

DATA NOTE

The old and grumpy biogas metagenome

Andreas Bremges^{1,2*}, Irena Maus¹, Alfred Pühler¹, Andreas Schlüter^{1†} and Alexander Sczyrba^{1,2†}

*Correspondence:

abremges@cebitec.uni-bielefeld.de

¹Center for Biotechnology,
Bielefeld University, Germany

Full list of author information is
available at the end of the article

[†]Equal contributor

Abstract

Background: a presentation of the interest or relevance of these data for the broader community

Findings: a very brief preview of the data type(s) produced, the methods used, and information relevant to data validation

Conclusions: a short summary of the potential uses of these data and implications for the field

Keywords: Biogas; Metagenome; Sequencing; Assembly; Annotation

Data description

Background

Biogas important energy source. Clean and awesome. Number of biogas plants in Germany/Europe/worldwide. Key is process optimization, not building more and more plants. Little is known about the microbial community responsible for everything.

Here, we report the first deeply sequenced metagenome (and metatranscriptome?) of an agricultural production-scale biogas plant on the Illumina platform [1]. We sequenced 27.3× and 19.3× deeper than previous studies relying on 454 [2] or SOLiD [3] sequencing. These data will enable a deeper exploration of the biogas-producing microbial community, a key step towards process optimization.

section not
final!

phrasing

Digester management

The biogas plant, located in North Rhine Westphalia, Germany, features a mesophilic continuous wet fermentation technology and was designed for a capacity of 537 *kW_{el}* combined heat and power (CHP) generation. The process comprises two digesters: a primary digester, where biogas and methane is produced, and a secondary digester, insignificant for production. The primary digester is fed hourly with a mixture of maize silage and liquid pig manure. The biogas and methane yields at the time of sampling were at 810.5 and 417.8 liters per kg organic dry matter (*l/kg oDM*), respectively. After a theoretical retention time of 55 days, the digestate is stored in the closed, non-heated final storage tank. Further metadata are summarized in Table 1.

Sampling and DNA isolation

Samples from the primary digester of the aforementioned biogas plant were taken in November 2010. Prior to sampling process, approximately 15 *L* of fermenter substrate were discarded before aliquots of 1 *L* were transferred into clean gastight sampling vessels and transported directly to the laboratory.

A 20 g aliquot of the fermentation sample was used for total community DNA preparation as described previously [4].

Metagenomic sequencing

we need
actual
numbers
somewhere

In total, we sequenced four different metagenome shotgun libraries with different insert sizes, resulting in over 23 gigabases scattered across 144 million reads. On Illumina's Genome Analyzer IIx system, we sequenced two libraries with an average insert size of 250 nt and 450 nt, respectively, applying the Paired-End DNA Sample Preparation Kit (Illumina Inc.) as described by the manufacturer. On Illumina's MiSeq system, we sequenced two further libraries with an average insert size of 190 nt and 690 nt, respectively, applying the Nextera DNA Sample Preparation Kit (Illumina Inc.) as described by the manufacturer. In all cases, we sequenced for approximately 300 cycles, applying the 2×150 nt paired-end protocol.

Eher kein 2x250: MiSeq v2 öffentlich 10/2012, Alex und ich hatten die MiSeq Daten in 07/2012!

Sequence quality control

cite if
needed

Quality control with FastQC, version 0.11.2. Adapter contamination in Lane 7.

mention at
all?

We used Trimmomatic [5], version 0.32, for adapter removal and moderate quality trimming. After adapter clipping, using Trimmomatic's *Truseq2-PE* and *Nextera-PE* templates, we removed leading and trailing low quality bases (below quality 3). Then, we performed an adaptive quality trimming, balancing read length against error rate (target read length of 100 bases, strictness of 0.2, thus favouring read length over error sensitivity). Finally, reads shorter than 36 bases were discarded.

section not
final!

Either another table or brief summary post-QC.

Metagenome assembly and quality assessment

2.3.1

We assembled the metagenome with Ray Meta [6], version 2.3.0, using a *k*-mer size of 31 and a minimum contig length of 1,000 bp. This resulted in a total assembly size of approximately 217 megabases in 55,563 contigs, with an N50 value of 8,137 bp. Table 2 summarizes our results.

2.2.4

We aligned the post-QC sequencing reads to the assembled contigs with bowtie2 [7], version 2.2.1, and used samtools [8], version 1.1, to convert SAM to BAM and thereafter sort the alignment file.

Run LAP
or ALE

Gene prediction and annotation

We then used MetaProdigal [9], version 2.6.1, to predict 239,412 protein-coding genes on the assembled contigs. Table 3 also includes these results.

We blasted all predicted genes against the KEGG database [10], release 72.0, using Protein-Protein BLAST [11], version 2.2.29+. Of the 239,412 predicted genes, 230,354 were found in the KEGG database. We determined the KEGG Orthology (KO) for each gene by mapping the top-scoring BLAST hit to its KO.

Coverage analysis

0.6.1p2

We counted aligned reads in predicted genes with HTSeq [12], version 0.6.1p1. Figure 1 relates the metagenome and the metatranscriptome.

work in
progress
and up for
discussion

Availability

Data accession

The datasets supporting the results of this article are available in the [repository name] repository, [unique persistent identifier and hyperlink to datasets in http://format].

Reproducibility

The complete workflow is organized in a single GNU Makefile and available on GitHub [13]. Starting from the raw read files, available from SRA and/or GigaDB, all data and results can be reproduced by a simple invocation of *make*. Excluding KEGG analysis, which relies on a commercial license of the KEGG database, all steps are performed using free and open-source software.

phrasing

Requirements

The assembly of the metagenome data sets with Ray Meta requires 70 GB of RAM. Using only 40 cores, it completed within 12 hours wall-clock time. Annotation with blast took, of course, forever.

log run-time and memory in final run!

Discussion

Potential use cases. Metagenomic (and metatranscriptomic?) profiling of the biogas-producing microbial community. Identification of metaproteomic data out there (cite Vera, in preparation).

Ultimate goal: process optimization by biological insights.

section not final!

Competing interests

The authors declare that they have no competing interests.

Author's contributions

AB conceived and performed all bioinformatic analyses and wrote the paper. IM investigated all metadata and drafted part of the data description. AP, ASch and will do something at some point. ASch conceived of many of the analyses and revised the paper. ASch and ASch jointly directed the project. All authors read and approved the final manuscript.

Acknowledgements

AB and IM are supported by a fellowship from the CLIB Graduate Cluster Industrial Biotechnology.

Author details

¹Center for Biotechnology, Bielefeld University, Germany. ²Faculty of Technology, Bielefeld University, Germany.

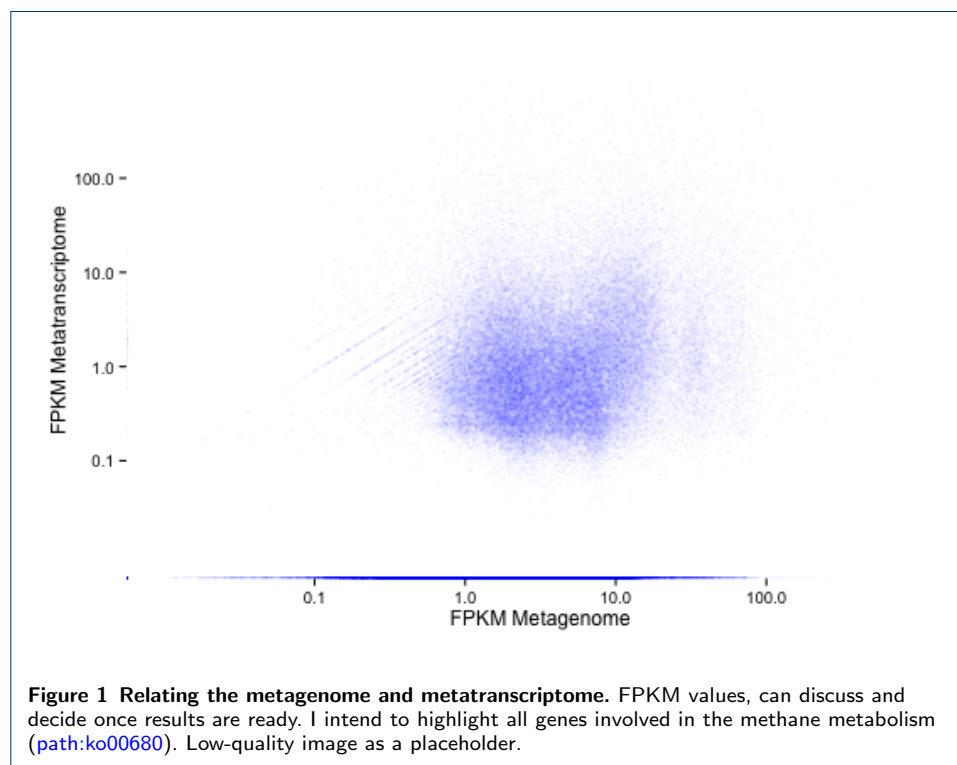
References

- Bremges, A., Maus, I., Pühler, A., Schlüter, A., Sczyrba, A.: Name of the GigaScience repository (2014). [DOI:foo/bar]
- Jaenicke, S., Ander, C., Bekel, T., Bisdorf, R., Dröge, M., Gartemann, K.H., Jünemann, S., Kaiser, O., Krause, L., Tille, F., Zakrzewski, M., Pühler, A., Schlüter, A., Goesmann, A.: Comparative and joint analysis of two metagenomic datasets from a biogas fermenter obtained by 454-pyrosequencing. *PLoS ONE* **6**(1), 14519 (2011). [PubMed Central:PMC3027613] [DOI:10.1371/journal.pone.0014519] [PubMed:21297863]
- Wirth, R., Kovács, E., Maróti, G., Bagí, Z., Rákhely, G., Kovács, K.L.: Characterization of a biogas-producing microbial community by short-read next generation DNA sequencing. *Biotechnol Biofuels* **5**, 41 (2012). [PubMed Central:PMC3395570] [DOI:10.1186/1754-6834-5-41] [PubMed:22673110]
- Schlüter, A., Bekel, T., Díaz, N.N., Dondrup, M., Eichenlaub, R., Gartemann, K.H., Krahn, I., Krause, L., Krömeke, H., Kruse, O., Mussgnug, J.H., Neuweiger, H., Niehaus, K., Pühler, A., Runte, K.J., Szczepanowski, R., Tauch, A., Tilker, A., Viehöver, P., Goesmann, A.: The metagenome of a biogas-producing microbial community of a production-scale biogas plant fermenter analysed by the 454-pyrosequencing technology. *J. Biotechnol.* **136**(1-2), 77–90 (2008). [DOI:10.1016/j.jbiotec.2008.05.008] [PubMed:18597880]
- Bolger, A.M., Lohse, M., Usadel, B.: Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**(15), 2114–2120 (2014). [PubMed Central:PMC4103590] [DOI:10.1093/bioinformatics/btu170] [PubMed:24695404]
- Boisvert, S., Raymond, F., Godzaridis, E., Laviolette, F., Corbeil, J.: Ray Meta: scalable de novo metagenome assembly and profiling. *Genome Biol.* **13**(12), 122 (2012). [PubMed Central:PMC4056372] [DOI:10.1186/gb-2012-13-12-r122] [PubMed:23259615]
- Langmead, B., Salzberg, S.L.: Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **9**(4), 357–359 (2012). [PubMed Central:PMC3322381] [DOI:10.1038/nmeth.1923] [PubMed:22388286]
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R.: The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**(16), 2078–2079 (2009). [PubMed Central:PMC2723002] [DOI:10.1093/bioinformatics/btp352] [PubMed:19505943]
- Hyatt, D., LoCascio, P.F., Hauser, L.J., Uberbacher, E.C.: Gene and translation initiation site prediction in metagenomic sequences. *Bioinformatics* **28**(17), 2223–2230 (2012). [DOI:10.1093/bioinformatics/bts429] [PubMed:22796954]
- Kanehisa, M., Goto, S., Sato, Y., Kawashima, M., Furumichi, M., Tanabe, M.: Data, information, knowledge and principle: back to metabolism in KEGG. *Nucleic Acids Res.* **42**(Database issue), 199–205 (2014). [PubMed Central:PMC3965122] [DOI:10.1093/nar/gkt1076] [PubMed:24214961]
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L.: BLAST+: architecture and applications. *BMC Bioinformatics* **10**, 421 (2009). [PubMed Central:PMC2803857] [DOI:10.1186/1471-2105-10-421] [PubMed:20003500]
- Anders, S., Pyl, P.T., Huber, W.: HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics* (2014). [DOI:10.1093/bioinformatics/btu638] [PubMed:25260700]
- Bremges, A.: GitHub (2014). [<https://github.com/abremges/2014-biogas>]

Funding
Andreas S.
Stadtwerke?

Less au-
thor
names,
remove
PMC?

Figures



Tables

Table 1 Characteristics of the studied biogas plant. Primary digester, sampling date: Nov 15, 2010.

Process parameter	Sample
Net volume	2041 m^3
Dimensions	6.4 m high, diameter of 21 m
Electrical capacity	537 kW_{el}
pH	7.83
Temperature	40 °C
Conductivity	22.10 mS/cm
Volatile organic acids (VOA)	5327 mg/l
Total inorganic carbon (TIC)	14397 mg/l
VOA/TIC	0.37
Ammoniacal nitrogen	2.93 g/l
Acetic acid	863 mg/l
Propionic acid	76 mg/l
Fed substrates	72 % maize silage, 28 % pig manure
Organic load	4.0 $kg\ oDM\ m^{-3}\ d^{-1}$
Biogas yield	810.5 $l/kg\ oDM$
Methane yield	417.8 $l/kg\ oDM$

TIC only
calculated,
might be a
few digits
off

Table 2 Metagenome assembly. Some assembly statistics, minimum contig size of 1,000 bp.

Assembly metric	Our assembly
Total size	216,554,757 bp
Number of contigs	55,563
N50 value	8,137 bp
Largest contig	319,083 bp

Table 3 Gene prediction and annotation. Whatever.

Annotation metric	Our annotaion
Predicted genes	239,412
of these, full-length	160,124 (66.9 %)
KEGG stuff	?

Todo list

section not final!	1
phrasing	1
we need actual numbers somewhere	2
Eher kein 2x250: MiSeq v2 öffentlich 10/2012, Alex und ich hatten die MiSeq Daten in 07/2012!	2
cite if needed	2
mention at all?	2
section not final!	2
2.3.1	2
2.2.4	2
Run LAP or ALE	2
0.6.1p2	2
work in progress and up for discussion	2
phrasing	3
log runtime and memory in final run!	3
section not final!	3
Funding Andreas S, Stadtwerke?	4
Less author names, remove PMC?	4
TIC only calculated, might be a few digits off	6