

Hot Start dNTPs - Pushing the Limits of PCR

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Abstract

In a field where the demands for speed, sensitivity, and specificity are high, PCR performance can be significantly improved by the use of Hot Start technologies. Hot Start dNTPs are a distinct approach that employs modified nucleoside triphosphates with a thermolabile protecting group. This modification blocks low temperature primer extension and is released at higher temperatures to allow for more specific DNA polymerase incorporation. Hot Start dNTPs can be used with a variety of thermostable enzymes and can be applied to dNTP analogs such as dUTP in UNG-mediated decontamination methods. The fast rate of dNTP deprotection allows for use in faster cycling PCR protocols. Furthermore, multiplex assays have also shown improved sensitivity and specificity for standard and fast PCR protocols. As this technology continues to develop, its application to other molecular biology assays has great potential.

Figure 1

Proposed activation mechanism of CleanAmp™ dNTPs

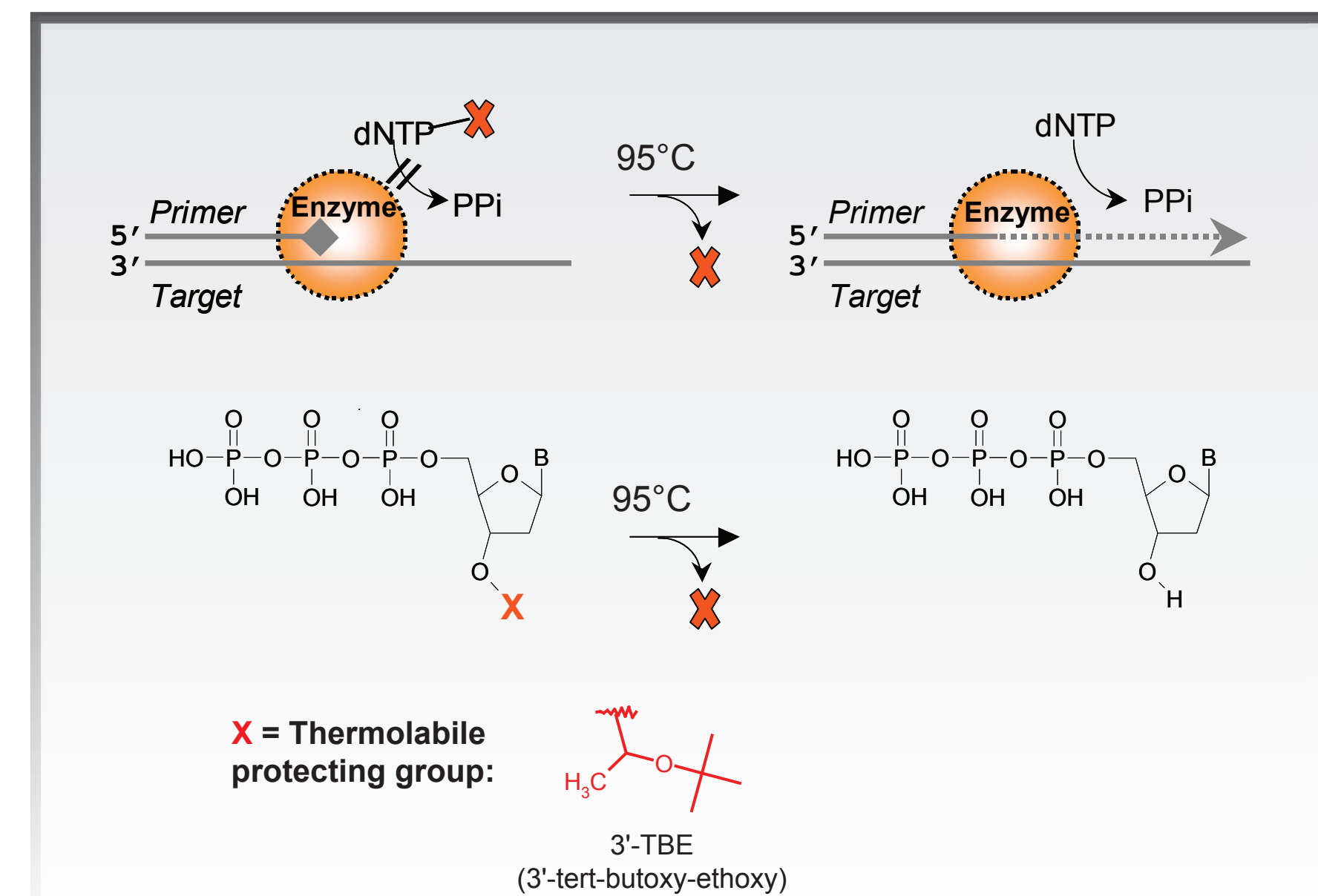
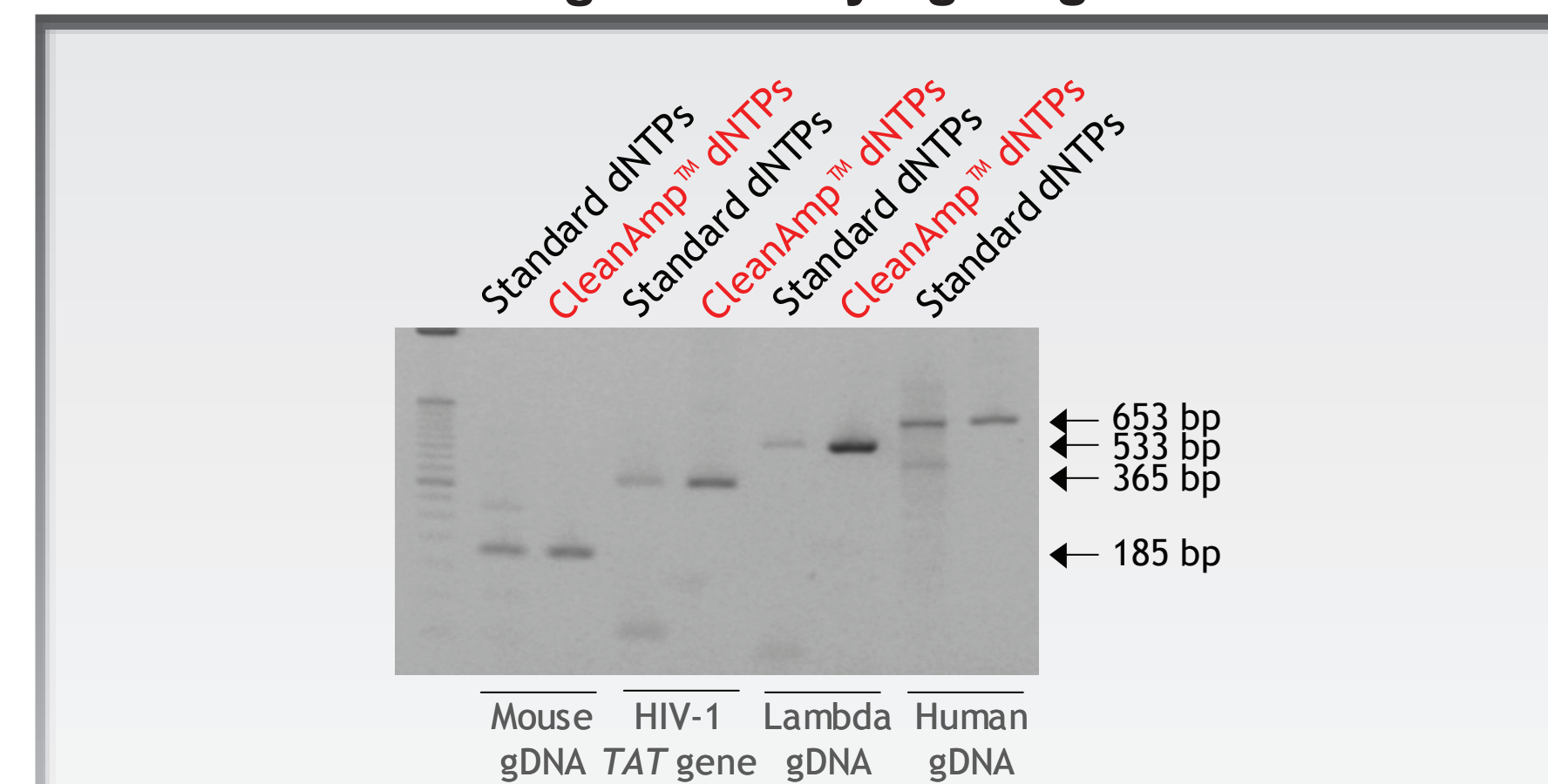


Figure 2

Comparison of Standard dNTPs and CleanAmp™ dNTPs in targets of varying lengths



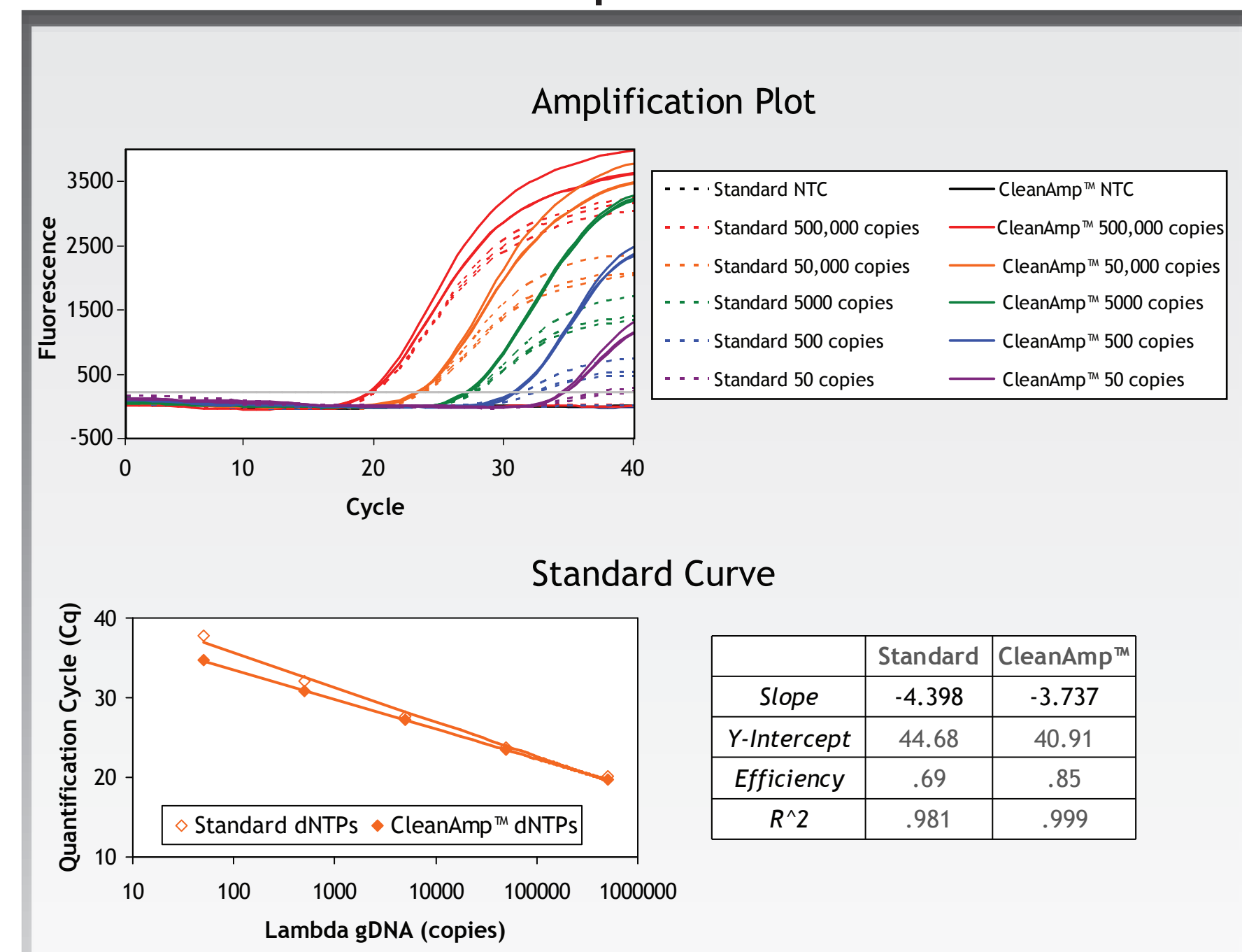
CleanAmp™ dNTPs improve PCR performance for different targets

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM MgCl₂), Primers (0.1-0.2 μM), 0.2-0.4 mM dNTPs (standard or 3'-TBE), 500 copies Lambda gDNA, 0.05 ng Mouse gDNA, 50 copies HIV-1 TAT gene, 0.5 ng Human gDNA, TaqMan® probe (0.1 μM), ROX, 1-1.25 U Taq DNA polymerase, 25 OR 50 μL.

Thermal cycling conditions:
Mouse gDNA: 95°C @ 10 min, cycle 40x(95°C @ 15 sec., 60°C @ 30 sec., 72°C @ 30 sec.) 3) 72°C @ 5 min.
HIV-1 TAT gene: 95°C @ 10 min, cycle 35x(95°C @ 40 sec, 58°C @ 30 sec, 72°C @ 1 min), 72°C @ 7 min
Human gDNA: 95°C @ 2 min, cycle 35x(95°C @ 30 sec, 60°C @ 1 sec, 72°C @ 45 sec), 72°C @ 7 min
Lambda gDNA: 95°C @ 10 min, cycle 40x (95°C @ 40 sec., 57°C @ 30 sec., 72°C @ 1 min), 72°C @ 7 min.

Figure 3

Evaluation of CleanAmp™ dNTPs in Real-time PCR



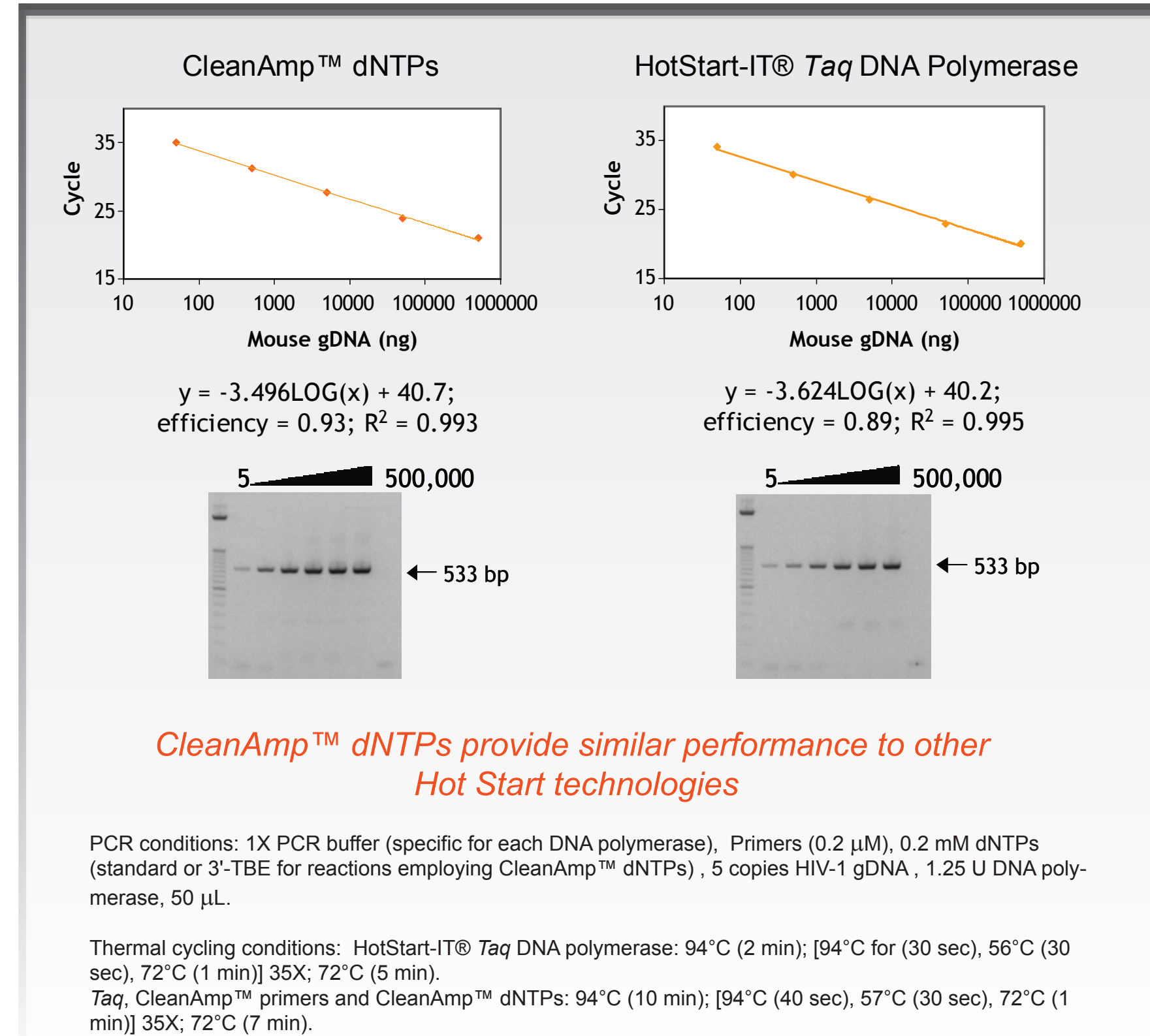
CleanAmp™ dNTPs improve Real-time PCR performance

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM MgCl₂), Primers (0.2 μM), 0.4 mM dNTPs (standard or 3'-TBE), 0-500,000 copies Lambda gDNA, TaqMan® probe (0.1 μM), ROX, 1.25 U Taq DNA polymerase, 25 μL.

Thermal cycling conditions: 95°C @ 10 min, cycle 40x (95°C @ 40 sec., 57°C @ 30 sec., 72°C @ 1 min), 72°C @ 7 min.

Figure 4

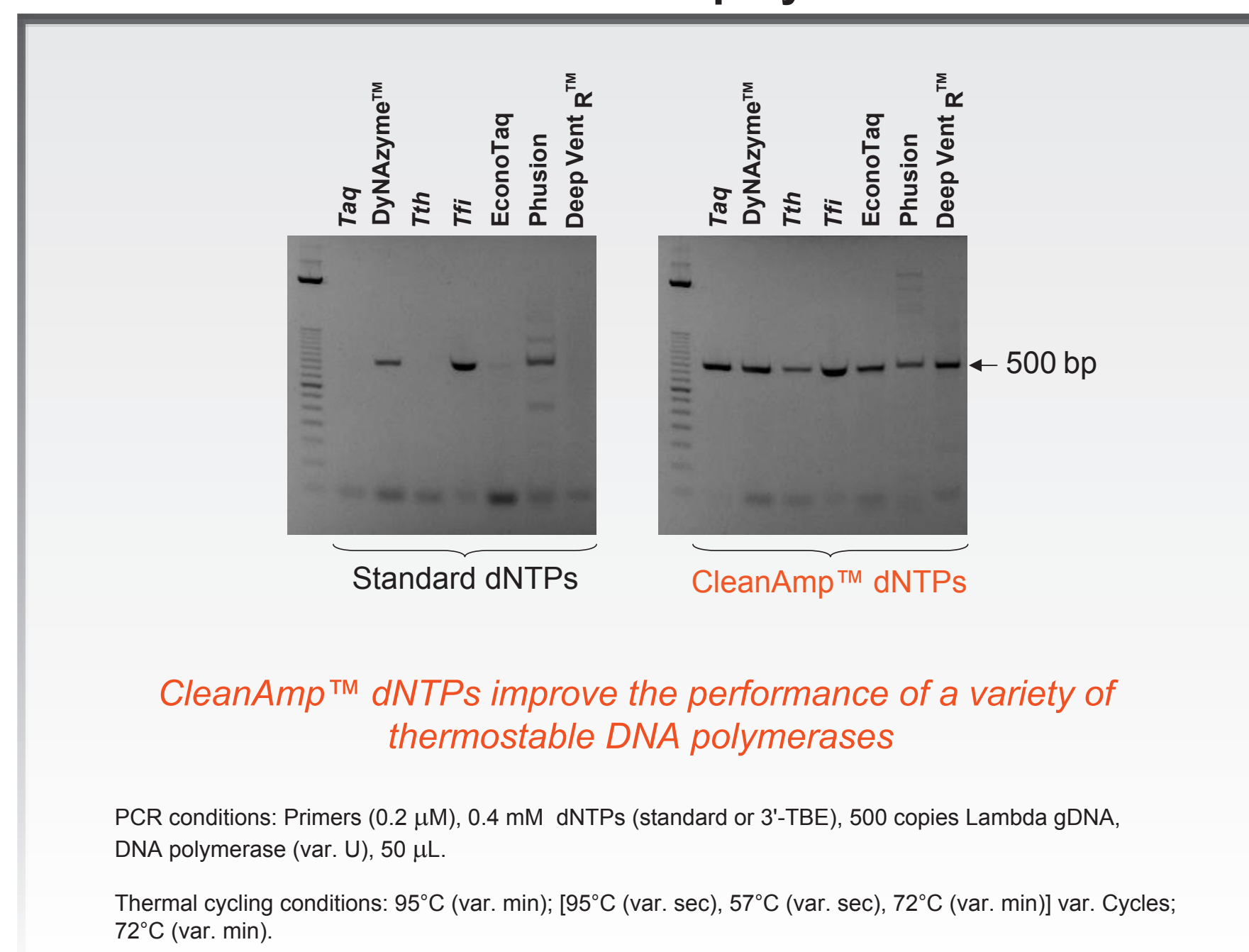
Comparison of CleanAmp™ dNTPs to another commonly used Hot Start technology



CleanAmp™ dNTPs provide similar performance to other Hot Start technologies

Figure 5

Evaluation of CleanAmp™ dNTPs for use with thermostable DNA polymerases



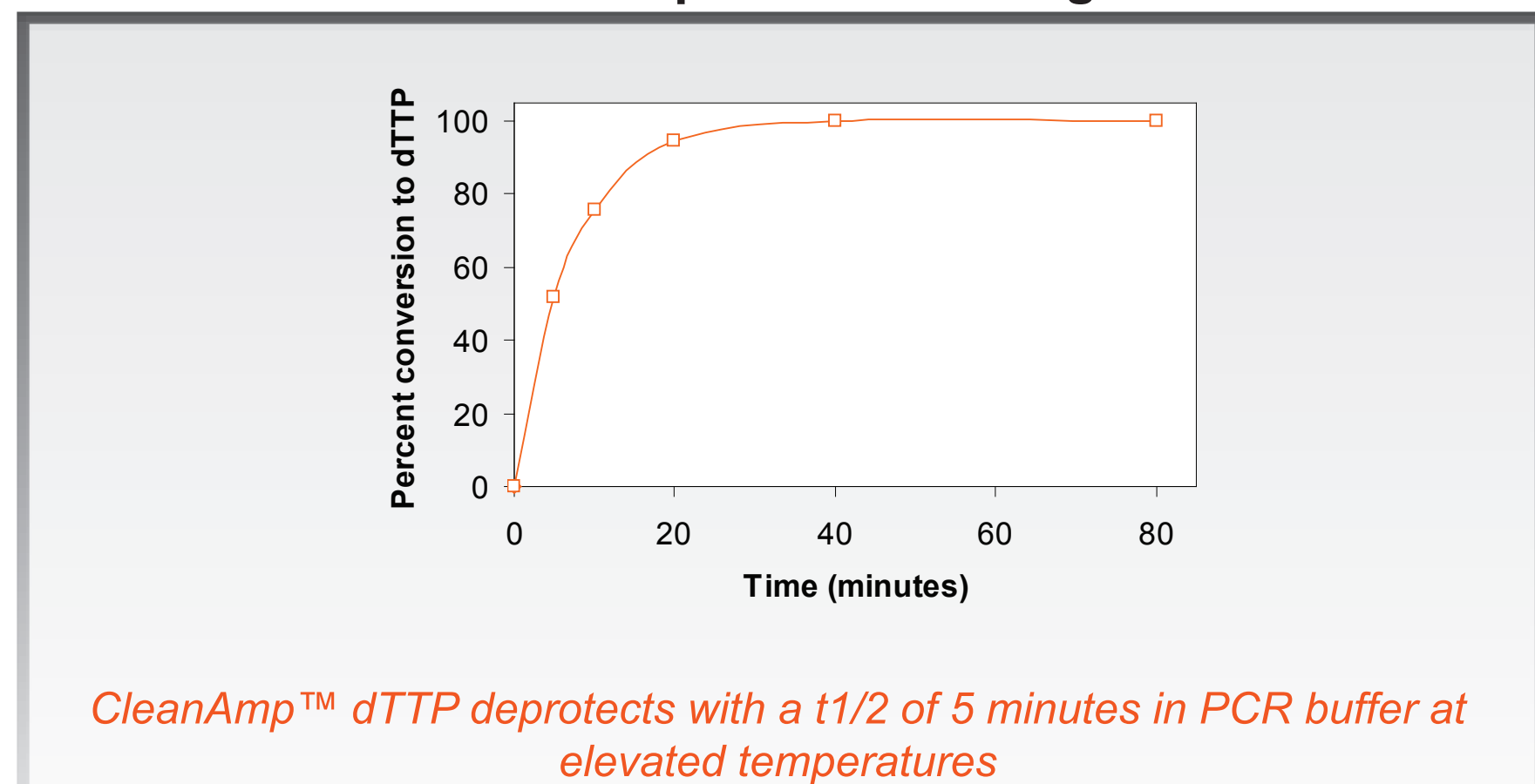
CleanAmp™ dNTPs improve the performance of a variety of thermostable DNA polymerases

PCR conditions: Primers (0.2 μM), 0.4 mM dNTPs (standard or 3'-TBE), 500 copies Lambda gDNA, DNA polymerase (var. U), 50 μL.

Thermal cycling conditions: 95°C (var. min); [95°C (var. sec), 57°C (var. sec), 72°C (var. min)] var. Cycles; 72°C (var. min).

Figure 6

Assessment of the deprotection kinetics of CleanAmp™ dNTP analogs

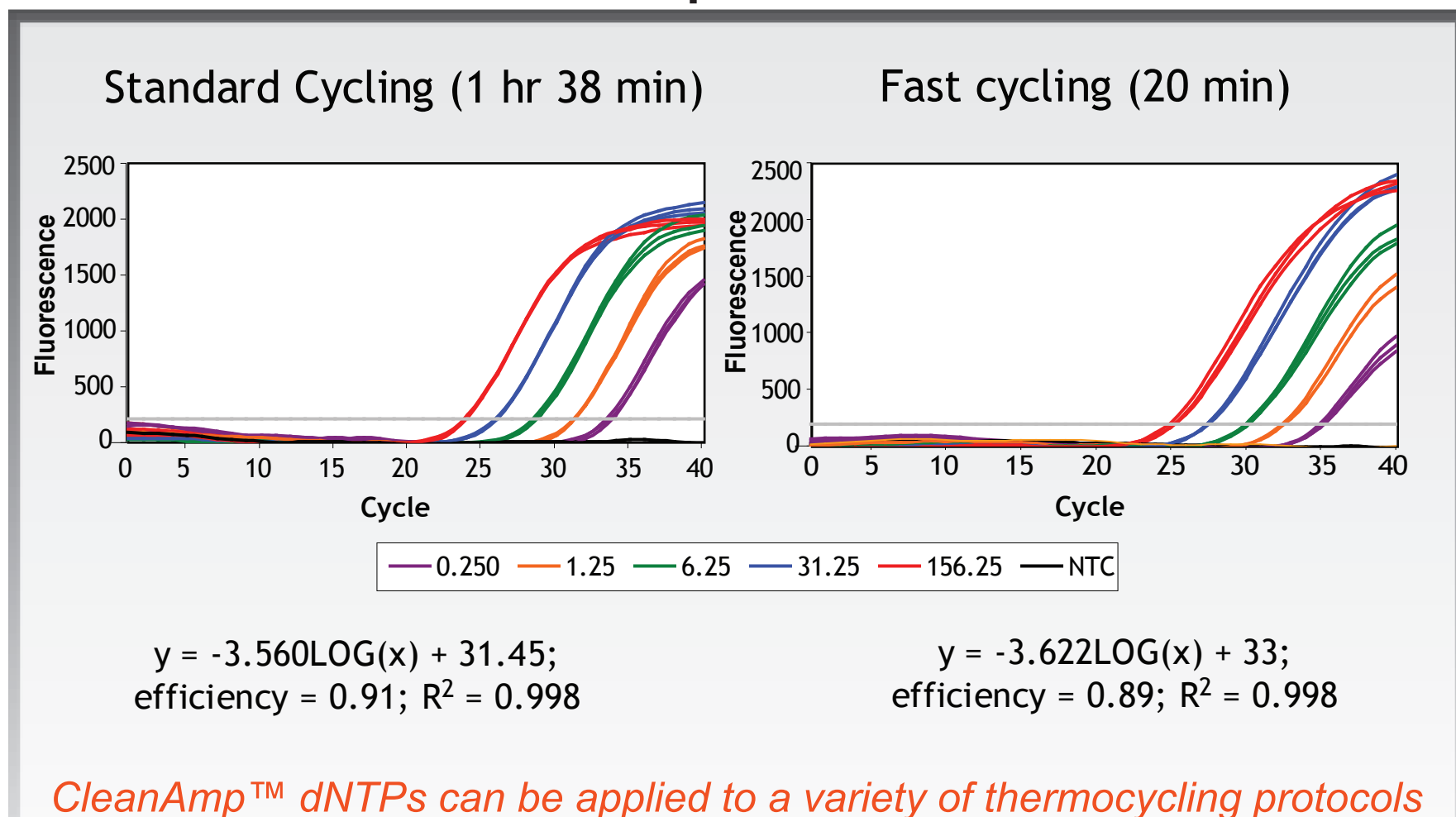


CleanAmp™ dTTP deprotects with a t_{1/2} of 5 minutes in PCR buffer at elevated temperatures

Deprotection conditions: 1X PCR buffer (50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris (pH 8.4 at 25°C)); 0.25 mM 3'-TBE-dTTP, 95°C incubation.

Figure 7

Evaluation of CleanAmp™ dNTPs in Standard and Fast PCR protocols



CleanAmp™ dNTPs can be applied to a variety of thermocycling protocols

Figure 8

Comparison of standard dNTPs and CleanAmp™ dNTPs in multiplex PCR

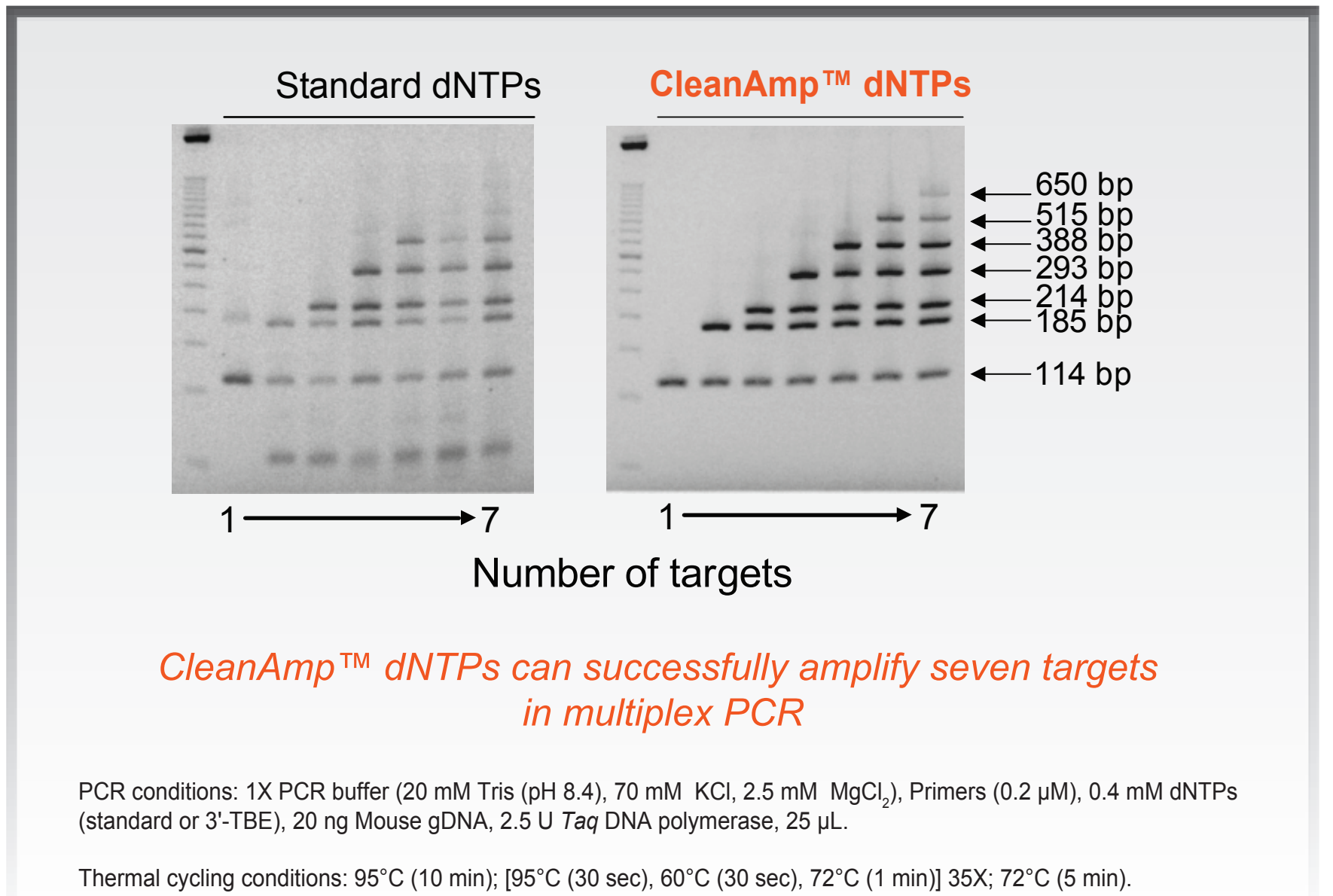
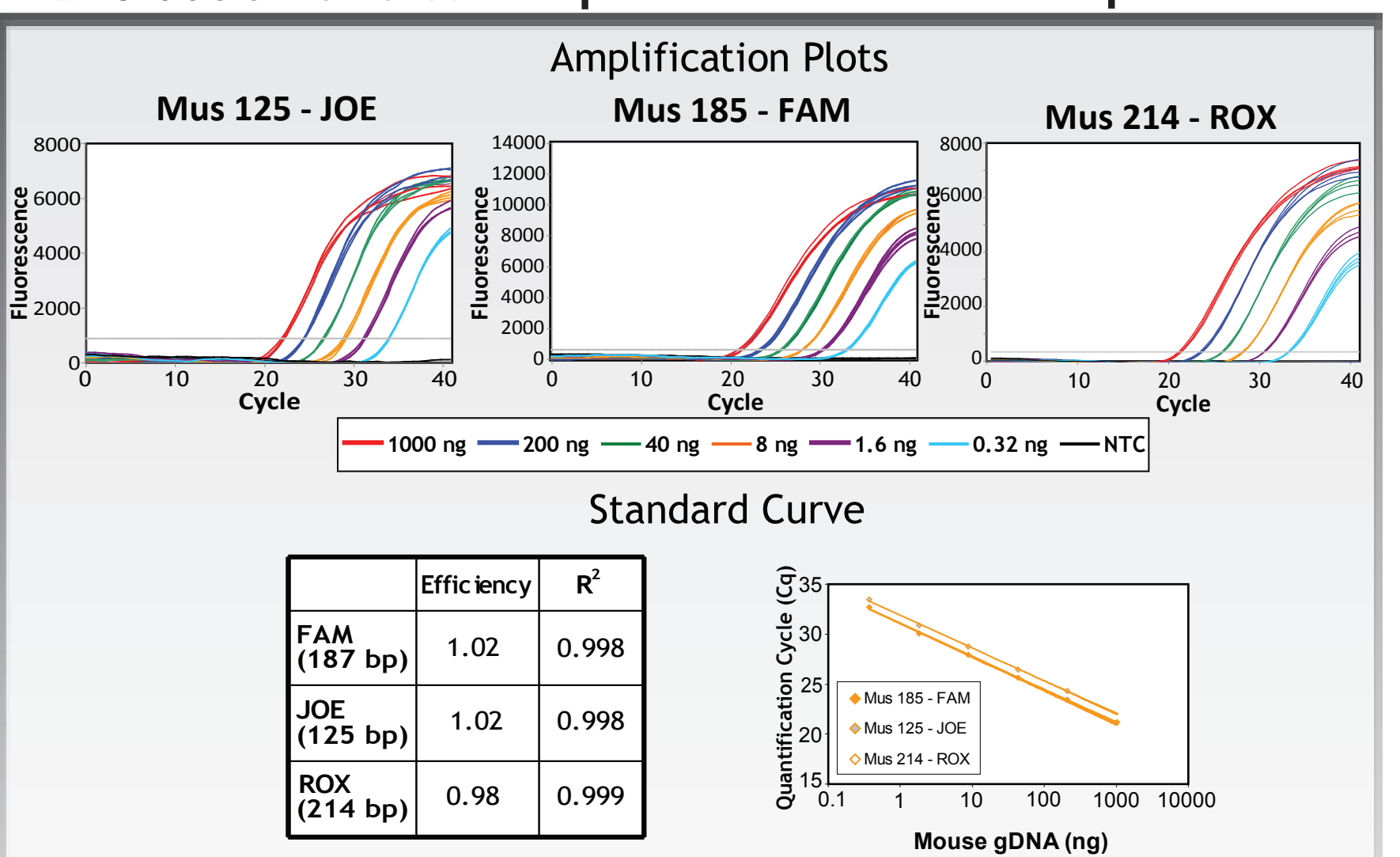


Figure 9

Evaluation of CleanAmp™ dNTPs in fast multiplex PCR



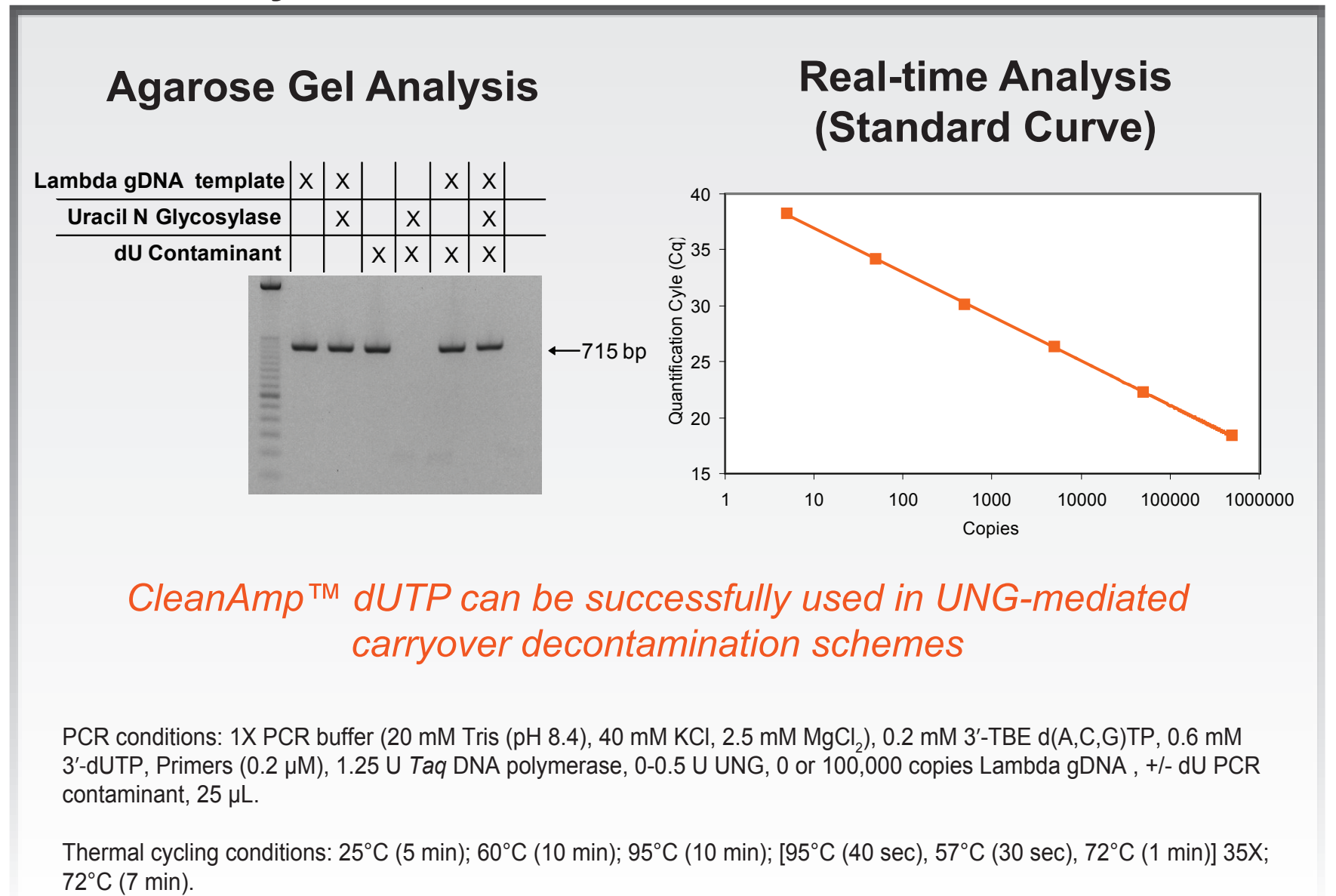
CleanAmp™ dNTPs allow simultaneous amplification of three targets in 27 minutes while maintaining similar efficiencies for all targets

PCR conditions: 1X PCR buffer, 4 mM MgCl₂, 70mM KCl total, 0.6 mM dNTP-TBE, Primer conc: 0.7 μM 214 primers, 0.5 μM, Hydrolysis probes 0.05 FAM and 0.2 μM ROX and JOE, 5 U/25μLx Enzymatics Taq DNA polymerase, 1000-0.32 ng Mouse gDNA, 15 μL.

Thermal cycling conditions: 98°C(30 sec); [95°C (5 sec), 65°C (20 sec)] 40x.

Figure 10

Development of CleanAmp™ dUTP for carryover decontamination PCR protocols



Conclusion

- CleanAmp™ dNTPs improve PCR performance relative to standard dNTPs for various targets of different lengths.
- CleanAmp™ dNTPs yield robust amplification of target in real-time PCR.
- CleanAmp™ dNTPs provide comparable results to another Hot Start DNA technology.
- CleanAmp™ dNTPs enrich PCR specificity when used with a variety of thermostable DNA polymerases.
- CleanAmp™ dNTPs can be applied to both standard and fast PCR cycling protocols.
- CleanAmp™ dNTPs enhance the specificity of multiplex PCR, allowing for amplification of up to seven targets.
- CleanAmp™ dNTPs can be used in fast multiplex PCR, amplifying three targets in 27 minutes.
- CleanAmp™ dUTP can be incorporated into UNG decontamination schemes.

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