Optimisation of 16S rDNA amplicon sequencing protocols for microbial community profiling of anaerobic digesters

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Optimisation of 16S rDNA amplicon sequencing protocols for microbial community profiling of anaerobic digesters

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**Introduction**

To understand the ecology network in anaerobic digesters it is necessary to produce a representative overview of the microbial community. In this study we develop a method for reliable and reproducible identification and quantification of microorganisms involved in biogas production. We test the effect of changing the parameters in a DNA extraction dependent approach to community profiling.

**Methods**

- **Sampling**
  - 3 biological replicates
  - Min. 3 technical replicates

- **Extraction**
  - 3 sample prep replicates
  - 3 sequencing replicates

- **Sample prep**
  - Total RNA extraction
  - cDNA synthesis
  - TruSeq DNA sample prep

- **Sequencing**
  - 384 well PCR of 16S rDNA
  - TruSeq adapters

- **Bioinformatic**
  - illumina TruSeq shotgun sequencing

**Primers**

- V1-3: 27F + 534R (506 bp)
- V3-4: 341F + 806R (465 bp)
- V4-6: 519F + 786R (522 bp)

**Replication**

- Min. 3 technical replicates
- 16S rDNA gene

**Validation**

- PCR independent assessment using illumina TruSeq shotgun sequencing

**Results**

- **Effect of bead beating on DNA yield**
  - All DNA extractions were done with 50 µL of AD sludge as input using the FastDNA® SPIN kit for Soil. The standard bead beating is 40 s.

- **Effect of bead beating on DNA integrity**
  - At high bead beating durations the DNA is fragmented.

**Conclusions**

- PCR independent validation is needed when conducting amplicon based studies!
- Four times the standard bead beating is recommended (160 s) in order to capture the microorganisms with relatively tough cell walls.
- The Sundberg et al. (2013) primer set seems promising for capturing the overall community composition of both bacteria and archaea.
- Every step of the protocol introduces variance, particularly the DNA extraction. However, the workflow gives good reproducibility.

**Conclusion**

- FastDNA® SPIN kit for Soil is not recommended for the standard bead beating is 40 s.
- All extractions were done with 50 µL of AD sludge as input using the FastDNA® SPIN kit for Soil. The standard bead beating is 40 s.

**References**

- Sundberg et al. (2013)
- Klindworth et al. (2013)
- McIlRoy et al. (2013)
- Rosselló-Mora and Amann (1996)
- Arumugam et al. (2011)
- McMinn et al. (2010)

**Fluorescence in situ hybridisation with archaea specific probes**

- Methanocorpusculum
- Methanosarcina
- Methanolinea

**Class level overview of the bacterial population**

- Chlorobium
- Bacteroida
- Bacteroidetes
- Actinobacteria
- Alphaproteobacteria
- Betaproteobacteria
- Gammaproteobacteria
- Deltaproteobacteria
- Epsilonproteobacteria
- Aerococci

**Class level overview of the archaean population**

- Methanocorpusculum
- Methanosarcina
- Methanolinea

**Amplicon sequencing protocols for microbial community profiling of anaerobic digesters**

- **Capture**
  - TruSeq adapters
  - PCR independent validation

- **Characterisation**
  - Bioinformatic analysis
  - Illumina TruSeq shotgun sequencing

- **Reproducibility**
  - Four times the standard bead beating is recommended (160 s) in order to capture the microorganisms with relatively tough cell walls.

**Bioinformatic**

- illumina TruSeq shotgun sequencing

**PCR independent assessment using illumina TruSeq shotgun sequencing**