

Extraction of metagenome from potable water to analyze microbial fingerprints

Abstract

Metagenomics approaches give an insight of uncultivable microorganisms which seems to be comprises about 99% of the total microbes present in an environment. The present work shows comparative analysis of the extraction process for potable and sewage water community DNA. Different methodologies have been implemented to extract the DNA pool from the sewage and potable water. The standard protocol has been compared with the commercial kits to check the purity and quantity of the DNA pool from both the samples. Prominent difference has been found in the sewage and potable water. Noticeable amount of the DNA pool has been extracted through the standardized protocol as compared to commercial kits.

Keywords: metagenomics, potable water, sewage water, standardized protocol, DNA pool, genetics, metagenomic dna isolation, purification, molecular biology

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Introduction

Metagenomics has emerged as a powerful tool as a combination of molecular biology and genetics wherein we can directly extract the genetic material from the environmental sample itself and apply that knowledge, regardless of that microorganism to grow in-vitro. The term "metagenomics" was first used by Jo Handelsman et al. and first appeared in publication 1998.¹ To extract DNA from the water sources, many protocols are available online, different protocols for different types of water like sea water, sewage water, fresh water sources.²⁻⁴ But we hardly find any protocol for the potable water available for the researchers though commercial kits are available. This study tries to put forth a standardized protocol to extract community DNA from the potable water. Our study indicates that despite of three steps of purification, there are still some microbes which are present in drinking water. Our lab work includes DNA extraction by three different methods, two kit based protocols and one standardized protocol, which has yield the comparable amount of DNA. Two kits include HiPurA™ Water DNA Purification Kit from Himedia and Metagenomic DNA Isolation Kit for Water from Epicentre. HiPurA™ Water DNA Purification Kit has two different procedures for the extraction of Gram positive and Gram negative Bacteria.

Material and methods

Materials

Fresh water samples has been collected in sterile bottles in sterile condition from Katraj and Dhankawadi area, Pune while sewage water samples has been collected in sterile condition from Katraj area drainage systems of Pune city, India. After repeated attempts, we were successful in standardization of the protocol for extraction of community DNA from potable water. To compare the volume of the DNA pool, sewage water has taken.

Chemical required

- Extraction buffer: 50mM NaCl
- 50mM Tris-HCL (pH 7.6)
- 50mM EDTA

- 5% SDS
- Dithiothrietol (1M)
- Phenol (Tris-saturated) pH-8 (keep in refrigerator)
- Chloroform: isoamyl alcohol (24:1)
- Chloroform
- Sodium acetate solution (3M) Sterile
- Isopropanol
- Ice cold 70% ethanol
- Microcentrifuge tubes
- For each sample-two 2ml sterile centrifuge tubes
- One 1.5ml sterile centrifuge tube
- Membrane filter 0.45µm

Method

- Filter 2L of drinking water on 0.45µm pore size filter paper.
- In sterilized condition cut the filter paper into pieces.
- Add 6-7 pieces of filter paper in 2ml sterile micro centrifuge tube which contain 0.5ml of glass beads.
- Add 1ml of extraction buffer.
- Add exactly 1µl of 1M Dithiothrietol into the lid of each sample. Mix by vortexing for 2-3 seconds.
- Centrifuge for 3 minutes at 13000rpm.
- Decant supernatant into fresh 2ml tube. This solution contains DNA. Discard the used tubes.
- Determine the volume of your extract, it should be approximately 900µl. Add half the volume of both phenol and chloroform/isoamyl alcohol. Vortex till the emulsion forms and the solution appears milky.

- I. Centrifuge for 3 minutes at 13000rpm. Until phases are well separated.
- J. The aqueous phase containing the DNA will be the upper phase with a sterile pipette tip, transfer the aqueous phase to a new 2ml tube.
- K. Extract again with an equal volume of chloroform (not chloroform/isoamyl alcohol) as above
- L. Centrifuge at 13000rpm, and then transfer the aqueous phase to a new 1.5ml tube.
- M. Determine the volume of your extract and add 0.1 volume of 3M sodium acetate solution and exactly 0.7 volume of Isopropanol. Mix well by inverting the tube several times. Do not vortex.
- N. Precipitate the DNA by centrifugation at 8000rpm for 30m in the refrigerated centrifuge at 10°C.
- O. Carefully discard the supernatant by aspirating the Isopropanol.
- P. Wash the pellet by adding 0.5ml ice cold 70% chilled ethanol by inverting the tube gently. Make sure that ethanol should contact all the wall of the tube.
- Q. Repellet again by centrifugation for 5 min at 10-15°C.
- R. Discard ethanol carefully.
- S. Allow pellet to dry for 2-3 minutes.
- T. Resuspend the pellet by adding exactly 50µl of DNase/RNase-free water and mix by flicking the tube with your figures until the pellet dissolve.
- U. Store at -20°C for longer duration.

With the help of this protocol, DNA pool has been successfully

extracted from the potable water sample. To compare our results, two separate kits; HiPurA™ Water DNA Purification Kit of Himedia (Code no. MB547) and Metagenomic DNA Isolation Kit for Water from Epicentre (Cat. No. MGD08420) were purchased. HiPurA™ Water DNA Purification Kit was purchased from Himedia Laboratories Pvt. Ltd., India. This kit is having two different protocols for Gram positive and negative bacteria. Here community population is bacteria. Metagenomic DNA Isolation Kit for Water was purchased from Epicentre, an Illumina company. The instructions were followed and community DNA pallet was extracted successfully. DNA concentration was calculated from the spectrophotometric reading.⁵

Results

To measure the concentration of nucleic acid in the obtained sample, O.D was taken at 260 and 280nm. Pure preparations of DNA and RNA have OD₂₆₀/OD₂₈₀ values of 1.8 to 2.0, respectively (Table 1).⁶ To identify the microbial fingerprints in any environmental samples, the first and very important step is extraction of community DNA. This technical note will support emerging researchers who intend to extract the community DNA from potable water. The standardization of protocol was the major issue. We tested almost 12 procedures for extraction of DNA from fresh water, but it was very difficult to extract, as it is already three steps purified, finally the result was made possible by one standardized protocol revised in lab. For the comparison with the standard protocol, two commercial kits were purchased, HiPurA™ Water DNA Purification Kit of Himedia and Metagenomic DNA Isolation Kit for Water of Epicentre for community DNA extraction. The main objective was to extract the community DNA from potable water, but to compare its yield, sewage water samples has been taken. The community DNA from potable water was extracted successfully with the help of standardized protocol. The purity as well as yield of the DNA is comparable with commercial kits. This will help the researchers to save money. It could be done at routine lab conditions.

Table 1 OD and concentration of community DNA from sewage water and potable water samples from three different protocols; 1. From standardized protocol, 2. From Himedia kit, 3. From Epicentre kit

S. no.	Sample	260nm	280nm	Ratio	Concentration of DNA µg/mL
Community DNA from standardized protocol					
1	Sewage water	0.018	0.009	2	0.89
	Potable water	0.019	0.01	1.9	0.95
Community DNA from hipurA™ water DNA purification Kit of himedia					
2	Sewage water (for gram -ve)	0.013	0.006	2.16	0.65
	Potable (for gram -ve)	0.025	0.012	2.08	1.25
	Sewage water (for gram +ve)	0.021	0.011	1.9	1.05
	Potable water (for gram +ve)	0.023	0.017	1.35	1.15
Community DNA from metagenomic DNA isolation Kit for water of epicentre					
3	Sewage water	0.031	0.013	2.38	1.55
	Potable water	0.029	0.014	2.07	1.45

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None.

Conflict of interest

The authors declare that there is no conflict of interest.

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