Hot Start Activation of DNA Ligase Chain Reaction Using CleanAmp™ Primers

TriLink BioTechnologies
The Modified Nucleic Acid Experts

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Abstract

Ligase Chain Reaction (LCR) could improve SNP detection in highly degraded and damaged nucleic acids in clinical, forensic and archaeological samples. LCR could also improve multiplex detection of nucleic acid sequences. Due to the high rate of occurrence of false positive signals LCR hasn't yet realized its potential.

A major source of false positive signals in conventional LCR is template-independent blunt-ended ligation of acceptor and donor probes. Another source is the inherent tolerance of most DNA ligases to some nucleotide mismatches at or near the ligation site. It is recognized that non-specific reactions typically occur as a result of "cold" enzymatic activity of the thermophilic ligase during sample preparation step under low stringency conditions.

Here we present a novel Hot Start employing CleanAmp™ technology approach that improves the specificity and sensitivity of LCR. We demonstrate that a CleanAmp™ modified acceptor probe does not form the of ligation product at low stringency conditions during sample preparation. The CleanAmp™ modification is heat sensitive and cleaves after the initial heat denaturing step (Hot Start) of LCR, releasing unmodified phosphodiester probe, the normal substrate for DNA ligase. The high stringency conditions under which this occurs enhances ligation specificity and significantly reduces formation of template-independent false positives.

Figure 1: CleanAmp™ Modified Acceptor Probe Structure & Activation

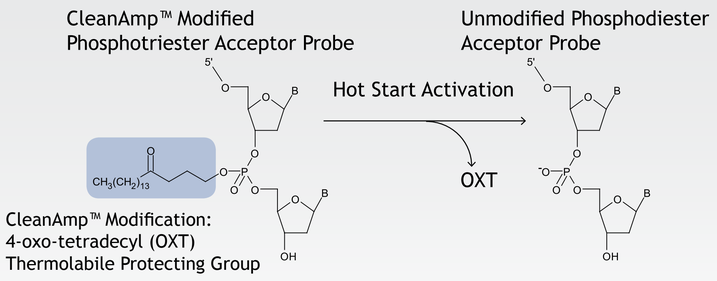


Figure 2: Hot Start Ligation Using CleanAmp™ Modified Acceptor Probes

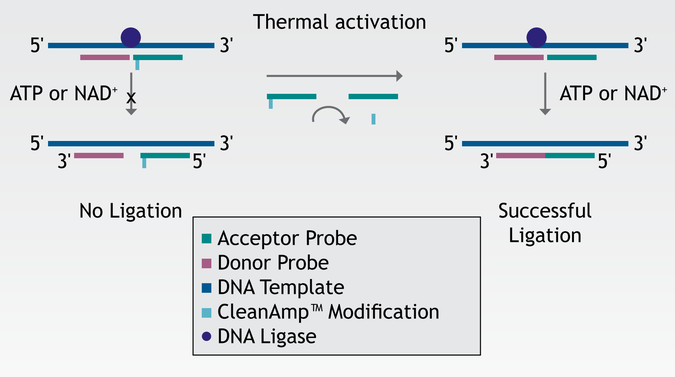
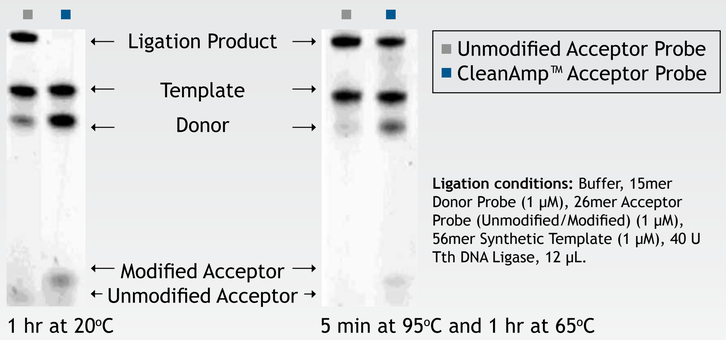


Figure 3: CleanAmp™ Acceptor Probe Blocks Ligation Until Hot Start Activation



Ligation conditions: Buffer, 15mer Donor Probe (1 µM), 26mer Acceptor Probe (Unmodified/Modified) (1 µM), 56mer Synthetic Template (1 µM), 40 U Tth DNA Ligase, 12 µL.

Figure 4: Ligase Chain Reaction Thermocylcing

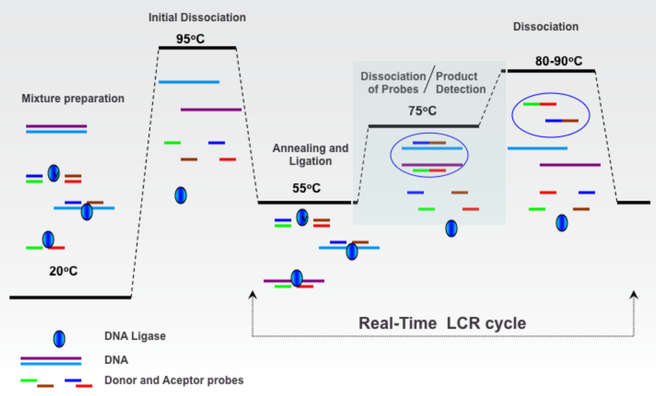


Figure 5: Template-Independent Blunt-Ended Ligation Results in False Positive

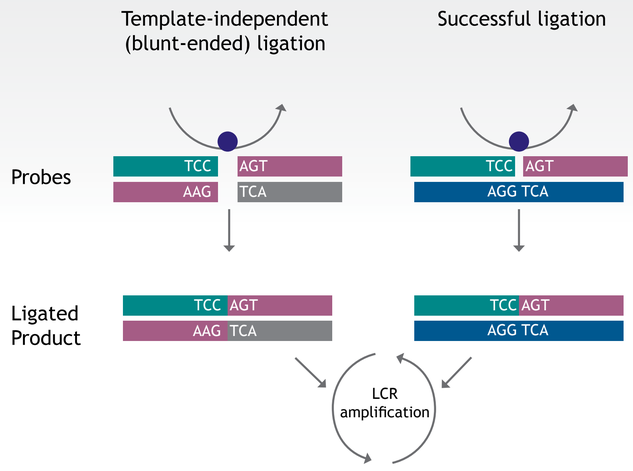
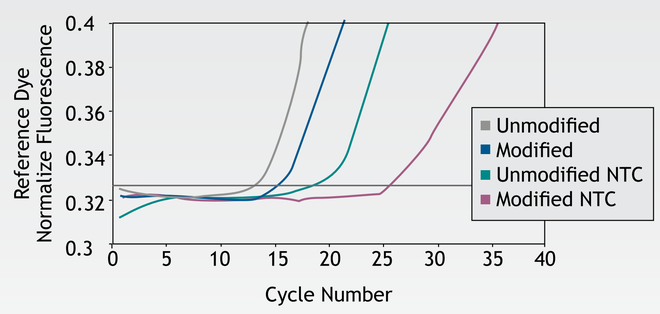
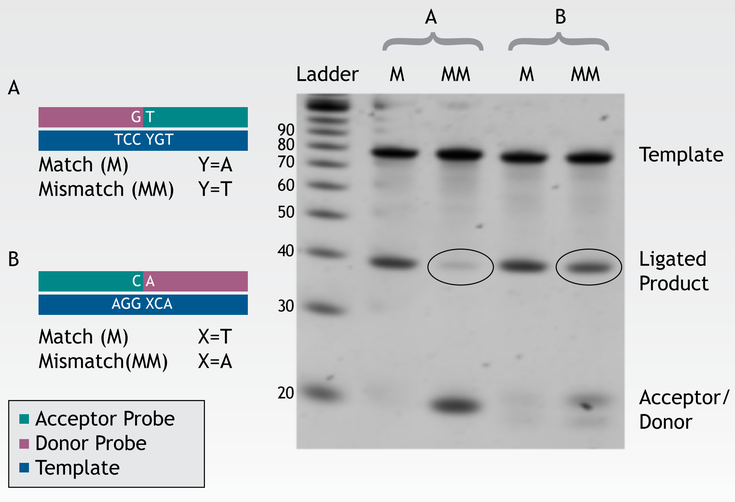


Figure 6: CleanAmp™ Acceptor Probes Reduce Template-Independent Blunt Ended Ligation in Real-Time LCR



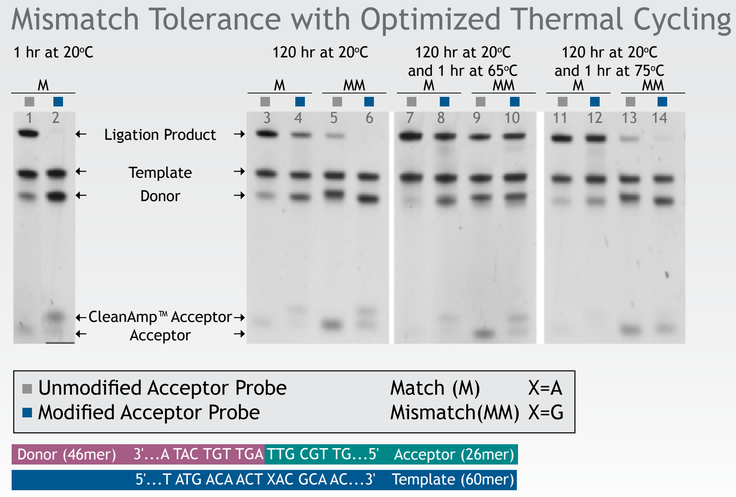
Ligation conditions: NEB 1.25X *Taq* DNA Ligase buffer, 3 x 106 copies of synthetic 77mer DNA template, 0.25 µM of each of donor and acceptor probes, 10 U *Taq* DNA ligase, volume: 20 µL; SYBR® Green detection. Thermal cycling: 95˚C (5 min); [95˚C (30 sec), 55˚C (5 sec), 74˚C (30 sec)] 50X.

Figure 7: Donor Probe Tolerates Ligation on Mismatched Template



Ligation conditions: 1 µM synthetic template, 1 µM of each of donor and acceptor probes, 40 U *Taq* DNA ligase, volume : 12 µL; 1 hr at 60˚C. Gel: TBE-Urea 15% polyacrylamide gel, run at 60-70ºC; SYBR® Gold stain.

Figure 8: CleanAmp™ Acceptor Reduces Donor Probe Mismatch Tolerance with Optimized Thermal Cycling



PAGE analysis of reaction with matched (A-T) DNA template (lanes: 1-4,7,8,11,12) or mismatched (G-T) DNA template (lanes: 5,6,9,10,13,14) and with unmodified or modified acceptor probe. All oligonucleotides are in equimolar ratio at 1 uM; Tth DNA ligase: 50U. Gel: TBE-Urea 15% polyacrylamide gel, run at 60-70ºC; SYBR® Gold stain.

Figure 9: Shift-ended Probes Reduce Mismatch Ligation

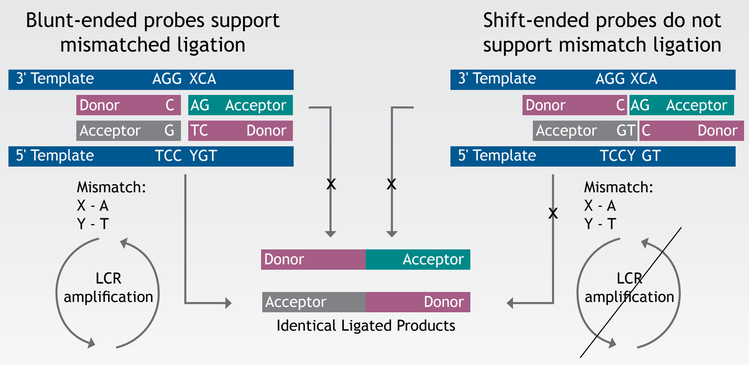


Figure 10: CleanAmp™ Acceptor Probes Improve Real-Time LCR Detection

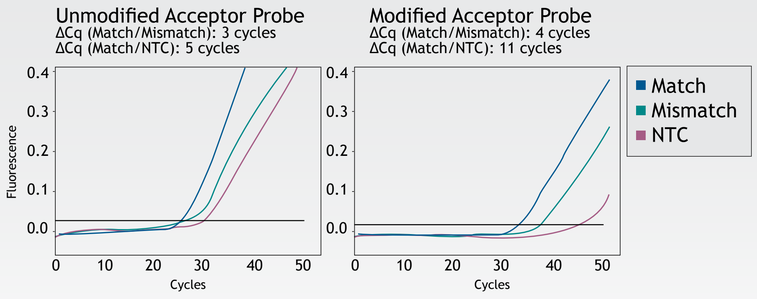
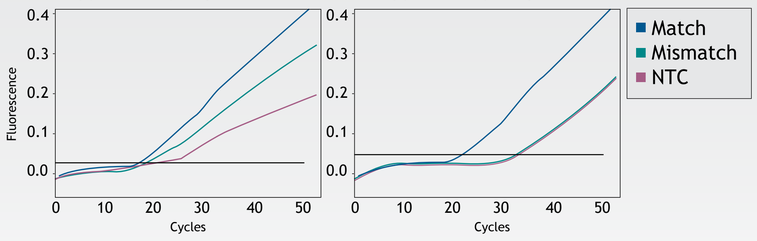


Figure 11: Shift-ended Ligation with CleanAmp™ Acceptor Probes Further Improves Detection



Ligation conditions: NEB 1X *Taq* DNA Ligase buffer, 3 x 106 copies of match (wild type) or mismatch (G551D mutant) synthetic DNA template, 0.1 uM of each of probe; 20 U *Taq* DNA ligase, 20 µL; SYBR® Green detection.Thermal cycling conditions: 95˚C (5 min); [95˚C (30 sec), 55˚C (5 sec), 74˚C (30 sec)] 50X.

Conclusion

- Template-independent (blunt-ended) ligation is the main source of false positive signal in Real-Time LCR
- Template-independent ligation and ligation in mismatched complexes occurs during sample preparation.
- Hot Start significantly improves specificity in Real-Time LCR
- CleanAmp™ modified acceptor probes efficiently suppress template independent ligation during LCR and improve specificity of SNP detection.
- CleanAmp™ acceptor probes allow for the preparation of LCR mixtures at room temperature without compromising the specificity of LCR.

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