

Chemically Modified Primers for Improved Hot Start PCR

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Abstract

PCR (polymerase chain reaction), a commonly used molecular biology technique, has intrinsic limitations due to frequently encountered problems such as primer dimer formation and off-target amplification (1). To improve the specificity of PCR, TriLink's novel approach to "Hot Start" PCR employs chemically modified primers. CleanAmp™ primers are available as either CleanAmp™ Turbo or Precision and can be easily prepared by standard solid-phase oligonucleotide synthesis. These primers differ in the rate of release of the chemical protecting group thereby allowing for greater control of primer extension. We show that the presence of these primer modifications significantly reduces the amount of primer dimer and mis-priming products formed, relative to corresponding unmodified primers. Turbo primer modifications have shown great advantage in fast cycling and in multiplex reactions. In reverse transcription PCR and low copy number detection, Precision primers provide optimal performance. Overall, this unique approach to "Hot Start" activation offers valuable improvements to PCR performance.

Fig 1: Proposed Activation Mechanism of CleanAmp™ Primers

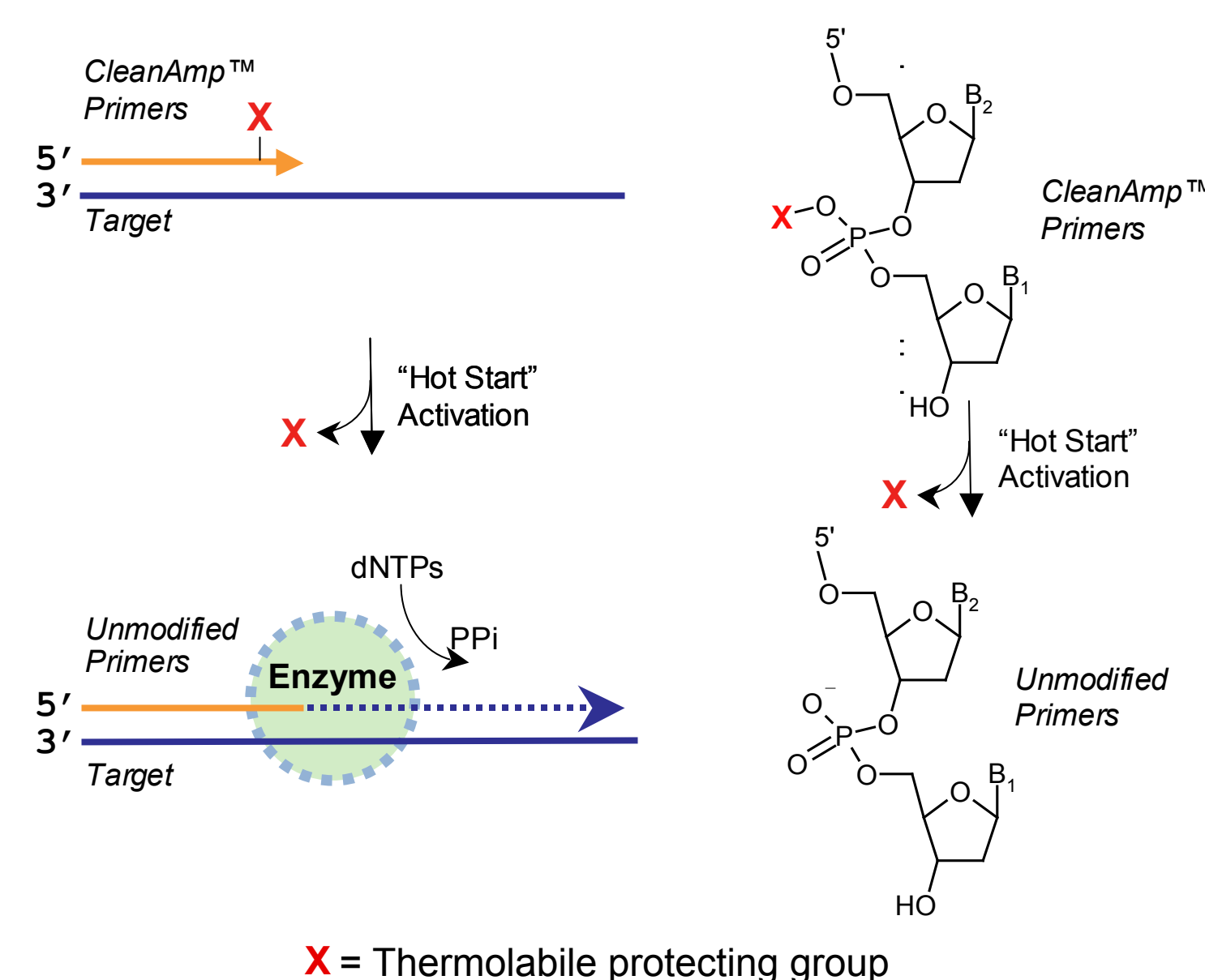
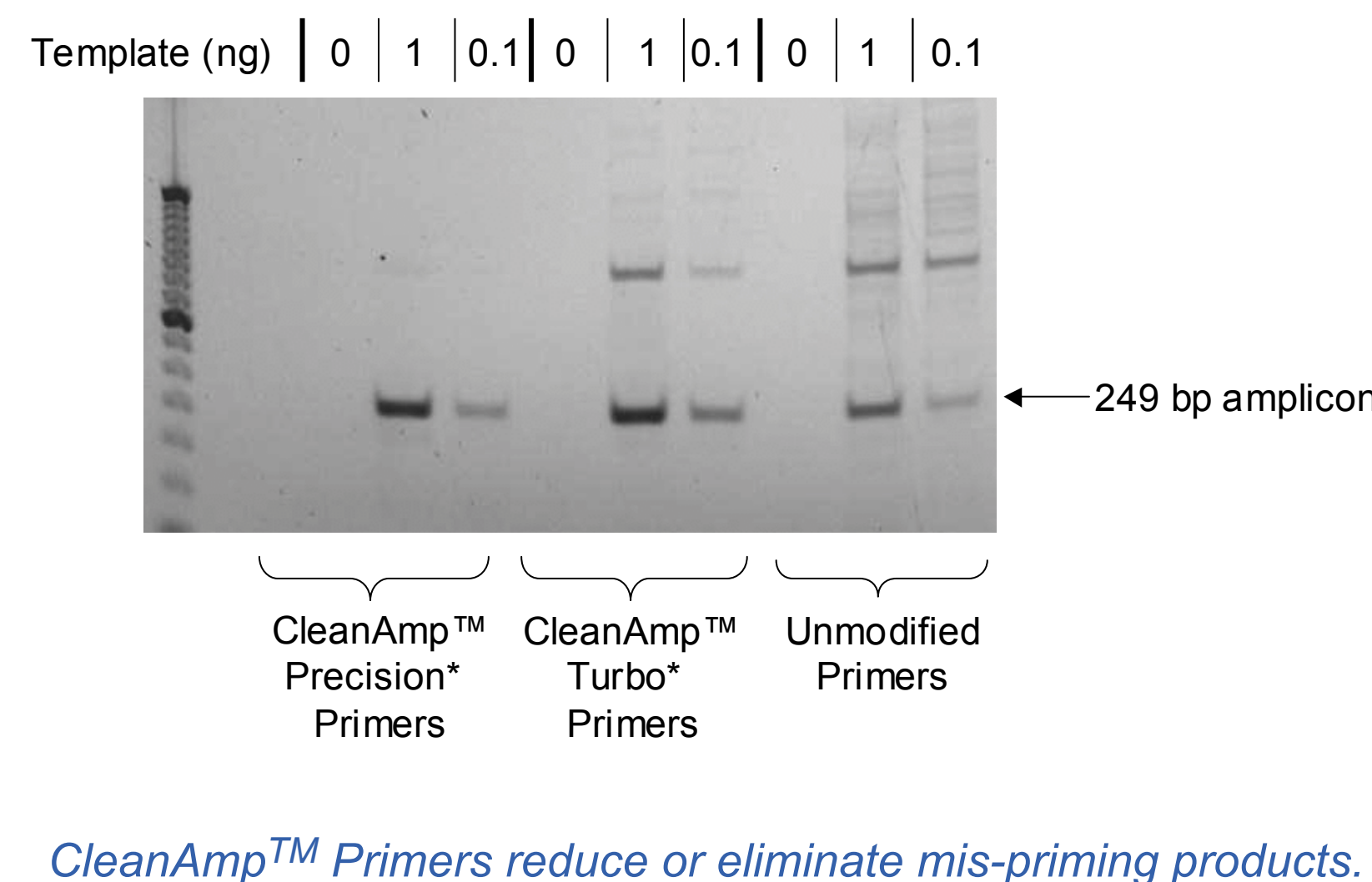


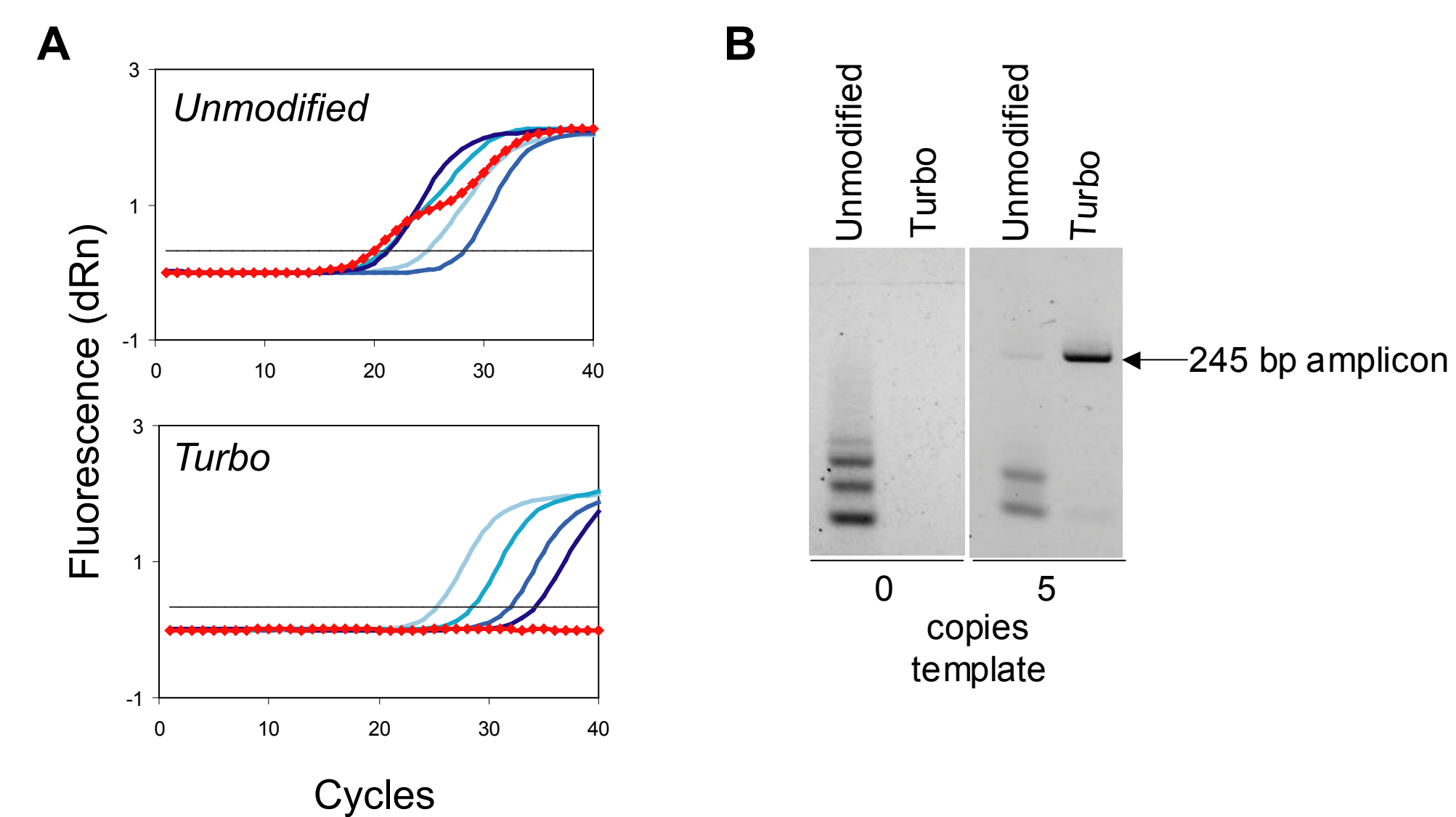
Fig 4: Endpoint PCR Evaluation of CleanAmp™ Primers in a Mis-priming System



PCR conditions:
1X PCR buffer (10 mM Tris (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂), Primers (0.5 μM), dNTPs (0.2mM), 0-1 ng of mouse gDNA, Taq DNA polymerase (1.25U), 50 μL.

Thermal cycling conditions:
95°C (10 min); [95°C (15 sec), 58°C (30 sec), 72°C (30 sec)]35X, 72°C (5 min)

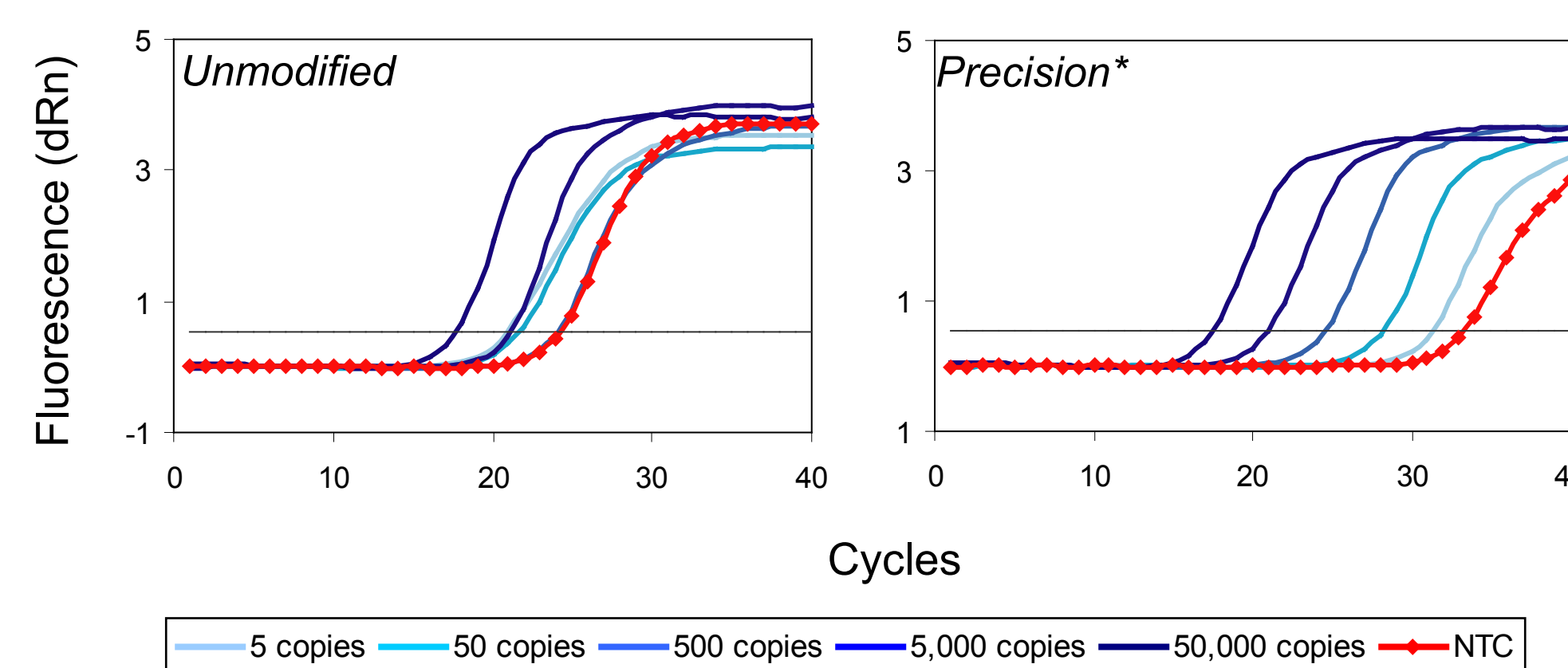
Fig 8: Fast Cycling PCR Assessment of CleanAmp™ Turbo Primers



PCR conditions:
1X PCR buffer (10 mM Tris (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂), Primers (2 μM), 0.8 mM dNTPs, 0-10,000 copies Lambda gDNA, 3.75 U Taq DNA polymerase, 25 μL, 1:2000 SYBR Green.

Thermal cycling conditions:
[95°C (10 sec), 66°C (30 sec)]40X

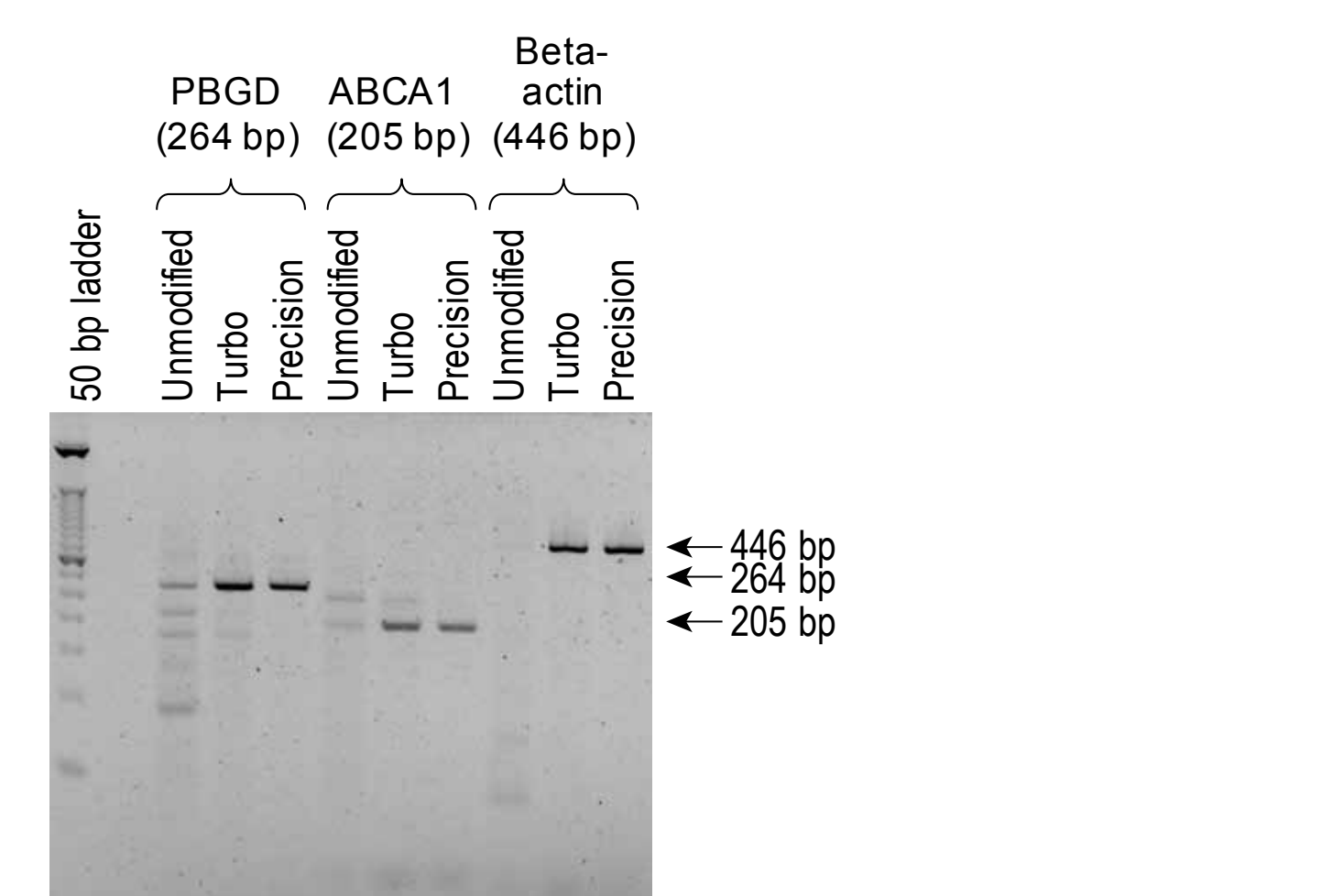
Fig 5: SYBR® Green Real-time Detection of Limiting Template Concentrations



PCR conditions:
1X PCR buffer 10 mM Tris (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, Primers (0.5 μM), SYBR® Green (0.15X), ROX (30 mM), dNTPs (0.2 mM), 0-50,000 copies Lambda gDNA, 1.25 U Taq DNA polymerase, 25 μL, reactions performed in duplicate.

Thermal cycling conditions:
95°C (10 min); [95°C (40 sec), 57°C (30 sec), 72°C (1 min)]40X

Fig 9: Performance of CleanAmp™ Primers in One-step RT-PCR

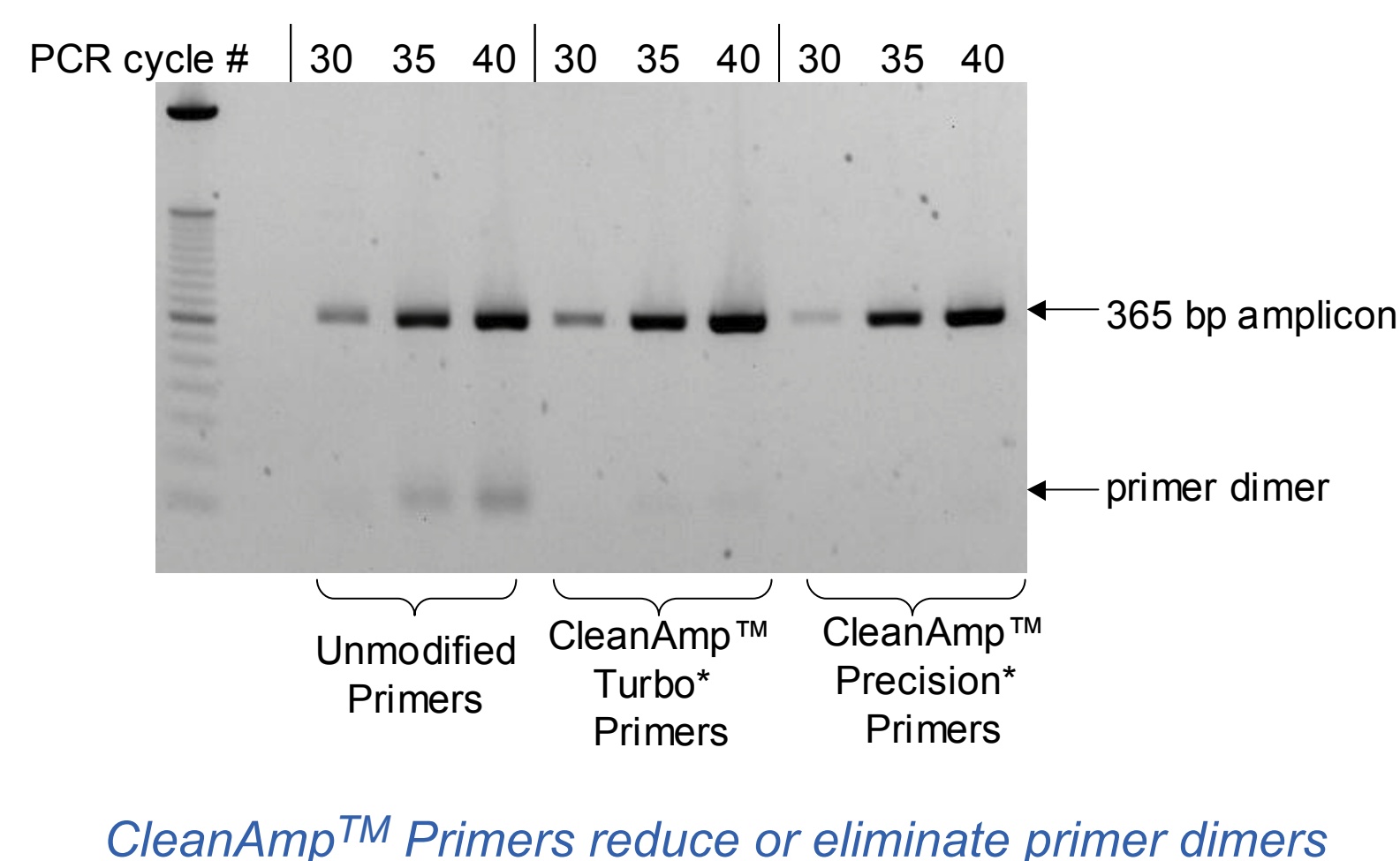


Because the presence of CleanAmp™ Primer's thermolabile modifications allow for selective primer utilization, the reaction can be completed in a one-step fashion.

PCR conditions:
1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂), Primers (0.5 μM), polydT primer (1 μM), 0.16 mM dNTPs, 0.25 μg Human liver total RNA, 25 U reverse transcriptase, 0.3 U Taq DNA polymerase, 25 μL.

Thermal cycling conditions:
42°C (30 min), 95°C (10 min), [95°C (30 sec) 60°C (30 sec) 72°C (30 sec)]30X, 72°C (5 min).

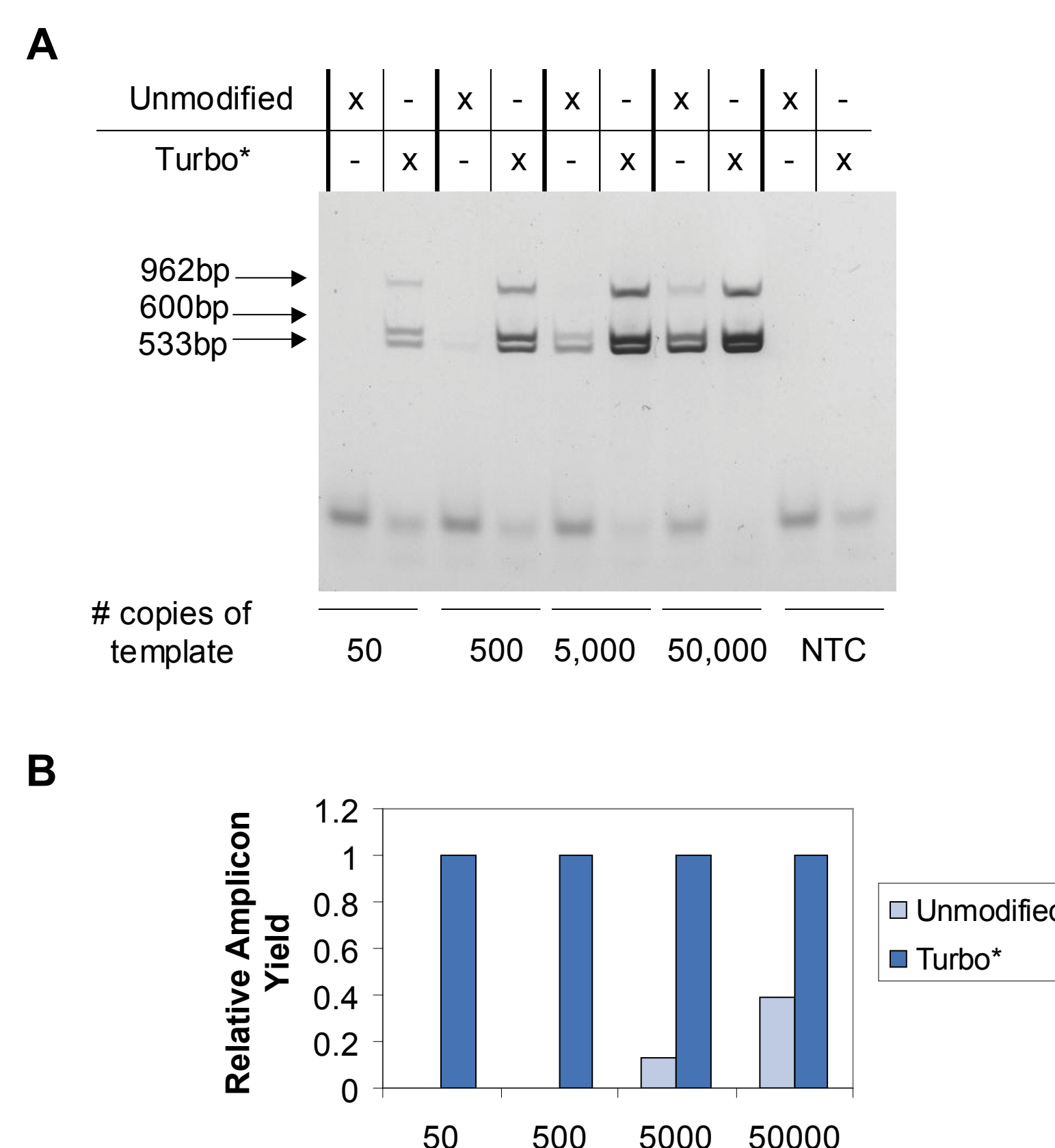
Fig 2: Endpoint PCR Evaluation of CleanAmp™ Primers in a Primer Dimer System



PCR conditions:
1X PCR buffer (10 mM Tris (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂), Primers (0.5 μM), dNTPs (0.2mM), 5 copies HIV-1 gDNA, Taq DNA polymerase (1.25U), 50 μL.

Thermal cycling conditions:
95°C (10 min); [95°C (40 sec), 56°C (30 sec), 72°C (1 min)]40X, 72°C (7 min)

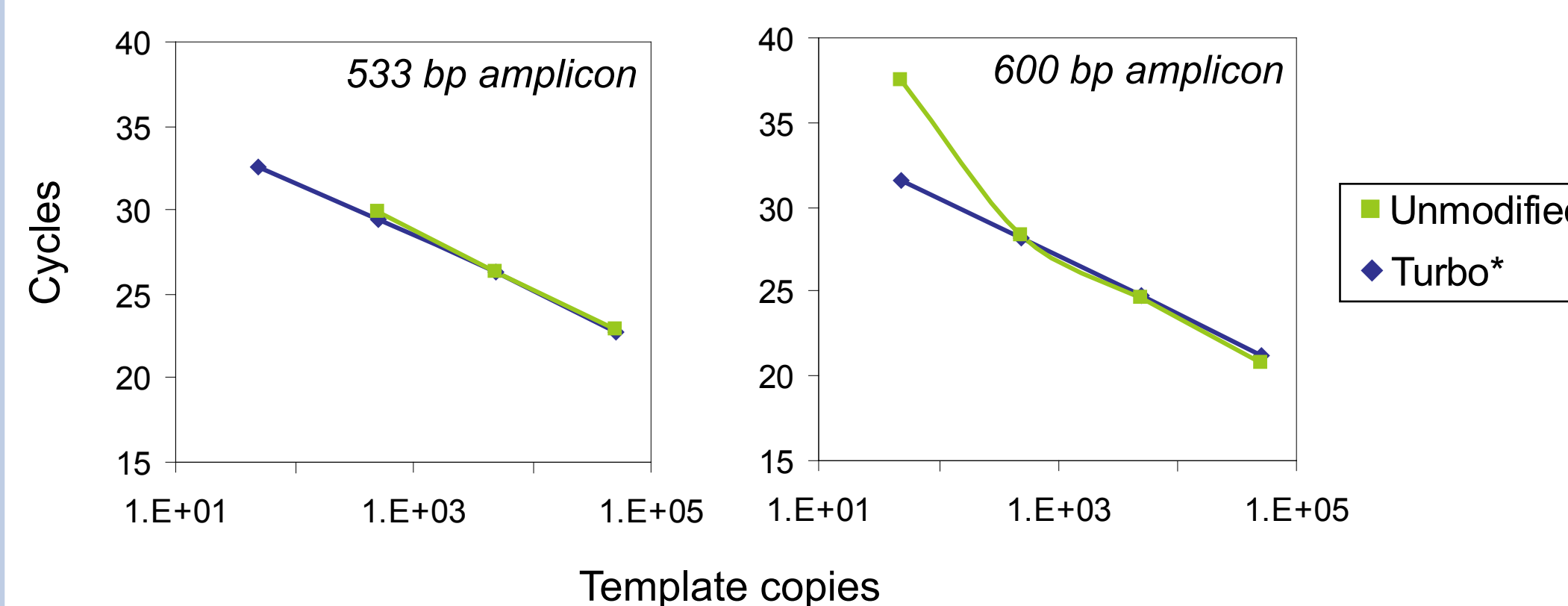
Fig 6: Endpoint Evaluation of CleanAmp™ Primers in Multiplexed PCR



PCR conditions:
1X PCR buffer (10 mM Tris (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂), Primers (0.5 μM), 0.2 mM dNTPs, 0-50,000 copies Lambda gDNA, 1.25 U Taq DNA polymerase, 50 μL.

Thermal cycling conditions:
95°C (10 min); [95°C (40 sec), 56°C (30 sec), 72°C (2 min)]35X, 72°C (7 min)

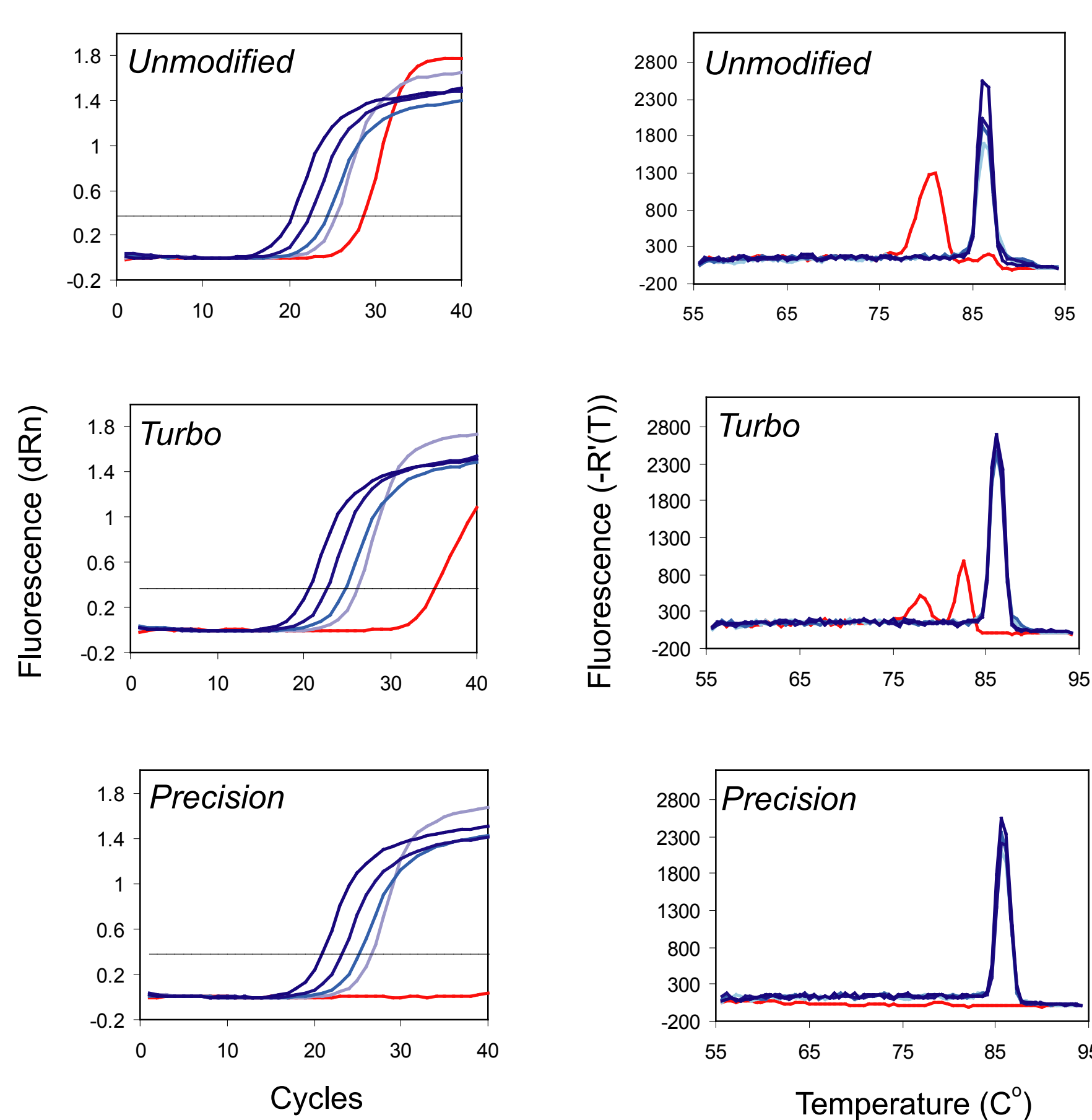
Fig 7: Real-time Multiplexed PCR Evaluation of CleanAmp™ Primers



PCR conditions:
1X PCR buffer (10 mM Tris (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂), CleanAmp™ Primers (2 μM), TaqMan® probe (0.1 μM), 0.2 mM dNTPs, 50-50,000 copies Lambda gDNA, 1.25 U Taq DNA polymerase, 25 μL, reactions performed in duplicate.

Thermal cycling conditions:
95°C (10 min); [95°C (40 sec), 56°C (30 sec), 72°C (2 min)]40X

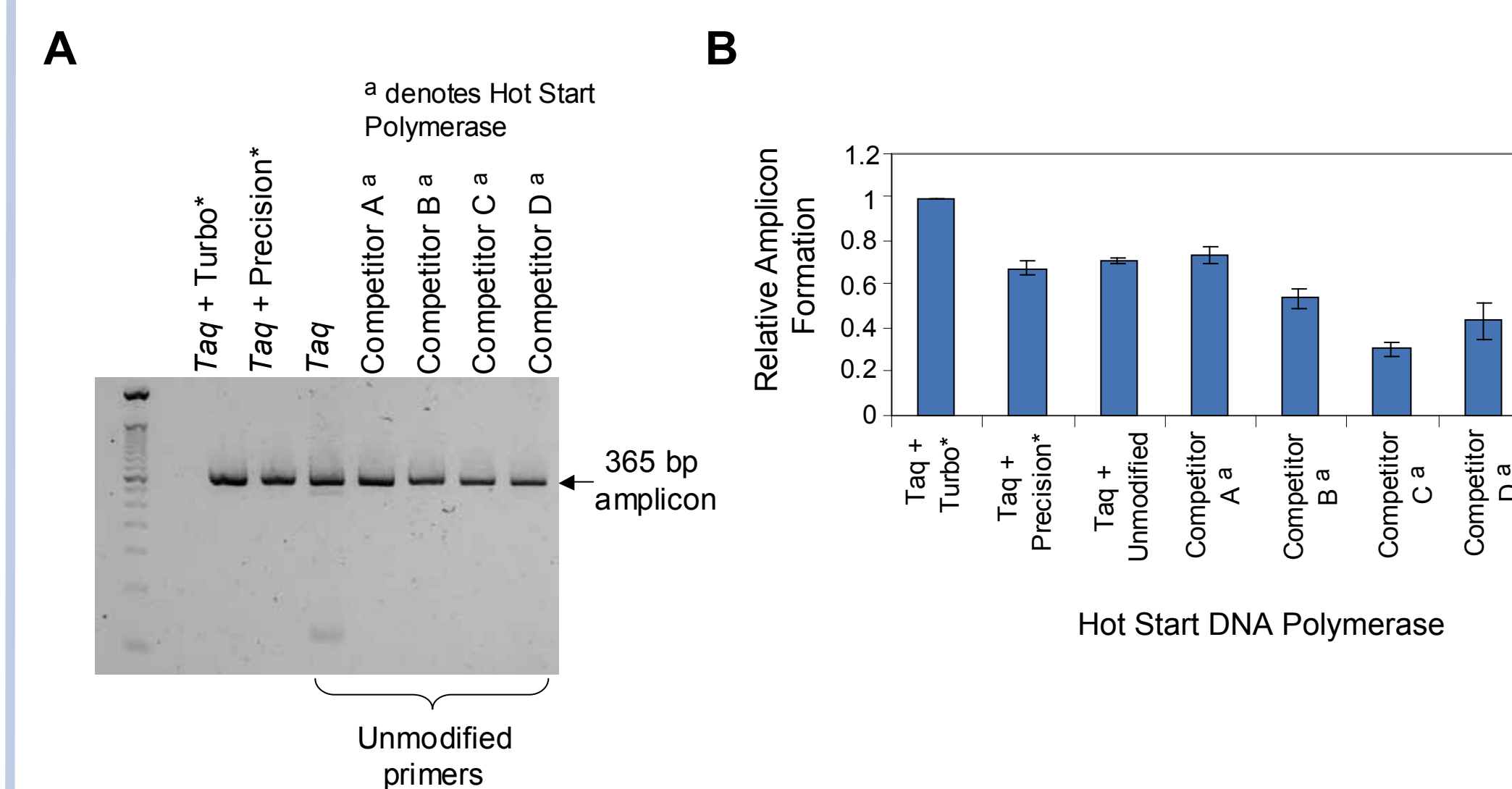
Fig 3: Real-Time PCR Evaluation of CleanAmp™ Primers in a Primer Dimer System



PCR conditions:
1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM MgCl₂), Primers (0.5 μM), 0.2 mM dNTPs, 0-125 copies HIV-1 gDNA, 1.25 U Taq DNA polymerase, 0.15X SYBR Green® I, 30 nM ROX passive reference dye, 25 μL.

Thermal cycling conditions:
95°C (2 min); [95°C (40 sec), 56°C (30 sec), 72°C (2 min)]40X.

Fig 10: Comparison of CleanAmp™ Primers to other Hot Start DNA Polymerases



PCR conditions:
1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM MgCl₂), Primers (0.4 μM), 0.2 mM dNTPs, 5 copies HIV-1 gDNA, DNA polymerases: (1.25 units), 50 μL.

Thermal cycling conditions:
95°C (10 min); [95°C (40 sec), 56°C (30 sec), 72°C (1 min)]35X, 72°C (7 min).

Conclusion

- CleanAmp™ Primers reduce or eliminate off target amplicon formation such as mis-priming and primer dimer formation.
- CleanAmp™ Precision Primers provide a lower limit of detection when compared to unmodified primers.
- CleanAmp™ Turbo primers are optimal for multiplex and fast-cycling PCR reactions by both lowering undesired off-target amplification and increasing overall desired amplicon yield.
- Using CleanAmp™ Precision primers in RT-PCR provides two benefits: 1) Increases specificity and yield, 2) Both RT and PCR steps can be combined into one tube as the thermolabile CleanAmp™ primers remain inactive during the first RT step.
- In systems plagued by primer dimer formation, CleanAmp™ Turbo primers provide a higher amplicon yield as compared to other commonly-used Hot Start DNA polymerases.

Acknowledgements

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References

(1) Chou, Q., et al. *Nucleic Acids Res.* (1992) 20, 1717-1723.

* Denotes CleanAmp™ Turbo II or CleanAmp™ Precision II primer modifications

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