Metagenome walking

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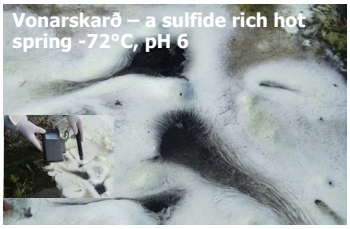
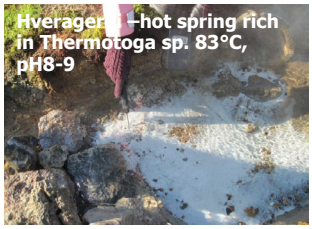
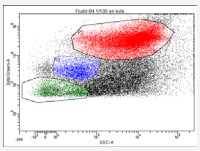


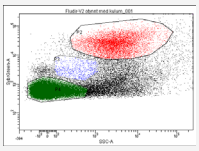


Introduction A strategy for improving metagenomes libraries, based on sequence capture, was developed in the EU-project "Amylomics". Thermophilic anaerobic and microaerophilic enrichments of environmental samples from geothermal habitats in Iceland were prepared, using starch derivatives and other carbohydrate substrates. The resulting metagenomes along with unenriched metagenome (YL1) 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.

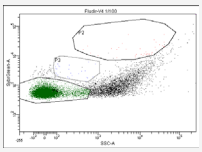
The density of microbial cells/particles in hot springs estimated applying flow cytometry



Diluted sample following filtration with bacterial filter

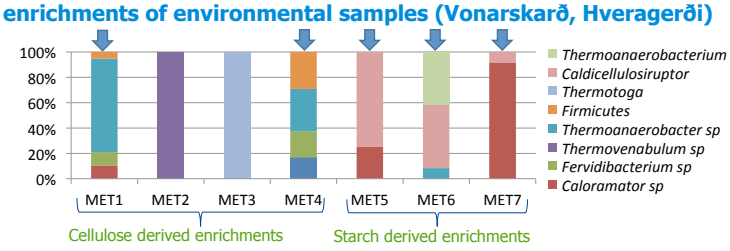


Sample following filtration with phage filter



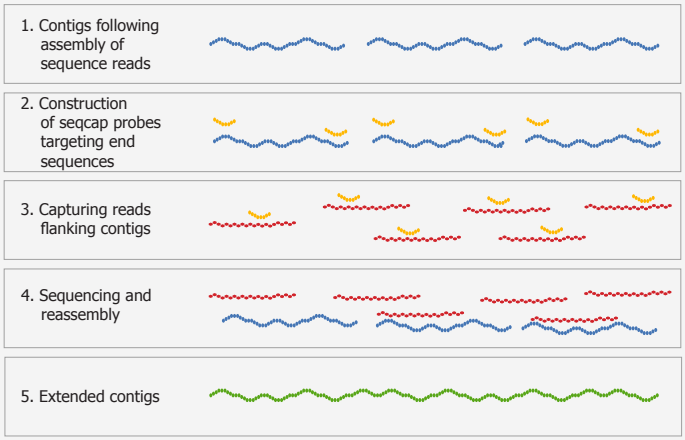
Sample following filtration with phage filter, diluted

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



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+ picotiter plate (PT plate)

Improving metagenome sequence data with sequence capture

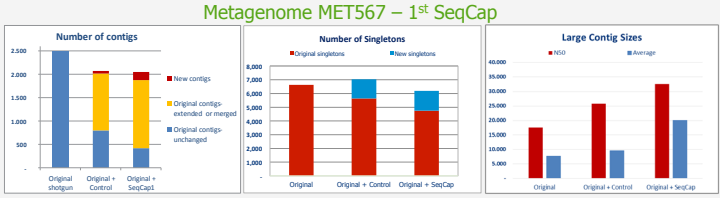


Improving metagenome sequence data applying sequence capture

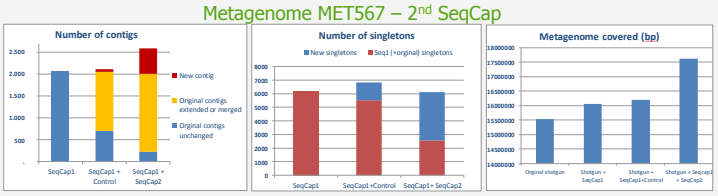
Metagenome walking - Sequence Capture - Results

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)

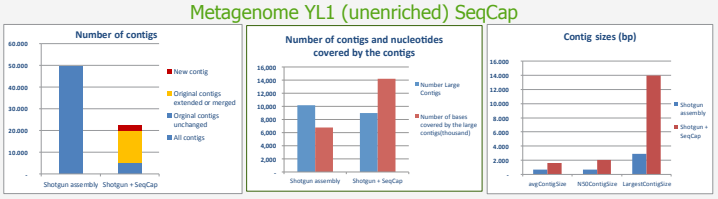
Metagenome MET567 – 1st SeqCap



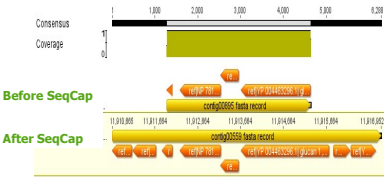
Metagenome MET567 – 2nd SeqCap

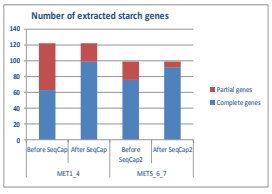


Metagenome YL1 (unenriched) SeqCap



Extended contigs /orfs – Retrieval of complete genes





Extension of contigs and reduction of gaps resulted in increased numbers of complete genes for retrieval and expression cloning

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

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