Enhanced Rolling Circle Amplification Performance with a Newly Engineered phi29 DNA Polymerase

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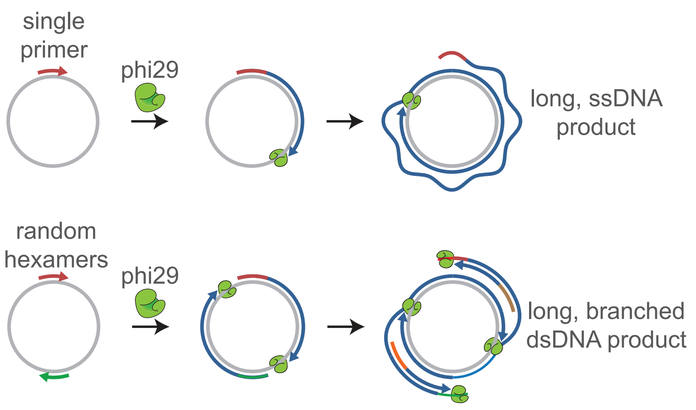
Introduction

Isothermal DNA amplification techniques exponentially increase the amount of DNA in a sample without the constraint of thermocycling. New England Biolabs (NEB) offers a wide range of innovative solutions in isothermal amplification. One of them, Rolling Circle Amplification (RCA) has become an invaluable tool for many biotechnology applications including: DNA sequencing, cell-free DNA synthesis, cell-free protein expression, biomolecular detection, and DNA hydrogel generation. The most widely used DNA polymerase for RCA is phi29, which possesses high processivity and fidelity, and strong strand-displacement activity. In the recently launched NEB phi29-XT RCA kit, we have incorporated an engineered phi29 DNA polymerase, phi29-XT, that shares the positive characteristics of the wild-type phi29, but also generates higher yield in less time than the wild-type enzyme. Additionally, while wild-type phi29 works optimally at 30°C, phi29-XT performs best at 42°C. We have used this kit downstream of DNA assembly technologies, such as GoldenGate and NEBuilder HiFi Assembly, to perform high-throughput cell- and vector-free protein expression. Furthermore, the kit also enabled high-throughput fosmid amplification directly from bacterial cells, followed by ONT sequencing for *de novo* fosmid DNA assembly. Taken together, these applications demonstrate the utility of the phi29-XT RCA kit for isothermal amplification of circular DNA templates.

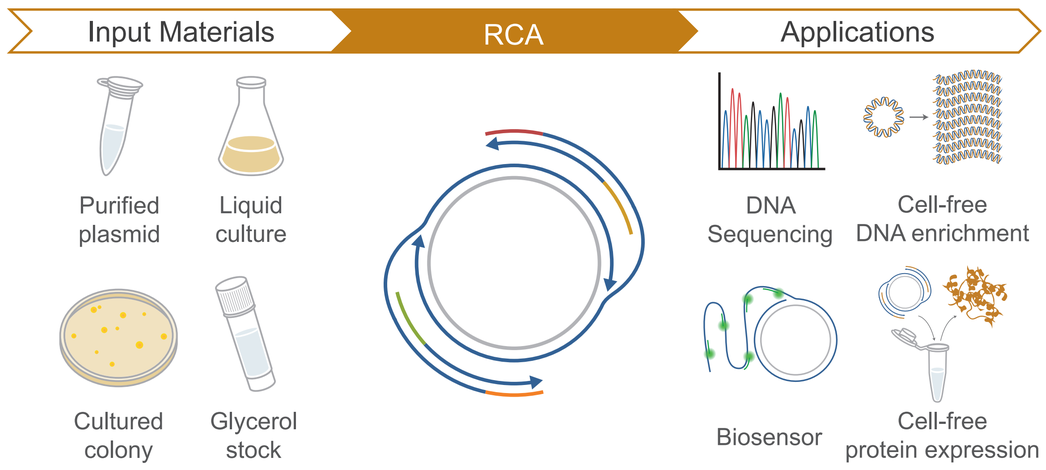
Rolling Circle Amplification with phi29 DNA Polymerase



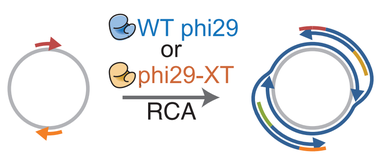
phi29 benefits

- Highly processive
- High fidelity
- Efficient at strand-displacement DNA synthesis

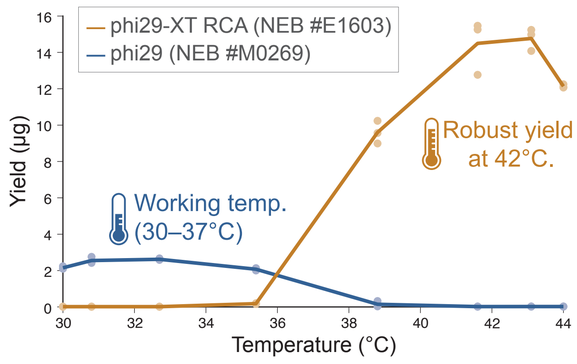
Highly versatile RCA assay



phi29-XT DNA Polymerase performance

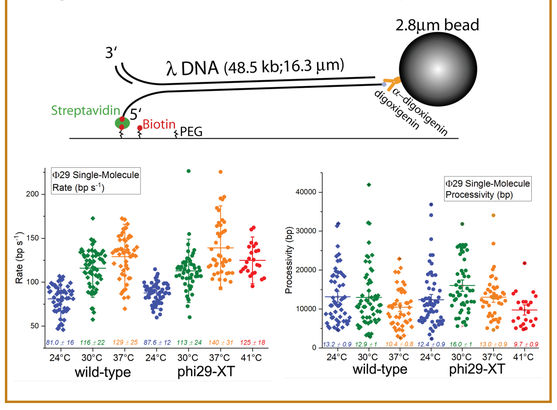


phi29-XT DNA Polymerase works optimally at 42°C and generates more product in less time than wild-type (WT) phi29



0.1 pg pUC19 input. 2-hour incubation at indicated temperature.

Single molecule characterization of phi29-XT

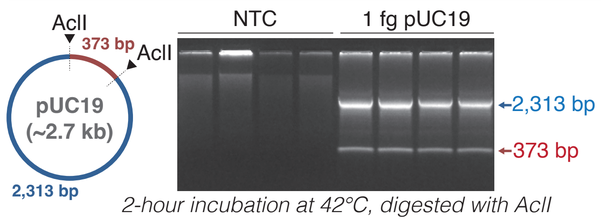


DNA Polymerase	Error Rate	Fidelity, Relative to *Taq*
Taq	1.6 x 10^{-4}	1
Wild-type phi29	1.5 x 10^{-5}	10.7
phi29-XT	9.0 x 10^{-6}	17.8

phi29-XT synthesizes with a rate, processivity, and fidelity comparable to wild-type phi29 DNA polymerase

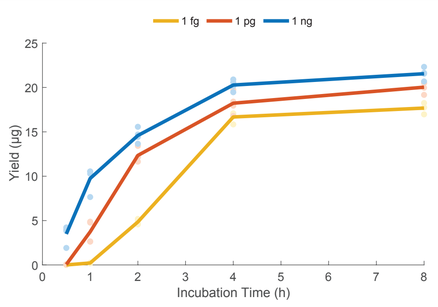
Note: Single molecule and fidelity experiments were not carried out with phi29-XT reaction buffer

phi29-XT DNA Polymerase sensitivity

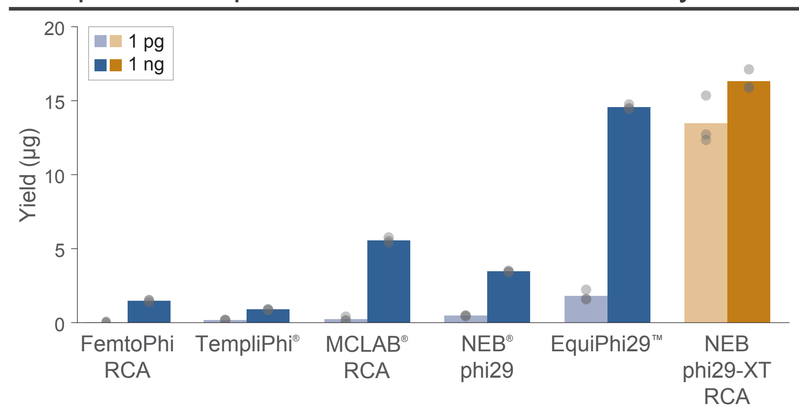


2-hour incubation at 42°C, digested with AclI

phi29-XT DNA Polymerase can amplify as little as 1 fg of 2.7 kb circular template



Comparison of phi29-XT to other commercially available phi29s

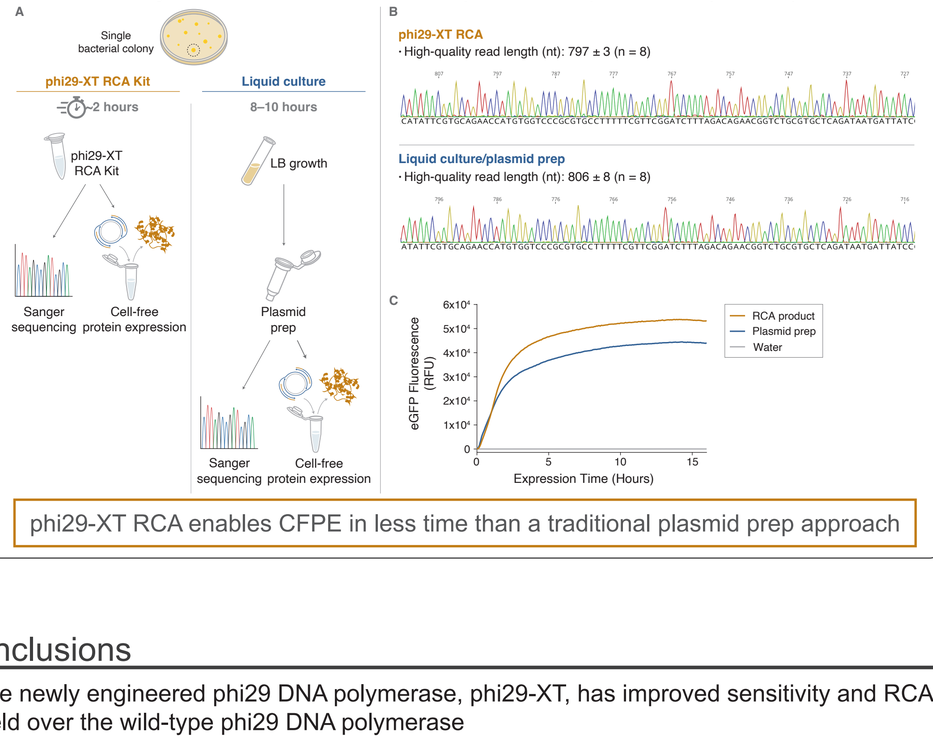


2-hour incubation with manufacturers' recommended conditions

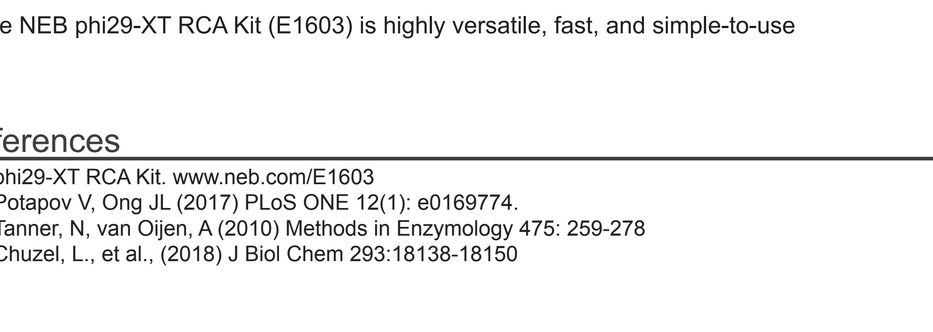
phi29-XT RCA Kit offers exceptional sensitivity and product yield

phi29-XT DNA Polymerase-mediated applications

phi29-XT RCA enables high-throughput metagenomics screening



Direct colony RCA enables cell-free protein expression (CFPE)



phi29-XT RCA enables CFPE in less time than a traditional plasmid prep approach

Conclusions

- The newly engineered phi29 DNA polymerase, phi29-XT, has improved sensitivity and RCA yield over the wild-type phi29 DNA polymerase
- The NEB phi29-XT RCA Kit (E1603) is highly versatile, fast, and simple-to-use

References

1. phi29-XT RCA Kit. www.neb.com/E1603
2. Potapov V, Ong JL (2017) PLoS ONE 12(1): e0169774.
3. Tanner, N, van Oijen, A (2010) Methods in Enzymology 475: 259-278
4. Chuzel, L., et al., (2018) J Biol Chem 293:18138-18150