Archives of Microbiology

Online Resources 1

Archaea, the unseen kingdom in the gut microbiome of *Anastrepha obliqua*

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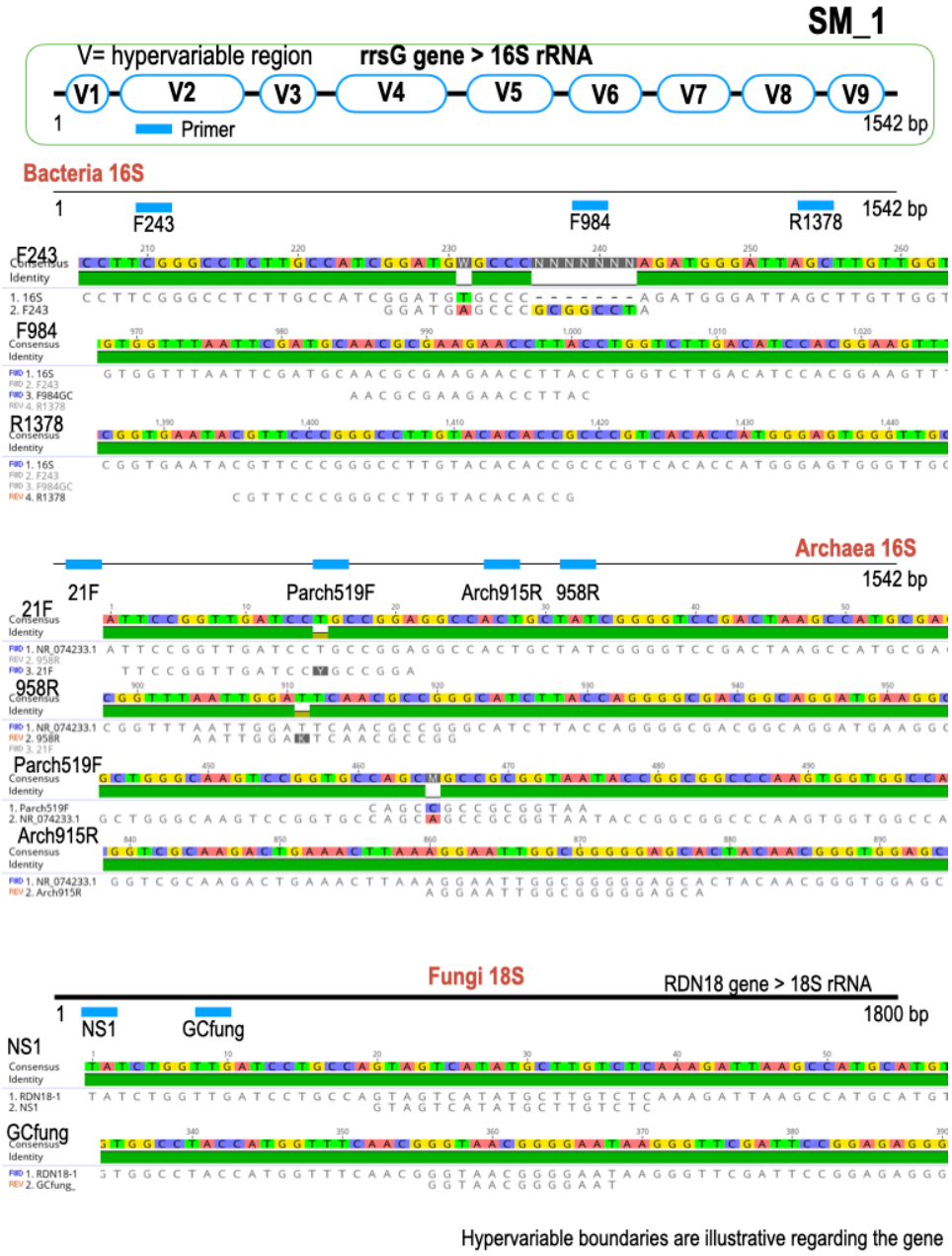

Online Resource 1. Primer annealing and PCR conditions.

The reaction mixture contained 0.20 ρmol of each primer, 1x buffer, 0.20 mM dNTPs (dinucleotide triphosphate), 1.5 mM $MgCl_2$ (2 mM Fungi), 1 U *Taq* DNA polymerase (Invitrogen, USA), and template DNA for a total volume of 20 µl. Amplification protocol in bacteria consisted in a denaturation at 94 °C for 5 min, followed by 30 cycles of 1 min at 95 °C, 1 min at 58 °C and 40 sec at 72 °C, with a final extension at 72 °C for 5 min. The Gram positive (actinomycetes) nested amplification used the same protocol for second amplification; the first annealing temperature was modified at 53 °C (Das, Royer, and Leff, 2007). Archaea nested PCR protocol for the first reaction was: denaturation at 94 °C for 5 min, followed by 30 cycles of 40 sec at 94 °C, 40 sec at 53 °C, 1 min at 72 °C, and a final extension at 72 °C for 5 min. For the second reaction, the alignment temperature was changed to 57 °C. Fungi PCR conditions consisted of denaturation at 95 °C for 2 min, followed by 35 cycles of 30 sec at 95 °C, 30 sec at 50 °C, and 1 min at 72 °C, with a final extension at 72 °C for 5 min (Nikolcheva, Cockshutt, and Bärlocher, 2003). Controls were designed as follow, to confirm the absence of Gram positive (actinomycetes) in PCR negative samples, we determined the minimum amount of *Streptomyces* sp DNA that renders an amplicon using the corresponding primers. This PCR was done using serial dilutions (14 ng to 0.0014 ng) of DNA. Negative control included ultrapure water, whereas *Escherichia coli* DH5-α, *Streptomyces* sp., and *Trichoderma* sp., DNA were used as positive controls for bacteria, actinomycete, and fungi, respectively. Total DNA from an anaerobic plant sludge wastewater treatment was used as PCR positive control. For alignments, *rrs*G sequence was downloaded from https://ecocyc.org/ for *E. coli* K12 MG1655; 16S rRNA sequence was downloaded at https://www.ncbi.nlm.nih.gov/ using the accession number NR_074233.1 *Methanocaldococcus jannaschii*; and RDN18 gene sequence of *Saccharomyces cerevisiae* was downloaded at https://www.yeastgenome.org/locus/S000006482. Images were generated using free license of Geneious (Kearse, et al., 2012). Nucleotide mismatches at F243 is due to the 16S target employed for the alignment (i.e., *E. coli*) showing the region to identify Gram-positive bacteria.