TempliAMP™ RCA Kit: Performance analysis against commercial RCA Kits for DNA yield and workflow time

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Introduction

Rolling Circle Amplification (RCA)[1-3] is an isothermal nucleic acid amplification technique often used to amplify circular DNA constructs such as plasmids. RCA utilizes phi29 DNA polymerase, which extends primers and continues to synthesize DNA around the circular template by displacing the previously synthesized DNA strand when encountered (Figure 1).

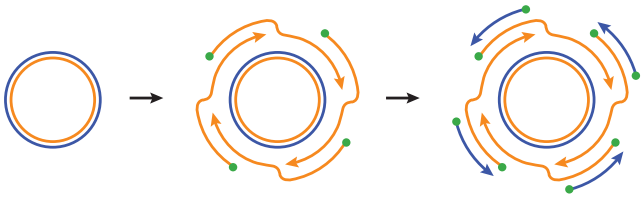


Figure 1: RCA DNA amplification process. Random primers (green dots) will anneal to multiple locations on both sense (blue) and anti-sense (orange) strands and amplify around the circle. This process will continue for the duration of the incubation, resulting in amplification of both strands of the target.

Key features of phi29 DNA polymerase include amplification at 30°C without thermal cycling, high processivity, strand displacement activity, and low error rate (1 x 10^{-6}-1 x 10^{-7}).[4-5] The method offers significant advantages, including:

1. **High sensitivity:** amplification from as little as 5 pg of DNA starting material
2. **Specificity:** phi29 DNA polymerase efficiency for a circular template reduces non-specific amplification
3. **Simplicity:** single-tube isothermal reaction requires minimal equipment

RCA is widely applicable for amplifying circular DNA. The TempliAMP™ RCA Kit from CELLSCRIPT™ offers a powerful solution for efficient and specific amplification of circular DNA, leveraging the high fidelity and strand displacement activity of phi29 DNA polymerase to minimize non-specific amplification. Traditional RCA methods, while effective, often suffer from long incubation times and inconsistent yields. In contrast, TempliAMP™ simplifies workflows by enabling direct amplification from bacterial colonies, glycerol stocks, or liquid cultures—eliminating the need for DNA extraction if desired. It also addresses common limitations such as low DNA yield, producing up to 30 µg of DNA from as little as 5 pg of input. The use of phosphorothioate-modified primers enhances primer stability and reduces degradation, while the high-fidelity phi29 polymerase ensures accurate and efficient replication. Together, these features reduce amplification time, increase specificity, and deliver consistent, high-quality results, making TempliAMP™ an ideal choice for plasmid preparation and sequencing applications.

In this application note, we examined the performance of CELLSCRIPT™'s TempliAMP™ RCA Kit against three commercially available RCA kits to demonstrate how TempliAMP™ provides consistent yields in less time while maintaining quality and specificity of the RCA DNA product.

Methods

Three other commercially available Rolling Circle Amplification company products were evaluated in comparison to CELLSCRIPT™'s TempliAMP™ RCA Kit under standardized conditions: EquiPhi29™ DNA Amplification Kit (Thermo Fisher Scientific), phi29-XT RCA Kit (New England Biolabs) and REPLI-g® Midi Kit (Qiagen). All reactions were performed in quadruplicate using a fixed input of 5 ng of plasmid DNA. RCA reactions were carried out following the respective kit instructions. Incubation times and temperatures were selected based on each manufacturers' recommended protocol: 4 hours at 30°C for the TempliAMP™ RCA Kit, 2 hours at 42°C for both the EquiPhi29™ DNA Amplification and phi29-XT RCA Kits, and 16 hours at 30°C for the REPLI-g® Midi Kit. After amplification, resultant amplified DNA was purified using AMPure® XP Bead-based Reagent (Beckman Coulter). The purified DNA was then digested with Xba I restriction enzyme (NEB), followed by a second round of purification using AMPure® XP beads. The plasmid used as template in the RCA reactions is ~8 kb and has a single Xba I restriction site, therefore amplification that is very specific will result in a single 8 kb band after digestion with Xba I.

Results

25 ng of each RCA product were visualized on a native 1% agarose gel. All products tested resulted in amplification of high molecular weight DNA (Figure 2A). Amplified DNA was digested overnight with Xba I and 25 ng was run on a native 1% agarose gel. The vast majority of Xba I digested RCA amplified DNA runs at the expected size (~8 kb), confirming that all kits produced the expected target and indicating successful and accurate amplification (Figure 2B).

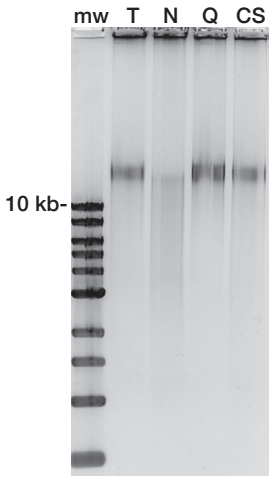
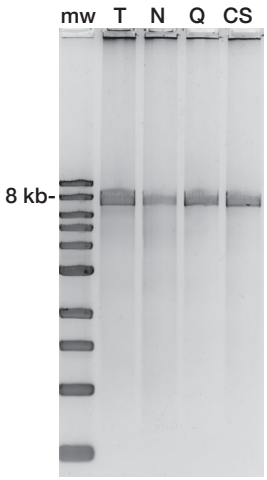


Figure 2A: Amplification of high molecular weight RCA DNA by products kits tested.



mw = 1 kb ladder (NEB)
T = EquiPhi29™ DNA Amplification Kit (Thermo Fisher)
N = phi29-XT RCA Kit (NEB)
Q = REPLI-g® Midi Kit (Qiagen)
CS = TempliAMP™ RCA Kit (CELLSCRIPT™)

Figure 2B: RCA amplified DNA size following overnight Xba I digestion.

CELLSCRIPT™'s TempliAMP™ RCA Kit and Qiagen's REPLI-g® Midi Kit produced the highest DNA yield compared to the other products tested (Figure 3A). When normalized against incubation time, CELLSCRIPT™'s TempliAMP™ RCA Kit generated more DNA template on a microgram per hour (µg/hr) basis compared to the other products tested (Figure 3B).

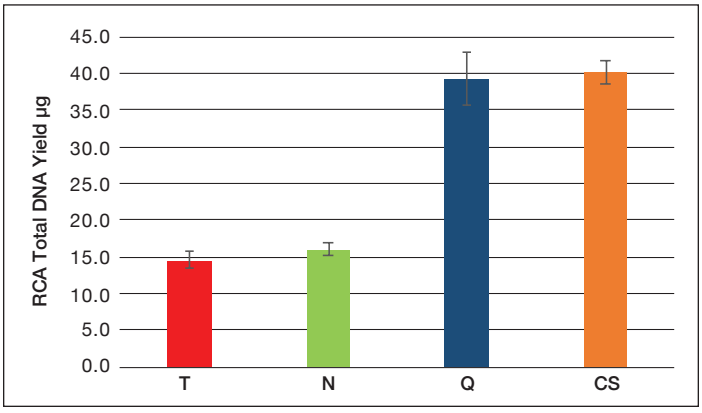


Figure 3A: RCA DNA yields in micrograms.

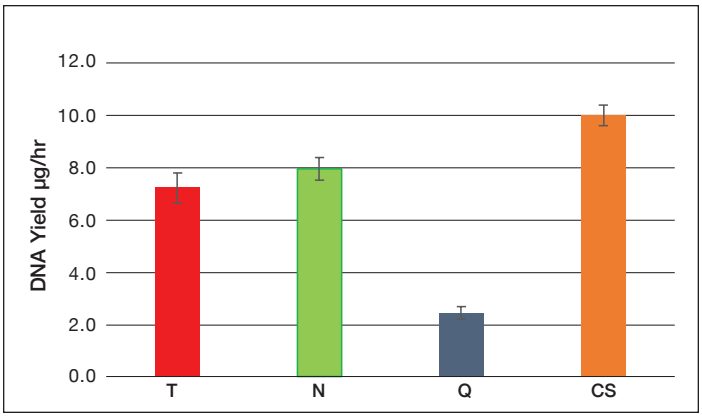


Figure 3B: RCA amplified DNA yield per hour of incubation.

Conclusion

The experimental results demonstrate that all four RCA products tested amplified DNA of comparable size, consistent with expected outcomes. Among the RCA company products tested, CELLSCRIPT™'s TempliAMP™ RCA Kit (Cat. No. TAR250325) delivers the most efficient yield-to-time ratio amongst the kits tested, underscoring its efficiency and suitability for time-sensitive workflows. Collectively, this work shows that while each of the evaluated RCA products can produce high-quality DNA suitable for downstream applications, CELLSCRIPT™'s TempliAMP™ RCA Kit offers a compelling combination of rapid processing and high specificity, making it a strong candidate for applications requiring efficient, precise and rapid DNA amplification.

References

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