Improving metagenome sequence data with sequence capture

Metagenome walking - Sequence Capture - Results
Reassembly of the original metagenome shotgun sequence reads from one PT plate with sequence capture reads from 1/8 of PT plate - for comparison assembly with the same number of new additional shot-gun sequence reads (control)

Conclusion:
- Sequence Capture improved Amylomics metagenome sequence databases by extending contigs and reducing gaps
- The subsequent data analysis and annotation became more streamlined and retrieval of genes encoding thermostable enzymes was facilitated

Metagenome MET567 – 1st SeqCap

Microbial diversity in selected anaerobic and microaerophilic enrichments of environmental samples (Vonarskarð, Hveragerði)

Improve metagenome sequence data with sequence capture

1. Contigs following assembly of sequence reads
2. Construction of secap probes targeting end sequences
3. Capturing reads flanking contigs
4. Sequencing and reassembly
5. Extended contigs

The density of microbial cells/particles in hot springs estimated applying flow cytometry

Diluted sample following filtration with bacterial filter
Sample following filtration with phage filter
Sample following filtration with phage filter, diluted

Metagenomes of a moderate complexity and enhanced evenness were generated by enriching starch or other carbohydrate utilizing organisms. Each pool (MET1,4 and MET5,6,7) was sequenced on one FLX+ picotitter plate (PT plate)

Introduction
A strategy for improving metagenomes libraries, based on sequence capture, was developed in the EU-project “Amylomics”. Thermophilic anaerobic and microaerophilic enrichments of environmental samples from geothermal habitats in Iceland were prepared, using starch derivatives and other carbohydrate substrates. The resulting metagenomes along with unenriched metagenome (YLI) were shotgun sequenced using the FLX+ sequencing platform (Roche). Subsequently, sequence capture was used to enrich DNA sequences absent in the original metagenome database by targeting and capturing fragments in existing metagenome DNA fragment libraries, flanking the contigs and singletons. Reassembly of the metagenome shotgun reads with additional sequence capture reads resulted in extended and merged contigs as well as new contigs. The sequence capture was repeated for a selected metagenomes. Thus, the overall procedure resembled a metagenome walking.

Metagenome MET567 – 1st SeqCap

Metagenome MET567 – 2nd SeqCap

Microbial diversity in selected anaerobic and microaerophilic enrichments of environmental samples (Vonarskarð, Hveragerði)

Cellulose derived enrichments
Thermoanaerobacterium
Clostridium sulfuranorum
Thermotoga
Firmicutes
Thermoanaerobacter sp
Thermoselenomonas sp
Fervidobacterium sp
Caldivirga maris

Starch derived enrichments

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Clostridium sulfuranorum
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改善メタゲノムのシーケンスデータの応用

メタゲノム走査 - シーケンスカッピング - 結果

改善のメタゲノムシーケンスデータの比較

3. セクショープの構築
4. シーケンシングと再集積
5. 伸長したコンチグ

改善したメタゲノムシーケンスデータのシーケンスカッピング

1. コンチグのシーケンスデータの組み立て
2. シーケンスデータの組み立て
3. シーケンスデータの組み立て
4. 伸長したコンチグ

改善したメタゲノムシーケンスデータの応用