

Next Generation Sequencing, Pipeline Development, and Data Analysis

Authors:

Jordan Taylor, Julian Pierre,
Amit Upadhyay, Bhanu Rekepalli

Overview

- Overview of Bioinformatics
- Next Generation Sequencing
- Data Analysis
- Genome Assembly
- Downstream Analysis
- Results/Comparisons
- Conclusion
- References

Overview of Bioinformatics



- Encompasses Multiple Scientific Disciplines
- Software Development
- Data Management
- Knowledge Discovery



Next-Generation Sequencing
See the difference

Image taken from Applied Biosystems



Image taken from 10th Cyanobacterial Molecular Biology Workshop



Image taken from Mobile Health 360

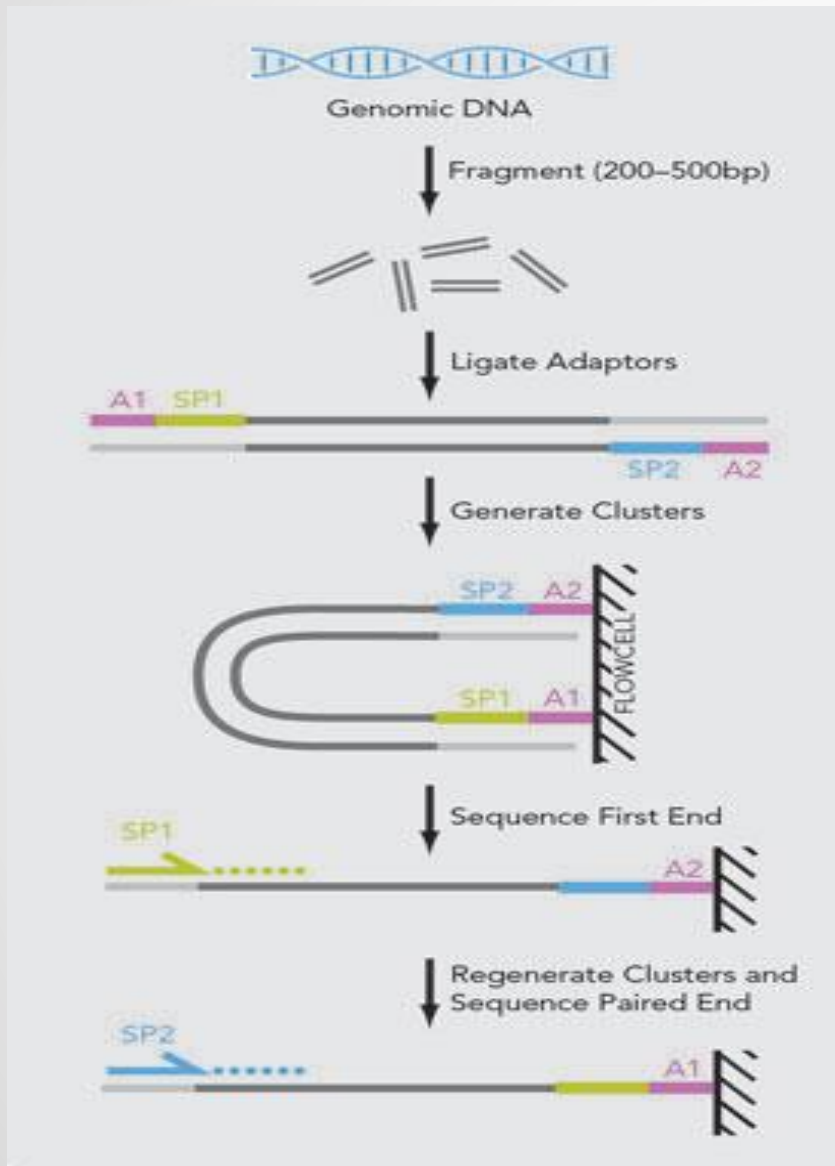


Image taken from Xconomy



Image taken from Industrial Exhibition 2004


Assembly



- Single End Sequence Assembly
- Paired End Sequencing Assembly
- Which is Preferred?


Data Analysis

Primary Analysis



- Analysis of hardware generated data, machine stats etc.
- Production of sequence reads and quality scores

Secondary Analysis



- QA filtering on raw reads
- Alignment/Assembly of reads
- QA and variant calling on aligned reads

Tertiary Analysis

“Sense Making”

- Multi-sample processing
- QA/QC of variant calls
- Annotation and filtering of variants
- Data aggregation
- Association analysis
- Population structure analysis
- Genome browser driven exploratory analysis

Downstream Analysis

- **BLAST (Basic Local Alignment Search Tool)**

contig09789 : NODE_52575_length_348_cov_3.548851, NODE_97374_length_772_cov_4.497409,
NODE_204370_length_106_cov_2.075472, NODE_284311_length_72_cov_4.611111,
NODE_514121_length_244_cov_2.102459

contig09788 : NODE_138945_length_196_cov_2.464286, NODE_246064_length_161_cov_2.614907

contig09783 : NODE_14623_length_1135_cov_3.317181, NODE_23403_length_416_cov_3.625000,
NODE_57393_length_270_cov_2.614815, NODE_160727_length_508_cov_3.454724,
NODE_198812_length_374_cov_3.762032, NODE_306930_length_186_cov_3.284946,
NODE_311476_length_111_cov_7.243243, NODE_346931_length_216_cov_2.680556,
NODE_511004_length_133_cov_2.015038, NODE_537359_length_317_cov_3.987382,
NODE_547876_length_181_cov_2.331492

- **MUMmer (Maximum Unique Matches)**

contig122151 : NODE_145039_length_491_cov_3.128309

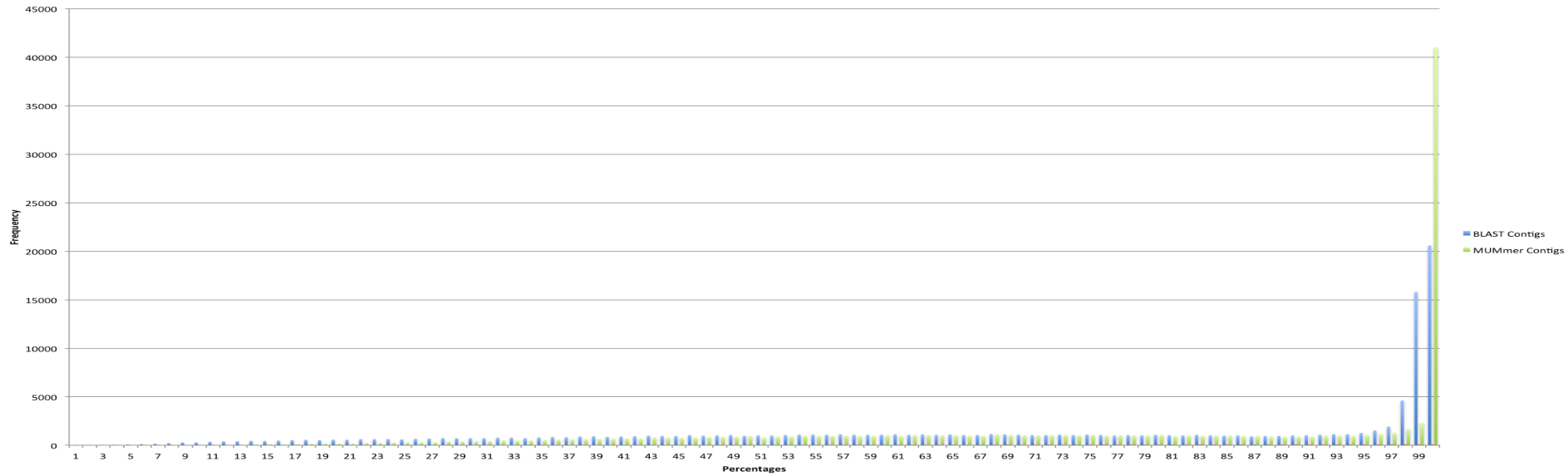
contig09789 : NODE_79919_length_122_cov_3.483607, NODE_204370_length_106_cov_2.075472,
NODE_173926_length_330_cov_3.427273, NODE_97374_length_772_cov_4.497409,
NODE_97374_length_772_cov_4.497409, NODE_97374_length_772_cov_4.497409

contig09788 : NODE_469389_length_516_cov_2.474806, NODE_259071_length_143_cov_2.237762,
NODE_373623_length_457_cov_2.916849, NODE_194630_length_141_cov_2.801419,
NODE_138945_length_196_cov_2.464286, NODE_138945_length_196_cov_2.464286,
NODE_138945_length_196_cov_2.464286

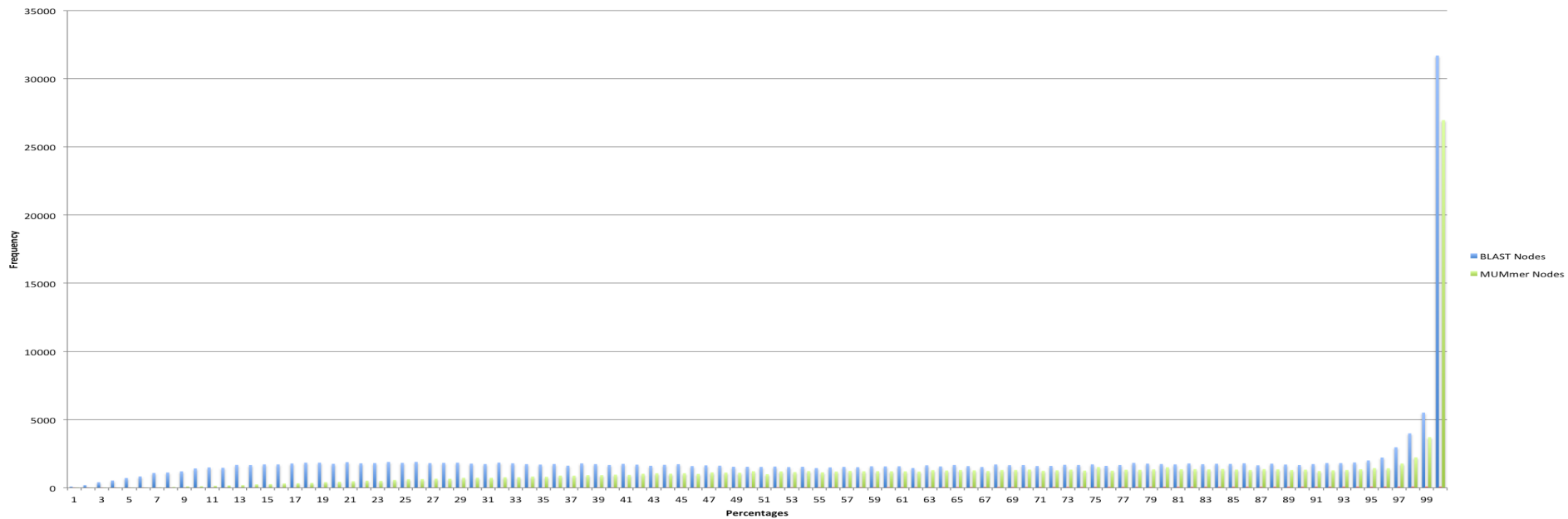
contig68189 : NODE_120143_length_513_cov_4.403509

Results/Comparisons

Comparison of Contig Coverage Frequency

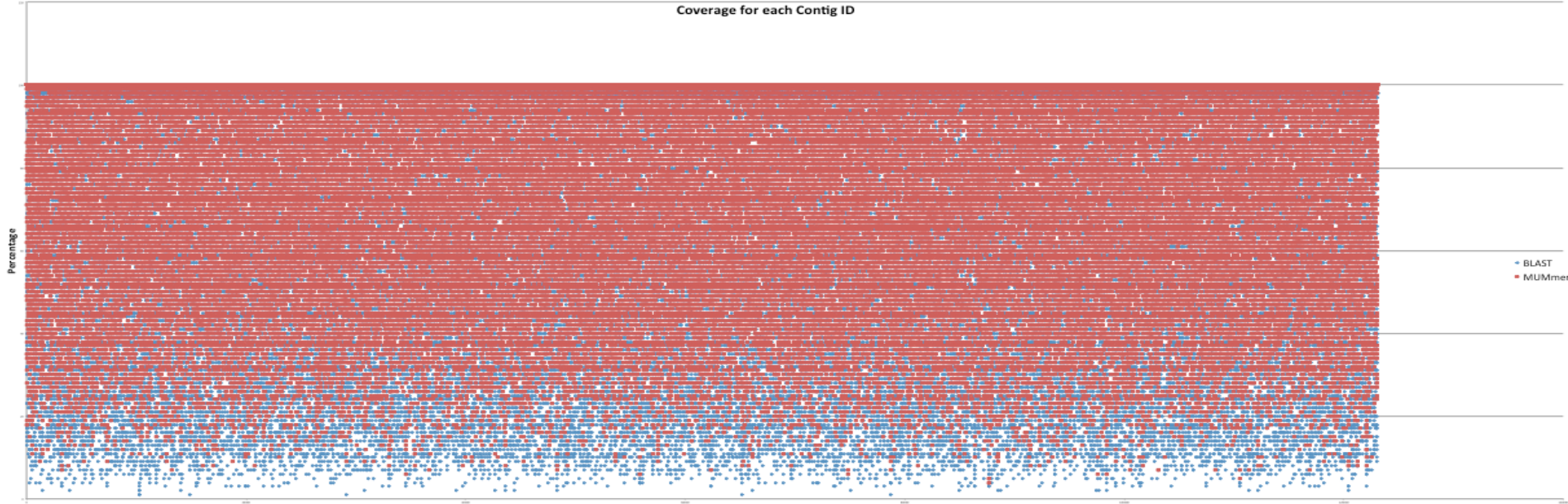


Comparison of Node Coverage Frequency

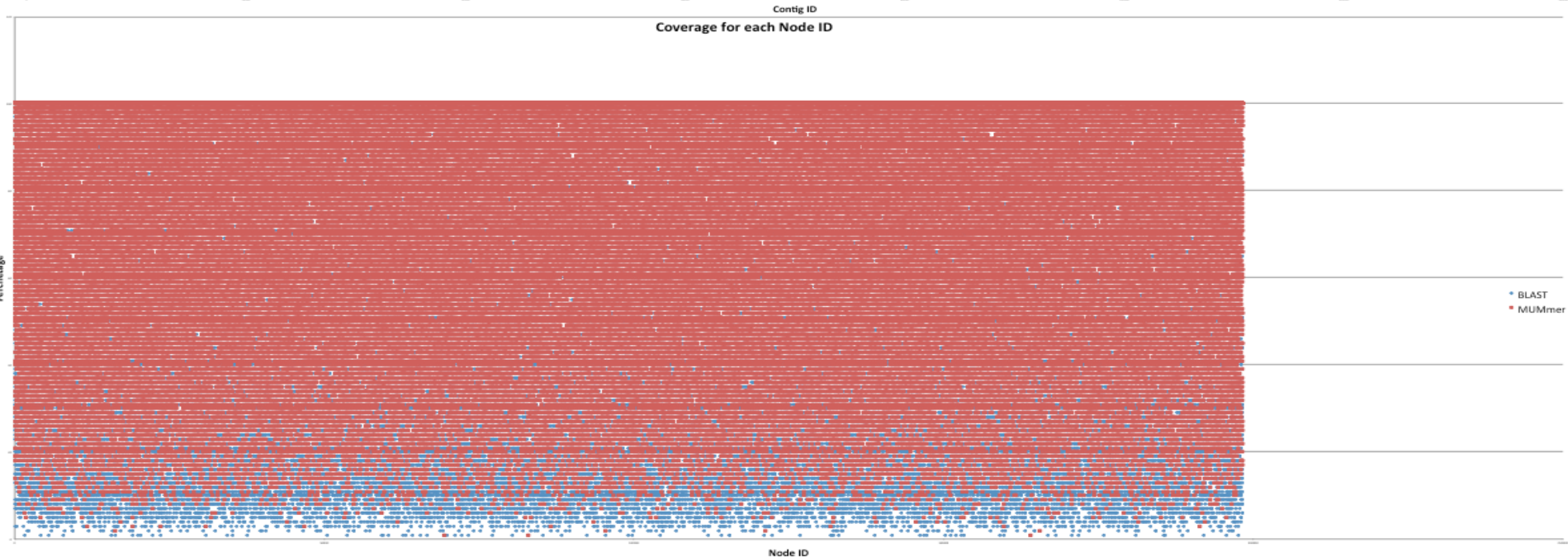


Results/Comparisons

Coverage for each Contig ID



Coverage for each Node ID



Conclusion

- Future of Next Gen sequencing
 - Personalized Medicine
 - Bio Fuels
 - Climate change



References

- Zhang W, Chen J, Yang Y, Tang Y, et al. (2011) A Practical Comparison of *De Novo* Genome Assembly Software Tools for Next-Generation Sequencing Technologies. PLoS ONE 6(3): e17915. doi:10.1371/journal.pone.0017915
- Goecks J, Nekrutenko A, Taylor J, and The Galaxy Team, Galaxy: a comprehensive for supporting accessible, reproducible, and a transparent computational research in the life sciences. *Genome Biol.* 2012 Aug 25;11(8):R86
- Misra N., Panda P. K., Parida B. K., Mishra B. K., Phylogenomic Study of Lipid Genes Involved in Microalgal Biofuel Production - Candidate Gene Mining and Metabolic Pathway Analyses, *Evolutionary Bioinformatics* 2012:8 545-564, doi: 10.4137/EBO.S10159
- <http://blast.ncbi.nlm.nih.gov/Blast.cgi>
- <http://mummer.sourceforge.net/>
- Gabe Rudy, (2010) *A Hitchhiker's Guide to Next-Generation Sequencing*, :1-9, Golden Helix
- John D. McPherson, (2009) *Next-Generation Gap*, 6:1-4, Nature Methods Supplement
- Michael L. Metzker, (2010) *Sequencing Technologies, - the next generation*, 11:1-5, Nature Reviews
- <http://www.sanger.ac.uk/resources/software/artemis/>
- Rutherford K, Parkhill J, Crook J, et al., *Bioinformatics (Oxford, England)* 2000;16;10;944-5
- <http://environment.nationalgeographic.com/environment/global-warming/biofuel-profile/>
- Radakovits R. et al. Draft genome sequence and genetic transformation of the oleaginous alga *Nannochloropsis gaditana*. *Nat. Commun.* 3:686 doi: 10.1038/ncomms1688 (2012)

Thank you!

Acknowledgements:

- CSURE (Computational Science for Undergraduate Research Experiences)
- University of Tennessee

Questions?