DATA NOTE

The old and grumpy biogas metagenome

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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.

section not final!

Here, we report the first deeply sequenced metagenome (and metatranscriptome?) of an agricultural production-scale biogas plant on the Illumina platform [1]. We sequenced $27.3\times$ and $19.3\times$ 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.

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

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 $2 \times 150\,nt$ paired-end protocol.

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

Sequence quality control

Quality control with FastQC, version 0.11.2. Adapter contamination in Lane 7 (?). Adapter removal and quality trimming with Trimmomatic [5], version 0.32, using some paramters.

1. Remove adapters, using the included Truseq2-PE and Nextera-PE adapter files.
2. Remove leading and trailing low quality or N bases (below quality 3).
3. 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).
4. Drop reads below 36 bases long.

> **Needed? k=31**

Either another table or brief summary post-QC.

> **section not final!**

> **2.3.1**

Metagenome assembly and quality assessment

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.

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.

> **2.2.4**

> **LAP/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 runtime 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.

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Less author names, remove PMC?

Figures

Figure 1 Relating the metagenome and metatranscriptome. Coverage or read counts, can discuss and decide once preliminary results are ready.

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	$22.10\,mS/cm$
Volative 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