Background: PCR amplification of nucleic acids is a fundamental technique used in many molecular biology laboratories. Despite its wide use, certain GC-rich regions of DNA, such as microsatellites and telomeric repeats, remain a challenge for amplification. Sequences high in GC content are particularly prone to secondary structure formation, which can interfere with the binding of Taq DNA polymerase and hence with the efficiency of amplification. Several methods have been developed to improve amplification of GC-rich targets, including the addition of thermostable protecting groups to the nucleotides. These groups prevent spontaneous annealing of the template strands during the PCR run, thereby improving the yield of the product. However, as the GC content increases, the combination of two or three such protecting groups is sometimes required.

Methods:

Here, we show how a new 7-deaza-dGTP is a commonly used molecule to amplify GC-rich targets, which can improve results when a thermostable protecting group is incorporated at the 7- position.

Results:

The 7-deaza-dGTP provides promising results when a thermostable protecting group is incorporated at the 7-position. The presence of the protecting group allows for the temperature to be increased without causing primer separation when the protecting group is removed, improving PCR yield as a result.

Conclusion:

The use of 7-deaza-dGTP modified with thermostable chemistry improves GC-rich amplification and provides a valuable solution that can improve disease diagnosis.

Figure 1 Proposed activation mechanism of CleanAmp™ dNTPs

Figure 2 Reduction of GC-Rich secondary structure formations

Figure 3 Assessment of 7-deaza-dGTP for the amplification of targets with 60% GC Content

Figure 4 Evaluation of 7-deaza-dGTP for amplification of targets with greater than 70% GC content

Figure 5 Comparison of common GC-rich additives to the CleanAmp™ 7-deaza-dGTP Mix

Figure 6 Amplification of different input target concentrations using standard 7-deaza-dGTP mix and CleanAmp™ 7-deaza-dGTP Mix

Figure 7 Analysis of real-time PCR performance with standard 7-deaza-dGTP mix and CleanAmp™ 7-deaza-dGTP Mix

Figure 8 Comparison of 7-deaza-dGTP Mix (standard and CleanAmp™) in multiplex PCR

Figure 9 Approach to improving dideoxy sequencing results by use of 7-deaza-dGTP in the pre-sequence PCR step

Figure 10 Investigation of CleanAmp™ 7-deaza-dGTP for amplification prior to dideoxy sequencing