Chemically Modified Primers for PCR and Ligation Applications

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Abstract

PCR is an essential tool with utility in a variety of advanced applications. To improve the specificity of PCR, a unique approach to "Hot Start" PCR employing primers containing thermostable modifications has been developed. These modified primers, named CleanAmp™ Primers, are amenable for use in Hot Start activation schemes as the modification is released after an initial denaturation step. CleanAmp™ Primers are available as either singly-modified CleanAmp™ Turbo or doubly-modified CleanAmp™ Precision. These two types of primers differ in the rate of release of unmodified primer thereby allowing for greater control of primer extension and an improvement in PCR amplification specificity. The faster deprotecting Turbo primers show a great advantage in multiplex PCR and low copy number detection. In reverse transcription PCR, the slower deprotecting Precision primers allow the user to perform reactions in a one-step, single tube format, reliably amplifying up to 5 targets simultaneously. The Precision primers also show benefit in the detection of ligation products by quantitative PCR, as they suppress nonspecific product formation for no template controls. Overall, this approach to "Hot Start" activation offers valuable improvements to PCR performance in multiple applications.

Figure 1
Proposed activation mechanism of CleanAmp™ Primers

Turbo Primers
- Improve amplification yield
- Retard off-target formation

Precision Primers
- Improve specificity and limit of detection
- Generate reduction in off-target formation
- Standard cycling
- One-step RT-PCR (singleplex and multiplex)
- Ligase PCR

Figure 2
Versatility of CleanAmp™ Turbo and Precision Primers

Turbo Primers
- Multiplex PCR
- Reduce off-target formation
- Fast cycling

Precision Primers
- Increase specificity and limit of detection
- Generate reduction in off-target formation
- Standard cycling
- One-step RT-PCR

Figure 3
Real-time analysis of multiplex PCR using CleanAmp™ Turbo Primers

Turbo Primers provide increased sensitivity in real-time PCR

Figure 4
Comparison of standard and CleanAmp™ Turbo primers in multiplex PCR

Figure 5
Singleplex and fourplex one-step RT-PCR using CleanAmp™ Precision Primers

Figure 6
Evaluation of CleanAmp™ Precision Primers in multiplex one-step RT-qPCR RNA quantification

Figure 7
Ligation PCR assay to screen for specificity enhancers

Figure 8
Evaluation of enhanced ligation conditions

Enhanced ligation conditions improve discrimination between match and mismatched targets

Figure 9
Performance of CleanAmp™ Precision Primers in ligation PCR

Figure 10
Real-time quantification of ligation fidelity using CleanAmp™ Precision Primers

Conclusion

1) CleanAmp™ Turbo Primers improve the PCR amplification of DNA targets:
   - Turbo Primers give optimal performance for multiple amplification of up to nine targets.

2) CleanAmp™ Precision Primers improve the RT-PCR amplification of RNA targets:
   - Precision Primers allow for both the RT and PCR steps of RT-PCR to be combined into a single reaction setup without sacrificing specificity.
   - Precision Primers allow for real-time RT-qPCR determination of relative gene expression in different tissues.
   - Precision Primers allow amplification of up to five targets at the same time and are compatible with other reverse transcriptase enzymes.

3) CleanAmp™ Precision Primers improve ligation PCR detection:
   - Enhanced ligation conditions improve the discrimination of single-nucleotide differences or a DNA target
   - Precision Primers allow for more precise quantification of ligation yields in real-time PCR.

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