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

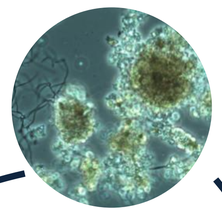
To understand the ecology network in anaerobic digesters it is necessary to produce a representative overview of the microbial community.
In this study we develop a method for reliable and reproducible identification and quantification of microorganisms involved in biogas production.
We test the effect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

Conclusions

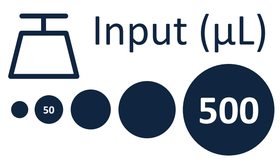
- PCR independent validation is needed when conducting amplicon based studies!
- Four times the standard bead beating is recommended (160 s) in order to capture the microorganisms with relatively tough cell walls.
- The Sundberg *et al* (2013) primer set seems promising for capturing the overall community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

Methods

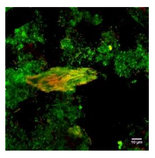
extraction



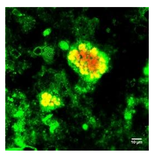
Input (µL)



Reactor temperature

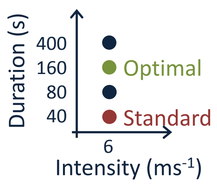


Mesophilic
Viborg



Thermophilic
Aalborg West

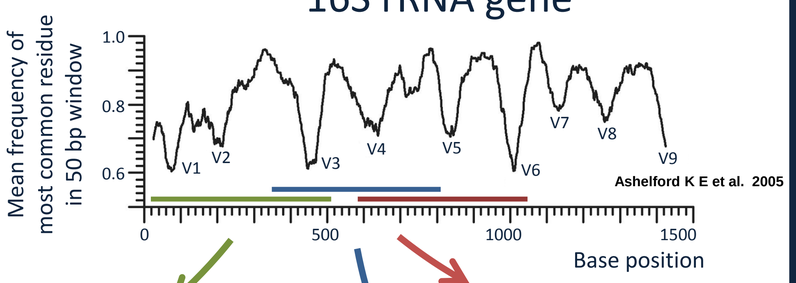
Bead beating



FastDNA® SPIN kit for Soil

primers

16S rRNA gene

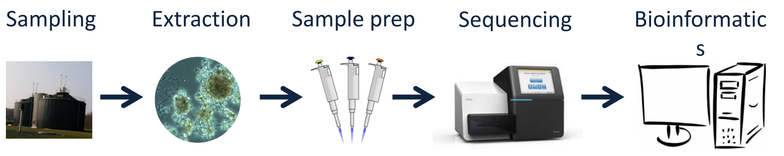


V1-3: 27F + 534R (≈506 bp)
Bacteria only
(Lane 1991, Muyzer et al 1993)

V3-4: 341F + 806R (≈465 bp)
Archaea and Bacteria
(Sundberg et al 2013)

V4-6: 519F + 1049R (≈522 bp)
Archaea only
(Klindworth et al 2013)

replication



5 biological replicates

Min. 3 extraction replicates

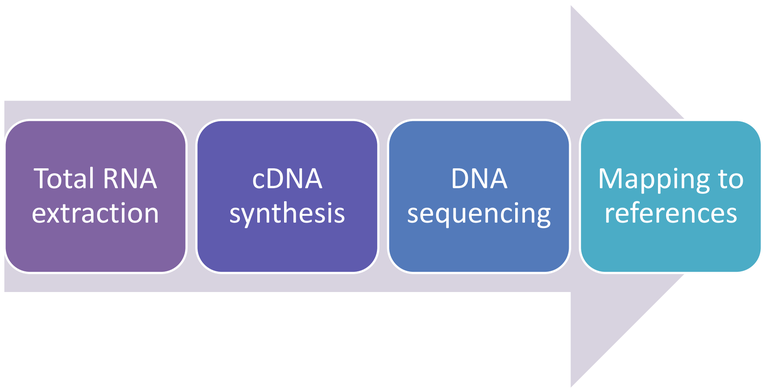
3 sample prep replicates

3 sequencing replicates

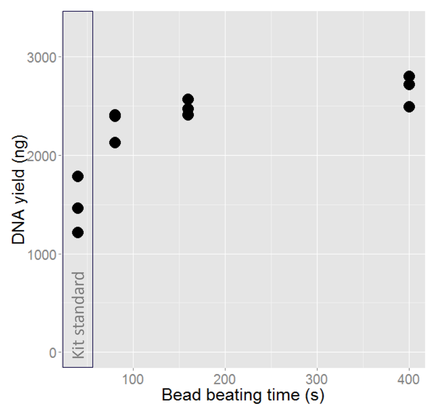
>50,000 overlapping 300 bp paired end reads pr. sample

validation

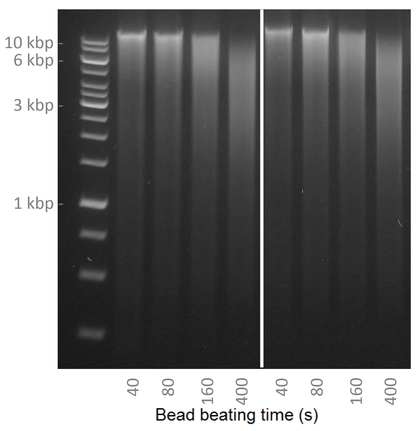
PCR independent assessment using Illumina TruSEQ shotgun sequencing



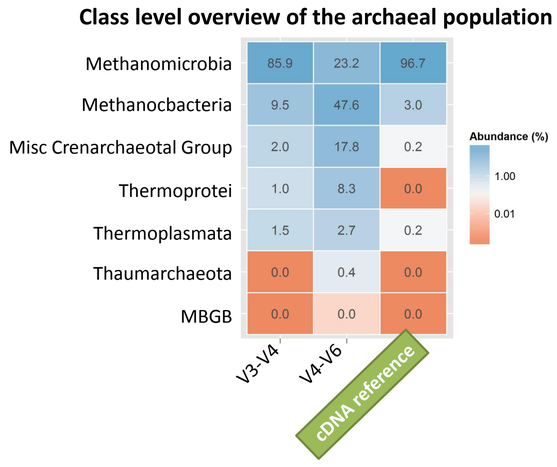
Results



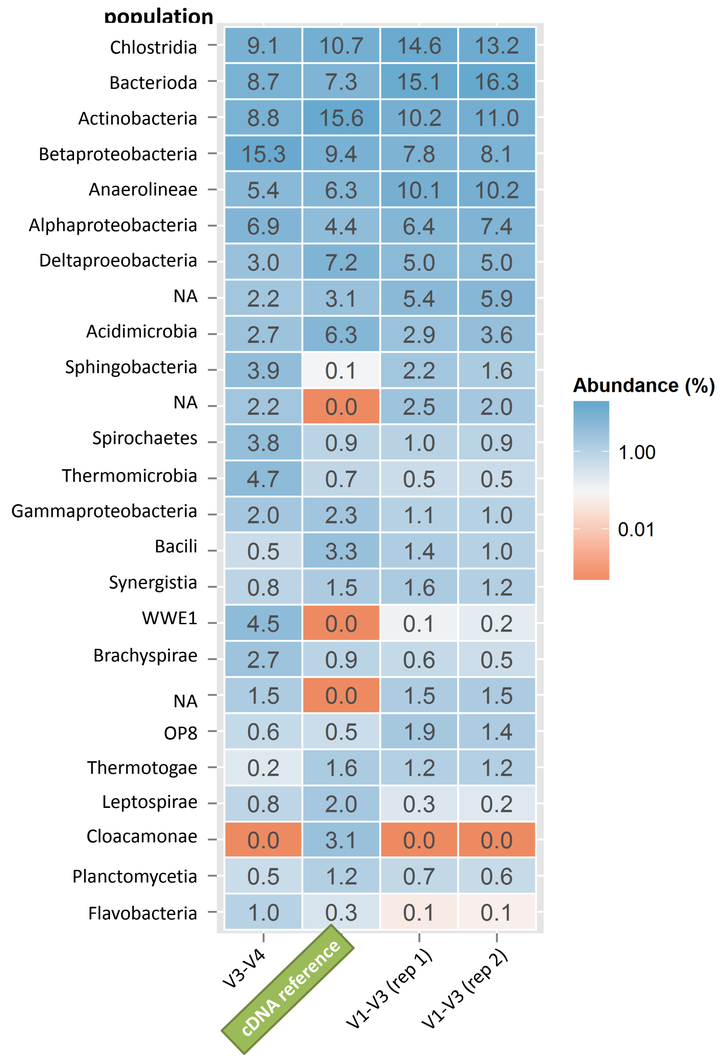
Effect of bead beating on DNA yield. All DNA extractions were done with 50 µL of AD sludge as input using the FastDNA® SPIN kit for Soil. The standard bead beating is 40 s.



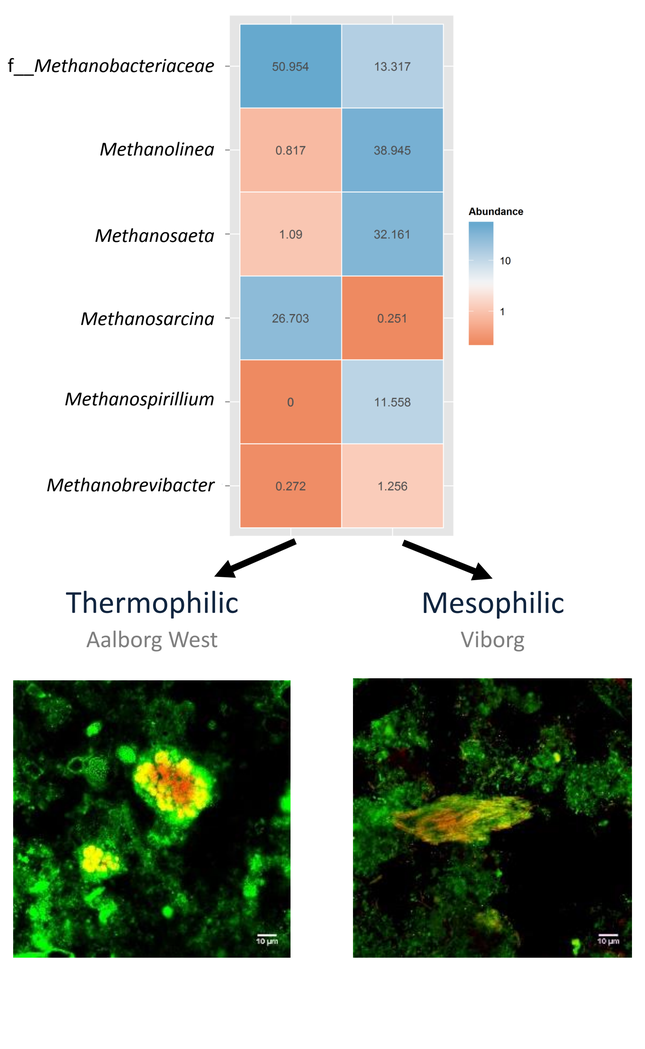
Effect of bead beating on DNA integrity. At high bead beating durations the DNA is fragmented.



Effect of primer set on the observed community structure. DNA extractions were done with 160 s. All results are relative abundance (%). The V3-V4 (Sundberg et al (2013)) primer set seems to cover the archaeal diversity seen with cDNA sequencing while the archaea specific V4-V6 (Klindworth et al (2013)) primers changes the composition dramatically. Note that the cDNA sequencing and 16S amplicon results are not directly comparable, but can be used as a rough guideline.



Effect of primer set on the observed community structure. DNA extractions were done with 160 s. All results are relative abundance (%). The V3-V4 (Sundberg et al (2013)) primer set seems to cover the diversity of the bacterial domain seen with cDNA sequencing. The V1-V3 amplicon illustrates that the entire amplicon workflow has a great level of reproducibility.



Fluorescence *in situ* hybridisation with archaea specific probes supports the amplcons. *Methanosarcina* is commonly observed in high abundance in thermophilic anaerobic digesters, typically forming microcolonies, while *Methanosaeta* are often present in abundance in mesophilic reactors as short filaments. Amplicon and FISH analyses of the thermophilic and mesophilic digester samples is consistent with these observations.

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