bio-101 www.bio-protocol.org/e3521
Bio-101: e3521.
DOI:10.21769/BioProtoc.3521

Different Methods of Soil DNA Extraction

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[Abstract] Soil is the major reservoir of microbial diversity. Only 1% of microbial diversity can be cultured while 99% is still not culturable. It is necessary to extract DNA from soil in order to explore the 99% microbial diversity, which will be useful to harness novel industrial enzymes and natural products. In the present study, six traditional and two kit-based methods were utilized to obtain total soil DNA from Garden soil. Quality (Absorbance ratio at A_{260}/A_{230}, A_{260}/A_{280} nm) of the extracted DNA was assessed and quantity was analyzed using the BioTek Epoch Microplate spectrophotometer. Quality of DNA is one of the important factors that should be taken in to account for downstream applications such as PCR or cloning experiments.
Keywords: Soil DNA, Non-culturable, DNA extraction, Garden soil

[Background] Soil is the largest terrestrial reservoir of microbial bio-diversity which significantly balance the critical cycle of carbon, nitrogen, and phosphorous; along with maintaining plant health, structure and fertility of the soil. 1 g of soil harbors 1,00,000-10,00,000 different bacterial and archaeal species (Satyanarayana, 2017). However, only 1% of the microbial communities can be cultured in the laboratory conditions while 99% are still unexplored because they are non-culturable. Total soil DNA extraction potentially onsets the journey of revealing hidden microbial diversity (Robe *et al.*, 2003; Fatima *et al.*, 2011 and 2014; Lamizadeh *et al.*, 2019).

Soil DNA extraction includes two major steps: microbial cell lysis followed by purification to get rid of inhibitory molecules of humic acid and fulvic acid. Cell lysis can be performed via physical, mechanical and chemical approaches; or a combination of all three methods. These methods involve the use of detergents such as Sodium Dodecyl Sulfate (SDS) along with heat treatment in buffers like Tris-HCl or sodium phosphate buffers, along with the introduction of chelating agents such Ethylenediaminetetraacetic acid (EDTA) to protect extracted DNA from DNases which are readily present in external environment. There are different components which add to the effectiveness of cell disruption such as enzymatic treatment with lysozyme, using strong chaotropic agents such as guanidium salts, physical and mechanical treatment such as using liquid nitrogen to grind soil samples, ultra-sonication, glass beads, Zirconia beads and bead-beating approach for cell lysis. Various combinations of these methods are utilized to improve the yield of isolated DNA. Purification steps are conducted after DNA extraction with the help of phenol:chloroform:isoamyl-alcohol method (PCI method), ethanol precipitation, precipitation via polyethylene glycol, isopropyl alcohol, and spin columns. Purification is a crucial step as it involves the removal of PCR and restriction digestion

Bio-101: e3521.
DOI:10.21769/BioProtoc.3521

inhibitors such as humic and fulvic acid which are co-extracted along with DNA. Hence, effective extraction procedure followed by stringent purification are crucial steps for isolating DNA which can be used for better understanding of microbial biodiversity (Fatima *et al.*, 2011; Bag *et al.*, 2016).

The present study was aimed to obtain a high yield of total soil DNA from garden soil by using different conventional methods of extraction as well as soil DNA kits (HiPura soil DNA kit and Dneasy power soil DNA kit). A comparative analysis was performed in terms of purity and yield using BioTek Epoch Microplate spectrophotometer for absorbance measurements.

Materials and Reagents

A. For soil collection and processing
   1. Sterile 50 ml Falcon (Tarson, catalog number: 546041)
   2. Sterile scapula
   3. Garden soil
   4. Ethanol
   5. Liquid Nitrogen

B. For Extraction of total soil DNA by different methods
   1. 0.1 mm Zirconia beads (BioSpec, catalog number: NC0362415)
   2. 2.7 mm Glass beads (BioSpec, catalog number: 11079127)
   3. 50 ml sterile Falcon (Tarson, catalog number: 546041)
   4. 2 ml Eppendorf (Tarson, catalog number: 500020)
   5. 1.5 ml Microcentrifuge tube (Tarson, catalog number: 5000010)
   6. Filter 0.45 µm (Jsil)
   7. 1,000 µl Microtips (Tarson, catalog number: 5210010)
   8. 200 µl Microtips (Tarson, catalog number: 521020)
   9. Disodium Hydrogen Phosphate anhydrous (Na_2HPO_4) (Molychem, catalog number: QB4Q640403)
   10. Sodium Dodecyl Sulfate (SDS, $CH_3(CH_2)_{11}OSO_3Na$) (Affymetrix, catalog number: 18220)
   11. Polyvinylpyrrolidone [PVPP, $(C_6H_9NO)n$] (Amresco, catalog number: 0507)
   12. Sodium Chloride (NaCl, Fisher Scientific, catalog number: 27605)
   13. Chloroform ($CHCl_3$) (Molychem, catalog number: 13620)
   14. Sodium acetate trihydrate ($CH_3COONa·3H_2O$) (Fisher Scientific, catalog number: 1390M)
   15. Tris (Hydroxymethyl)Aminomethane Hydrochloride (Tris-HCl, $C_4H_{11}NO_3.HCl$) (SRL, catalog number: 99438)
   16. Ethylenediaminetetraacetic acid (EDTA, Qualigens, catalog number: 12635)
   17. Phenol:Chloroform:Iso-amylalcohol 25:24:1 (PCI) (Sigma, catalog number: P2069)
   18. Sodium Dihydrogen orthophosphate Dihydrate ($NaH_2PO_4·2H_2O$) (Molychem, catalog number: 25700)

19. Cetyl trimethylammonium bromide (CTAB) (Molychem, catalog number: 22560)
20. Mannitol (HiMedia, catalog number: RM9914)
21. Lysozyme (Sigma, catalog number: 89833)
22. Proteinase K (Sigma, catalog number: P2308)
23. Sodium Lauroyl Sarcosine (Amersco, catalog number: 0719)
24. Isopropyl alcohol (Molychem, catalog number: 1750)
25. Iso-amyl alcohol (SRL, catalog number: 69931)
26. Guanidine Hydrochloride (Sigma, catalog number: G3272)
27. Glycine (MP Biomedicals, catalog number: 194825)
28. Sodium Hydroxide (Molychem, catalog number: 25800)
29. Polyethylene Glycol (6000) (LOBA, catalog number: Art.5312)
30. Hydrochloric acid (Molychem, catalog number: 23540)
31. RNase (Invitrogen PureLink Genomic DNA mini kit, catalog number: K1820-01)
32. Proteinase K (Invitrogen PureLink Genomic DNA mini kit, catalog number: K1820-01)
33. Spin Column (Invitrogen PureLink Genomic DNA mini kit, catalog number: K1820-01)
34. Wash buffer1 (Invitrogen PureLink Genomic DNA mini kit, catalog number: K1820-01)
35. Wash buffer2 (Invitrogen PureLink Genomic DNA mini kit, catalog number: K1820-01)
36. Elution buffer (Invitrogen PureLink Genomic DNA mini kit, catalog number: K1820-01)
37. Ethanol 99.9% pure (Changshu Hongsheng Fine Chemicals, catalog number: 1170)
38. Stock solution (see Recipes)
    1 M NaCl
    1 M NaH_2PO_4
    1 M Na_2HPO_4
    0.2 M Na_2HPO_4
    1 M Tris-HCl
    0.2 M EDTA
    1 M HCl
    50x TAE buffer
39. Working stock solution (see Recipes)
    DNA extraction buffer 1
    50% PEG (6000)
    0.6 M NaCl
    Chloroform:Isoamyl alcohol (24:1)
    3 M sodium acetate
    $T_{10}E_1$ buffer
    PCI
    0.1 M Phosphate buffer saline
    DNA extraction buffer 2
    10% CTAB + 0.7 M NaCl

TEN buffer (pH 8.0)
TEN buffers (1 ml) supplied with 0.2 mg Lysozyme
20% SDS
$T_{50}E_1$
Lysozyme (10 mg/ml)
Guanidine-HCl (5 M)
10% Sodium Lauryl Sarcosine
TENP
Lysozyme solution
SDS solution
1x TAE buffer
70% Ethanol

C. Commercial kits for soil DNA extraction
1. HiPurA soil DNA kit (HiMedia, catalog number: MB542)
2. Dneasy Power Soil (Qiagen, catalog number: 12888-50)

D. For agarose gel electrophoresis
1. Tris-Base (MP Biomedicals, catalog number: 103133)
2. EDTA (Qualigens, catalog number: 12635)
3. Glacial Acetic acid (Molychem, catalog number: 21020)
4. Ethidium bromide (Sigma-Aldrich, catalog number: E7637)
5. 1 kbp ladder (Generuler, catalog number: SM0313)
6. 6x gel Loading dye (Frementas, catalog number: R0611)
7. Agarose (MP Biomedicals, catalog number: 218072090)

Equipment

1. Sieve (Mesh size of 0.22 mm, Sumeet Royal Interchangeable sieve)
2. Mortar and pestle (diameter of Mortar: 6 cm)
3. Pipettes 100-1,000 µl, 20-200 µl, 0.5-10 µl, 2-20 µl (Glison)
4. Water bath (Metalab, model: MSI14)
5. Centrifuge (Eppendrof, model: 5424R)
6. Micro-centrifuge (Pfact, model: 5804K)
7. Incubator Shaker (Hexatech, model: HIPL-035C)
8. Ice-machine (Wensar, model: LMIF series)
9. -20 °C refrigerator (Thermo)
10. Refrigerator (LG, model: GL-365YVQG5)
11. Sonicator (Cole-Parmer, model: 08895-22)

12. Weighing machine (Shimadzu, model: AUX220)
13. Horizontal gel electrophoresis apparatus (Techno Source, model: Sleek Gel)
14. Gel Documentation system (Bio-Rad, model: Universal Hood II)
15. Epoch Microplate spectrophotometer with Take3 plate accessories (BioTek, serial No.: 401554)
16. Microwave (LG microwave, model: MS-2347BS)

Software

1. Gen-5 Data Analysis Software (version 3.03)
2. Image Lab version 5.2.1 build11

Procedure

A. Soil sample Collection

1. Dig the soil surface up to 3 cm depth. Top soil is a precious natural resource as it contains humus (a rich source of nutrients), minerals required for growth of plants and microbial flora. Top soil is mainly rich in microbial diversity. **The guidelines for Soil sampling depth is given in Table 1.**

Table 1. The guidelines for sampling depth (Fery *et al.*, 2018) and Reference 13

Crop	Sampling depth (cm)
Grasses and grasslands	Up to 5 cm
Shallow rooted crops (*e.g.*, rice, groundnut)	Up to 15 cm
Deep-rooted (*e.g.*, Cotton, sugarcane)	Up to 22 cm

2. Collect three soil samples at different spots (at a distance of 3 m) from the same field.
3. Collect the soil in labeled sterile 50 ml Falcons with the help of sterile spatula.
4. Sieve the soil sample with the help of 0.22 mm mesh.
5. Mix the sieved soil sample.
6. Aliquot 10,000-15,000 mg of above-mixed soil in 50 ml sterile Falcons (maximum 15,000 mg, *i.e.*, one aliquot is enough to carry out all soil DNA extraction methods).
7. Store it in -20 °C for long term storage.

B. Soil sample processing

Two types of processing carried out for each method mentioned below: liquid nitrogen grinding and direct.

Note: Clean mortar and pestle with 100% ethanol.

1. Soil sample 1 (S1): Soil sample processed by using Liquid Nitrogen.
   a. Weigh 10,000 mg of the soil sample.

b. In mortar with pestle, grind the soil sample by carefully pouring liquid nitrogen. The treatment should be continued until it turns in to powder (label this soil sample as S1). The soil sample processed with liquid nitrogen will be used for all the conventional methods listed below. Since 1 g of soil harbor 10^{10} bacterial cells and 4 x 10^3 to 5 x 10^4 species diversity, each method requires 500 mg to 1 g of soil sample (Raynaud and Nunan, 2014).

2. Soil sample 2 (S2): Direct: Without liquid nitrogen processing. The soil sample processed without liquid nitrogen will be used for all the conventional and kit-based methods listed below:
   a. Weigh 10,000 mg of the soil sample.
   b. In mortar with pestle, grind it without liquid Nitrogen to make powder as fine as possible.

Notes:

1. *The following methodology is carried out for both samples, i.e., S1 as well as S2, except for kit-based method where only S2 is used.*
2. *After extraction of DNA by different methods, immediately store it at -20 °C.*

C. Protocols for total soil DNA extraction

Figure 1 summarizes the above procedure and total soil DNA extraction methods 1 to 8.

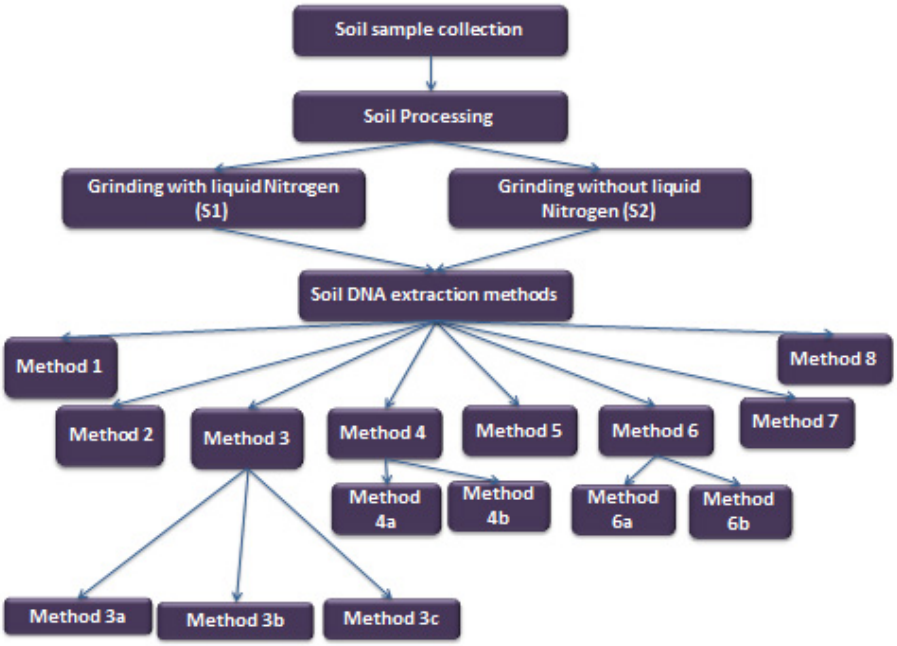

Figure 1. Workflow of soil DNA extraction protocol. The details of different steps and methods 1-8 are described under Methods section.

1. Method 1: PEG Method
   a. Weigh 1,000 mg of the soil sample in a 50 ml sterile centrifuge tube and add 10 ml of DNA extraction buffer 1.
   b. Incubate it at 65 °C for 1 h. in a water bath.

c. Centrifuge it at 7,000 *x g* 10 min at 4 °C.
d. Collect the supernatant in another centrifuge tube.
e. Add 1/2 volume 50% PEG (6000) and 1 volume 0.6 M NaCl (*e.g.*, if the volume of supernatant collected is 5 ml, then add 2.5 ml of 50% PEG (6000) and 5 ml of 0.6 M NaCl). Mix the solution gently by inverting the tubes 3-4 times.
f. Add 1 volume of Chloroform:isoamylalcohol (24:1). Mix the solution gently by inverting the tubes 3-4 times.
g. Centrifuge at 13,000 *x g* 10 min at 4 °C.
h. Collect the supernatant.
i. Add 1/10th volume of 3 M sodium acetate (pH 5.2).
j. Add 2 volumes of ice-cold ethanol (100%). Mix the solution gently by inverting tubes 3-4 times. Incubate overnight at -20 °C, which results in high yield of DNA.
k. Centrifuge at 13,000 *x g* 10 min at 4 °C.
l. Remove the ethanol and air dry the pellet for 15-20 min.
m. Dissolve the pellet in 100 µl of $T_{10}E_1$ buffer.

2. Method 2: Phenol:Chloroform:Isoamylalcohol (PCI) method
   a. Follow Steps C1a-C1d of the Method 1.
   b. Add equal volume of Phenol:Chloroform:Isoamylalcohol (25:24:1) to the supernatant. Mix the solution gently by inverting the tubes 3-4 times.
   c. Centrifuge 13,000 *x g* 10 min at 4 °C.
   d. Collect the aqueous fraction.
   e. Add 2 volumes of ice-cold ethanol and 1/10th volume of 3 M sodium acetate (pH 5.2). Mix the solution gently by inverting tubes 3-4 times. Incubate overnight at -20 °C.
   f. Centrifuge at 13,000 *x g* 10 min at 4 °C.
   g. Remove the supernatant.
   h. Air dry the pellet up to 15-20 min to remove ethanol.
      Note: Ethanol can hinder in downstream applications. Hence it is necessary to remove it completely. Be careful not to dry pellet completely.
   i. Dissolve the pellet in 100 µl of $T_{10}E_1$ buffer.

3. Method 3
   Note: In this method there are three sub-methods naming it as 3a, 3b and 3c. However, steps 'C3a to C3g' are common to all three methods.
   a. Weigh 1,000 mg of the soil sample in a 50 ml sterile centrifuge tube and add 5 ml of 120 mM Phosphate Buffer Saline (PBS, pH 7.4).
   b. Shake the mixture at 150 rpm 10 min at 4 °C in Hexatech shaker.
   c. Centrifuge at 7,000 *x g* for 10 min at 4 °C.
   d. Discard the supernatant and wash the pellet with PBS.
   e. Again, centrifuge at 7,000 *x g* for 10 min at 4 °C.
   f. Discard the supernatant and suspend the pellet in 10 ml DNA extraction buffer 2.

g. Incubate at 65 °C for 1 h.

i. Method 3a: Mannitol-PBS-PEG/NaCl

1) Follow Steps C3a to C3g.
2) Centrifuge the soil suspension at 8,000 *x g* 10 min at 4 °C.
3) Collect the supernatant.
4) Add 1/2 volume PEG (50%) and 1 volume of 0.6 M NaCl.
5) Incubate at 4 °C overnight.
6) Centrifuge at 13,000 *x g* 4 °C 10 min.
7) Discard the supernatant and resuspend the pellet in 3 ml TE buffer.
8) Proceed with Steps C2b to C2i.

ii. Method 3b: Mannitol-PBS-PCI

1) Follow Steps C3a to C3g.
2) Centrifuge the soil suspension at 7000 *x g* 10 min at 4 °C.
3) Collect the supernatant.
4) Proceed with Steps C2b to C2i.

iii. Method 3c: Mannitol-PBS-CTAB

1) Follow Steps C3a to C3g.
2) Centrifuge the soil suspension at 7,000 *x g* 10 min at 4 °C.
3) Collect the supernatant.
4) Add 50 µl 1 M NaCl and 50 µl 10% CTAB (prepared in 0.6 M NaCl).
5) Incubate for 15 min at 4 °C.
6) Proceed with Steps C2b to C2i.

4. Method 4

Note: There are 2 sub-methods of method 4: 4a and 4b. Steps 'C4a-C4r' are common for both methods.

a. Weigh 500 mg of soil and add 1 ml of TEN buffer (Tris-Cl-EDTA-NaCl buffer).
b. Vortex for 1 min.
c. Centrifuge at 10,000 *x g* 10 min RT.
d. Decant the supernatant and wash the pellet with 1 ml TEN.
e. Centrifuge at 10,000 *x g* 10 min RT.
f. Discard the supernatant. Resuspend the pellet in 1 ml TEN (add 0.2 mg lysozyme) (see Recipe: TEN buffers supplied with 0.2 mg Lysozyme).
g. Incubate for 1 h at 37 °C.
h. Keep it on ice for 10 min and then 20 min at 65 °C.
i. Add 100 µl of 20% SDS.
j. Vortex for 1 min.
k. Incubate for 30 min **at RT**.
l. Centrifuge at 10,000 *x g* 10 min at RT.
m. Collect the supernatant.

n. Add 500 µl of 3 M sodium acetate.
o. Incubate for 5 min **at 65 °C.**
p. Incubate on ice for 20 min.
q. Centrifuge at maximum '14,000 *x g*' for 30 min at 4 °C.
r. Collect the supernatant
   i. Method 4a
      1) Follow Steps C4a-C4r.
      2) Proceed with C2b to C2i.
   ii. Method 4b
      1) Follow Steps C4a-C4r.
      2) Load the supernatant on Invitrogen PureLink Genomic DNA mini kit spin column.
      3) Centrifuge at 16,000 *x g* 2 min RT.
      4) Discard the flow-through.
      5) Add 500 µl of wash buffer1 into the column.
      6) Centrifuge at 16,000 *x g* 1 min RT.
      7) Discard the flow-through.
      8) Add 500 µl of wash buffer2.
      9) Centrifuge at 16,000 *x g* 1 min RT.
      10) Discard the flow-through.
      11) Centrifuge at 18,407 *x g* 3 min RT.
      12) Discard the collection tube.
      13) Place column in new sterile 1.5 ml Eppendorf.
      14) Add 50 µl of elution buffer.
      15) Incubate for 2 min at RT.
      16) Centrifuge at 18000 *x g* 2 min at RT.
      17) Store at -20 °C.

5. Method 5
   a. Weigh 1,000 mg of soil sample and add 400 µl of 50 mM Tris-HCl and 1 mM EDTA.
   b. To the above mixture add 4 sterile glass beads (2.5 mm).
   c. Vortex for 1 min until it is homogenized.
   d. Remove glass beads with sterile tweezer.
   e. Add 50 µl lysozyme (10 mg/ml) (see Recipe: Lysozyme (10 mg/ml))
   f. Incubate it for 1.5 h. at 37 °C.
   g. Add 250 µl of Guanidine Hydrochloride (4 M).
   h. Mix gently for 45 s.
   i. Add 300 µl Sodium Lauryl Sarcosine.
   j. Vortex for 10 min at 37 °C.
   k. Incubate **for 1 h at 70 °C**.
   l. Add around 300 mg 0.1 mm zirconia beads.

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m. Vortex for 20 min.
n. Add 15 mg PVPP.
o. Gently vortex it.
p. Centrifuge at 14,000 *x g* 4 °C for 5 min.
q. Transfer the supernatant in another tube.
r. Wash the pellet with 200 µl TENP. Centrifuge at 14,000 *x g* 4 °C for 5 min. Collect the supernatant carefully.
s. Pool supernatant of Steps C5q-C5r.
t. Add 2 volumes of 100% ethanol.
u. Invert the tubes
v. Incubate at RT for 5 min.
w. **Centrifuge at 14,000 *x g* 4 °C for 5 min.**
x. Remove the supernatant.
y. Air dry for 15 min.
z. Resuspend the pellet with 450 µl of PB supplemented with 50 µl 3 M sodium acetate.
aa. Incubate at 4 °C for 1 h.
bb. Add 2 µl **each** of RNase and Proteinase K.
cc. Incubate **for 30 min** at 37 °C.
dd. Add 50 µl of sodium acetate (3 M) and 1 ml ethanol.
ee. Centrifuge at 14,000 *x g* 10 min 4 °C.
ff. Remove supernatant and wash pellet with 70% ice-cold ethanol.
gg. Air dry the pellet.
hh. Resuspend pellet in 100 µl of TE buffer.

6. Method 6

Note: There are 2 sub-methods of method 6: 6a and 6b. Steps 'C6a-C6n' are common for both methods.

a. Weigh 1,000 mg of soil.
b. Add 100 mM sodium phosphate buffer (pH 8).
c. Add 1 ml of lysozyme solution (see Recipes section below: Lysozyme solution).
d. Incubate at 37 °C for 2 h.
e. Add 1 ml of SDS solution (see Recipes section below: SDS solution: 10% SDS + 1 M NaCl + Tris-HCl).
f. Mix the above mixture well.
g. Incubate it at RT for 5 min.
h. Incubate it on ice for 2 min.
i. Keep it at 65 °C for 20 min
j. Again on ice for 2 min.
k. Add 1 ml of 3 M sodium acetate.
l. Vortex vigorously.

m. Centrifuge at 6,000 *x g* for 10min.

n. Collect the supernatant.

i. Method 6a

1) Follow Steps C6a-C6n.

2) Proceed with C2b to C2i.

ii. Method 6b

1) Follow Steps C6a-C6n.

2) Add equal volume of ethanol.

3) Centrifuge at 14,000 *x g* 10 min at 4 °C.

4) Remove supernatant.

5) Air dry the pellet.

6) Resuspend the pellet in 100 µl TE buffer.

7. Method 7: HiPura soil DNA kit

Weigh 250 mg* of soil and follow manufacturer's instructions given in the manual. The procedure is briefly explained here. The steps and its importance of soil DNA extraction by using HiPurA soil DNA kit are also listed below.

**Note: If it is the sediment sample, then weigh approximately 500 mg. Yield of total soil DNA depends upon the sample type and number of micro-organisms present in the sample.*

a. Cell lysis

Bead beating and soil lysis solution (SL), along with vigorous vortexing for 10 min, can help in lysing the microbial cells. Separation of the soil particles from lysed microbial cells is done by centrifugation at 13,000 *x g* for 1 min. Collect the supernatant, and if there are still few soil particles in the collected supernatant, again centrifuge it at 13,000 *x g* for 1 min and collect the supernatant.

b. Removal of inhibitors

Add Inhibitor Removal Solution (IRSH) to remove inhibitors such as humic acid. Collect the supernatant; which has nucleic acid, proteins, and other lysed cellular components; by performing a round of centrifugation at 10,000 *x g* for 1min. Supernatant should be transparent (brownish color of supernatant indicates high humic acid content) and free of soil particles, otherwise it will hinder in purification steps by clogging the membrane.

c. Purification and elution

Apply the collected supernatant on to spin column to purify total genomic DNA with the aid of silica membrane (present in the spin column format) by using binding, washing and elution buffers. Binding buffer provides optimum pH environment for nucleic acids to bind on to silica membrane, washing buffers helps to remove proteins and other contaminants, and elution buffer efficiently elute total soil DNA.

8. Method 8: Dneasy Power Soil

Weigh 250 mg of soil and follow manufacturer instructions given in the manual. Briefly the steps and its importance of soil DNA extraction by using Dneasy Power Soil is given below.

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a. Cell lysis

Total soil DNA is extracted by lysis of microbial cells via cell lytic buffer and bead beating method. Hence mechanical and chemical methods are used to obtain efficient cell lysis of microbial cells. Separation of the soil particles from lysed microbial cells is done by centrifugation at 10,000 *x g* for 30 s. Collect the supernatant, and if there are still few soil particles in the collected supernatant, again centrifuge it at 10,000 *x g* for 1 min and collect the supernatant.

b. Removal of inhibitors

Add inhibitor removal solution to remove inhibitors present. Solution should be transparent. Brownish-yellow color solution is a visual indication of the presence of humic acid. Hence solution should be preferably transparent and free of soil particles otherwise it will lead to inefficient extraction of DNA.

c. Purification and Elution of genomic DNA

It is performed by using silica-coated spin column by following number of steps which involves addition of binding buffer in order to bind nucleic acid to the membrane, washing buffers to remove other contaminants such as proteins and elution buffer for eluting total genomic DNA.

D. Visualization of extracted total soil DNA

Prepare 0.8% gel

1. Weigh 800 mg of Agarose.
2. Dissolve it in 100 ml of 1x TAE.
3. Microwave it for 2 min till it is completely dissolved.
4. Let it be warm and add 4 µl of EtBr (10 mg/ml).
5. Pour it in a casting tray and allow it to solidify.
6. Take 5 µl of the extracted DNA + 2 µl 6x gel loading dye. Mix it well.
7. Load it on the gel.
8. Load 1 kbp ladder and lambda DNA HindIII digest.
9. Let it resolved in 1x TAE at 100 volts for 45 min.
10. Visualize it in Gel Documentation system and Image Lab software. The gel images of total soil DNA that was extracted by using Methods 1 to 3, 4 to 6, 7 and 8 are shown in Figures 2, 3, 4, and 5 respectively.

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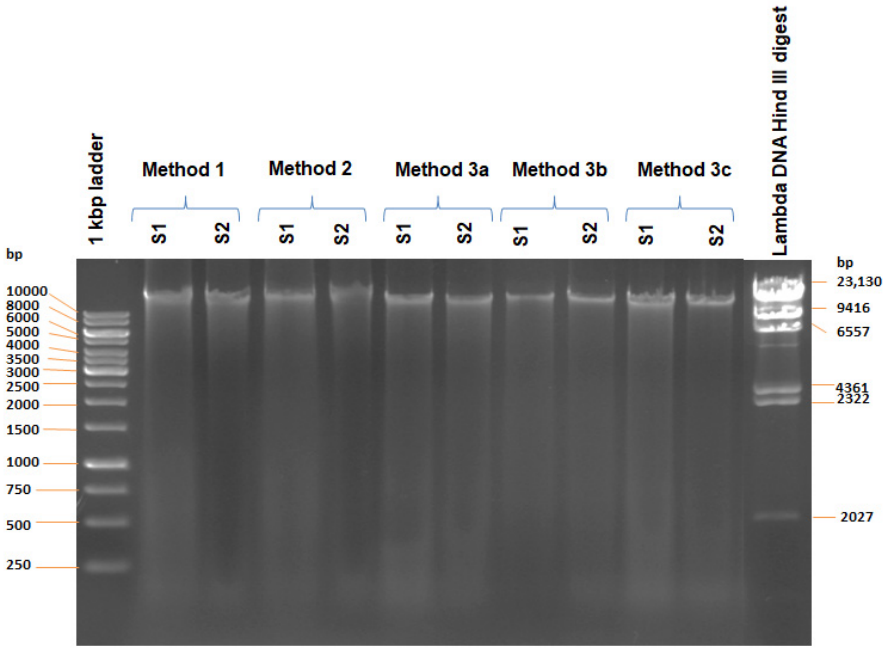

Figure 2. Gel electrophoresis of total soil DNA. The total soil DNA extracted by using methods 1 to 3 were resolved by 0.8% agarose gel electrophoresis and visualized with the help of Gel Documentation system and Image Lab software. S1: sample processed with liquid nitrogen, S2: sample processed without liquid nitrogen.

Note: High molecular weight DNA (approximately 23 kbp) was extracted using Methods 1 to 3.

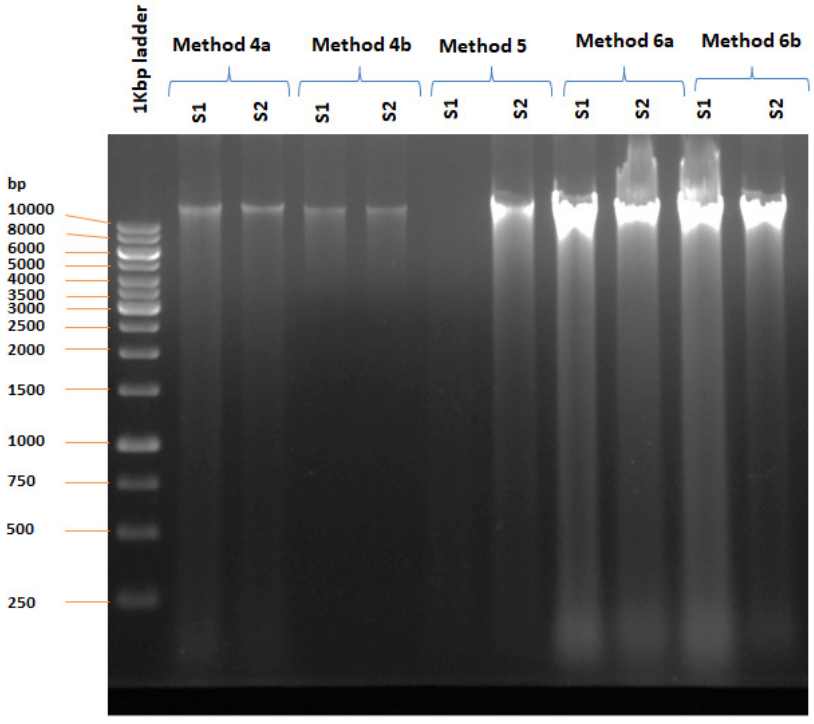

Figure 3. Gel electrophoresis of total soil DNA. The total soil DNA extracted by using methods 4 to 6 were resolved by 0.8% agarose gel electrophoresis and visualized with the help

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of Gel Documentation system and Image Lab software. S1: sample processed with liquid nitrogen, S2: sample processed without liquid nitrogen.

Note: High molecular weight DNA (> 10 kbp) was extracted using Methods 4 to 6.

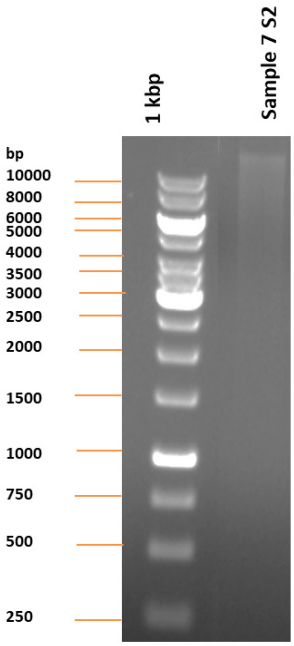

Figure 4. Gel electrophoresis of total soil DNA. The total soil DNA extracted by using method 7 were resolved by 0.8% agarose gel electrophoresis and visualized with the help of Gel Documentation system and Image Lab software. S1: sample processed with liquid nitrogen, S2: sample processed without liquid nitrogen.

Note: High molecular weight DNA (> 10 kbp) was extracted using Method 7.

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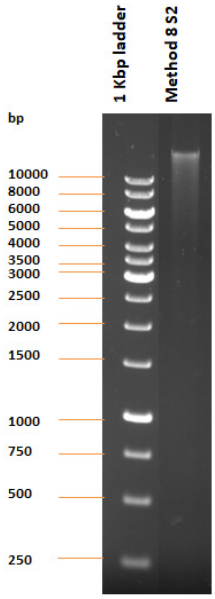

Figure 5. Gel electrophoresis of total soil DNA. The total soil DNA extracted by using method 8 were resolved by 0.8% agarose gel electrophoresis and visualized with the help of Gel Documentation system and Image Lab software. S1: sample processed with liquid nitrogen, S2: sample processed without liquid nitrogen.

Note: High molecular weight DNA (> 10 kbp) was extracted using Method 8.

E. BioTek Epoch Microplate spectrophotometer

1. Clean Take-3 plate reader with ethanol.
2. Dilute the samples if necessary (1:100 or 1:200 dilution) by using elution buffer as diluent.
3. Blank should be elution buffers used for different extraction procedures.
4. Add 2 µl on the plate and read it with the help of BioTek ELISA plate and support by Gen 5 Software for providing user-friendly interface for analysis. The quantification of total soil DNA is collectively shown in Table 2 and Figure 6.

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Table 2. Quantification of total soil DNA using BioTek Epoch Microplate spectrophotometer

Sample	ng/µl	A_{260}/A_{280}	A_{260}/A_{230}
Method 1 S1	9217.8	1.438	0.534
Method 1 S2	6380.6	1.487	0.482
Method 2 S1	4277.8	1.478	0.577
Method 2 S2	6003.4	1.46	0.616
Method 3a S1	3704.6	1.534	1.174
Method 3a S2	5767.7	1.458	1.416
Method 3b S1	6576.6	1.516	0.711
Method 3b S2	2814	1.515	0.627
Method 3c S1	4298	1.472	0.626
Method 3c S2	4601.7	1.507	0.677
Method 4a S1	856.3	1.19	0.689
Method 4a S2	681.2	1.442	0.745
Method 4b S1	34.1	2.33	0.048
Method 4b S2	14.8	3	0.023
Method 5a S1	104	2.33	0.184
Method 5a S2	563.8	1.468	0.649
Method 6a S1	616.9	1.732	0.691
Method 6a S2	1825.8	1.604	1.433
Method 6b S1	1567.5	1.699	1.427
Method 6b S2	453.1	1.561	1.391
Method 7 S2	11.045	1.851	1.052
Method 8 S2	19.608	1.87	1.789

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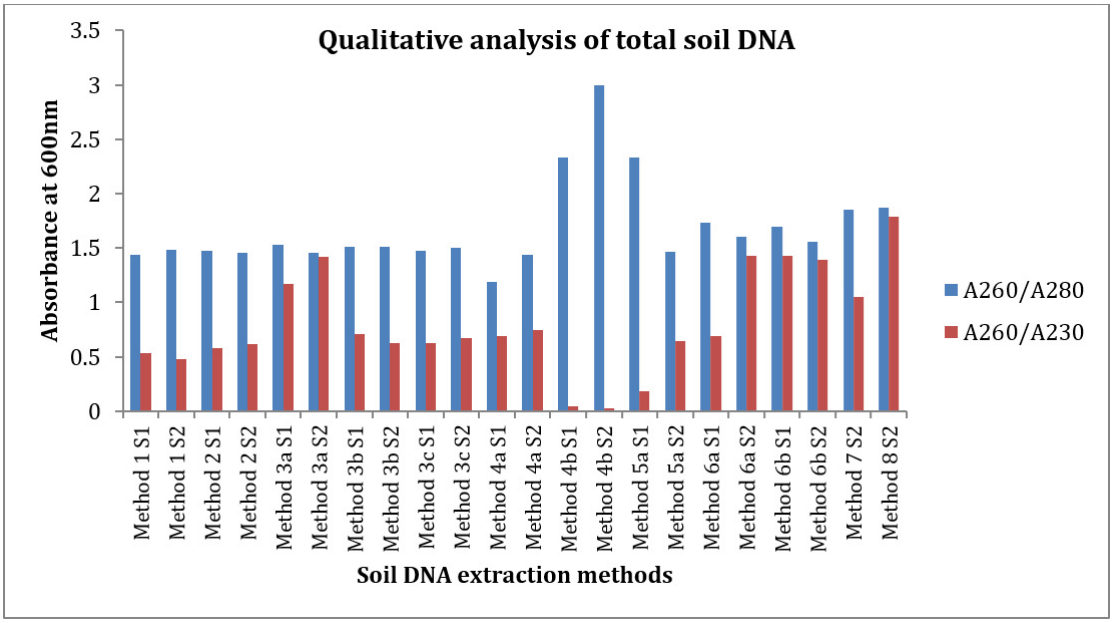

Figure 6. Qualitative analysis of total soil DNA: A ratio of A_{260}/A_{280} and A_{260}/A_{230} indicates the quality of extracted DNA. If the ratio of A_{260}/A_{280} is in between 1.8 and 2, it indicates high quality DNA. If it is less than 1.8, it indicates protein or other aromatic compounds contamination. If the ratio of A_{260}/A_{230} is less than 2 it indicates the presence of organic components such as humic acid. Humic acid present in soil samples can co-extract with the total soil DNA during the extraction procedure since both have the same charge and characteristics. Humic acid has the ability to interfere in downstream applications such as PCR (Humic acid binds to DNA polymerase and interfere in its activity) (Fatima *et al.*, 2014)

Conclusion: In the present study, conventional as well commercial kit based methods were used to extract total genomic DNA from soil. The quality and quantity of the extracted total DNA were visualized and analyzed by using the BioTek Epoch Microplate spectrophotometer (A_{260}/A_{280} and A_{260}/A_{230} ratio) and Gel electrophoresis technique. Commercial kit-based methods aid to obtain high quality of DNA as compare to conventional methods. Soil DNA extraction is the first step to explore culturable (accounts for 1% of microbial communities) as well as non-culturable microbial diversity (accounts for rest 99% of microbial communities) in a given soil sample. The DNA extracted by above-mentioned protocols can be used to perform soil metagenomics studies in order to discover novel biomolecules such as novel metabolites, enzymes, antibiotics (Kapoor *et al.*, 2015; Farias *et al.*, 2018; Castillo Villamizar *et al.*, 2019). It can also be used to study microbial diversity in different soil samples using 16S rRNA gene metagenomics. This will expand the existing microbial databases and also help to understand the effect of various environmental factors on soil microbial population and diversity (Leite *et al.*, 2014; Liu *et al.*, 2019; Matsushita *et al.*, 2019).

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Recipes

A. Stock solution

1. 1 M NaCl
   a. Add 2,922 mg of NaCl in 40 ml of MilliQ-water and then make up the volume to 50 ml with MilliQ-water
   b. Store at RT
2. 1 M NaH_2PO_4
   a. Weigh 7,800.5 mg in 40 ml of MilliQ-water. Mix well. Make up the volume to 50 ml with MilliQ-water
   b. Store at RT
3. 1 M Na_2HPO_4
   a. Add 7,080 mg of Na_2HPO_4 in 40 ml of MilliQ-water. Mix well. Make up the volume to 50 ml with MilliQ-water
   b. Store at RT
4. 0.2 M Na_2HPO_4
   a. Add 4 ml of 1 M Na_2HPO_4 in 16 ml of MilliQ-water
   b. Store at RT
5. 1 M Tris-HCl
   a. Add 7,880 mg of Tris-HCl in 40 ml of MillIQ-water
   b. Adjust the pH to 8
   c. Make the volume to 50 ml with MilliQ-water
   d. Store at RT
6. 0.2 M EDTA
   a. Add 3,725 mg of EDTA in 30 ml of MilliQ-Water. Keep this mixture on the magnetic stirrer
   b. Add few pellets of NaOH and let it stir until EDTA completely dissolves. Check the pH of the solution. It should be pH 8 since EDTA dissolves at pH 8
   c. Store at RT
7. 1 M HCl (1 L)
   Add 83 ml of concentrated HCl and adjust the volume to 1 L with MilliQ-water
8. 50x TAE buffer
   2 M Tris-Base
   1 M Glacial acetic acid
   50 mM EDTA
   For total volume 1 L:
   a. Add 242,000 mg of Tris-Base
   b. Dissolve it in 750 ml of deionized water
   c. Carefully add 57.1 ml of Glacial Acetic Acid
   d. Add 100 ml of 0.5 M EDTA (pH 8)

e. Adjust the solution to final volume of 1 L
f. Store at RT

B. Working stock solution

Note: Filter all following preparations with 0.45 µm filter.

1. DNA extraction buffer 1

   Final concentration: 120 mM Na_2HPO_4 + 5% SDS (w/v) + 0.1 g PVPP)

   For 50 ml total volume follow the recipe given below:

   a. Add 6 ml of 1,000 mM Na_2HPO_4 + 0.1 mg PVPP. Mix it well
   b. Then add 2,500 mg of SDS. Mix it well and keep it at 50 °C to completely dissolve
   c. Let the froth settle down
   d. Make the volume to 50 ml with MilliQ-water

2. 50% PEG (6000)

   For 10 ml total volume

   a. Add 5,000 mg of PEG 6000 in 7 ml of MilliQ-water. Dissolve it properly. Keep it at 50-60 °C water bath until dissolve
   b. Make the volume to 10 ml with MilliQ-water

3. 0.6 M NaCl

   For total volume 25 ml

   a. Add 15 ml of the 1 M NaCl in 7 ml of MilliQ-H_2O
   b. Dissolve it by vortexing
   c. Make up the volume to 25 ml by using MilliQ-water

4. Chloroform:Isoamyl alcohol (24:1)

   For total volume 25 ml

   a. Add 24 ml of Chloroform in 1 ml of isoamylalcohol
   b. Mix it well
   c. Store at 4 °C

5. 3 M sodium acetate

   Total volume 50 ml

   a. Add 12,304.5 mg of Sodium acetate in 30 ml of MilliQ-Water
   b. Adjust the pH to 5.2
   c. Make up the volume to 50 ml with MilliQ-water

6. $T_{10}E_1$ buffer

   Total volume 50 ml

   a. Add 0.5 ml of 1 M Tris-HCl + 0.25 ml of 0.2 M EDTA + 49.25 ml of MilliQ-water
   b. Mix it well and store at 4 °C

7. PCI

   a. Add Equilibration buffer in to the content as per manufacturer's instructions
   b. pH of the phenolic phase is between 7.8-8.2

c. Mix it well and store at 4 °C

8. 0.1 M Phosphate buffer saline

   0.02 M NaH_2PO_4

   0.08 M Na_2HPO_4

   9% NaCl

   For 50 ml total volume:

   a. Add 5 ml of 1 M NaH_2PO_4 +20 ml of 0.2 M Na_2HPO_4 + 4,500 mg of NaCl

   b. Adjust the pH 7.4

   c. Make up the volume to 50 ml with MilliQ-water

9. DNA extraction buffer 2

   For 10 ml DNA extraction buffer-2:

   0.1 M Tris-HCl (pH 8.0)

   0.2 M EDTA (pH 8.0)

   10% SDS

   0.2 M Mannitol

   1 M NaCl

   2% CTAB

   Follow the steps given below:

   a. Add 2 ml of 1 M Tris-HCl+ 1 ml of 0.2 M EDTA+ 1 mg SDS

   b. Vortex to mix it

   c. Keep it at 60 °C to dissolve the mixture completely

   d. Let the foam settle down

   e. Add 0.3643 mg mannitol. Dissolve it properly

   f. Add 0.2 mg of CTAB. Vortex it vigorously. Again let the foam settles down

   g. Keep it at 60 °C until dissolve

   h. Add 0.5 g of NaCl. Vortex. Again keep it at 60 °C to dissolve completely

   i. Filter the solution through 0.45 µm filter

10. 10% CTAB + 0.7 M NaCl

    For total volume 25 ml:

    a. Pre-warm 20 ml of MilliQ-Water

    b. Add 2.5 mg of CTAB + 1.0227 mg NaCl in pre-warm water

    c. Make up the volume to 25 ml with MilliQ-water

11. TEN buffer (pH 8.0)

    100 mM Tris-HCl

    50 mM EDTA

    500 mM NaCl

    Add 5 ml of 1 M Tris-HCl + 12.5 ml of 0.2 M EDTA + 25 ml of 1 M NaCl + 42.5 ml of MilliQ-water

12. TEN buffers supplied with 0.2 mg Lysozyme

    For total volume 1 ml

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a. 1 ml of TEN buffer + 0.2 mg of Lysozyme
b. Vortex it to dissolve
c. Keep it completely in water-sonicator

13. 20% SDS

For 50 ml total volume

a. Weigh 10,000 mg of SDS. Add it in 45 ml of MilliQ-water
b. Vortex it
c. Keep it at 40 °C in water bath until dissolve

14. $T_{50}E_1$

For total volume 5 ml

a. Add 0.25 ml of 1 M Tris-HCl + 0.025 ml of 0.2 M EDTA + 4.725 MilliQ-water
b. Store at RT

15. Lysozyme (10 mg/ml)

a. Weigh 10 mg of Lysozyme in 1 ml of MilliQ-water
b. Vortex it until dissolve

16. Guanidine-HCl (5 M)

For total volume 25 ml

a. Weigh 11,941 mg of Guanidine-HCl in 20 ml of MilliQ-water. Vortex it
b. Make up the volume to 25 ml with MilliQ-water

17. 10% Sodium Lauryl Sarcosine

a. Weigh 5,000 mg of Sodium Lauryl Sarcosine
b. Add 45 ml of MilliQ-water
c. Make up the volume to 50 ml with MilliQ-water

18. TENP

50 mM Tris-HCl

20 mM EDTA

100 mM NaCl

1% PVPP

Add 2.5 ml of 1 M Tris-HCl + 5 ml of 0.2 M EDTA + 5 ml of 1 M NaCl

19. Lysozyme solution

150 mM Tris-HCl

100 mM EDTA

Lysozyme (15 mg/ml)

For total volume 25 ml:

a. Add 3.75 ml of 1M Tris-HCl + 12.5 ml of 0.2 M EDTA + 375 mg of lysozyme
b. Make up the volume to 25 ml with MilliQ-water

20. SDS solution

a. 1 ml of 1 M NaCl + 5 ml Tris-HCl + 1 g SDS (10%). Mix well
b. Let the foam settle down

c. Make up the volume to 10 ml with MilliQ-water

21. 1x TAE buffer (1 L)
    Add 20 ml of 50x TAE + 980 ml of MilliQ-water
22. 70% Ethanol
    a. Add 70 ml of 100% ethanol + 30 ml of MiliQ-water
    b. Store at 0 °C

Acknowledgments

The present study was supported by SVKM's NMIMS intramural seed grant. We would like to acknowledge research papers authored by Fatima *et al*., 2011 and 2014; Bag *et al*., 2016.

Competing interests

The authors declare no conflict of interest.

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