGreen supermix (1x final), and water to a final reaction volume of 20 µl per well. All samples were run in triplicate. Assays under optimal conditions were run at 60ºC (annealing/extension), whereas assays run under suboptimal conditions were run at 68ºC (annealing/extension). Assay annealing/extension times varied from 60 sec to 1 sec.

Results

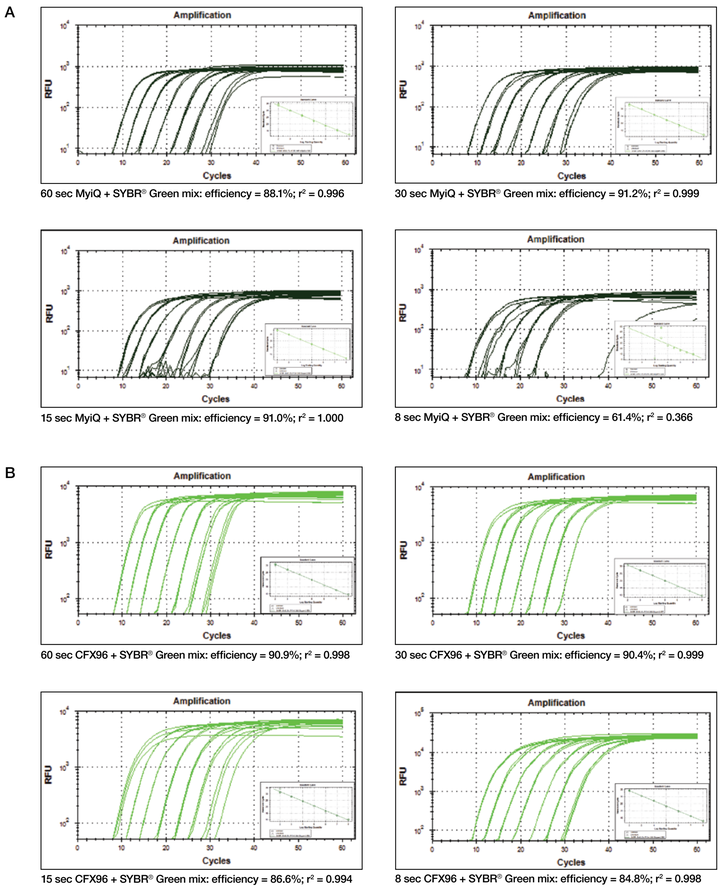

Fig 1. Extension time study of a 10-fold dilution series (10^8–10^2 copies) of GAPDH amplified with an annealing/extension temperature of 60ºC using iQ™ SYBR® Green supermix. A, an identical GAPDH template dilution series was run on the MyiQ system with annealing/extension incubations of 60, 30, 15, and 8 sec. The assays maintained performance and dynamic range from 60–15 sec. In the 8 sec annealing/extension assay, the two lower dilutions disintegrated. **B**, the same assays as above were run on the CFX96 system; with this system the assays maintained performance and dynamic range from 60–8 sec. RFU, relative fluorescence units.

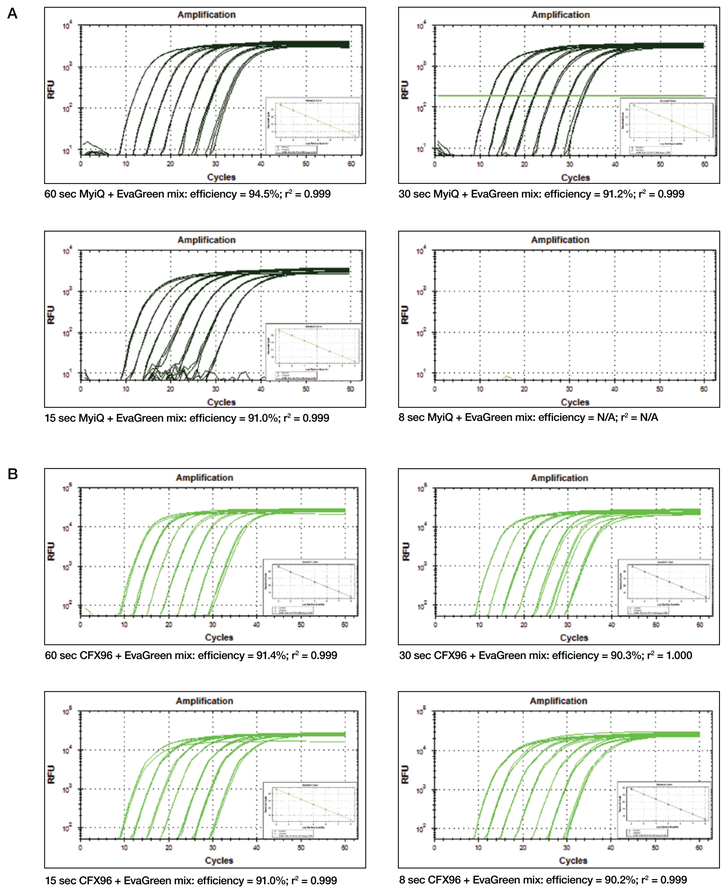

Fig 2. Extension time study of a 10-fold dilution series (10^8–10^2 copies) of GAPDH amplified with an annealing/extension temperature of 60ºC using SsoFast EvaGreen supermix. A, an identical GAPDH template dilution series was run on the MyiQ system with annealing/extension incubations of 60, 30, 15, and 8 sec. Excellent results were obtained with 60–15 sec annealing/extension conditions. The 8 sec assay failed because the MyiQ instrument requires more than 8 sec to acquire a complete amplification signal when using very strong dyes. **B**, the same assays as above were run on the CFX96 system; nearly identical results were obtained, independent of the length of annealing/extension. Efficiency remained stable as well. RFU, relative fluorescence units.

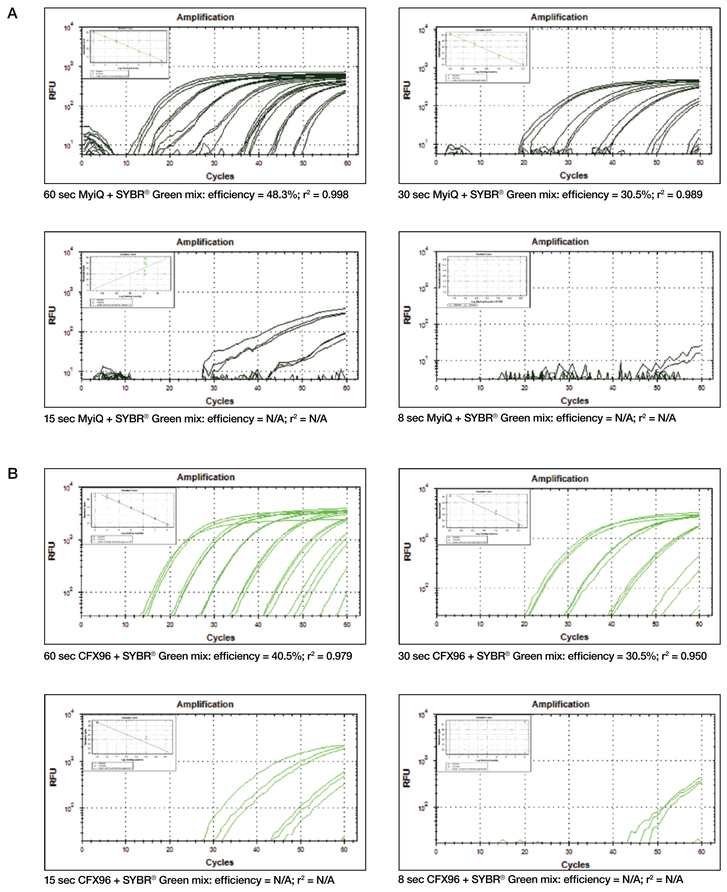

Fig 3. Extension time study of a 10-fold dilution series (10^8–10^2 copies) of GAPDH amplified with an annealing/extension temperature of 68ºC using iQ™ SYBR® Green supermix. A, an identical GAPDH template dilution series was run on the MyiQ system with annealing/extension incubations of 60, 30, 15, and 8 sec. Efficiency immediately dropped to 48% and the assays quickly disintegrated because they were run 4°C above the upper limit of the optimal annealing range. **B**, the same assays as above were run on the CFX96 system with similar results; however, replicate samples displayed greater uniformity on the CFX96. RFU, relative fluorescence units.

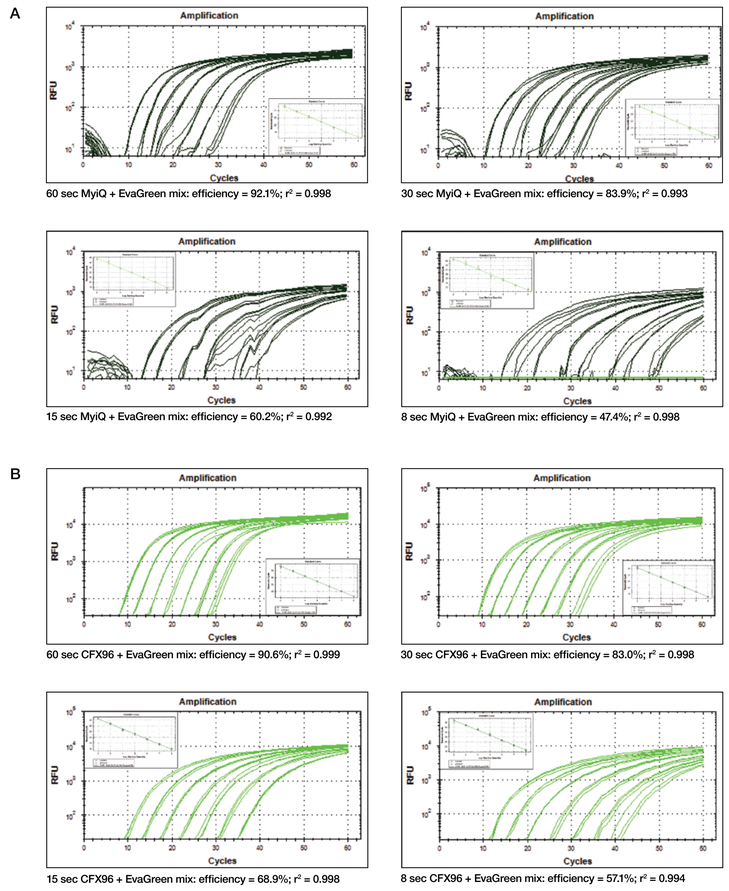

Fig 4. Extension time study of a 10-fold dilution series (10^8–10^2 copies) of GAPDH amplified with an annealing/extension temperature of 68ºC using SsoFast EvaGreen supermix. An identical GAPDH template dilution series was run on (**A**) the MyiQ system or (**B**) the CFX96 system, with annealing/extension incubations of 60, 30, 15, and 8 sec. Under these suboptimal annealing conditions, SsoFast EvaGreen supermix performed surprisingly well. With a 60 sec incubation, initial efficiencies were similar to those yielded by assays that were run under optimal conditions. As expected, when the incubation time was significantly reduced, efficiencies dropped accordingly. Replicate samples run on the CFX96 system yielded more reproducible results. RFU, relative fluorescence units.

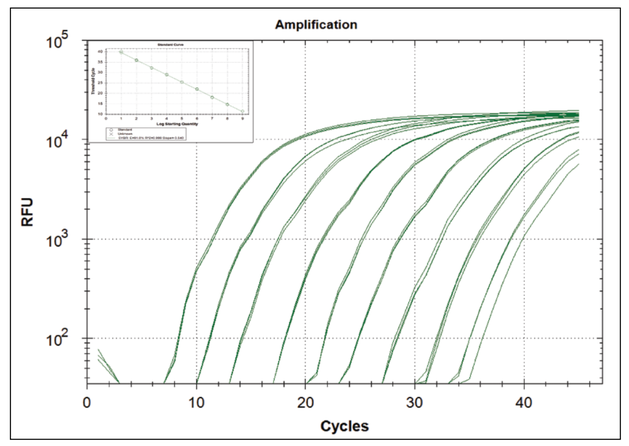

Fig 5. 1 sec programmed annealing/extension of GAPDH 169 bp amplicon using SsoFast EvaGreen supermix on the CFX96 system. GAPDH was amplified over a dynamic range of 10^9 to 10 copies per well in a 20 µl reaction using the following protocol: 98°C for 0:30, 45 x 95°C for 0:01/60°C for 0:01 + Plate Read, 60°C for 0:30, and Melt Curve 65 to 95°C, increment 0.5°C/0:05 + Plate Read. Total time including instrument lid warm-up was 46 min on the CFX96 system. These data were generated 2 months after the original data (Figures 1–4). Even when run at these extreme speeds, amplification efficiency was 91.6%, similar to the 90.2–91.4% efficiencies of previous assays that were run with annealing temperatures of 60°C on the CFX96 system (Figure 2, bottom charts). These results clearly demonstrate the speed and reproducibility of SsoFast EvaGreen supermix and the robustness and uniformity of the CFX96 system. RFU, relative fluorescence units.

Conclusions

When we ran assays under optimal annealing temperatures, they worked very well regardless of the instrument platforms or reagents we used. As expected, the legacy MyiQ single-color real-time PCR detection system and iQ™ SYBR® Green supermix continue to generate excellent results.

Under very robust and optimal conditions, both reagents worked well on both platforms using incubation times from 60–15 sec. At shorter incubation times (8 sec), when using either reagent, the CFX96 outperformed the MyiQ system. With SsoFast EvaGreen supermix, overall assay performance surpassed that of assays that were run with iQ™ SYBR® Green supermix.

Under suboptimal annealing temperatures, assays using SsoFast EvaGreen supermix yielded earlier threshold cycles and more reproducible data than assays using iQ™ SYBR® Green supermix. As incubation times were reduced, the iQ™ SYBR® Green assays disintegrated, whereas the SsoFast EvaGreen supermix assays remained intact. Again, with shorter assays (30 and 15 sec), runs on the CFX96 platform amplified better than the assays run on the MyiQ system.

Overall, the combination of the CFX96 real-time PCR detection system and SsoFast EvaGreen supermix provided the best results.

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