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Terminal restriction fragment length polymorphism (T-RFLP)

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Eva Petrova[1], Roey Angel[1]

[1]Soil and Water Research Infrastructure

SoWa RI Anaerobic and Molecular Microbiology (public)
Tech. support email: **eva.petrova@bc.cas.cz**

Eva Petrova
Soil and Water Research Infrastructure

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Protocol status: Working
We use this protocol and it's working

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Abstract

Optimized protocol for T-RFLP analysis of complex bacteria and archea communities.

Attachments

TRFLP-Run conditions...

24KB

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24KB

1 Extract and purify DNA from the sample. DNA should be at about 10-20 ng/µl.

2 Amplify the sample using PCR (refer to PCR conditions and mixes.doc). Primers need to be fluorescently labeled at their 5'. Labeling should be of at least one of the primers though both can be labeled (in which case the two primers should have different label colors).
Depending on the available size standard the optimized primers for bacteria are 27F^M^I – 908R^I or 341F^I-908R^I and for archea are 109F^I-934R^I.
It is recommended to perform 3 different PCR reactions for each sample and combine them. PCR should be performed at minimum number of cycles and minimum annealing temp. We optimized the analysis for 3 replicates of 50 µl each at 45°C for 24 cycles (bac) and 25 cycles (arc).

3 Combine the samples and concentrate to about 50µl.

4 Perform Mung bean exonuclease digestion according to the manufacturer's instructions. Refer to Egert & Friedrich 2003, Simpson et al. 1999, or Jensen and Straus 2007 for more information.

5 Immediately clean the digested PCR reaction using PCR purification kit. Elute with 50 µl.

6 Run 3 µl of each samples on agarose gel to ensure successful cleanup and no overdigestion by the exonuclease.

7 Divide the remaining volume to 3 aliquots of 15 µl and digest overnight with 20U of restriction enzymes. For BAC we used the following: TaqI, HhaI, and RsaI. For ARC we use: TaqI, Tru1I, and MboI. For new primers refer to MICA's Enzyme resolving power analysis.

8 Desalt the samples with ethanol precipitation and resuspend in 17 µl of PCR grade DDW. Refer to Ethanol precipitation of small DNA fragments.doc

9 Run the samples on an automated microcapillary sequencer.