DNase I Treatment

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Abstract

This protocol describes the preparation of and treatment with DNase I to degrade DNA in solutions containing iron chloride, EDTA–Mg ascorbate buffer, and cesium chloride. DNase I treatment, CsCl purification, and sucrose purification methods were compared using replicated viral metagenomics in Hurwitz et al. 2012. We use this protocol primarily to remove free host and other contaminating DNA from phage preparations prior to extracting nucleic acid from the phage particles.

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Guidelines

Resuspending DNase I from powder

Determine number of units per mg powder: Roche grade II (cat.no. 10 104 159 001) contains approximately 2,000 Kunitz units per mg and comes in 100 mg size which equals 200,000 U. Resuspend in 5ml NEB recommended storage buffer, 10 mM Tris-Cl pH 7.5, 2 mM CaCl2 in 50% glycerol which equals 40,000 U/ml and store at -20°C .

Unit definition

One unit is the amount of enzyme which will completely degrade 1µg of pBR322 DNA in 10 min at 37°C in DNase I reaction buffer.

Reaction Buffer

Prepare a 10x reaction buffer containing MgCl2 and CaCl2 which are required for enzymatic activity. Final 1x buffer contains 10 mM Tris-HCl pH 7.6, 2.5 mM MgCl2, 0.5 mM CaCl2.

DNase I reaction conditions

DNase I was tested for ability to degrade DNA in solutions containing iron chloride, EDTA-Mg ascorbate buffer and cesium chloride (see BTP Exp. 72) under conditions of 37°C for 1 hr, room temperature for 2 hr and 4°C overnight. The DNA concentration (1 Kbp DNA ladder) in each solution was 200 ng/µl. The DNase I (40,000 U/ml) was diluted 1:40 in 10x reaction buffer with final concentration of 1,000U/ml (=1 U/µl) in 10x buffer. The diluted DNase I was used at a 1:10 dilution in the DNA solutions: 1 µl DNase I in 9 µl DNA. The DNA was degraded in each solution at all the reaction conditions tested.

DNase I Storage Buffer: 10 mM Tris-HCl, pH 7.5, 2 mM CaCl2 in 50% glycerol

100 µl 1 M Tris-HCl, pH 7.5 (autoclaved or filter-sterilized stock)
20 µl 1 M CaCl2 (filter-sterilized stock)
10 ml 50% Glycerol (autoclaved)

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10X DNase I Reaction Buffer: 100 mM Tris-HCl, pH 7.6, 25 mM MgCl2, 5 mM CaCl2
1 ml 1 M Tris-HCl, pH 7.5 (autoclaved or filter-sterilized stock)
250 µl 1 M MgCl2 (filter-sterilized stock)
50 µl 1 M CaCl2 (filter-sterilized stock)
8.7 ml Q-water (autoclaved)

Citation: Hurwitz, B. L., Deng, L., Poulos, B. T., & Sullivan, M. B. (2012). Evaluation of methods to concentrate and purify ocean virus communities through comparative, replicated metagenomics. Environmental Microbiology. doi:10.1111/j.1462-2920.2012.02836.x

Protocol

DNaseI Treatment

Step 1.
DNase I made up to 40,000 U/ml in storage buffer (-20°C freezer door) = stock solution.

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Step 2.
Dilute stock solution 1:40 in 10x reaction buffer = 1,000 U/ml = 1 U/ul = working dilution.

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Step 3.
Use at a 1:10 dilution: 1µl per 9 µl solution to be treated.

DNase Treatment

Step 4.
Incubate at room temperature for 2hr.

DURATION
02:00:00

DNase Treatment

Step 5.
Prepare 1M EDTA disodium salt dihydrate.

PROTOCOL

. EDTA disodium salt dihydrate

CONTACT: Bonnie Poulos

Step 5.1.
Weigh out 3.72 g EDTA and place in 5 ml molecular biology grade water.

Step 5.2.
Add a few NaOH pellets to get to pH 9 and dissolve EDTA (can warm to 45°C with stirring to aid in dissolution).

Step 5.3.
Measure volume and determine molarity: 1M = 3.72g/10 ml, therefore, measured volume/10 ml = final molarity.

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Step 6.
Prepare 1M EGTA tetrasodium salt.

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PROTOCOL

. EGTA tetrasodium salt

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Step 6.1.

Weigh out 4.68 g and place in 5 ml molecular biology grade water.

AMOUNT

5 g Additional info:

REAGENTS

EGTA E8145 by Contributed by users

Step 6.2.

Stir until dissolved.

Step 6.3.

Measure volume and determine molarity: 1M = 4.68g/10 ml, therefore, measured volume/10 ml = final molarity.

DNase Treatment

Step 7.

Inactivate DNase by adding 100 mM EDTA/100 mM EGTA final concentration.

NOTES

Bonnie Poulos 16 Jul 2015

The working DNaseI solution may be 0.02 µm filtered if being used on samples that will be used for metagenomic studies. Syringe filters are available through GE Healthcare: Anotop 25 cat. no. 6809-2102.

Although the DNaseI is active using other reactions conditions, room temperature for 2hr is recommended when working with marine viruses. Alternatively, 4C overnight may be used. Avoid using higher temperatures (eg, 37C for 1hr) as marine virual capsids may destabilize at higher temperatures.

The EDTA and EGTA stop the enzyme reaction by chelating magnesium and calcium ions that are necessary for DNaseI activity.

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