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► To cite this version:

J. Denonfoux, C. Wahl, Jean-Pierre Gauthier, Y. Laurent, Lionel Lebreton, et al.. Optimized and standardized 192-plex solution for 16S rDNA gene sequencing on Illumina Miseq platform to assess soil biodiversity. 1. Global Soil Biodiversity Initiative Conference (GSBI), Dec 2014, Dijon, France. 703 p., 2014. hal-01208772

HAL Id: hal-01208772

<https://hal.science/hal-01208772>

Submitted on 3 Jun 2020

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[P1.264]

Optimized and standardized 192-plex solution for 16S rDNA gene sequencing on Illumina MiSeq platform to assess soil biodiversity

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The growing need to survey the tremendous microbial diversity in a culture independent manner, has led to the development of molecular methods through sequence profiling of conserved genes such as 16S rDNA. Next-generation sequencing technologies are now used routinely to assess bacterial communities composition in complex environmental samples. Recently, the improvement of the Illumina MiSeq platform to a 2×300 bases paired-end version made it much more attractive for 16S rDNA amplicons sequencing enabling the analysis of a longer region thus facilitating taxonomic assignation. Additionally, the Illumina V3 chemistry enables high-throughput metagenomics analysis at the greatest coverage yet possible with a lower cost per sequence. Nevertheless, known limitations associated with the sequencing of low sequence diversity samples have hampered harnessing its true potential to sequence 16S rDNA gene amplicons. Moreover, validated Illumina 16S rDNA amplicon sequencing protocols do not support more than 96 samples per run, which underutilized the overall capacity of a sequencing run.

We therefore worked on the development of a standardized and optimized high-multiplexed metagenomic solution for the exploration of complex environments. We developed an integrated solution combining several technical and bioinformatical strategies that are (i) a custom and standardized amplification protocol for 16S rDNA library preparation to minimize as full as possible technical biases; (ii) a custom 192-index strategy within a single run; (iii) the inclusion of a nucleotide sequence variability at the first sequencing cycles; (iv) an accurate paired-end reads assembly process for full length amplicon analysis and (v) a dedicated pipeline for taxonomic affiliation of 16S rDNA sequences and microbial communities analysis.

Our solution validated within soil samples provides high sequencing accuracy and technical reproducibility with no index biases based on taxonomic distribution analysis. The association of technical and bioinformatical improvements yields substantial cost reductions and provides greater target flexibility to assess soil biodiversity as well as functions of microbial communities through carbon, phosphorus or nitrogen cycles in future developments.

Keywords: Metagenomic, next-generation sequencing, soil biodiversity