

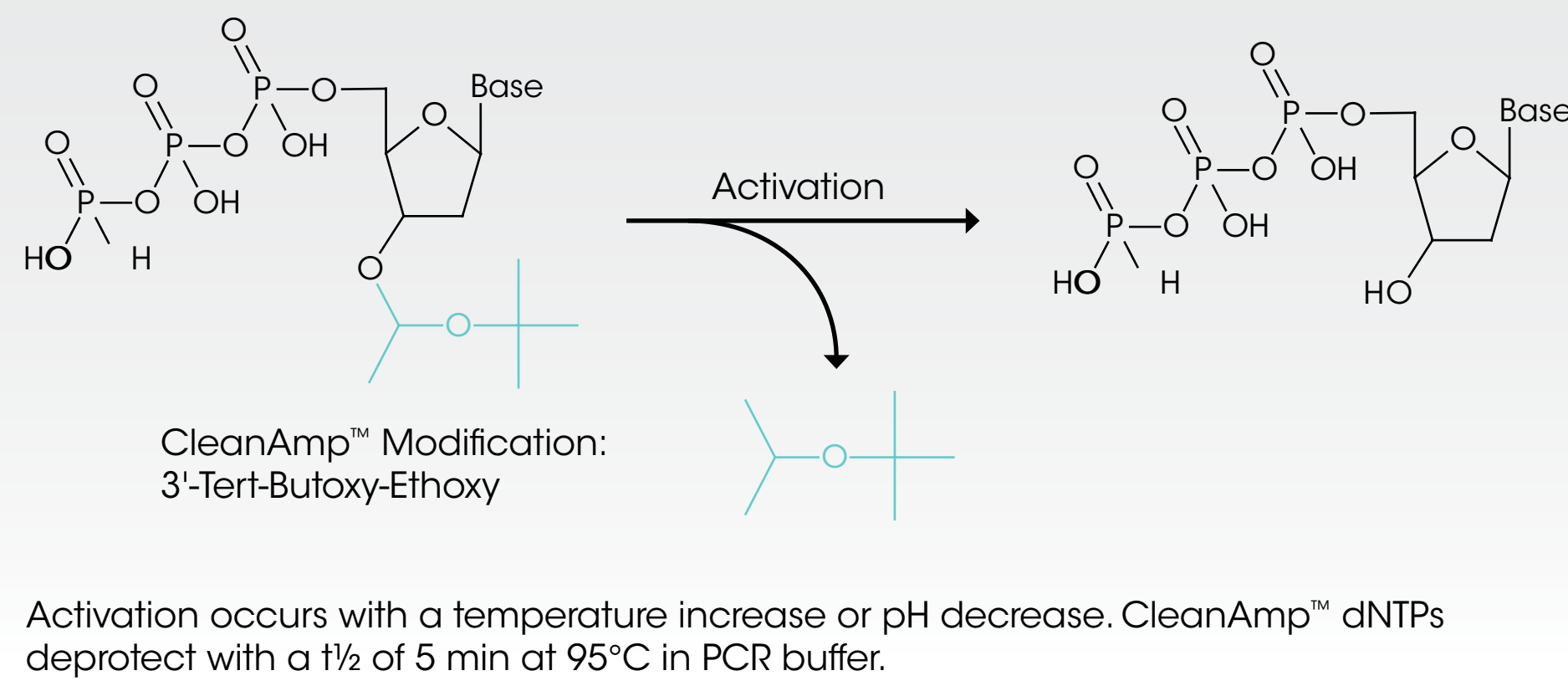
# The Universal Hot Start Tool: CleanAmp™ Hot Start dNTPs

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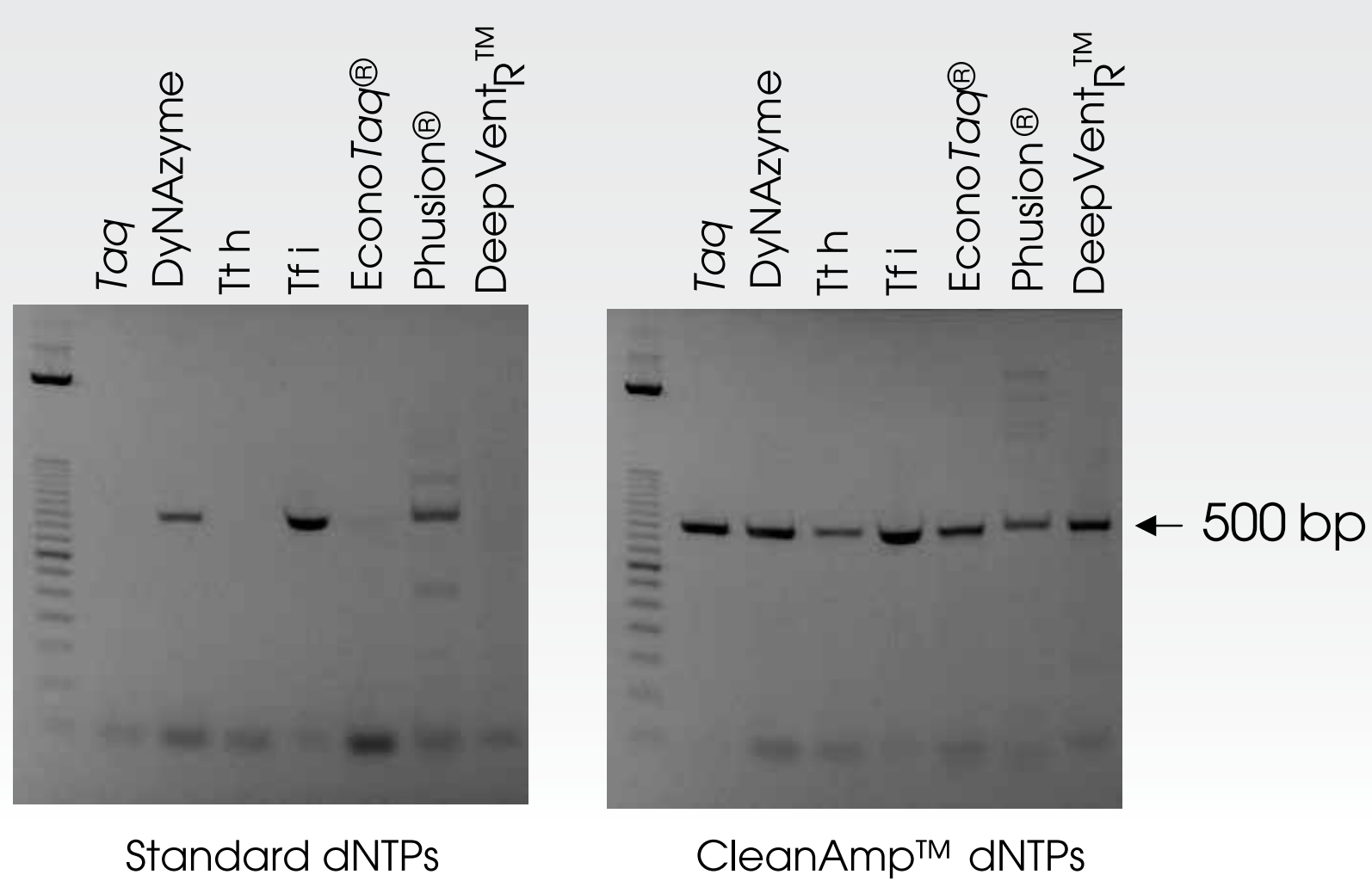
## Abstract

PCR is a powerful tool which is being consistently refined to push the limits of speed, sensitivity, and specificity. A Hot Start method is imperative in any PCR technique to improve performance by reducing primer dimers and to prevent mispriming. Many Hot Start technologies are limited to specific assay types or by higher temperature requirements for activation. In contrast, Hot Start dNTPs are a universal approach that offers a flexible mode of activation which relies on a combination of temperature, pH, and time. A thermolabile protecting group inhibits dNTP incorporation at ambient temperatures when many side reactions occur and is gradually released as temperature is elevated or pH is lowered. Hot Start dNTPs have demonstrated that they improve the performance of most reverse transcriptase and DNA polymerase enzymes by increasing yields and specificity in various assay types. The CleanAmp™ thermolabile protecting group can be applied to dNTP analogs such as 7-deaza-dGTP and dUTP. CleanAmp™ 7-deaza-dGTP improves GC-rich target amplification for targets up to 85% GC-rich and allows for more accurate and longer reads for downstream Sanger dideoxy sequencing. CleanAmp™ dUTP is used in carry over prevention assays in conjunction with Cod UNG (Uracil-DNA Glycosylase). CleanAmp™ dNTPs are an ideal tool for RT-PCR where they provide Hot Start functionality for both RT and PCR steps. At standard RT temperatures (45-65°C) only a fraction of dNTPs are available for cDNA synthesis which limits the ability of off target reactions from occurring. Once PCR temperatures (95°C) are reached, an abundance of dNTPs are available for efficient amplification. In RT-PCR assays Hot Start dNTPs exhibit improved yield and specificity when compared to other RT-PCR kits and can effectively amplify multiple targets at once. CleanAmp™ dNTPs are a universal Hot Start tool that can be integrated into a variety of assays to enhance performance.

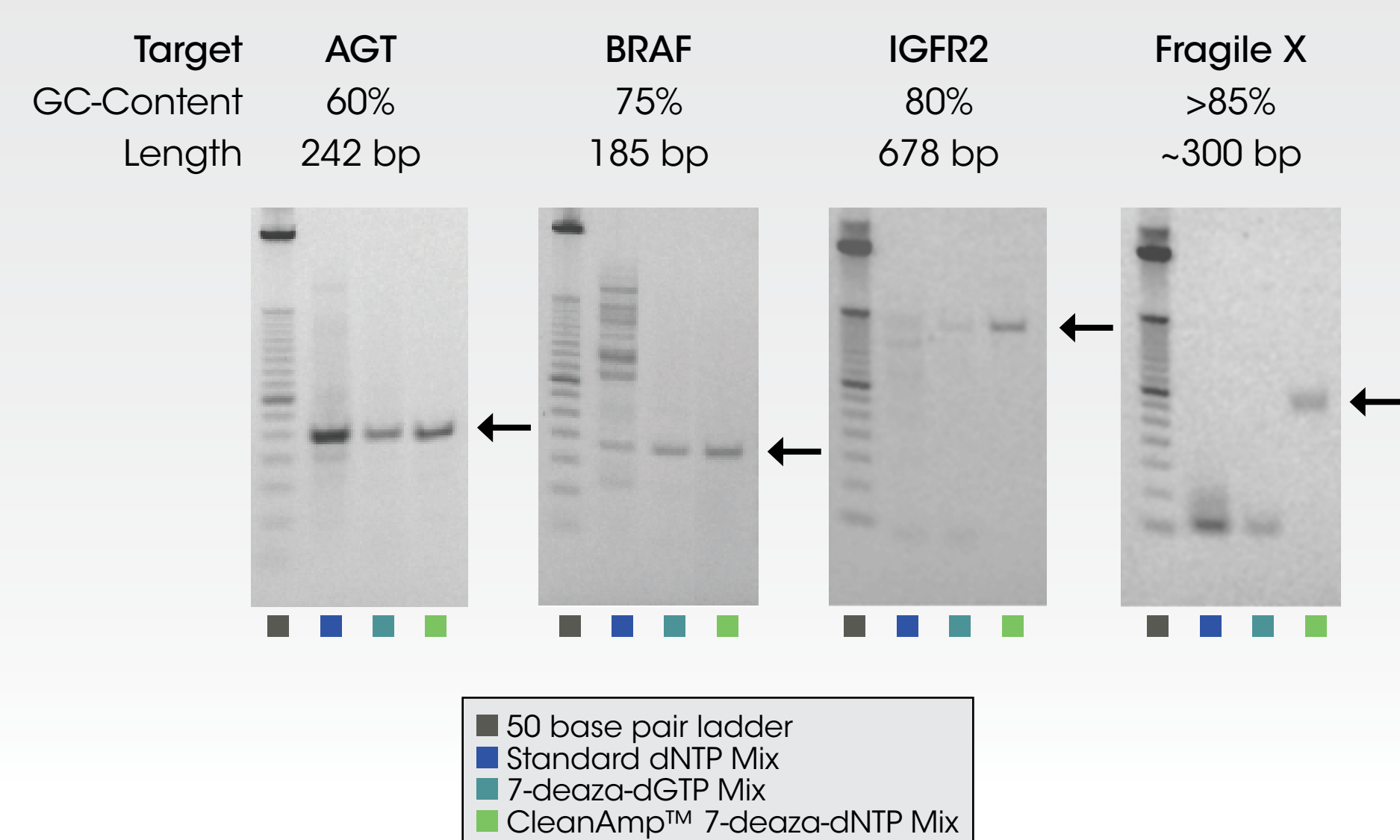
## Figure 1: CleanAmp™ Hot Start dNTP Structure & Activation



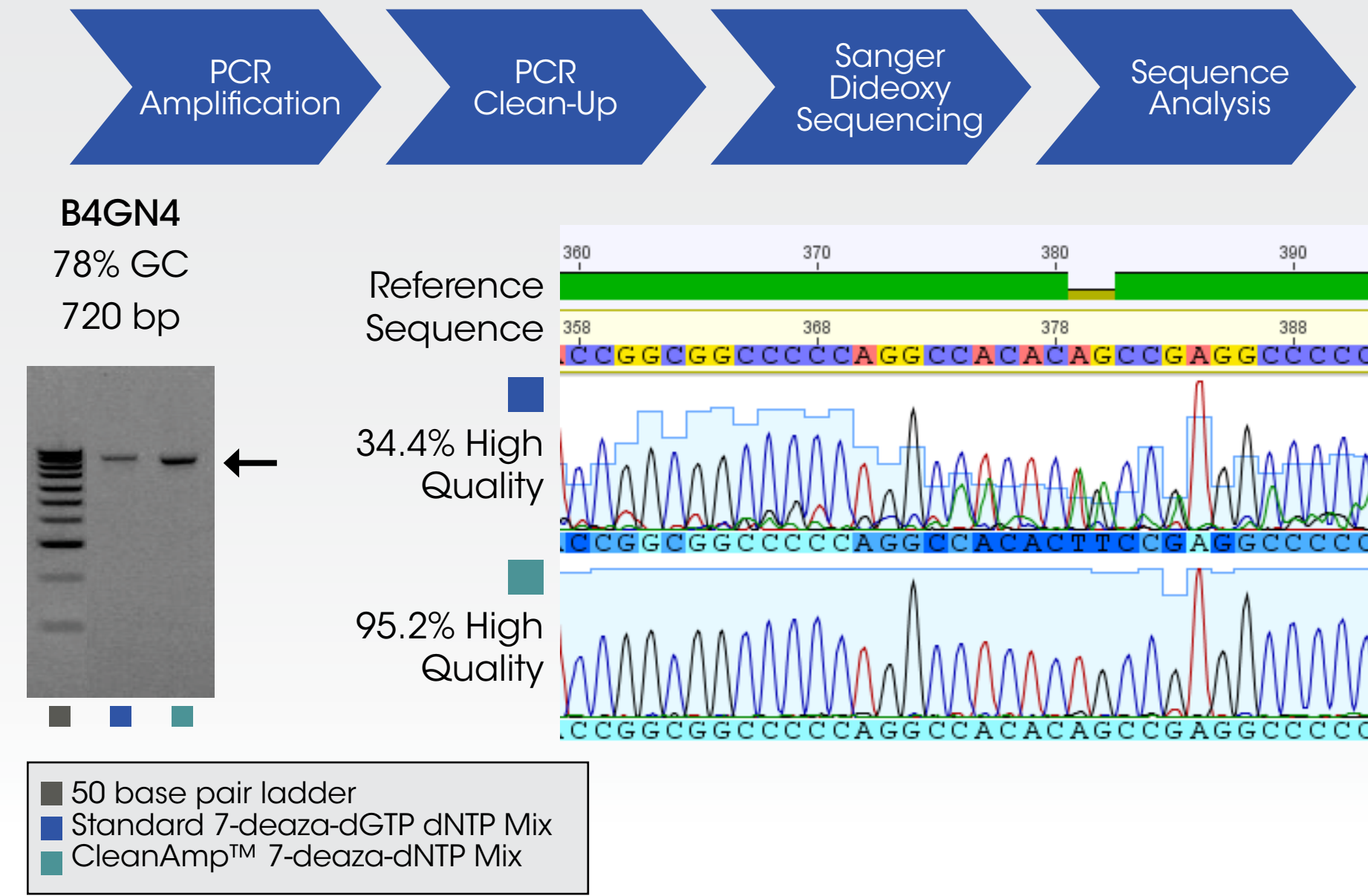
## Figure 2: CleanAmp™ dNTPs Improve the Performance of DNA Polymerases



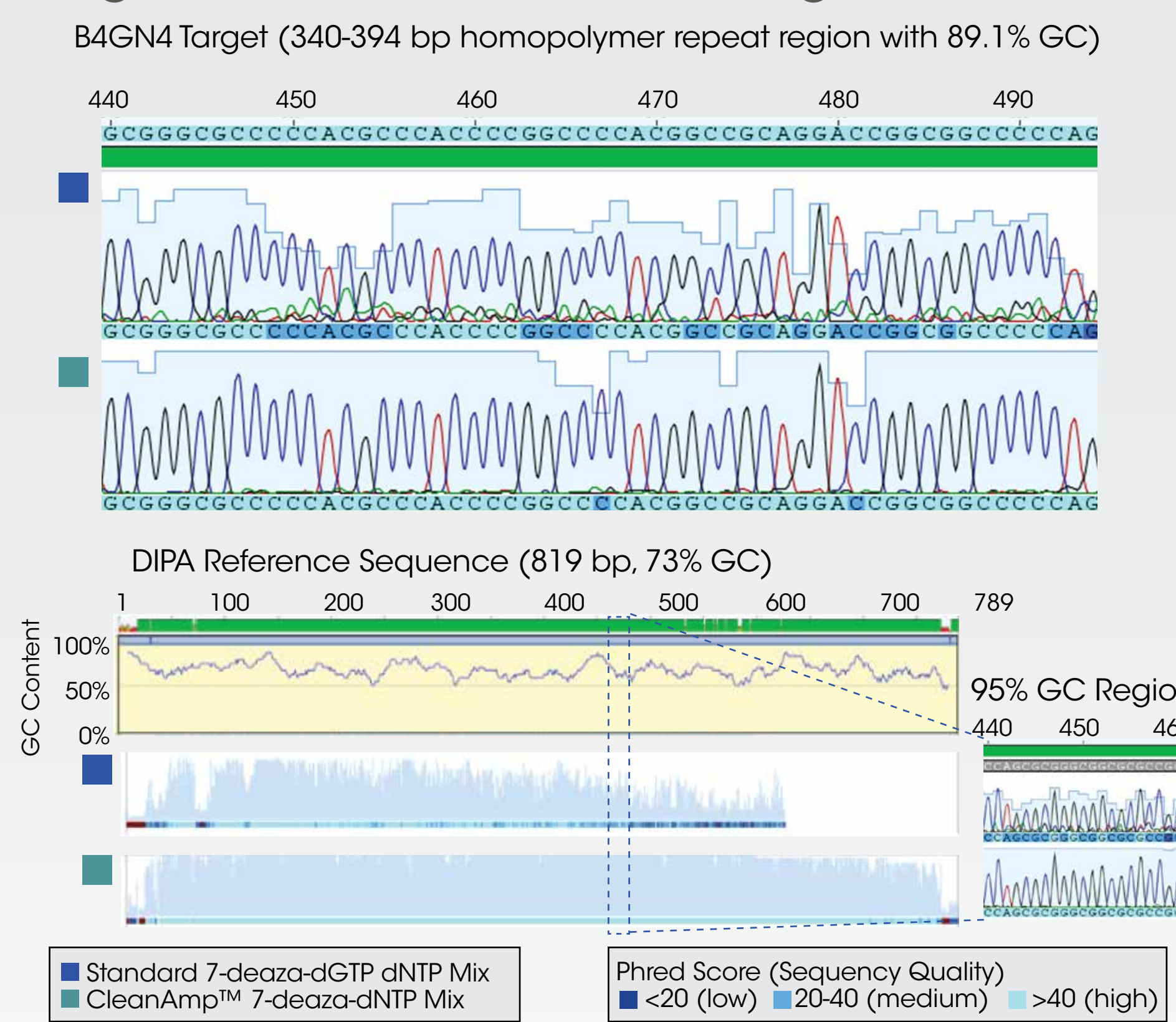
## Figure 3: Improved Amplification Yield and Specificity for Targets Up to 85% GC-Content



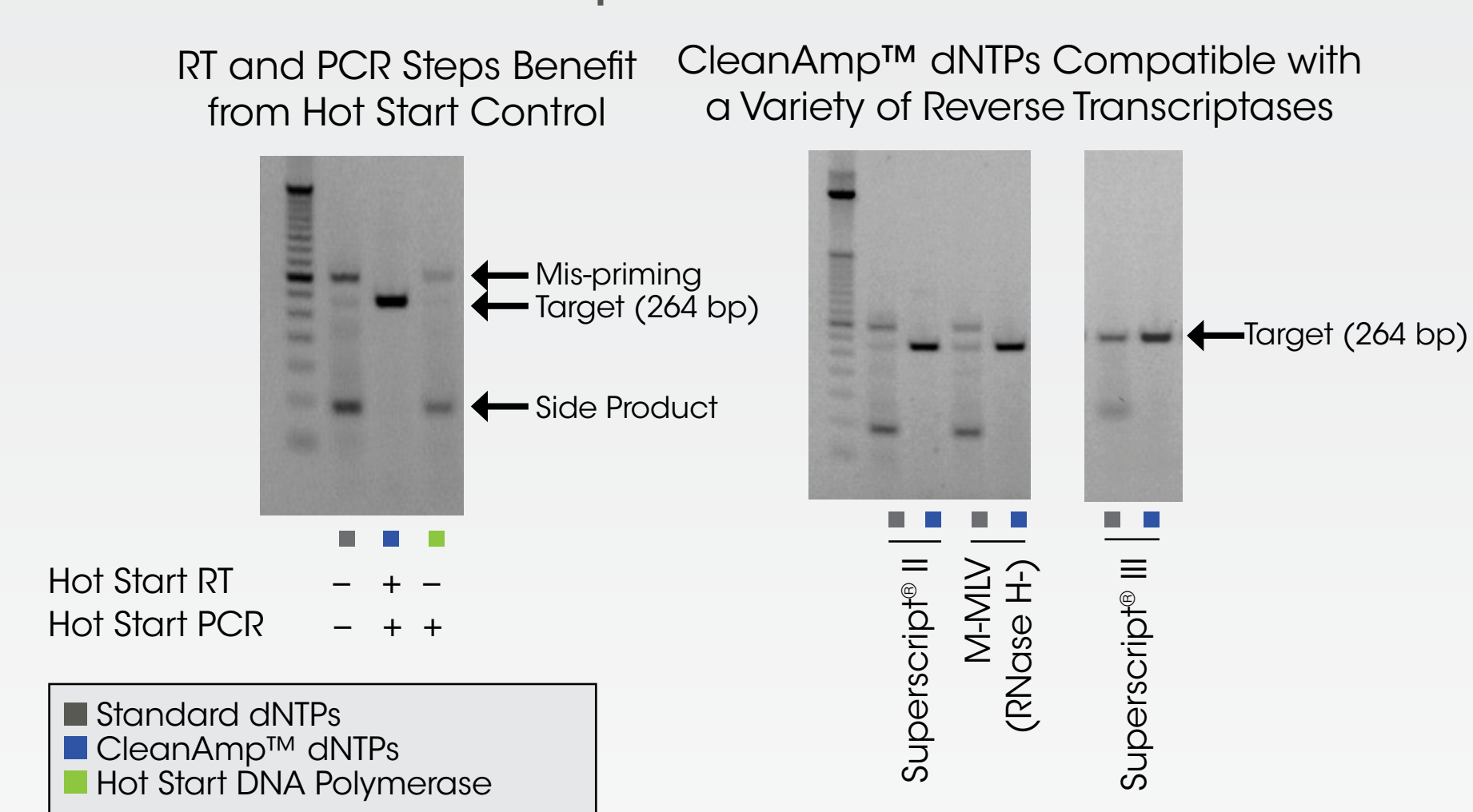
## Figure 4: PCR Amplification with CleanAmp™ 7-deaza-dGTP Improves Accuracy of Dideoxy Sequencing in GC-Rich Targets



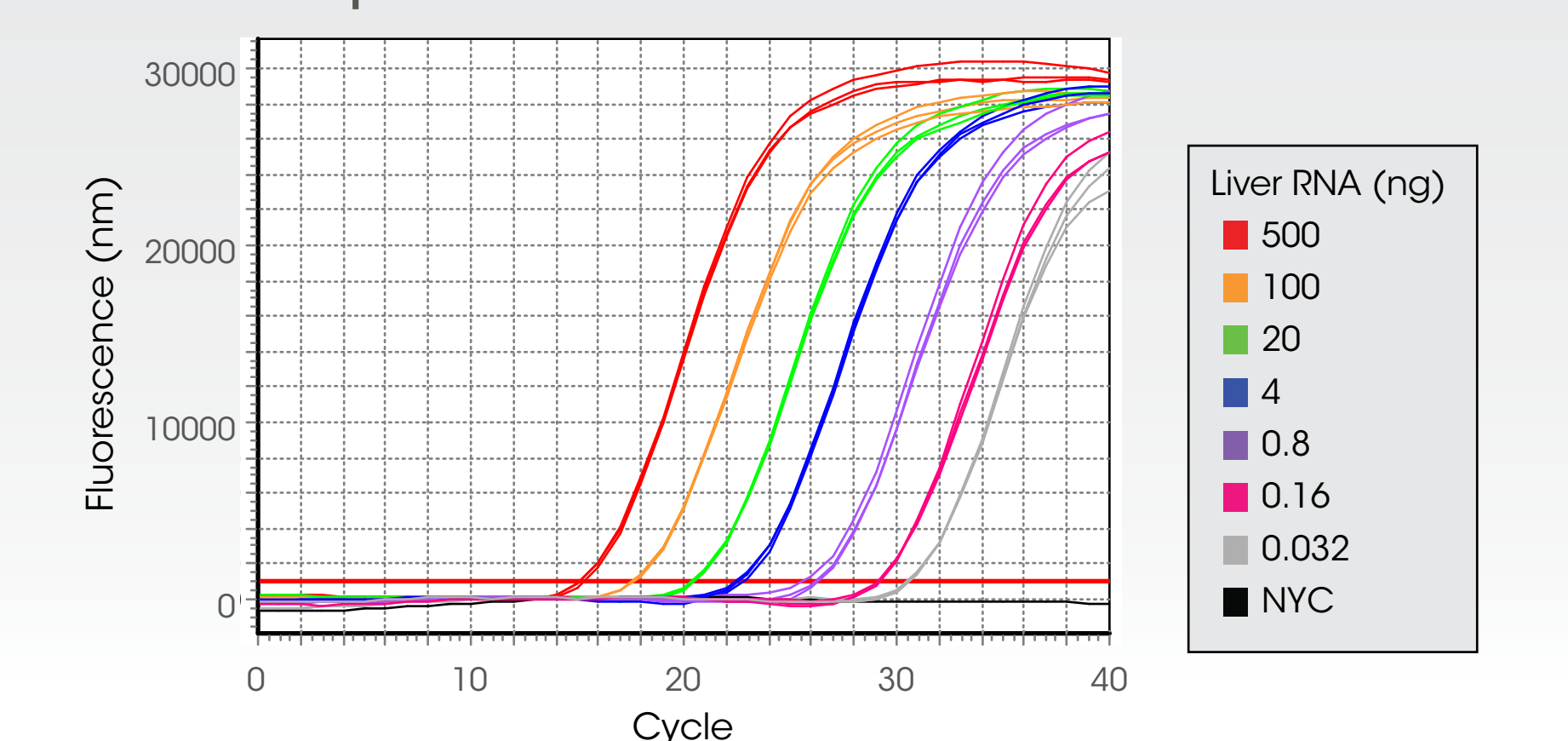
## Figure 5: PCR Amplification Improves Sequencing Accuracy of Homopolymer Regions and Allows for Longer Reads



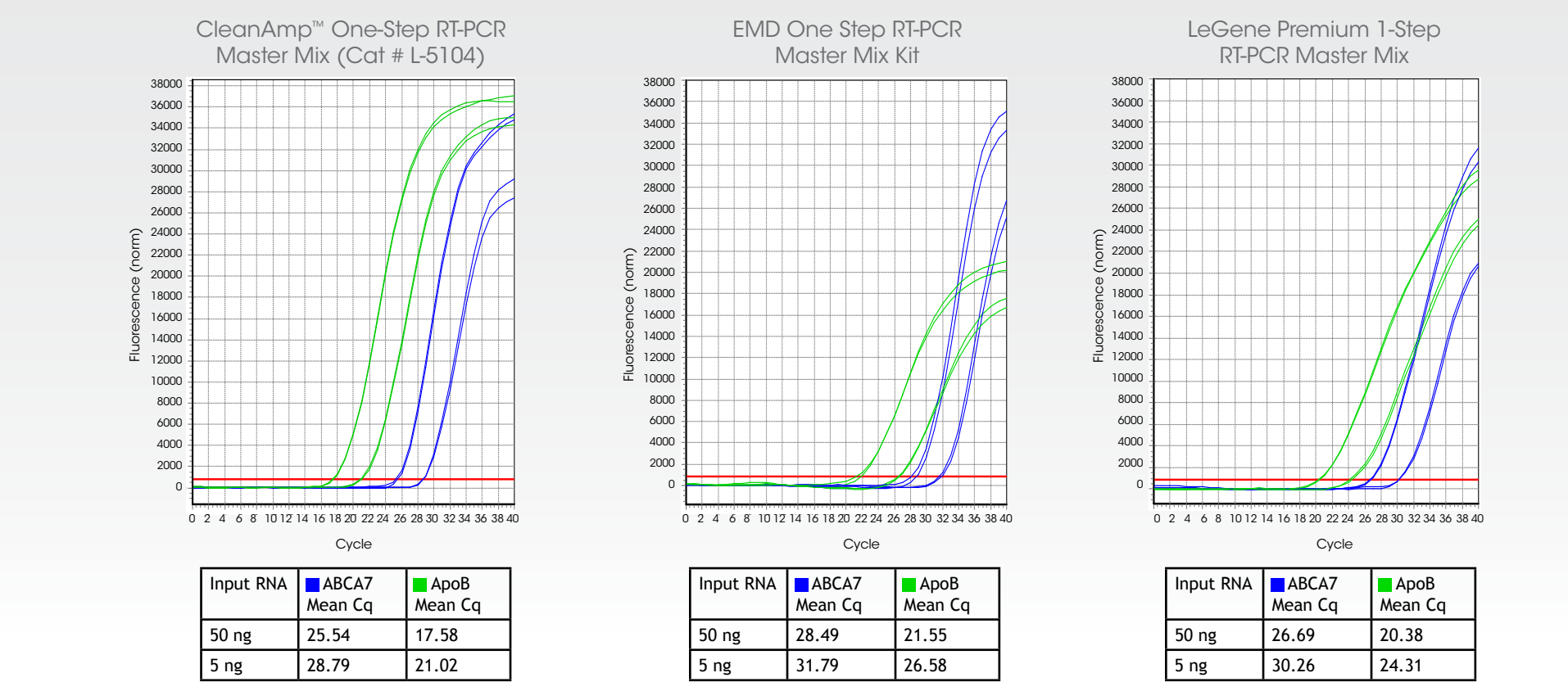
## Figure 6: One-Step RT-PCR Specificity is Improved by Hot Start Activation of the RT and PCR Steps



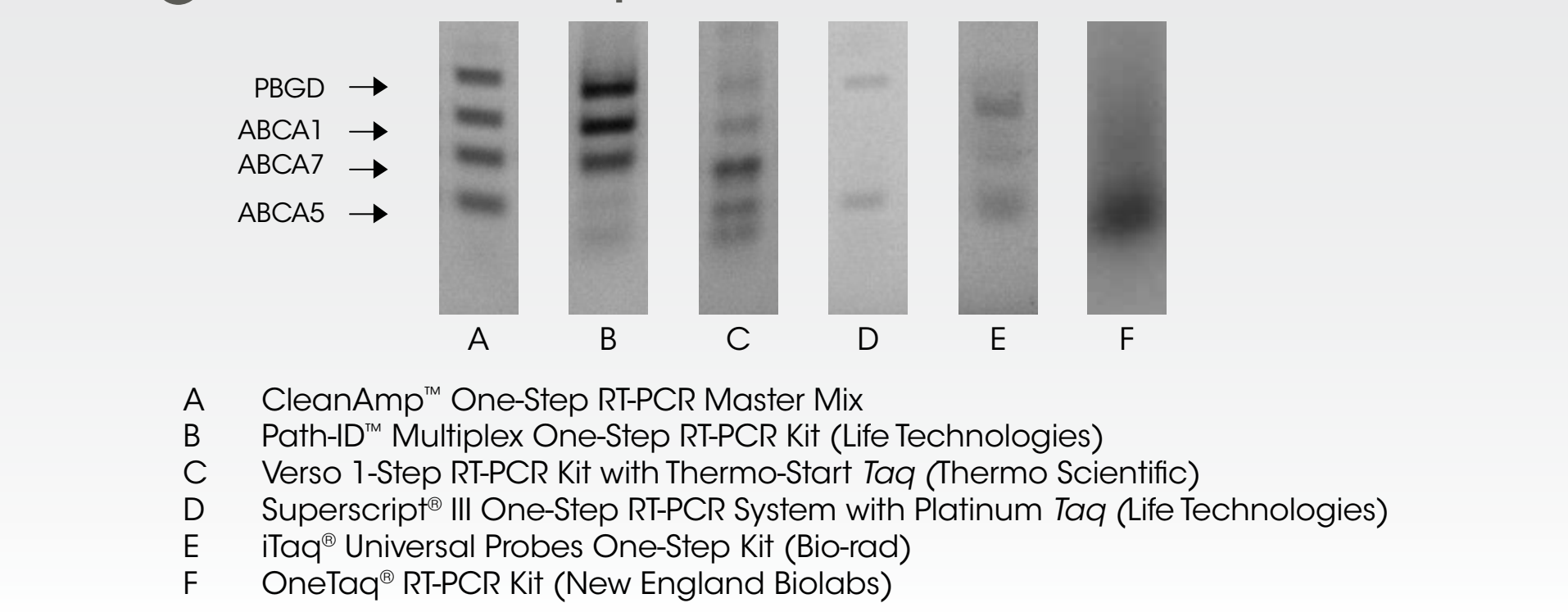
## Figure 7: Quantification of RNA Expression Levels by Real-Time Hot Start One-Step RT-PCR



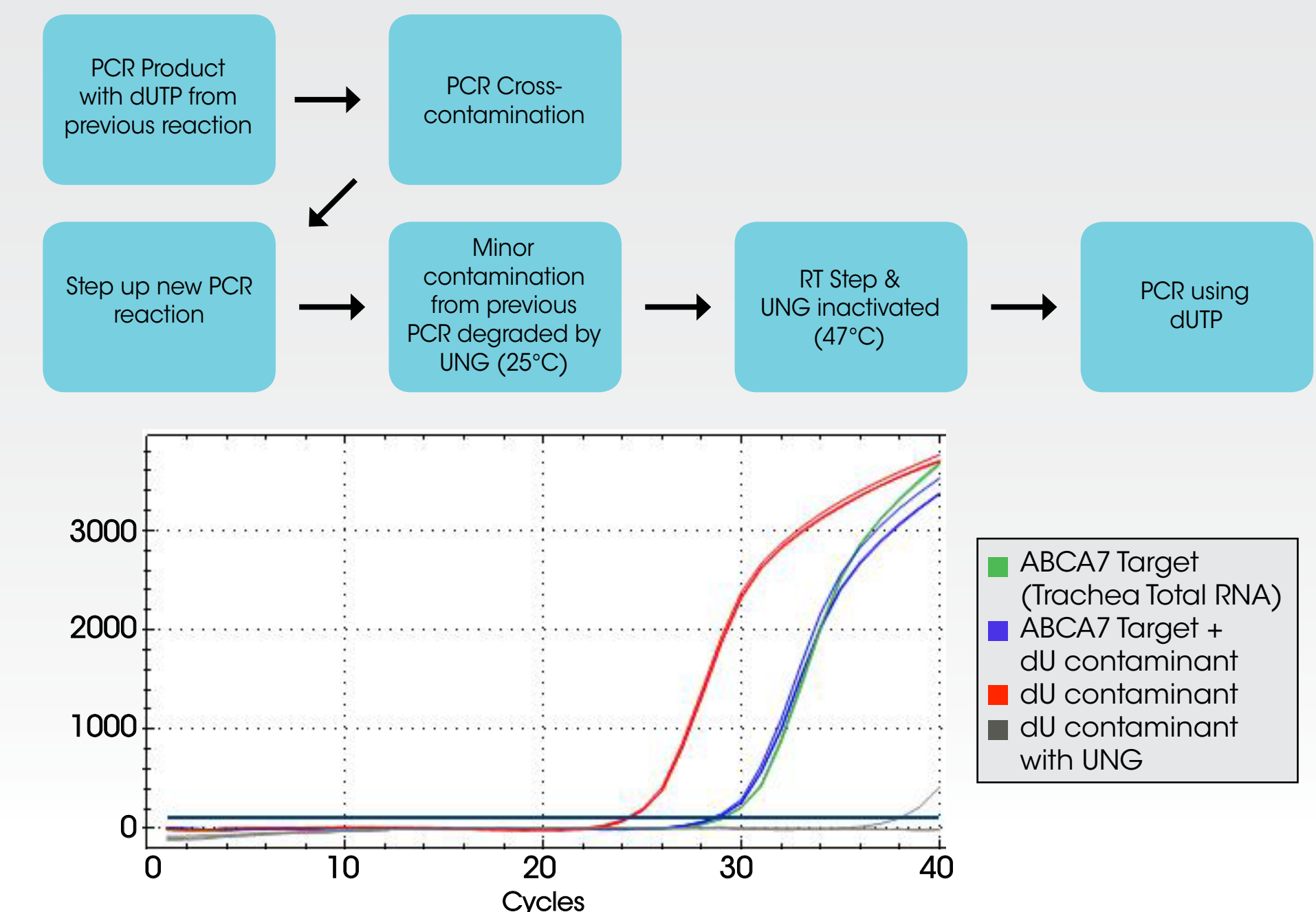
## Figure 8: CleanAmp™ One-Step RT-PCR Generates Lower Cq Values



## Figure 9: CleanAmp™ One-Step RT-PCR Master Mix Successfully Amplifies Four Targets in Multiplex RT-PCR



## Figure 10: One-Step RT-PCR with CleanAmp™ dUTP and UNG Prevents Carry Over Contamination



## Conclusion

- CleanAmp™ dNTPs are compatible with a variety of enzymes and molecular biology assays.
- CleanAmp™ dNTPs enhance the specificity of multiplex PCR where amplification of at least 50 targets has been demonstrated (data not shown).
- CleanAmp™ dNTPs can be employed in fast PCR cycling protocols to achieve high quality data in as little as 24 minutes (data not shown).
- CleanAmp™ 7-deaza-dGTP provides robust amplification and sequencing of GC-rich targets.
- CleanAmp™ 7-deaza-dGTP improves sequencing of GC-rich targets and allows longer sequence reads.
- CleanAmp™ dNTPs improve one-step RT-PCR specificity by introducing Hot Start control to both the RT and the PCR steps.
- CleanAmp™ One-Step RT-PCR Master Mix outperforms other commercial RT-PCR kits and master mixes.
- CleanAmp™ dUTP with UNG protects against PCR cross-contamination.

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Reaction conditions can be found at [www.trilinkbiotech.com/posters](http://www.trilinkbiotech.com/posters)

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