

T-RFLP FRAGSORT; a Computer Program to Correlate Multiple 16S rRNA Gene T-RFLP Profiles with Corresponding *in silico* Amplification and Digestions of Ribosomal Database Project II Alignments

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T-RFLP of 16S rRNA genes is an established method for profiling microbial communities. The technique involves nucleic acid extraction, PCR amplification with fluorescently labeled primers, restriction digestion, and automated sizing of labeled terminal restriction fragments (TRFs). T-RFLP has advantages over other community analysis methods in that phylogenetic information can be obtained without direct sequencing of individual 16S rRNA gene fragments. However several different organisms may have terminal fragments of a similar size. Few methods exist to rapidly compare TRFs from multiple digestions to the TRFs expected based on *in silico* amplification and digestions of ribosomal database (RDP II) sequences. Although the TAP-TRFLP tool of the RDP II permits *in silico* T-RFLP experiments, interpretation of experimentally generated profiles must be done manually. T-RFLP FRAGSORT (<http://www.oardc.ohio-state.edu/trflpfragsort/>) is a computer program that compares the TRFs obtained from samples processed with widely used primers (8F, 907R, 11F, 226F, 1111R or EF4) and restriction enzymes (*MspI*, *HhaI*, *RsaI*, *HaeIII*, and *BfaI*) to TRFs from corresponding *in silico* amplification and digestions of RDP II alignments (version 8.0). Fragment sizing error can be either a constant value or a variable value based on fragment size. Output is a list of microorganisms and TRF sizes that correlate with multiple experimentally generated TRFLP profiles in descending order from the greatest to the least normalized TRF peak areas. Validation experiments showed that at least three different digestions must be used to accurately identify pure cultures and members of defined bacterial communities. Analysis of samples from twelve agricultural soils showed that a majority of individual TRF sizes obtained from environmental samples correspond to sizes predicted by *in silico* digestion. However, the TRF areas corresponding to organisms consistent with three different digestions comprised less than 40% of the total PCR product. In conclusion, FRAGSORT is a useful tool for rapidly analyzing multiple digestion T-RFLP data.