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 thermolabile 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 five 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

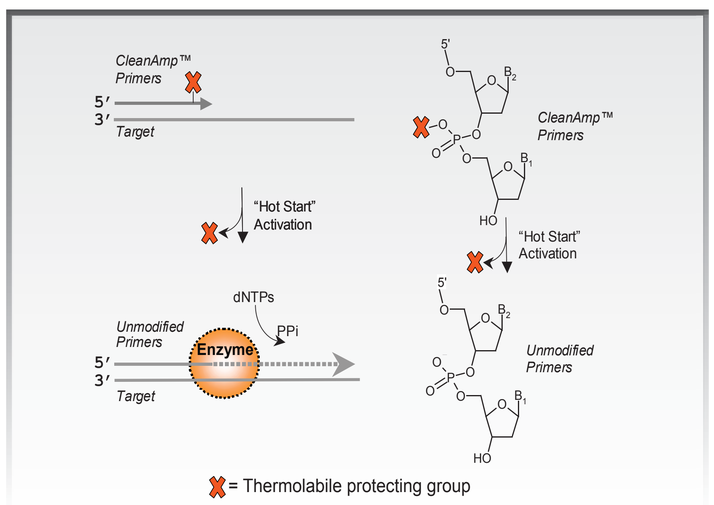
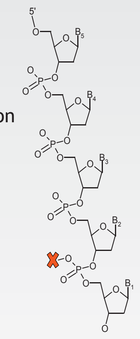


Figure 2

Versatility of CleanAmp™ Turbo and Precision Primers

Turbo Primers
- Improve amplicon yield
- Reduce off-target formation

Applications:
- Fast cycling
- Multiplex PCR



Precision Primers
- Improve specificity and limit of detection
- Greatest reduction in off-target formation

Applications
- Standard cycling
- One-step RT-PCR (singleplex and multiplex)
- Ligation PCR

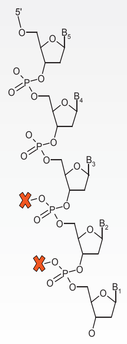
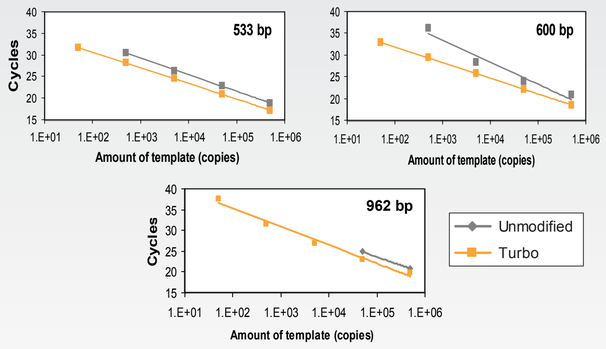


Figure 3

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



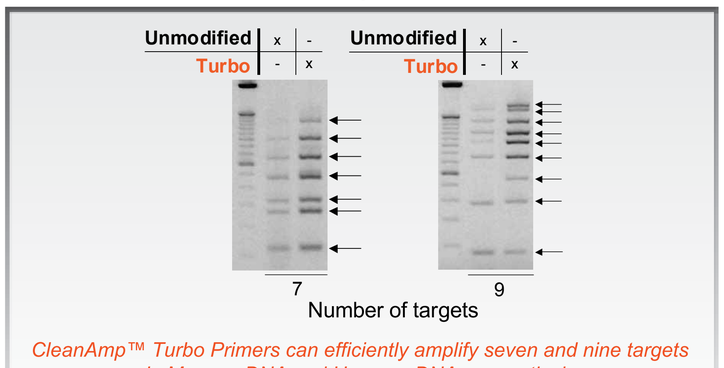
Turbo Primers provide increased sensitvity in real-time PCR

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl), $MgCl_2$ (2.5 mM)), CleanAmp™ Turbo Primers (0.2 µM), TaqMan® Probe (0.1 µM), dNTPs (0.2 mM), 50 to 50,000 copies Lambda gDNA, 1.25 U *Taq* DNA polymerase, 25 µL; reactions performed in triplicate.

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

Figure 4

Comparison of standard and CleanAmp™ Turbo primers in multiplex PCR



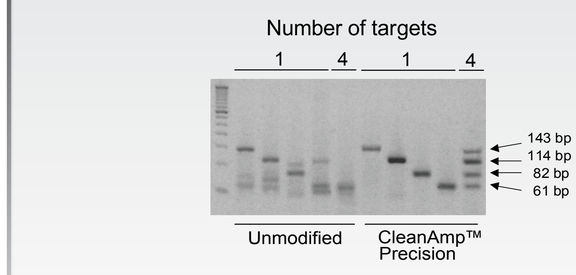
CleanAmp™ Turbo Primers can efficiently amplify seven and nine targets in Mouse gDNA and Human gDNA, respectively

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50mM KCl, 2.5 mM $MgCl_2$), Turbo Primers (0.2 µM), 0.4 mM dNTPs, additional 1.5 U MgCl (total 4 mM $MgCl_2$), additional 40 mM KCl (Total 90 mM KCl)), 20 ng Mouse gDNA or 500 copies Lambda gDNA, 2.5 U *Taq* DNA polymerase, 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).

Figure 5

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



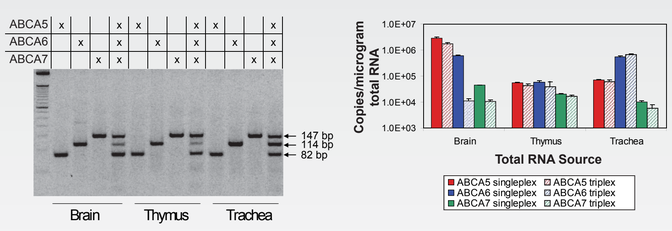
CleanAmp™ Precision Primers demonstrate superior amplicon yield in multiplex one-step RT-PCR relative to unmodified primers

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl), 1.5 mM $MgCl_2$, ABCA7, ABCA6,ABCA5,ABCC10 Precision Primers (0.5 µM), $oligo(dT)_{18}$ primer (1 µM), 0.16 mM dNTPs, 0.5 µg Human trachea total RNA, 50 U/50 µL MMLV reverse transcriptase, 0.6 U *Taq* DNA polymerase, 50 µL.

Thermal cycling conditions: 42°C (30 min); 95°C (10 min); [95°C (15 sec), 60°C (1 min)] 45X; 72°C (5 min).

Figure 6

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



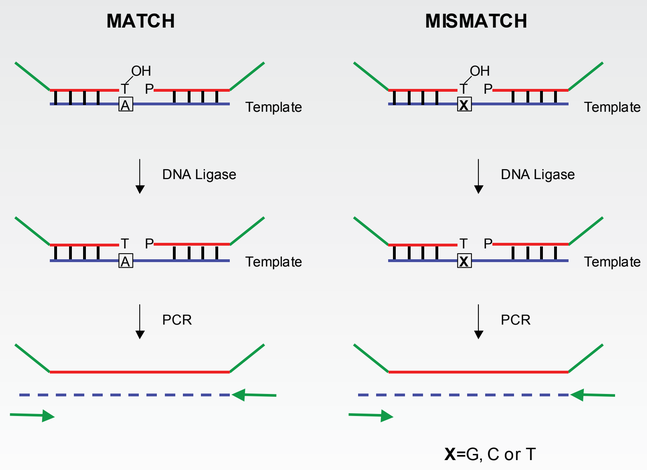
CleanAmp™ Precision primers allow for reliable quantification of three targets individually and simultaneously in different tissues

RT-PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl), $MgCl_2$ (1.5 mM), ABCA7, ABCA6, ABCA5, Primers (0.5 µM), $oligo(dT)_{18}$ primer (1 µM), dNTPs (0.16 mM), RNase Inhibitor (5 U), TaqMan® Probes (0.1 µM), ROX (0.03 µM), 0.5 µg Human brain, thymus or trachea total RNA, 50 U MMLV IVGN Reverse transcriptase, 2.5 U Taq DNA polymerase, 50 µL.

Thermal cycling conditions: 42°C (30 min); 95°C (10 min); [95°C (30 sec), 60°C (1 min)] 45X.

Figure 7

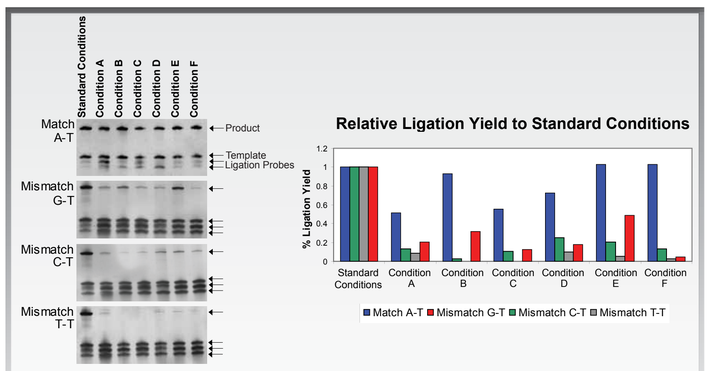
Ligation PCR assay to screen for specificity enhancers



Goal: Identify reaction conditions that suppress or eliminate ligation and PCR amplification with mismatched template

Figure 8

Evaluation of enhanced ligation conditions



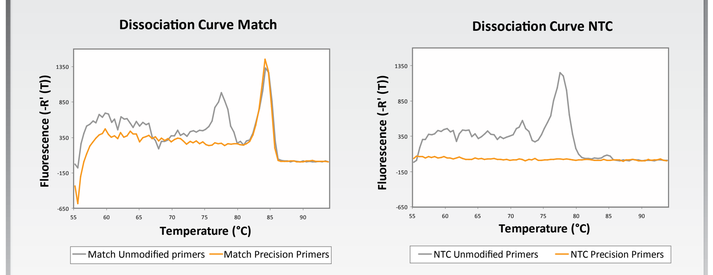
Enhanced ligation conditions improve discrimination between match and mismatched targets

Ligation conditions: 1X T4 Ligase buffer, Ligation probes (1 µM), Template (1 µM), T4 DNA Ligase (0.5 Weiss Units), 20 µL (Non competitive ligation: used only one template per reaction).

Thermal cycling conditions: 22°C (1 hour), 65°C (10 min).

Figure 9

Performance of CleanAmp™ Precision Primers in ligation PCR



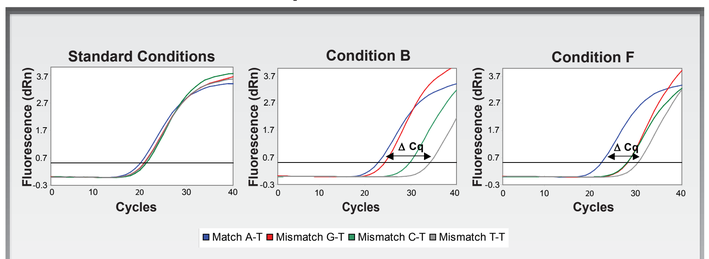
CleanAmp™ Precision Primers allow real-time PCR detection of ligation product formation by suppressing primer dimer formation

PCR conditions: 1X PCR buffer (20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$), Precision Primers (0.1 µM), 0.2 mM dNTPs, 5 µL Ligation product (Diluted 1:10^{10}), 1.25 U IVGN *Taq* DNA polymerase, SYTO®9 green (2 µM), ROX (0.03 µM), 25 µL.

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

Figure 10

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



	Match A-T		Mismatch G-T		Mismatch C-T		Mismatch T-T	
	Cq (dRn)	ΔCq	Cq (dRn)	ΔCq	Cq (dRn)	ΔCq	Cq (dRn)	ΔCq
Standard Conditions	20.15	0	20.96	1	21.48	1	21.1	1
Condition B	22.97	0	24.4	4	29.84	10	34.31	14
Condition F	22.52	0	28.14	8	28.09	8	30.45	10

CleanAmp™ Precision Primers allow for detection of improved mismatch discrimination

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$), Precision Primers (0.1 µM), 0.2 mM dNTPs, 5 µL Ligation product (Diluted 1:10^6), 1.25 U IVGN *Taq* DNA polymerase, SYTO®9 green (2 µM), ROX (0.03 µM), 25 µL.

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

Conclusion

1) CleanAmp™ Turbo Primers improve the PCR amplification of DNA targets.
   - *Turbo Primers give optimal performance for multiplex amplification of up to nine targets*
   - *Turbo Primers improve the limit of detection in multiplexed real-time PCR*

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 set-up 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 transcriptases*

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

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