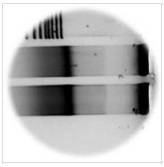

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phi29 DNA polymerase protocol

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High molecular weight DNA extraction from all kingdoms | Long Read Club


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ABSTRACT

Isothermal amplification of a few nanograms of DNA with phi29 DNA polymerase in the presence of trehalose allows for a more stringent amplification temperature that prevents unspecific primer amplification in the water control reaction.

Spiess, A. N., Mueller, N. & Ivell, R. Trehalose is a potent PCR enhancer: lowering of DNA melting temperature and thermal stabilization of taq polymerase by the disaccharide trehalose. Clin Chem 50, 1256-1259

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KEYWORDS

null, isothermal amplification, phi29, DNA, nanopore, sequencing

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MATERIALS TEXT

phi29 DNA Polymerase - 250 units **New England Biolabs Catalog #M0269S**

D-( )-Trehalose dihydrate **Sigma Aldrich Catalog #T5251** : Make a 1.2 M stock in deionized sterile water, final volume 50 mL. Make a few aliquots in 1.5 mL tubes, and store at -20ºC

dNTP Set 100 mM Solutions **Thermo Fisher Scientific Catalog #R0182**

8 mM dNTP mix (I use Thermofisher and mix them at equal molar ratios in sterile deionized water and store aliquots at -20ºC)

Exo-Resistant Random Primer **Thermo Fisher Catalog #SO181** dissolved in TE buffer (sterile 10 mM Tris-HCl pH 7.5, 1 mM EDTA) or a custom made random hexamer with two PT-modified links at the 3'-end 5 ' -NNNN*N*N- 3 '

10 mM Tris-HCl pH, 1 mM $MgCl_2$, pH 8.0, sterile

Deionized water, sterile

0.2 mL PCR tubes

1.5 mL microcentrifuge tubes

PCR machine

1% agarose gel in 1XTAE buffer, and gel box with power pack to run it

1 Kb Plus DNA Ladder **Invitrogen - Thermo Fisher Catalog #10787018** diluted 1:10 in TE buffer and 1X loading buffer

1X TAE buffer: 40 mM Tris, 20 mM acetic acid, and 1 mM EDTA pH 8.3

SYBR SAFE DNA stain **Life Technologies Catalog #S33102** diluted 3 µL in 50 mL 1X TAE buffer, in a plastic box protected from the light

Extra plastic box with a lid

Gel documentation system

Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #Q32854** with appropriate 0.5 mL tubes and fluorometer

BEFORE STARTING

Clean pipettes, tube racks, bench surfaces, tip boxes, etc., with 70% ethanol
Use sterile tips, with or without aerosol barriers

Isothermal amplification 1d

1 Set up the following profile in a PCR machine, 1 cycle only, lid at 105ºC:

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95ºC 3 min → 40ºC 1 min → 38ºC 12.5 h → 65ºC 10 min → 10ºC 10 min → stop

2 Prepare the 1X amplification mix and keep it on ice. Besides the DNA reaction, include a water control, and an extra reaction:

0.6 M trehalose; 1X NEB phi29 Reaction Buffer, 0.8 mM dNTPs, 2 Units phi29 DNA polymerase

A	B
Reagent	3 reactions (1 µL)
Deionezed sterile water	5.4
10X phi29 DNA polymerase reaction buffer	6
1.2 M Trehalose	30
8 mM dNTP mix	6
phi29 DNA polymerase 10 U/uL	0.6

3 Prepare the annealing mix, including a control (water only):

A	B	C
10 mM Tris-HCl, 1 mM MgCl2, pH 8	2 µL	2 µL
PT-random hexamer, 10 pmol/µL	1 µL	1 µL
Genomic DNA ~10 ng/µL	1 µL	-
Deionized sterile water	-	1 µL

4 Mix and incubate in PCR machine
Run the 95°C and 40°C steps, pause the machine

5 Add 16 µL of 1X amplification mix. Vortex the tube and quickly spin down for few seconds. Return the tube to the PCR machines and continue with the amplification profile

If concerned with such a long incubation, you can add a drop of mineral oil before resuming the PCR profile

6 Spin down the tube and transfer the contents to a 1.5 mL tube. Rinse the PCR tube with 10 µL sterile deionized water and transfer it to the 1.5 mL tube. Mix briefly. Final volume ~ 30 µL

Quantification and quality check 2h

7 Quantify the amount of DNA using 1 µL of the amplification reaction and the HS dsDNA Qubit kit
Quantify 1 µL of the water control reaction as well

8 Depending on the yield, load 50 - 100 ng of amplified DNA in one lane of a 1% agarose gel in 1X TAE. Load ALL the water control reaction in another well. Load 4 µL of the 1 Kb Plus ladder in a separate lane
Run the gel at 140 V for 30 min

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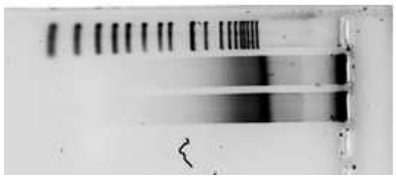
8.1 When finished, transfer the gel to the staining box containing 50 mL 1X TAE buffer and 3 µL SybrSafe. Close the box and shake gently for 10 min at room temperature

Take a photo of the gel. If the bands are too fain, place the gel on a new box with tap water, close the box and shake gently for 2-4 min to destain the gel slightly and take the photo again

Expected result

9



phi29 DNA polymerase, 0.6 M trehalose, 12.5 h @ 38°C	
	Yield ng
1 kb+	
Hoki Liver Bblob	78.5
Hoki Liver Bblob 30G	105
water	5.4

Alternatively, the sample can be analyzed in either the BioAnalyzer or the Fragment Analyzer, where you only need 2-5 ng

The amplified sample can be trusted only if the water control is blank

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