

## Soil microbial diversity: an ISO standard for soil DNA extraction

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Soil carries out functions that are crucial for the environment and life on earth and is therefore an essential non-renewable resource for mankind. Recently, the European Soil Framework Directive proposal (COM(2006)232) indi-

cated that soil is under increasing environmental pressure mostly due to the intensification of human activities, which are damaging the capacity of soil to continue to perform in full its broad variety of crucial functions. Most of these soil

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functions are dependent on microorganisms inhabiting the soil. The diversity of soil microorganisms is the highest on earth with estimates of several thousand to several million different genomes per gram of soil (Whitman et al. 1998; Torsvik et al. 2002; Zhang and Xu 2008). However, fundamental understanding of the diversity and ecology of microbial communities carrying out soil functions has been hampered by our inability to grow most microorganisms under laboratory conditions.

Since the early eighties, direct DNA-based methods have been developed to circumvent the biases resulting from the low number of microorganisms that could be cultured, thus hampering our understanding of microbial diversity in soil (Torsvik 1980; Porteous et al. 1991; Tsai et al. 1991; Smalla et al. 1993; Zhou et al. 1996; He et al. 2005). These methods were based on direct extraction of the DNA from micro-organisms living in the soil, using various lysis treatments. Since then, numerous articles have been published describing either new or improved methods for soil DNA extraction and at least ten companies are commercializing “Soil DNA” extraction kits. The ongoing success of these direct molecular methods for studying soil micro-organisms is reflected by the fact that more than 1,000 articles are now published yearly using some type of soil DNA extraction method. Unfortunately, the wide use of these methods has resulted in a huge number of laboratory or even user-specific protocols, which contain minor to major modifications of the existing methods or kits.

However, the choice of DNA extraction method is far from being inconsequential as it affects the picture of the microbial diversity present in a soil sample (Frostegard et al. 1999; Krsek et al. 1999; Martin-Laurent et al. 2001; He et al. 2005). To tackle this crucial problem and ensure comparable data, a standard soil DNA extraction method should be used. This is becoming all the more important because studies of soil microbial diversity based on soil DNA extraction are generating an exponential amount of sequence data thanks to rapid advances in sequencing technologies.

For this purpose, we proposed in 2006 the standardization of soil DNA extraction to the International Organization for Standardization (ISO), with support from the French Standards Association and the French Environment and Energy Management Agency. The need for an international standard for soil DNA extraction has been rapidly recognized by ISO members as a priority and formally agreed upon. The ISO 11063 *Soil quality method to directly extract DNA from soil samples* is now being prepared by the Technical Committee ISO/TC 190, Subcommittee SC4, *Biological methods*. The standard was developed based on the publication made by Martin-Laurent et al. (2001). In the first phase, the technical scope of the future standard has been defined by a working group, consisting of expert researchers from countries

interested in the subject matter. It was decided that since DNA purification is dependent on soil type, only the actual DNA extraction step, which is likely to generate the main differences between methods, would be subject to standardization. After a successful French inter-laboratory assay, we were requested during the ISO/TC 190 meeting in Delft in November 2008 to set up an international ring test for a final validation of the method. This task involved ten different laboratories from seven countries. Six soils collected in different European Countries were chosen for this study. The final report of this ring test was included in the revised of the standard and sent to the ISO in January 2010.

After a 5 months ballot, more than two thirds of the votes cast by the national standards bodies members were positive and the ISO standard for Soil DNA extraction has been approved. The normative document will now be disseminated within the scientific community. However, information and international cooperation will be needed for the acceptance and success of ISO/CD 11063 standard. We hope that this standardization of a soil DNA extraction method will be an important first step towards the unification of soil microbiologists.

## References

- Frostegard A, Courtois S, Ramisse V, Clerc S, Bernillon D, Le Gall F et al (1999) Quantification of bias related to the extraction of DNA directly from soils. *Appl Environ Microbiol* 65:5409–5420
- He JZ, Xu ZH, Hughes J (2005) Pre-lysis washing improves DNA extraction from forest soils. *Soil Biol Biochem* 37:2337–2341
- Krsek M, Wellington EMH (1999) Comparison of different methods for the isolation and purification of total community DNA from soil. *J Microbiol Meth* 39:1–16
- Martin-Laurent F, Philippot L, Hallet S, Chaussod R, Germon JC, Soulas G et al (2001) DNA extraction from soils: old bias for new microbial diversity analysis methods. *Appl Environ Microbiol* 67:2354–2359
- Porteous LA, Armstrong JL (1991) Recovery of bulk DNA from soil by a rapid, small-scale extraction method. *Curr Microbiol* 22:345–348
- Smalla K, Cresswell N, Mendoncahagler LC, Wolters A, Vanelsas JD (1993) Rapid DNA extraction protocol from soil for polymerase chain reaction-mediated amplification. *J Appl Bacteriol* 74:78–85
- Torsvik VL (1980) Isolation of bacterial-DNA from soil. *Soil Biol Biochem* 12:15–21
- Torsvik VL, Ovreas L, Thingstad TF (2002) Prokaryotic diversity—magnitude, dynamics, and controlling factors. *Science* 296:1064–1066
- Tsai YL, Olson BH (1991) Rapid method for direct extraction of DNA from soil and sediments. *Appl Environ Microbiol* 57:1070–1074
- Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: the unseen majority. *Proc Natl Acad Sci USA* 95:6578–6583
- Zhang L, Xu ZH (2008) Assessing bacterial diversity in soil: a brief review. *J Soils Sediments* 8:379–388
- Zhou JZ, Bruns MA, Tiedje JM (1996) DNA recovery from soils of diverse composition. *Appl Environ Microbiol* 62:316–322