JOURNAL OF PURE & APPLIED MICROBIOLOGY, Nov. 2014. Vol. **8**(Spl. Edn. 2), p. 69-75

# Comparison of Genomic DNA Extraction Commercial Kits Based on Clayey and Paddy Soils

**Peng Li[1,2†], Xuefeng Su[1,2†], Jinbin Wang[1,2], Shifang Yang[1,2], Guogan Wu[1,2], Meifeng Lin[1,2], Kai Zhao[1,2], Carlos Bezuidenhout[3] and Xueming Tang[1,2]***

[1]Biotech Research Institute of Shanghai Academy of Agricultural Sciences,
[2]Shanghai Key Laboratory of Agricultural Genetics and Breeding,
[3]Unit for Environmental Sciences and Management, North-West University, South Africa.

(Received: 26 March 2014; accepted: 15 June 2014)

**In this study, the relative efficacy of three commercial DNA extraction kits (the ISOIL for Beads Beating kit (IS), the FastDNA® SPIN Kit for Soil (FD) and the ExtroSpin® Soil Kit (ES)) were evaluated. Further, PCR-DGGE technique was assessed for its feasibility in detecting differences in clayey and paddy soils bacterial fingerprint profiles. Sufficient amounts of DNA could be successfully extracted from the clayey and paddy soils using both IS and FD kits, while it failed to extract detectable amount of DNA by the ES kit. PCR products of bacterial 16S rRNA genes were achieved by all three kits. Results showed that higher amounts of DNA and bacterial diversity in DGGE fingerprints were obtained by the IS and FD kits than by the ES kit. The IS and FD kits were appropriate for DNA extraction, displaying no significant differences in experimental results. When time and cost were considered, the FD kit contributed in cost by about two-fold reduction compared with the IS kit. In summary, the FD kit was the most cost-effective and time-efficient technique to extract DNA from the clayey and paddy soils.**

**Key words:** Extraction kit, Paddy soil, Clayey soil, PCR-DGGE, Microbial community.

Soil microorganisms play important roles in soil quality and plant productivity. The development for studying the diversity, distribution, and behavior of microorganisms in soil habitats is essential for a broader understanding of soil health. Traditionally, the analysis of soil microbial communities relies on culturing techniques using a variety of culture media designed to maximize the recovery of diverse microbial populations. However, only a small fraction (<0.1%) of the soil microbial community is accessible with this approach (Hill *et al*, 2000).

* To whom all correspondence should be addressed.
E-mail: pengli13@fudan.edu.cn

Because of the inherent limitations of culture-based methods, soil microbial ecologists are turning increasingly to culture-independent methods of community analysis. Based on the technology of 'culture independent' (Handelsman, 2004), circumventing the disadvantages of microbial isolation and culture, the direct use of molecular biology methods have become valuable tools for the study of soil microbial populations and communities. Thus, data derived from these molecular biology methods provide a more complete analysis of the microbial communities. A molecular fingerprinting technique that combines PCR-amplification of 16S rRNA gene and separation of amplicons using Denaturing Gradient Gel Electrophoresis (PCR-DGGE) has produced successful results in monitoring variations in microbial community in various environmental samples (Ovreas *et al*, 1997; Nakatsu, 2007).

The analytical success of molecular techniques, including PCR-DGGE, are greatly affected by the reliance on cell lysis efficiency and the quality of DNA recovered from the environmental samples (Merlin *et al*, 2010). Therefore, the prerequisite for the molecular methods in environmental samples is the extraction of high quality DNA suitable for downstream applications like the PCR-DGGE. Different DNA isolation methods that cause to insufficient cell lysis or shearing of DNA may result in bias in PCR amplification (Holland *et al*, 2000; McOrist *et al*, 2002). Extraction DNA methods are often hampered by the co-extraction of organic substances such as humic and fulvic acids extracted from soils along with DNA, which interfere with the PCR amplification (Von Wintzingerode *et al*, 1997). Clayey soils are particularly problematic when extracting DNA from microbial communities (Yankson and Steck, 2009). Currently, various commercial DNA extraction kits have been developed to simplify and speed up the extraction process. However, regarding the extraction efficiency, time-efficiency, cost-effectiveness and laboratory requirements available to isolate nucleic acids, these extraction kits need further evaluation.

The aim of this study was to compare three commercial DNA extraction kits (the ISOIL for Beads Beating kit (IS) (Nippon Gene, Toyama, Japan), the FastDNA® SPIN Kit for Soil (FD) (MP Biomedicals, Irvine, CA) and the ExtroSpin® Soil Kit (ES) (Lvjia Agro-tech Co., Ltd, Shanghai, China) in extracting microbial genomic DNA from clayey and paddy soils that are typically problematic in DNA extractions, high organic matter and high clay content. These kits were selected due to their availability, cost, ease of use, popularity and differences in cell lysis methods. Although FD kit had been tested separately by different researchers on various biological samples (Knauth *et al*, 2013; Merlin *et al*, 2010), our study further extended the knowledge by direct comparison and application of ES kit, IS kit and FD kit to PCR-DGGE. The average price of kit was received from respective provider. The approximate time to completion was calculated according to 'Instruction Manual'. The quantity and purity of the DNA extracts were evaluated by agarose gel electrophoresis and UV spectroscopy. PCR-DGGE fingerprinting was conducted to investigate the banding patterns of amplified 16S rRNA genes, and cluster analysis was further used to study similarities of the banding patterns, comparing the information obtained by fingerprints based on the DNA extracts.

## MATERIALS AND METHODS

### Soil properties and sampling

The paddy and clayey soil samples were collected from the Yuejing Farm of Chongming and Zhuanghang Base of Fengxian district of Shanghai (31°30' N, 121°31' E; 30°49' N, 121°29' E), respectively. The physical and chemical characteristics of the soils were shown in Table 1. Samples (three replicates) for DNA extractions were taken from the upper 5 cm section of root-free bulk soil, manually homogenized, and stored at -20°C before further processing.

### DNA Extraction

The following three commercial DNA extraction kits were evaluated: the ISOIL for Beads Beating kit (IS) (Nippon Gene, Toyama, Japan), the FastDNA® SPIN Kit for Soil (FD) (MP Biomedicals, Irvine, CA) and the ExtroSpin® Soil Kit (ES) (Lvjia Agro-tech Co., Ltd, Shanghai, China). The main features of the extraction kits were given in Table 2. In experiment, 0.5 g of the paddy and clayey soil samples was used to extract DNA in triplicate using the three kits according to 'Instruction Manual'. The DNA was eluted in 50 ¼l 1×TE buffer. All DNA extracts were stored at -20°C.

### Assessment of quality, quantity and composition of metagenome DNA

Genomic DNA (three replicates) was analyzed by 2% agarose gel electrophoresis. The results were photographed after ethidium bromide (EB) staining. DNA concentration was determined by NanoDrop® ND-1000 and DNA yield was calculated. To evaluate the purity of the extracted DNA, OD (optical density) values were measured under wavelengths of 230, 260 and 280 nm (A230, A260 and A280, respectively), and the ratios A260/280 and A260/230 were calculated. Absorption values of DNA solution were measured by full spectrum scan at a wavelength range of 220–320 nm. The average ratio of A260/280 was calculated for each set of triplicate samples, and used to estimate the purity of extracted nucleic acid: samples with mean A260/280 of 1.8-2.0 were presumed to be free of contamination; those with

A260/280 < 1.8 were presumed to contain protein or other contaminants; and those with A260/280 > 2.0 were presumed to be due to the presence of RNA (ND-1000 Spectrophotometer V3.1 User's Manual, 2005).

**Statistical Analysis**

All Genomic DNA extractions were performed in triplicate to account for analytical variability. Means of DNA yield and differences between samples were analyzed using SAS (version 9.1; SAS Institute, Cary, NC) by one-way and two-way ANOVA. Data were expressed as means ± SE. Differences were considered as significant when P was < 0.05.

**PCR-Denaturing gradient gel electrophoresis (DGGE)**

For bacterial DGGE analysis, 16S rRNA gene fragments were amplified with the primers F968-GC and R1401-1B. The PCR program and the subsequent DGGE analysis were performed as described by Jolanda K Brons (Jolanda *et al*, 2008). The mixed PCR products from three replicates PCRs were used twice for DGGE analysis to minimize deviation. PCR products were confirmed by electrophoresis on 2% agarose gels stained with ethidium bromide. Banding patterns of the DGGE profile were analyzed by the Quantity One software (version 4.5, Bio-Rad, Hercules, USA). The position and intensity of each band was determined automatically (by the program). The intensity value of each band was divided by the average band intensity one of the sample in order to minimize the influence of differences in DNA concentration between samples.

The DGGE banding patterns were used to calculate the Shannon-Wiener diversity index (*H*). The index was calculated with the following equations: $H = \text{“}Pi \ln Pi$, where *Pi* was calculated as follows: $Pi = ni / N$, where *ni* is the height of a peak and *N* is the sum of all peak heights in the densitometric curve. The species richness (*R*) was a simple count of the number of bands found in a community. The species evenness (*E*) was calculated using the equation $E = H / \ln R$ (Liu et al, 2007). Cluster analysis of DGGE banding patterns was performed with the unweighted-pair group method using the NTSYS-pc software package. Significant ($P < 0.05$) differences were analyzed by the Tukey's *t*-test with SPSS 13.0.

## RESULTS AND DISCUSSION

**Yield and purity of DNA extracts**

To examine the purity of the DNA extraction, the UV absorptions at 230, 260, and 280 nm were measured and the ratios of A260/230 and A260/280 were calculated (Table. 3). As shown in Table 3, on average, regardless of the soil type, FD kit provided the higher final DNA yield than other kits. Nevertheless, the DNA quantity of FD kit and IS kit was not significantly different. Meanwhile, FD and IS kits provided a higher DNA yield for paddy soil, compared to clayey soil, suggesting that DNA extraction was especially difficult for clayey soil. Although the A260/280 value of DNA extracts for the three kits approximate to 1.8, accepted as 'pure' for DNA; The A260/230 value of the DNA extraction of FD kit was 1.52 and the A260/230 value of the DNA extraction of the other methods showed lower, which may indicate the presence of co-purified contaminants (ND-1000 Spectrophotometer V3.1 User's Manual, 2005). Nevertheless, the ratios obtained with the FD kit showed higher values, indicating a higher purity of DNA extracts.

Agarose gel electrophoresis of the genomic DNA extracted by the three kits was shown in Fig. 1. The results suggested that the IS and FD kits succeeded in extracting sufficient amounts of DNA to be detected by agarose gel electrophoresis, whereas DNA extracted with the ES kit could not be detected by agarose gel electrophoresis. The low extraction efficiency of the ES kit resulted in lower amounts of extracted DNA.

**Analysis of PCR**

The PCR results were shown in Fig. 2. PCR products of the 16S rRNA gene were equally

**Table 1.** Physical and chemical characteristics of clayey and paddy soils

| Soil types | PH | Organic carbon | Total N (g/kg) | Total P (g/kg) | Total K (g/kg) |
|---|---|---|---|---|---|
| paddy soil | 6.07 | 6.23 | 1.86 | 0.98 | 23 |
| clayey soil | 5.35 | 2.84 | 2.74 | 0.76 | 7.59 |

achieved for the above three kits. All the three DNA quality was shown to be qualified for subsequent use in molecular applications.

**Comparative Analysis of DGGE Fingerprint Profiles**

In this study, DGGE was performed to evaluate the potential bias of DNA extracts obtained with different kits on fingerprinting methods (Fig. 3). Cluster analysis was carried out to compare the banding patterns of the DGGE fingerprints (Fig. 4). As shown in Fig. 3, the intensity of the majority of single bands was consistent among the three kits, regarding the same type of soil sample. Only few differences were observed between paddy soil and clayey soil as indicated by arrows. The resulting dendrograms of the DGGE patterns (Fig. 4) showed two distinct clusters, which were identified correlating with the two different soil types, respectively.

**Table 2.** Comparison of the recommended DNA extraction protocols based on technical booklets.

| Extraction kit/steps | IS kit | FD kit | ES kit |
|---|---|---|---|
| Soil wt (mg) | 0.5 | 0.5 | 0.5 |
| Beads | Unknown beads | 1.4 mm ceramic spheres, 0.1 mm silica spheres, 4 mm glass beads | 1.0mm zirconium beads |
| Cell lysis by chemical buffer | Lysis solution BB, lysis solution 20S | Sodium phosphate buffer, MT buffer | Sodium phosphate buffer, MT buffer |
| Cell lysis by mechanical method | Beads Beating disruption apparatus | Fast Prep Instrument | Horizontal vortexer |
| DNA binding | Precipitation solution | Binding Matrix E | Binding solution B |
| Washing, purification steps | Wash solution, Ethanol | spin™ Filter, 1× SEWS-M solution | Adsorption column |
| Average time until completion | 70-90 min | 70-90 min | 70-90 min |
| Average cost of kit (50 times) | $600 | $330 | $200 |

**Table 3.** Determination of quantity and quality of the genomic DNA isolated from the soil samples using the three extraction kits

| Soil | DNA quantity (¼g/g soil) | | | Absorption ratio$A_{260/280}$ | | | $A_{260/230}$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | IS kit | FD kit | ES kit | IS kit | FD kit | ES kit | IS kit | FD kit | ES kit |
| Padddy soil | 2.15± 0.11[a] | 2.26± 0.12[a] | 0.5± 0.1[b] | 1.92± 0.25[a] | 1.84± 0.06[a] | 1.82± 0.08[a] | 1.32± 0.29[a] | 1.52± 0.11[a] | 0.19± 0.11[b] |
| Clayey soil | 1.02± 0.11[a] | 1.45± 0.1[a] | 0.3± 0.1[b] | 1.77± 0.21[a] | 1.74± 0.11[a] | 1.69± 0.09[a] | 1.17± 0.21[a] | 1.23± 0.09[a] | 0.08± 0.06[b] |

Values are means ± SE.
Different letters (a, b) indicate a significant difference at $P < 0.05$

**Table 4.** Shannon-Wiener diversity index, richness, and evenness of bacteria as determined from DGGE bands patterns of paddy soil and clayey soil. The IS kit (lanes 1-4); FD kit (lanes 5-8); ES kit (lanes 9-12); the paddy soil samples (lanes 1-2, 5-6, 9-10); The clayey soil samples (lanes 3-4, 7-8, 11-12)

| DGGE Lanes | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | 38 | 38 | 33 | 33 | 39 | 39 | 33 | 33 | 32 | 32 | 25 | 25 |
| H’ | 2.67 | 2.69 | 2.33 | 2.37 | 2.73 | 2.75 | 2.35 | 2.35 | 2.29 | 2.32 | 2.08 | 2.05 |
| E | 0.73 | 0.74 | 0.67 | 0.68 | 0.75 | 0.75 | 0.67 | 0.67 | 0.66 | 0.67 | 0.65 | 0.64 |

The Shannon–Wiener diversity index (*H'*), richness (*R*), and evenness (*E*) of the bacterial community were different obtained by the three kits between paddy soil and clayey soil (Table 4). Regardless of the soil type, the common index (*H'*, *R* and *E*) for rhizosphere soil was lowest obtained by ES kit – and highest obtained by FD kit.

**Average time to completion and cost of kit**

When analysing the costs of the three extraction kits, based on prices from our local providers, we were able to define the information summarised in the following Table 2. IS Kit was the most expensive out of all the extraction kits. It was almost two times and three times more expensive than the FD kit and ES kit, respectively. All the three kits require about the same time to be performed, taking on average about 70-90 min per sample extracted.

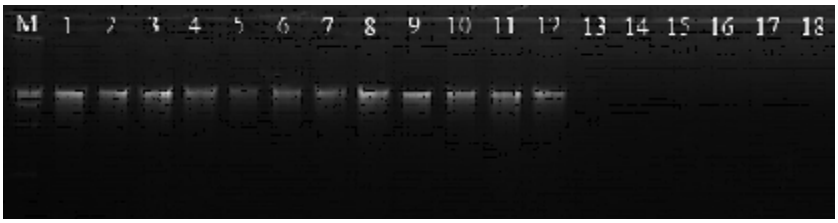


**Fig 1.** Agarose gel electrophoresis of the genomic DNA extracted by the three kits. IS kit (lanes 1-6); FD kit (lanes 7-12); ES kit (lanes 13-18); M: DL15000 Marker; The paddy soil samples (lanes1-3, 7-9, 13-15); The clayey soil samples (lanes 4-6, 10-12, 16-18).

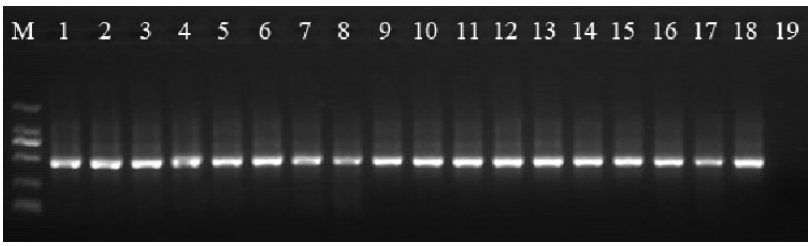


**Fig 2.** Agarose gel electrophoresis of PCR amplification of the 16s rRNA gene extracted by the three kits. IS kit (lanes 1-6); FD kit (lanes 7-12); ES kit (lanes 13-18). M: DL2000 Marker. The paddy soil samples (lanes1-3, 7-9, 13-15); The clayey soil samples (lanes 4-6, 10-12, 16-18).

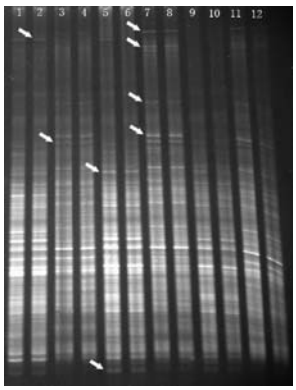


**Fig 3.** DGGE fingerprints of 16S rRNA genes of bacteria. IS kit (lanes 1-4); FD kit (lanes 5-8); ES kit (lanes 9-12); The paddy soil samples (lanes 1-2, 5-6, 9-10); The clayey soil samples (lanes 3-4, 7-8, 11-12). Arrows indicated bands of different intensity.

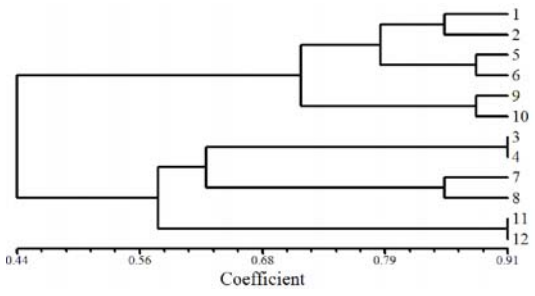


**Fig 4.** Cluster analysis of DGGE profiles for 16S rRNA genes of bacteria. IS kit (lanes 1-4); FD kit (lanes 5-8); ES kit (lanes 9-12); The paddy soil samples (lanes 1-2, 5-6, 9-10); The clayey soil samples (lanes 3-4, 7-8, 11-12); The dendrogram was calculated on the basis of UPGMA.

## DISCUSSION

Table 3 showed that FD kit provided the higher final DNA yield than other kits. The reason could be different sizes beads of FD kit (1.4 mm ceramic spheres, 0.1 mm silica spheres, one 4 mm glass bead) (Table 2) guaranteeing that the soil microbial cells were combined with beads and lysed thoroughly. Further, ES kit provided lower final DNA yield than other kits, the reason of which could be a consequence of the consecutive application of two steps of adsorption column for the DNA purification. The amount of eluted DNA reduced after purification on such adsorption column, and thus a second application might cause higher loss of DNA. The only size of bead of ES kit compared to that of the IS and FD kit might also be the reason for less DNA quantity that microbial cells were not lysed thoroughly. Meanwhile, FD and IS kits provided a higher DNA yield for paddy soil, which is consistent with previous studies (Andersen et al, 1998; Braid et al, 2003). The problem with extraction of DNA from clayey soil is that DNA binds to clay sorption sites with strong phosphor bindings.

Once quality and quantity of DNA obtained was evaluated, a PCR to investigate amplification of the 16S rRNA gene was performed on DNA extracts of all soils samples with the three kits. From the result of Fig.2, it was concluded that DNA extracts obtained by ES kit could also meet the requirements of PCR reaction, although ES kit provided lower final DNA yield and purity than other kits.

Denaturing Gradient Gel Electrophoresis (DGGE) is a culture-independent molecular fingerprinting method for the study of soil microbial communities dynamics. Individual PCR products are separated within a denaturing gel matrix and the DGGE profiles correspond to the microbial community composition (Muyzer *et al*, 1995). Theoretically, all profiles should have been identical since DNA is extracted from the same homogenized samples. Therefore, the differences in band resolution are best illustrated in comparisons of profiles of the same sample using different DNA extraction kits. Regarding the same type of soil sample, there was little difference in DGGE profiles generated from DNA extracts using IS kit and FD kit. On the other hand, the profile from ES kit was the least similar to the others. From the UPGMA dendrogram, the DGGE patterns obtained by the three kits for the same type of soil sample clustered together, indicating that the effect of soil types outweigh that of extraction kits on the soil microbial communities (Fig. 4).

Species richness (*R*) and species evenness (*E*) are two aspects to estimate bacterial diversity in an environmental sample. Shannon–Wiener index (*H'*) integrates these two parameters to indicate the diversity directly (Molles, 2000). Table 4 showed that FD kit and IS kit provided higher bacterial diversity than ES kits, suggesting that FD kit and IS kit could detect more predominant members in the bacterial communities. The reason could be less DNA quantity and purity obtained by ES kit affecting the subsequent PCR-DGGE results. So it was concluded that FD kit and IS kit could be preferably used to study the microbial community. Meanwhile, the result showed that paddy soil provided higher bacterial diversity than clayey soil, which is consistent with the above result of less DNA obtained from clayey soil.

## CONCLUSION

In this study, we provided a comparison of the three kits that are commercially available for the genomic DNA extraction of microbes from clayey and paddy soils. In summary, there were no statistically significant differences between the quality and quantity of DNA extracted by the IS and FD kits. When cost derived for each kit was accounted, the FD kit was the most cost-effective and time-efficient technique to isolate DNA from the two sorts of soil samples.

This work was financially supported by the Key Technologies Program of Shanghai Agricultural Commission, Grant No. 2011-4-4, 2011-1-8, the Public Research Platform Foundation of SSTC, Grant No. 13DZ2290700, the International Cooperation Foundation of China of MOST, Grant no. CS06-L10, and the Key R&D Program of MOST, Grant no. 2012ZX07101-009, the Science and Technology Development Pragram of SAAS, grant no. 2013(03); 2013(14) and Shanghai Agriculture Committee Young Talent Foundation, grant no. 1-20 (2014).

## REFERENCES


1. Andersen J B, Sternberg C, Poulsen L K, *et al*. New unstable variants of green fluorescent protein for studies of transient gene expression in bacteria. *Appl Environ Microbiol.* 1998; **64** (6):2240-2246
2. Braid M D, Daniels L M, Kitts C L. Removal of PCR inhibitors from soil DNA by chemical flocculation. *J Microbiol Methods*. 2003; **52**(3): 389-393
3. Handelsman J. Metagenomics: application of genomics to uncultured microorganisms. *Microbiol Mol Biol Rev.* 2004; **68**(4):669-85
4. Hill G T, Mitkowski N A, Aldrich-Wolfe L, *et al*. Methods for assessing the composition and diversity of soil microbial communities. *Applied Soil Ecology.*2000; **15**(1) :25-36
5. Holland J L, Louie L, Simor A E, et al. PCR detection of Escherichia Coli O157:H7 directly from stools: evaluation of commercial extraction methods for purifying fecal DNA. *J Clin Microbiol*. 2000; **38**(11):4108-4113
6. Jolanda K. Brons, Jan Dirk van Elsas. Analysis of Bacterial Communities in Soil by Use of Denaturing Gradient Gel Electrophoresis and Clone Libraries, as Influenced by Different Reverse Primers. *Appl Environ Microbiol.* 2008; **74**(9): 2717-2727
7. Knauth S, Schmidt H, Tippkötter R. Comparison of commercial kits for the extraction of DNA from paddy soils. *Letters in Applied Microbiology.* 2013; **56**(3):222-8
8. Liu B, Gumpertz M L, Hu S, *et al*. Long-term effects of organic and synthetic soil fertility amendments on soil microbial communities and the development of southern blight. *Soil Biol Biochem*, 2007, **39**: 2302-2316.
9. McOrist A L, Jackson M, Bird A R. A comparison of five methods for extraction of bacterial DNA from human fecal samples. *J Microbiol Methods.* 2002; **50**(2):131-139
10. Merlin W Ariefdjohan, Dennis A Savaiano, Cindy H Nakatsu. comparison of DNA extraction kits for PCR-DGGE analysis of human intestinal microbial communities from fecal specimens. *Nutrition Journal.* 2010; **9**: 23
11. Molles M C. Ecology: concepts and applications. McGraw-Hill companies, Inc., Berlin, 2000
12. Muyzer G, Teske A, Wirsen C O. *et al*. Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Arch Microbiol.* 1995;**164**: 165-172
13. Nakatsu C H. Soil microbial community analysis using Denaturing Gradient Gel Electrophoresis. *Soil Sci Soc Am J*. 2007; **71**(2):562-571
14. ND-1000 Spectrophotometer V3.1 User's Manual. NanoDrop Technologies, Inc.:2005, 5-2
15. Ovreas L, Forney L, Daae F, *et al*. Distribution of bacterioplankton in meromictic Lake Sælenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Appl Environ Microbiol.* 1997; **63**(9): 3367-3373
16. Von Wintzingerode F, Göbel U B, Stackebrandt, E. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol Rev.* 1997; **21**(3): 213-229
17. Yankson K K, Steck T R. Strategy for extracting DNA from clay soil and detecting a specific target sequence via selective enrichment and Real-Time (quantitative) PCR amplification. *Appl Environ Microbiol.* 2009; **75**: 6017-6021