Title
Finishing of New Technology Only Microbes and Fungi

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Publication Date
2009-05-27

Finishing of New Technology Only Microbes and Fungi

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Current Workflow

Receive draft genome containing 454 shotgun titanium and 454 large insert paired end data

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Separate gaps into subprojects and utilize unassembled 454 data to close gaps

Assemble Illumina data using Velvet

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Add fakes from resolved subprojects and velvet assembly to main project

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Perform reactions for remaining subprojects

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Repeat performing reactions and data addition until all gaps are closed and all repeats resolved (if possible)

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Quality improvement using Illumina and Sanger where necessary

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Completed genome ready for annotation

Current Approach:

With the onset of new technology JGI has shifted its scope of work for microbes to 454 standard titanium, 454 paired end titanium, and Illumina data for gap closure and quality improvement (polishing). Raw reads are assembled by Newbler to create a draft assembly that will be passed to finishers. An in-house developed software tool creates subprojects for each gap. In-silica attempts are made to close gaps using existing unassembled pyrosequence and Illumina data. Any remaining gaps are tackled by PCR based methods. These include standard PCR, bubble PCR, multiplex PCR, combinatorial PCR, and long range PCR. Once products are generated they can be sequenced, cloned or shattered as needed. Currently gap closing data is still generated using Sanger. Once a genome is closed, Illumina data is used to polish the genome. Any areas that are still substandard are subjects for resequencing.

Future developments:

Eventually gaps may be pooled and sequenced using Illumina or 454. Other future developments include improvements in repeat resolution software and working with 454 to provide feedback and improvements for Newbler. Future fungal genome improvements may include targeted finishing, a limited number of rounds of reactions, in-silica only improvements, or complete finishing. Improvements to draft genomes will be discussed by Alla Lapidus.

Illumina Data Use:

1. Genome quality improvement using in-house tools (polisher.pl and acePolisher)- These scripts create files containing corrections and unsupported areas, which are used to make corrections, tag an acefile with various flags (polishTarget, unsupported, solexaCorrected), and design primers for areas with targeted areas. See image A below for a sample tagged acefile.

2. Alignment to consensus sequence- This can be done to see the Illumina data for areas which need manual inspection. See image B for a Illumina data aligned to a reference using mosaik.

3. De-novo assembly of data- We standardly use Velvet for de-novo assembly of Illumina data. If Velvet contigs hit the main assembly, fakes are created and added to projects to close gaps See image C for a fake generated from a Velvet contig.

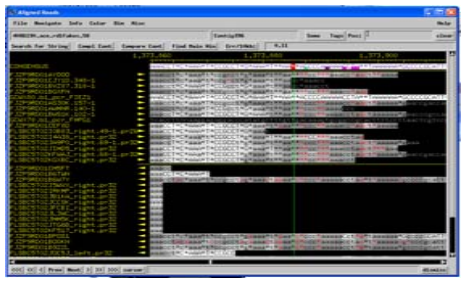


Image A: Tags generated from acePolisher. The green tag represents an insertion or deletion. The purple tag is a polishTarget whose area was addressed with a Sanger polishing read. The magenta tags represent "unsupported" bases where there isn't enough coverage to clearly make a base change.

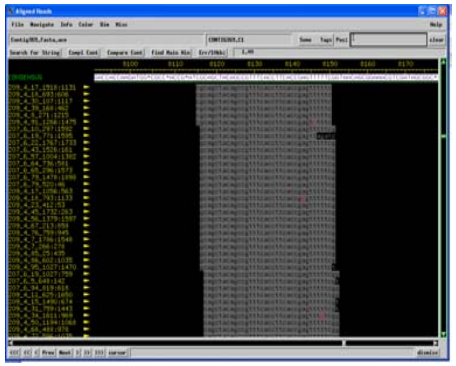


Image B: Illumina data aligned to a reference sequence using mosaik. These alignments can be used to manually inspect areas where depth of coverage wasn't enough for automatic correction.

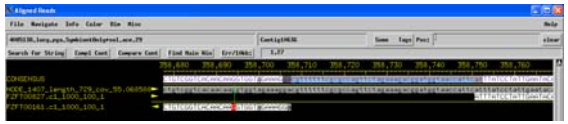


Image C: A fake created from a Velvet assembled Illumina contig is used to close a gap.

454 Data Use:

1. De-novo assembly and draft creation- 454 standard titanium and a large insert 454 paired end library are assembled by Newbler to create a draft genome.

2. Subproject creation-For each gap a separate project is created (using gapResolution.pl). Unique reads and pairs, as well as unassembled data, are assembled to try and resolve the gap in-silica.

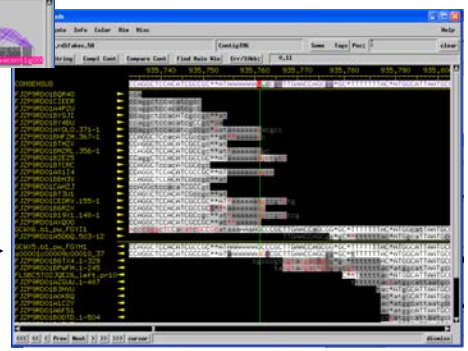
3. Genome quality improvement using in-house tools (polisher.pl)- 454 reads can be aligned and suggest corrections and improvements.

Sanger Data Use:

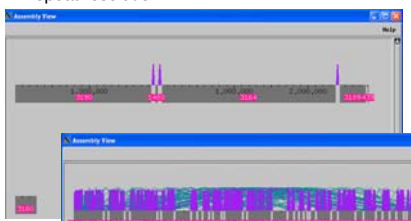
1. Gap closing reactions-PCR based methods are used to create templates and sequenced with Sanger to close gaps See images below for genome improvements using Sanger.

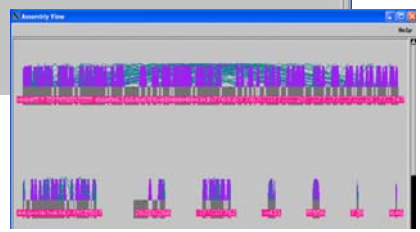
2. Assembly verification-PCR based methods are used to verify/resolve repeats as necessary.

3. Larger and more complex projects may require fosmid libraries for clone walking and repeat resolution.

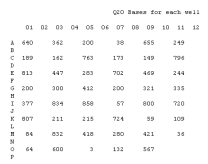


Above is an assembly view image of a subproject created by our gapResolution software. It was created by pooling unique areas and mate pairs surrounding a gap. This gap was closed using bubble PCR. The image to the right shows the fake from the subassembly incorporated back into the main assembly. For more information on gapResolution software please see poster by Kurt LaButti and Stephan Trong.





The upper image to the current state Acidovorax delafieldii 2AN and the lower is the draft version. Most gaps have been closed with bubble PCR sequenced by Sanger. Where bubble PCR sequencing failed, attempts were made to close gaps with pairwise primers using the bubble PCR product as a template. To the right is sequencing data for this approach. For more information on bubble PCR see poster by Hope Tice.



PCR Based Methods

New technology only projects have no templates, so we employ different PCR kits depending on the need.

PCR methods	kit	company
bubble PCR	Failsafe PCR System	Epicentre
long range PCR	LongRange PCR Kit	Qiagen
multiplex	iProof High Fidelity Kit	Bio-Rad Laboratories
standard pairwise or combinatorial	Failsafe or iProof	Epicentre/Bio-Rad

LLNL-POST-413452

This work was performed under the auspices of the US Department of Energy's Office of Science, Biological and Environmental Research Program, and by the University of California, Lawrence Berkeley National Laboratory under contract No. DE-AC02-05CH11231, Lawrence Livermore National Laboratory under Contract No. DE-AC52-07NA27344, and Los Alamos National Laboratory under contract No. DE-AC02-06NA25396.