

# A practical introduction to microbial community sequencing

Mini-Review

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**Abstract:** The use of molecular methods is gaining popularity throughout the field of microbial community ecology studies thanks to their flexibility of application, which ranges from community structure to function and trait determination. Nonetheless, there are environmental microbiologists, who are new in the field and are just starting to get to grips with the genetic tool box. It is for them that this practitioner's mini-review was compiled. The methods available for microbial community structure analysis are discussed, after which, the reader is introduced to sequencing, as this tool is the most appropriate and has seen the greatest development in recent years. A focus on the practical aspects of the methodology is maintained throughout. The sample preparation procedure from extraction to sequencing is described. Different applications and considerations of sequencing are briefly explained, including clone library sequencing vs. amplicon library sequencing, shotgun-metagenomics vs. metatranscriptomics and the 'double RNA approach'.

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## 1. Introduction

The great popularity of methods based on the analysis of genetic material which has unquestionably been observed over the last decades has come about as a result of their quickness, their flexibility of application and trends in research. Many scientists in fields other than molecular biology who have not yet added these methods to their lab tools are now considering doing so. It has now become a standard practice in research to use molecular tools in studying microbial communities. This article is intended for scientists whose experience of the genetic tool box has so far been limited. The main topic is sequencing, one of the molecular methods which can be used in the study of microbial communities. It is particularly useful in describing their biodiversity and has gained a great deal of attention in recent years.

### 1.1 Studying the structure of microbial communities

What are the methods available for studying the structure of microbial communities? They are based on either the cultivation of microbes *per se* or on biochemical analyses, in other words, the isolation and/or detection

of their cell compounds, namely, lipids, proteins and nucleic acids.

#### 1.1.1 Cultivation

Culture selections are widely disapproved of because only a limited fraction of the total cell number grows in an artificial environment; in the case of soil, about 5% of the culturability is commonly the upper limit [1]. Some researchers have argued, though, that the conditions established in order to achieve culturing need to be optimised [1]. With the use of diffusion chamber-based methods, average culturability has been increased fivefold, reaching approximately 35% as an average and 50% as a maximum of total seawater or soil bacteria, including many new taxonomic units. However, several important groups, such as *Actinomycetes* and *Acidobacteria*, were missing [2]. Cultivation is still more a tool for those who are interested in describing new species and wish to devote their energies to isolating single cell lines or high cell biomass is needed, eg. for biotransformations. In Biolog plates, which were used to investigate functional diversity of communities [3], 31 to 95 carbon substrates are employed. Does this variety of substrates increase the actual culturability on Biolog

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