DNase treatment with Invitrogen kit

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MAY 10, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.14egn3yml5dy/v1

Protocol Citation: Anna JSzékely 2023. DNase treatment with Invitrogen kit. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn3yml5dy/v1

MANUSCRIPT CITATION:
Mechanisms determining the fate of dispersed bacterial communities in new environments
AJ Székely, M Berga, S Langenheder
The ISME journal 7 (1), 61

Protocol status: Working
We use this protocol and it's working

Created: Oct 04, 2016

Last Modified: May 10, 2023

ABSTRACT

We use this protocol to remove DNA from DNA/RNA mix isolated with Easy-DNA Kit (see Simultaneous DNA/RNA extraction for filtered water samples protocol).

PROTOCOL integer ID:
3827

GUIDELINES

SAFETY: One of the kit components contains the following chemicals:

- 8-10% of human urine (potential biohazard)
- 0.1-0.3% bovine serum albumin (harmful)
- ≤0.09% sodium azid (very toxic).

As both the amount and the concentration of the very toxic compound (sodium azid) is very low, the risk is also low.
Avoid contact with eyes, skin and other mucous membranes. Wash thoroughly after handling. Avoid breathing vapor/mist/spray.

QUALITY ASSURANCE:

1. **Controls:** Run a negative DNase treatment sample for every batch of DNase treatment to control for potential contaminations of reagents or during the treatment procedure.
2. **Kit properties:** Always write down the LOT number of the kit you use. Avoid using expired kits or reagents.
3. **Test PCR for treatment**: always perform test PCR (step 10-11.) to confirm the success of the DNase treatment.

VERY IMPORTANT: RNA is much more sensitive to degradation than DNA, therefore contamination with RNase has to be avoided (the main source is your own skin) and the samples have to be kept on ice all the time to slow degradation. The following steps help avoid degradation before and during RNA work:

1. Before starting to work clean bench, pipettes and the racks with MQ water (against DNA and RNA contamination), 70% ethanol (desinfection) and **RNaseAway spray**.
2. Use only nuclease-free filter tips and tubes.
3. Keep thawed RNA samples on ice all the time.
4. Use new gloves to touch samples and RNase-free pipettes and racks (cleaned in point 1). If you touch anything else (for example freezer handle), immediately change gloves. Change gloves regularly anyway during work with RNA, at least after every few steps. Before starting to work, spray RnaseAway on your gloves.

Special care should be applied until RNA is completely transcribed to DNA (end of RT process).

MATERIALS

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DNase I, Amplification Grade **Invitrogen - Thermo Fisher Catalog #18068015**

- Clean all surfaces (bench, pipettes, racks) before starting to ensure removal of contaminants. To do this wipe sequentially with a combination of (1) MQ, (2) 70% ethanol and (3) **RNAse Away.**
- Get ice
- Take out from the freezer and thaw: 10xBuffer, EDTA (included in kit)
- Get samples from -80ºC
- Label tubes or strips, one for each sample and one as negative control.
- Leave DNase in freezer until use. DNase is sensitive to physical denaturation, therefore **never vortex it!**
- You can also apply 10 minutes UV exposure in the UV-box for pipettes, tips, racks, etc.

Mix preparation

1 Prepare incubation mix (for few samples) and continue at step 5:

		Amount (µL)
	DEPC-treated water (not MQ)	0-7
	10xbuffer	1
	DNase I	1
	Sample or diluted sample	1-8
	Total	10

There are no exact indications for the amount of sample to use but I usually start with the maximum amount (8 µl) and reduce it only for the samples were the test PCR is positive.

For more samples prepare master mix (MM) of 10x buffer and DNase I (never vortex!), put back to freezer after preparing MM).

		For 1 sample (µL)	For n sample (µL)
	10xbuffer	1	1*n*1.15 (e.g. for 20 samples, add 23 µL)
	DNase I	1	1*n*1.15

2 Add RNAse-free water to tubes if needed.

Calculate the amount like this: (8µL - amount of sample) * n * 1.15
For higher DNA/RNA content samples use less than 8µL.

3 Mix gently and spin MM, and distribute in labelled PCR tubes/strips.

4 Add 1-8 µL of DNA/RNA sample or diluted sample to each tube, keep tubes on ice.

5 Mix gently and spin tubes.

Digestion

6 Incubate tubes in PCR machine at 25ºC for 15’ (DNA digestion).
00:15:00

Inactivation

7 Move tubes back to ice and add **1 µL EDTA** to each, vortex and spin.

8 Incubate tubes in PCR machine at 65ºC for 10’ (DNase inactivation).
00:10:00

9 Spin and store tubes at -20ºC until next step.

Test PCR

10 Perform test PCR. Always use a **positive control** and a negative control!

We do everything the same way as we will generate the amplicons for Illumina sequencing with the trancribed cDNAsing except that we do only one longer PCR. We also use only half amount of template (0.5 µl) as the cDNA will also be diluted during the RT reaction.

Primers:
Illumina adapter-N4-341F:
3'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCTACGGGNGGCWGCAG-5'
Illumina adapter-805NR:
3'-AGACGTGTGCTCTTCCGATCTGACTACNVGGGTATCTAATCC-5'

PCR reactions

	Components	Working conc.	Final conc.	1 reaction (20 µl)	(N) reactions	
	5xQ5 Reaction Buffer	5X	1X	4 µl		
	Forward Primer (illu-ada-341F)	10 µM	0.25 µM	0.5 µl		
	Reverse Primer (illu-ada-805NR)	10 µM	0.25 µM	0.5 µl		
	dNTPs	2 mM	200 µM	2 µl		
	Q5 HF DNA polymerase	2 U/µl	0.02 U/µl	0.2 µl		
	Template DNA			0.5 µl		
	Nuclease-Free water			12.3 µl		

	Σ			20 µl		

DNase-test PCR program

	STEP	TEMP.	TIME
	Initial Denaturation	98ºC	30 seconds
	35 cycles	98ºC	10 seconds
	48ºC	30 seconds	
	72ºC	30 seconds/kb	
	Final Extension	72ºC	2 minutes
	Hold	6ºC	∞

11 Check PCR products in 1% Agarose gel.
If there is product only in the postivie control but not in the others, continue with RT protocol.
If there is product in a sample, repeat the DNAse treatment for that product with less template or diluted template.