EZassay

Rolling Circle Amplification (RCA) Kit

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Catalog Code: RCA-50
RCA-500

CONTENTS

Product name

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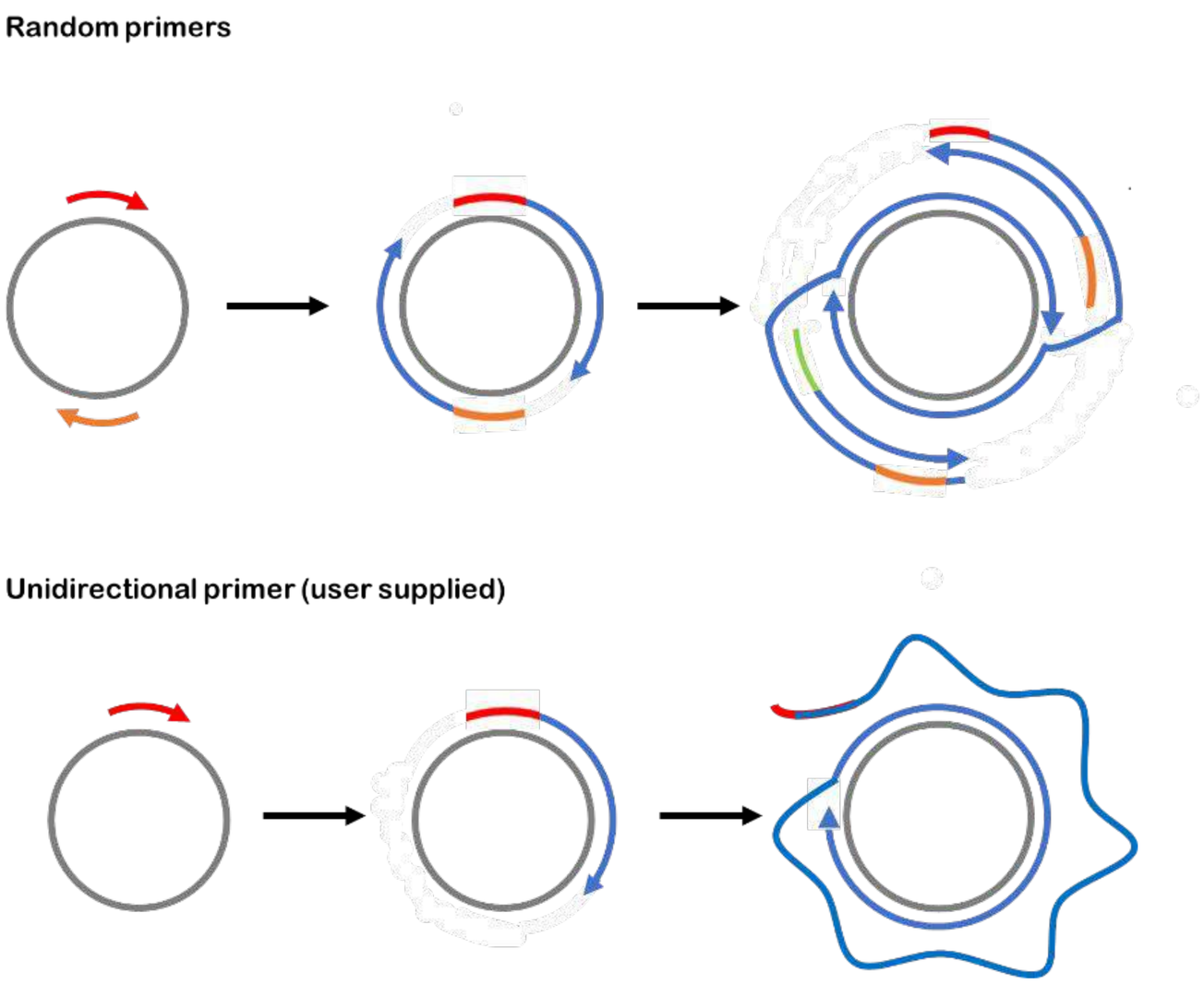
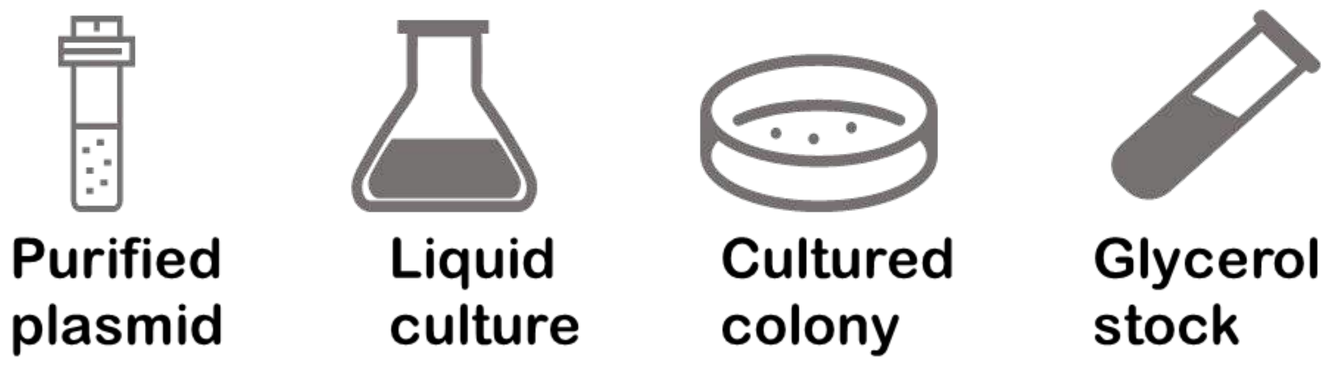
Introduction

Rolling circle amplification (RCA) is an isothermal amplification method to continuously amplify circular DNA by generating long, repetitive copies of the circular sequence. The kit features phi29 DNA polymerase-v2, an engineered polymerase with improved thermostability and sensitivity, and generates higher yield in a shorter reaction time than wild-type phi29 DNA Polymerase. Also included are dNTPs and exonuclease-resistant random primers (containing phosphorothioate bonds) to universally amplify circular DNA sequences. Also specific RCA can be performed by adding custom primers.



Input material can be purified circular DNA (single or double-stranded) or direct from liquid media



culture, agar plate colonies, and glycerol stocks without the need for DNA extraction.

RCA products can be used directly in downstream applications such as DNA sequencing, cell-free DNA enrichment, cell-free protein expression, and DNA biosensors.

Materials supplied

Item	RCA-50	RCA-500
Phi29 DNA polymerase-v2	10 U/μl *25 μl	50 U/μl *50 μl
Reaction Buffer (10X)	100 μl	1 ml
Random hexamer primers (Exo-Resistant) (100μM)	50 μl	500 μl
10 mM dNTPs	50 μl	500 μl
Enhancer Buffer	25 μl	250 μl
Diluent buffer	100 μl	1 ml

Storage

-20°C. Suggest to aliquot after receiving. Avoid repeated freeze-thaw.

Example

Input Material: purified genomic DNA from bacteria.
1. Prepare reactions as described in the table below.

Components	Volume
Nuclease-free Water	X μl
Reaction Buffer (10X)	1 μl
Enhancer Buffer	0.5 μl
Random hexamer primers (Exo-Resistant) (100μM)*	1 μl
Template DNA (4ng)*	Y μl
Total Volume	8 μl

*Specific primers can be used to target specific sequence of interest.
**Template input amount can be adjusted as needed.

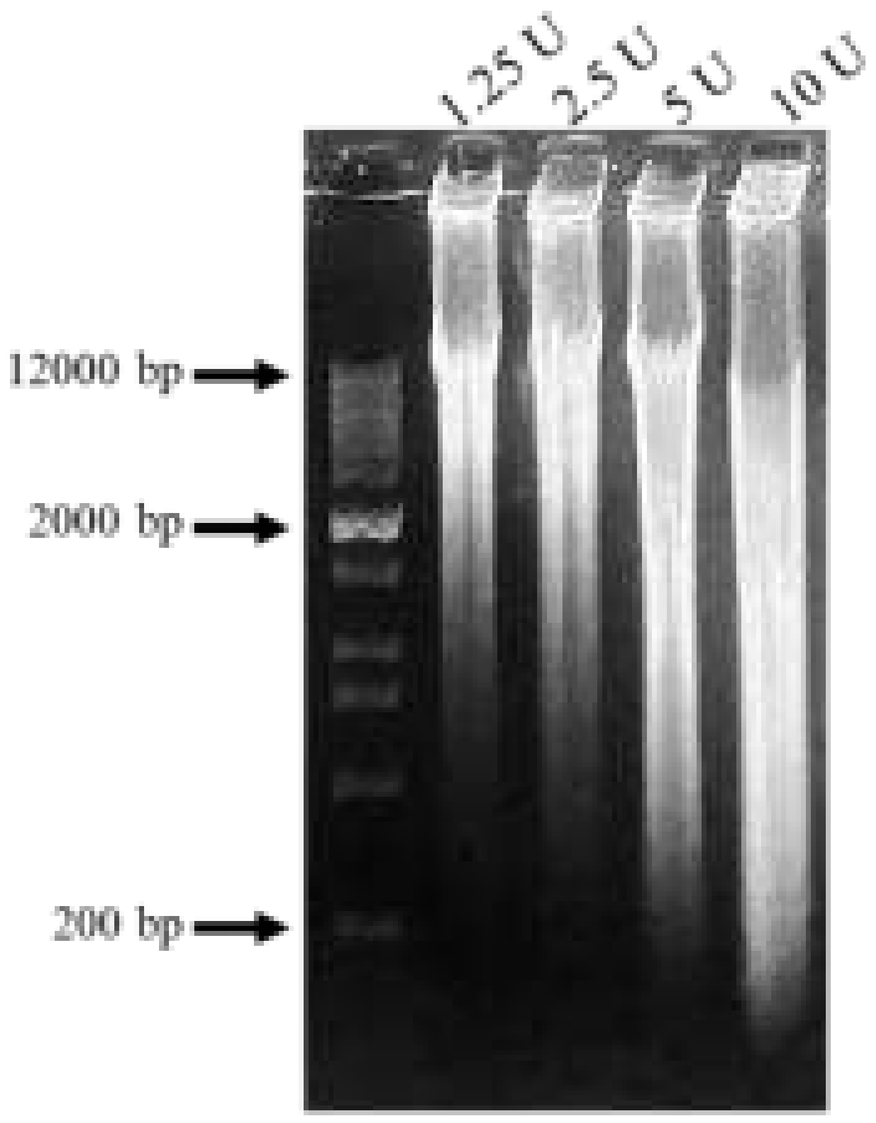
2. Incubate at 95°C for 3 min and immediately place on ice to cool for 5 min.

3. Prepare amplification as described in the table below.

Components	Volume
Product from last step	8 µl
dNTP (10 mM)**	1 µl
Phi29 DNA Polymerase (5U/µl) ***	1 µl
Total Volume	10 µl

** dNTP optimization range 100~500µM;
***Phi29 DNA polymerase optimization range: 1~10 U/10µl reaction.

4. Vortex and briefly centrifuge. Then incubate at 30°C overnight.
5. Incubate at 65°C for 10 min to deactivate Phi29 DNA polymerase. The amplified product could be used for sequencing or downstream application after purification.



DNA agarose gel 0.7%, 100V, 40mins

Notes

1、 For DNA samples that require denaturation, incubate at 95 °C for 5 minutes, followed by 4°C or an ice bath for 2 minutes.
2、 For whole-genome amplification, Phi29 DNA polymerase, reaction buffer, and ddH2O can be pre-mixed with the DNA sample and incubated at 30°C for 30 minutes. The exonuclease activity of Phi29 DNA polymerase was used to remove linear DNA. Then random primers and dNTP are added to start the amplification reaction.
3、 A thermostatic water bath or heat block is recommended for the reaction. If using a hot-lid thermal cycler, adjust the hot-lid temperature to 40°C to avoid enzyme inactivation.
4、Yeast Inorganic Pyrophosphatase (Cat.#.:PPAS-0100) can be used to further enhance the amplication of DNA.