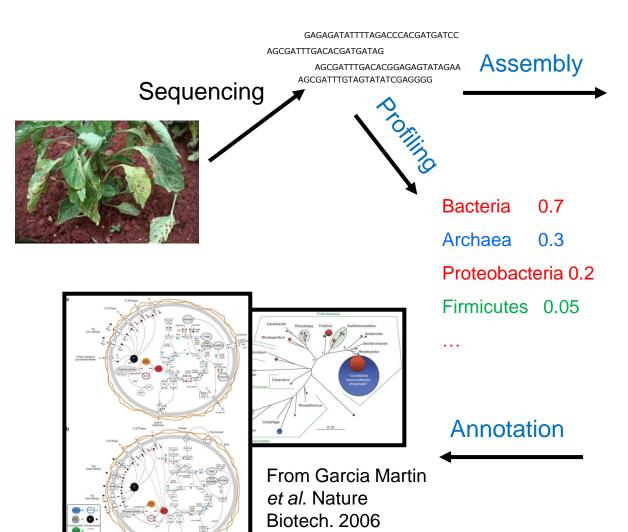


Critical Assessment of Metagenome Interpretation

Alice C. McHardy
Computational Biology of Infection Research
Helmholtz Centre for Infection Research

and the CAMI Initiative

Computational Metagenomics



 ${\tt CCTCGACGGGGGTGCGCTCGTTGGCCCCGTTCGGCTCCTCTTGGAGCACGGCGCTCGCCTGGATCTCGGC}$

GCTCTTCTCCTGTGGAACGGCTCCAGGAAATCGGCGAGGTCGTCGCCCGAACCGCTCGGCGTCGCG TAGAAGAAGCCGAGGAGGCGCCTCGTGCAGCCGCCCGCGCTCGGGGAACCGATCCTCCGGGTTCTTCCGGG GCATCGTGCTCACGACGTCGGCGACCGACCGGGCACGTCGGGGCCACGAGCGCGAGACGGCGATACTC

GCTCGCCTGCAGCCGCCGCATGATCTCGAACGCCGTCGGCGCCGCGAACGGGTTCGACCCGGCGAGCATC
GATGGTCGTGTTCCGCGGGATGAGCTTGAAGCTCACGCCGCCGCCGGTCTCGACGCCGACGCGACAGCGGC

GGTCTCGAGCGACGACGAGACCTGCTTGCCTTCTCGGCCGCCTCCTTGAGCCGCTGCAGCGCCAGC CGATCCGGGCGAGACCGATGCGGCTCTTCCCCTCGAACTGCTGGCGGAGACGGATGCAGTCGATGCGCTGGC CGAAGTCCTGCCGCCGCGAGGTGCGTGTCGCCGCCGGTCGCCTTGACGCTGAGACCCCGCTCGCGATCTC GAGGATCGAATATCCAACGTGCCTCCGCCGAGATCGTGAGACCGCGATCGTCGGCCTTCACCTGTGG L..



 ${\tt CCTCGACGGGGGTGCGCTCGTTGGCCCCGTTCGGCTCCTCTTGGAGCACGGCGCTCGCCTGGATCTCGGC}$

GCTCTTCTCCTGGTGGAACGGCTCCAGGAAATCGGCGAGGTCGTTCGCCCCCGAACCGCTCGGCCGTCGGG TAGAAGAAGCCGAGGAGCGCCTCGTGCAGCCGCCCGGCGTCGGGGAACCGATCCTCCGGGTTCTTCTCGCGA GCATCGTGCTCACGACGTCGGCGAGCGACCGGGGCACGTCGGGGGCGCACAGACGGGAGAGGGGGGATACTC

GCTCGCCTGCAGCCGCCGCATGATCTCGAACGCCGTCGGCGCGCGAACGGGTTCGACCCGGCGAGCATC
GATGGTCGTGTTCCGCGGGATGAGCTTGAAGCTCACGCCGCCGCCGCTCTCGACGCCGAGCGACCACACGCGC

GGTTCGAGCGACGAACGAGGTCGTGCTTCGCCTTCTCGGCCGCCTCCTTGAGCCGCTGCAGCGCCATG CGATCCCGGCCCAGATCGATCGCGCTTCTCCCTCGAACTCGTCGGCGAGCAGGTCGATGATCCGCTGGT CGAAGTCCTCGCCGCCGAGTGCGTGTCGCCGCCGGTCGCCTTGACGGTGAAGACCCCGCTCGCGATCT GAGGATCGAGATATCGAACGTGCCTCGCCCGAATCGTGAACCGCGATCGTCTCGGCCTTTCACCTTGTGG [...]

The Critical Assessment of Metagenome Interpretation (CAMI) competition

27 Jun 2014 | 6:36 PM | Posted by Tal Nawy | Category: Bioinformatics, Computational, Guest Post, Metagenomics

Alice McHardy, Alex Sczyrba and Thomas Rattei announce a new initiative for assessing metagenomics methods in this guest post.



Alice McHardy FOLKER MEYER



Alex Sczyrba

A. SCZYRBA



Thomas Rattei

In just over a decade, metagenomics has developed into a powerful and productive method in microbiology and microbial ecology. The ability to retrieve and organize bits and pieces of genomic DNA from any natural context has opened a window into the vast universe of uncultivated microbes. Tremendous progress has been made in computational approaches to interpret this sequence data but none can completely recover the complex information encoded in metagenomes.

A number of challenges stand in the way. Simplifying

Towards a comprehensive and objective evaluation of computational metagenomics software



PROTEINS: Structure, Function, and Genetics 23:301-317 (1995)

A Critical Assessment of Comparative Molecular Modeling of Tertiary Structures of Proteins*

Steven Mosimann, Ron Meleshko, and Michael N.G. James

Medical Research Council of Canada, Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

ABSTRACT In spite of the tremendous increase in the rate at which protein structures are being determined, there is still an enormous gap between the numbers of known DNA-derived sequences and the numbers of three-dimensional structures. In order to shed light on the biological functions of the molecules, researchers often resort to comparative molecular modeling. Earlier work has shown that when the sequence alignment is in error, then the comparative model is guaranteed to be wrong. In addition, loops, the sites of insertions and deletions in families of homologous proteins, are exceedingly difficult to model. Thus, many of the current problems in comparative molecular modeling are minor versions of the global pro-

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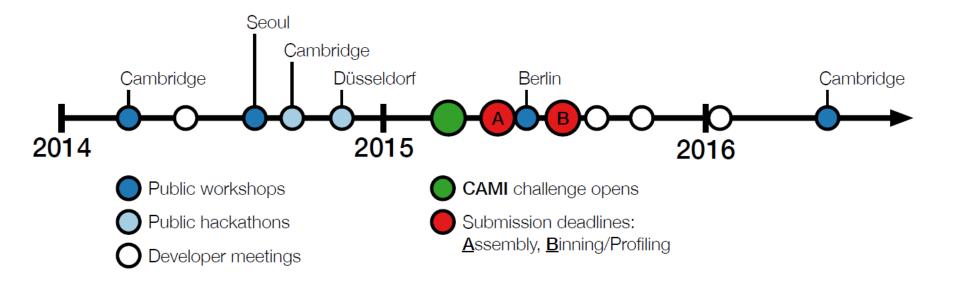
Principles

- Design decisions made by the community (data sets, evaluation measures and principles)
- Extensive, high-quality benchmark datasets from unpublished data
- Evaluation measures: informative to developers and the applied community
- Reproducibility: data generation, programs, evaluation
- Benchmark assembly, (tax.) binning and taxonomic profiling software





Timeline 1st CAMI Challenge





Sczyrba et al., Nature Methods 2017





Reproducibility and Standardization

- Output formats for binning and profiling
- Standard interfaces
- Docker-based Bioboxes for programs and metrics
- Semi-automatic benchmarking in future



Belmann et al., Gigascience 2015



CAMI Challenge Datasets

- Common experimental setups and community types
- Strain-level variation
- Different ev. distances to public genomes
- Non-bacterial sequences (archaea, plasmids, viruses)

| CAMI_low | |
|------------------------|--|
| 1 sample | |
| 15 Gb | |
| 2 x150 bp | |
| Insert size: 270 bp | |

| CAMI_medium (differential abundance) |
|--|
| 2 samples |
| 40 Gb |
| 2 x150 bp |
| Insert sizes: 270 bp & 5kbp |

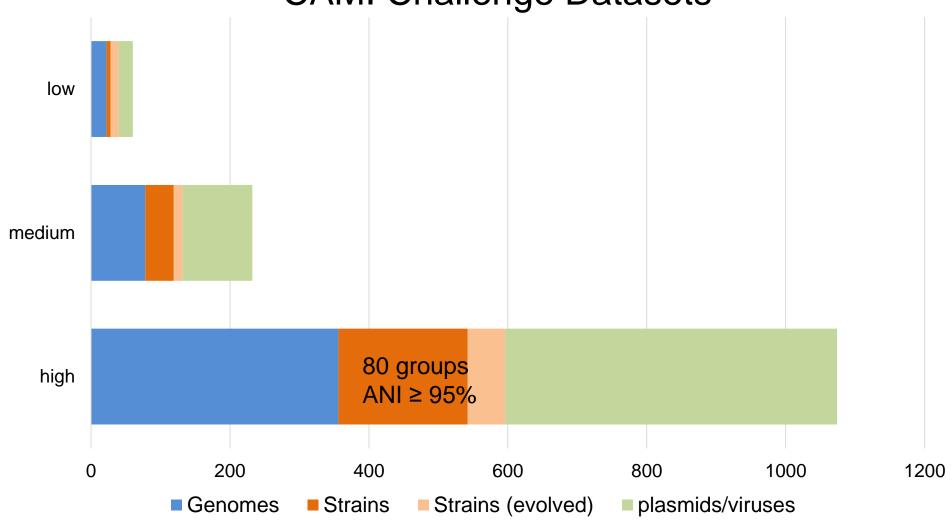
| CAMI_high (time series) | | | | |
|----------------------------|--|--|--|--|
| 5 samples | | | | |
| 75 Gb | | | | |
| 2 x150 bp | | | | |
| Insert size: 270 bp | | | | |

Simulated from ~700 novel microbial genomes, 600 novel viruses, plasmids and other circular elements

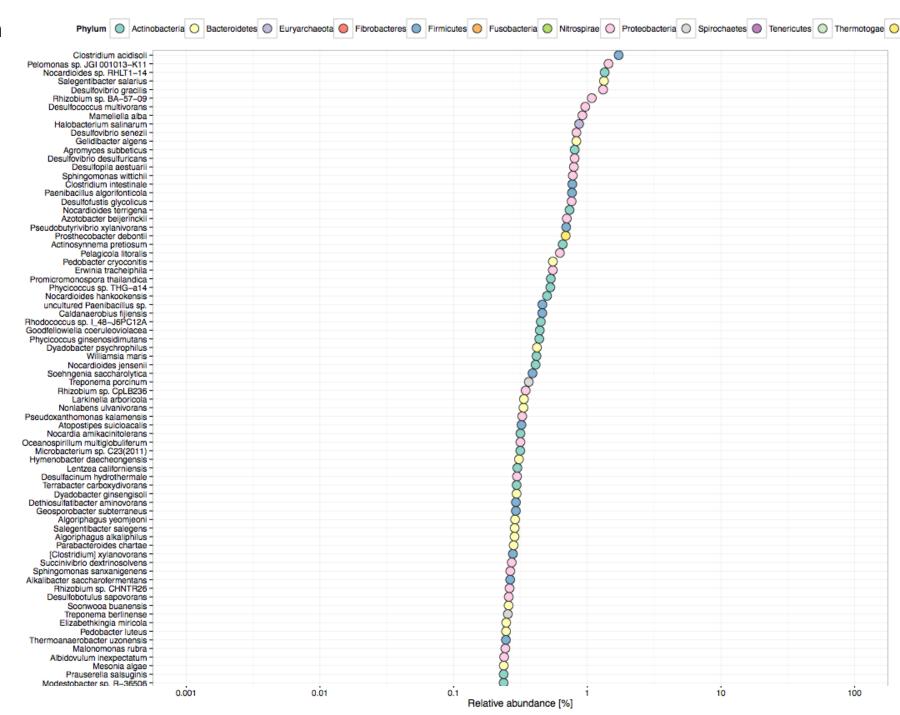








High





Challenge Participants



https://data.cami-challenge.org

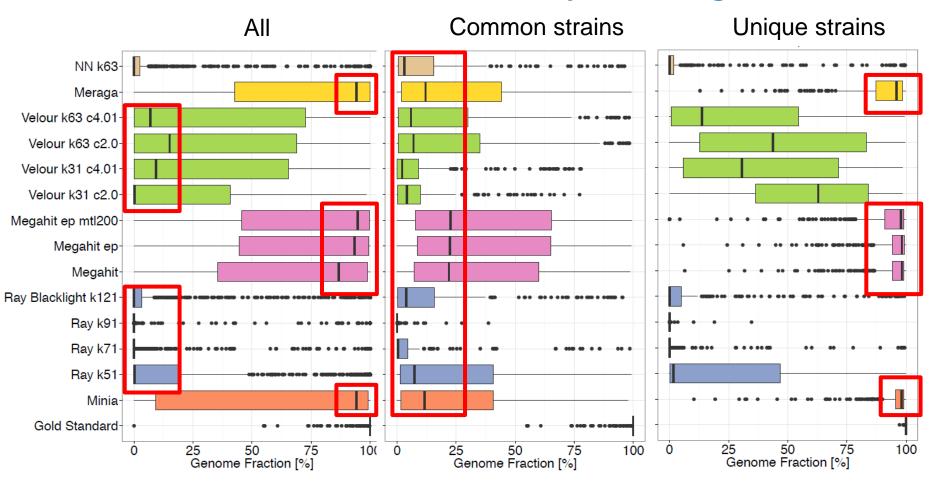


Submissions to the CAMI Challenge

With consent to publish: 215 submissions;16 teams; 25 programs; 36 bioboxes

| Assemblers | | Genome binners and taxonomic binners | | | |
|-----------------------|---|--|---|---|--|
| Megahit ⁴ | Metagenome assemble succinct de Bruijn grap | CONCOCT ¹¹ | Binner using differential coverage, tetranucleotide frequencies, paired-end linkage Binner using multi-sample coverage, tentranucleotide | | |
| Ray Meta ⁶ | Distributed de Bruijn gr | MaxBin 2.08 | | | |
| Meraga | Meraculous ⁴³ + MEGA | | frequencies | | |
| Minia ⁵ | B B " 1 | Krakan ¹⁵ | Tavonomic hinner using long | | |
| A*44 | MetaPhyler ²⁵ | Phylogenetic marker genes | ted assignments | | |
| | mOTU ²⁶ | Phylogenetic marker genes | 3 | ence similarities and | |
| Velour ₁ | Quikr/ARK/SEK ²⁷⁻²⁹ | k-mer based nonnegative least squares | | | |
| | Taxy-Pro ³⁰ | Mixture model analysis of protein signatures | | /erage, tetranucleotide ge | |
| | TIPP ³¹ | Marker genes and SATÉ pl | rker genes and SATÉ phylogenetic placement | | |
| | CLARK ²⁰ | Phylogenetically discrimina | tive k-mers | requencies and differential | |
| | Common Kmers/MetaPalette ²¹ | Long k-mer based nonnegative least squares | | uencies, multi-sample nylogenetic marker genes | |
| | | | | r frequencies (4-6mers), | |
| | DUDes ²² | Read mapping and deepes | t uncommon ancestor | | |
| | FOCUS ²³ | k-mer based nonnegative least squares | | ence homology and tax. | |
| | MetaPhIAN 2.0 ²⁴ | Clade specific marker gene | es | | |
| | | | | # HELMHOLTZ | |

Results of the Assembly Challenge

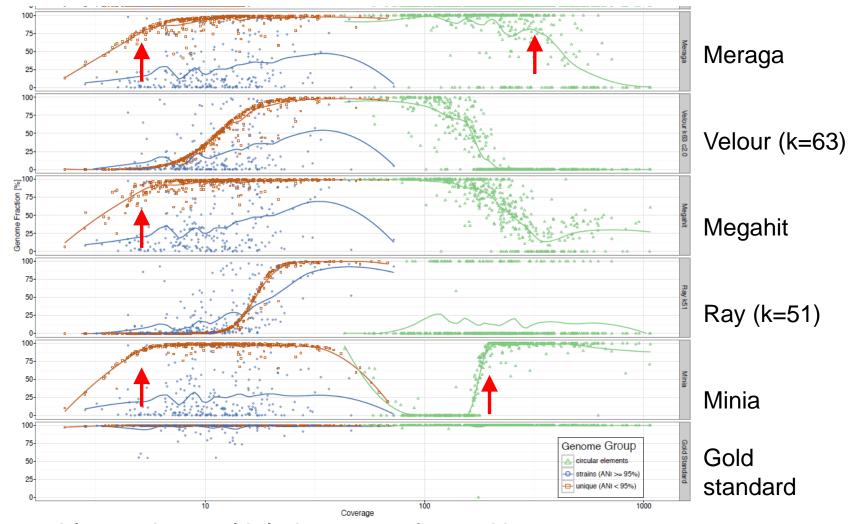


Good performance in genome assembly for unique strains

Subspecies diversity is a challenge!



Genome Recovery at different Coverages

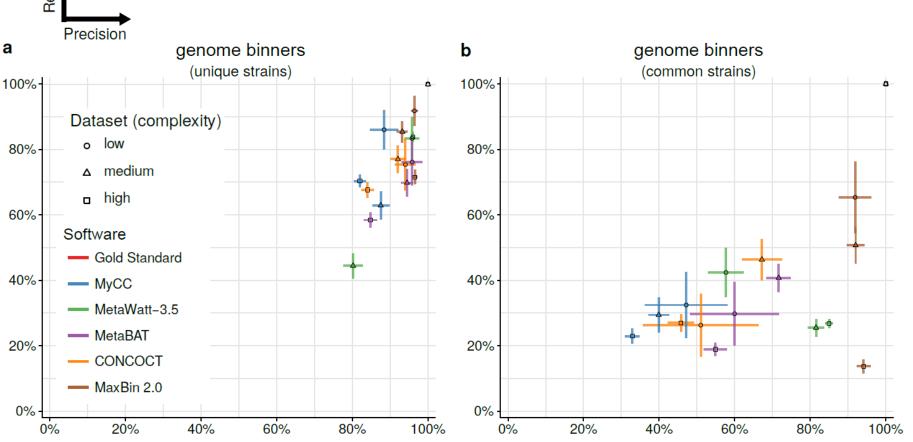


- Assemblers using multiple kmers performed better
- Minia (and Meraga) are good in plasmid assembly





Results for Genome Binners



- Good performance in genome reconstruction for unique strains
- Subspecies diversity is a challenge!



Metric

— Accuracy

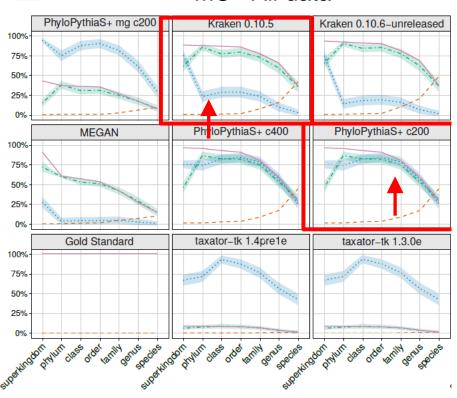
Results for Taxonomic Binners

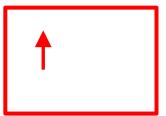
- Av. Precision

Misclassification

- Av. Recall

MC - All data

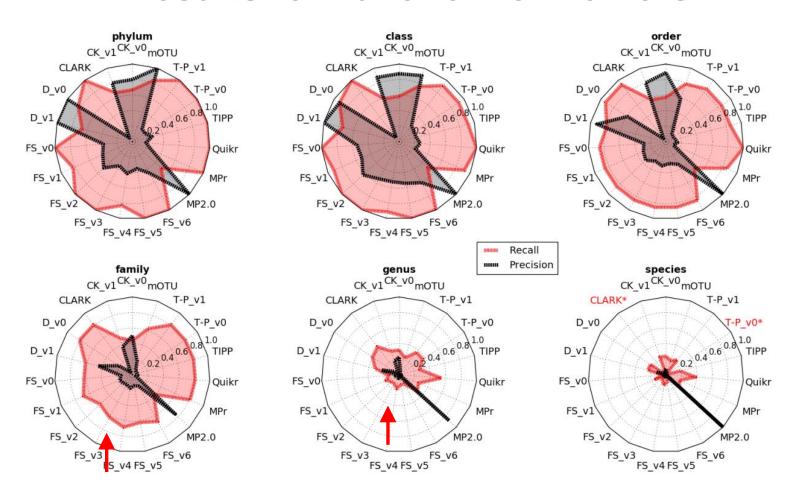




- Good performance until family rank and substantial decrease below
- Small predicted bins are oftentimes false positives



Results for Taxonomic Profilers



- Performance decreases substantially below family rank
- Virus and plasmid data affect abundance estimates



Main Conclusions

- Good assembly and genome binning of "single strain" species
- Strain-level diversity is a challenge
- Taxonomic profiling and taxon binning good until family level
- Reproducibility is very important, large variability of program performances with parameter settings

What is next?

- Benchmark programs on CAMI challenge datasets with CAMI benchmarking platform (<u>www.data.cami-challenge.org</u>)
- 2nd CAMI challenge
 - Illumina/PacBio/ONP data sets
 - specific environments
 - strain madness
 - workflows
- Get in touch: alice.mchardy@helmholtz-hzi.de;contact@camichallenge.org





Participants of CAMI Workshop at INI in Cambridge May 2016



Contributors 1st CAMI Challenge

P. Hofmann, P. Belmann, D. Koslicki, T. Woyke, N. Shapiro, S. Janssen, M. Barton, P. D. Blood, S. Majda, J. Dröge, I. Gregor, J. Fiedler, E. Dahms, R. Garrido-Oter, A. Bremges, A. Fritz, M. Z. DeMaere, C. Quince, T. Sparholt Jørgensen, L. Hestbjerg Hansen, S. J. Sørensen, Y. Bai, D. Turaev, M. Beckstette, M. Balvociute, F. Meyer, N. Nagarajan, B. Chia, B. Denis, J. L. Froula, Z. Wang, R. Egan, D. Don Kang, J. Cook, R. Chikhi, C. Deltel, C. Lemaitre, P. Peterlongo, G. Rizk, D. Lavenier, Y.-W. Wu, S. Singer, C. Jain, M. Strous, H. Klingenberg, P. Meinicke, T. Lingner, H.-H. Lin, Y.-C. Liao, G. Gueiros Z. Silva, D. A. Cuevas, R. A. Edwards, S. Saha, V. C. Piro, B. Y. Renard, M. Pop, H.-P. Klenk, M. Goeker, M. Balvociute, N. Kyrpides, J. Vorholt, P. Schulze-Lefert, E. M. Rubin, A. E. Darling, T. Rattei, A. Sczyrba





46 institutions







Why CAMI? CAMI

Tool development for shotgun metagenome data sets is a very active area: Assembly, (tax.) binning, taxonomic profiling

- Method papers present evaluations using many different metrics, simulated data sets (snapshots) and are difficult to compare
- It is unclear to everyone which tools are most suitable for a particular task and for particular data sets
- Comparative benchmarking requires extensive resources and there are pitfalls

Towards a comprehensive, independent and unbiased evaluation of computational metagenome analysis methods



PROTEINS: Structure, Function, and Genetics 23:301–317 (1995)

A Critical Assessment of Comparative Molecular Modeling of Tertiary Structures of Proteins*

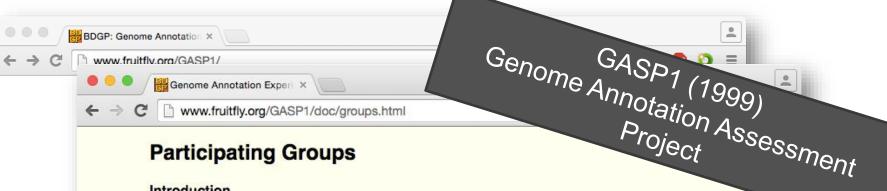
Steven Mosimann, Ron Meleshko, and Michael N.G. James

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Participating Groups

Introduction

Following groups are participating:

1. Team Gaasterland, program name: MAGPIE

Terry Gaasterland group leader, Laboratory for Computational Genomics, Rockefeller Alexander Sczyrba Laboratory for Computational Genomics, Rockefeller Elizabeth Thomas Laboratory for Computational Genomics, Rockefeller Gulriz Kurban Laboratory for Computational Genomics, Rockefeller Institute for Marine Biosciences, Canada Paul Gordon Christoph Sensen Institute for Marine Biosciences, Canada

2. Computational Genomics Group, The Sanger Centre, program name: FGenes/FGenesH

Victor Solovyev, group leader Asaf Salamov

3. Genome Annotation Group, The Sanger Centre, program name: Wise2/GeneWise

Ewan Birney, group leader

4. Chair for Pattern Recognitions, The University of Erlangen/Nuremberg, program name: LME/MCPromoter

Uwe Ohler, group leader George Stemmer Stefan Harbeck Heinrich Niemann

Computational Biosciences, Oakridge National Laboratory, program name: GRAIL

Richard J. Mural, group leader Douglas Hyatt Frank Larimer Manesh Shah Morey Parang

HOLTZ TRE FOR CTION RESEARCH A large-scale evaluation of computational protein function prediction

Critical Assessment of protein Predrag Radivojac1, Wyatt T Clark1, Tal Ronnen Oron2, Ale Artem Sokolov4,5, Kiley Graim4, Christopher Funk6, Karin Jeffrey M Yunes¹⁰, Ameet S Talwalkar¹¹, Susanna Repo^{8,12} Function Annotation Rita Casadio14, Zheng Wang15, Jianlin Cheng15, Hai Fang Jussi Nokso-Koivisto¹⁷, Liisa Holm¹⁷, Domenico Cozzetto¹⁸, Danies David T Jones¹⁸, Bhakti Limaye¹⁹, Harshal Inamdar¹⁹, Avik Datta¹⁹, Sunitha Meghana Chitale²⁰, Daisuke Kihara^{20,21}, Andreas M Lisewski²², Serkan Erdin²², Eric ve Robert Rentzsch²³, Haixuan Yang²⁴, Alfonso E Romero²⁴, Prajwal Bhat²⁴, Alberto Paccanaro Rebecca Kaßner²⁵, Stefan Seemayer²⁵, Esmeralda Vicedo²⁵, Christian Schaefer²⁵, Dominik Achten²⁵, Florian Auer²⁵, Ariane Boehm²⁵, Tatjana Braun²⁵, Maximilian Hecht²⁵, Mark Heron²⁵, Peter Hönigschmid²⁵, Thomas A Hopf²⁵, Stefanie Kaufmann²⁵, Michael Kiening²⁵, Denis Krompass²⁵, Cedric Landerer²⁵, Yannick Mahlich²⁵, Manfred Roos²⁵, Jari Björne²⁶, Tapio Salakoski²⁶, Andrew Wong²⁷, Hagit Shatkay^{27,28}, Fanny Gatzmann²⁹, Ingolf Sommer²⁹, Mark N Wass^{30,31}, Michael J E Sternberg³⁰, Nives Škunca³², Fran Supek³², Matko Bošnjak³², Panče Panov³³, Sašo Džeroski³³, Tomislav Šmuc³², Yiannis A I Kourmpetis^{34,35}, Aalt D J van Dijk34,36, Cajo J F ter Braak34, Yuanpeng Zhou37, Qingtian Gong37, Xinran Dong37, Weidong Tian37, Marco Falda³⁸, Paolo Fontana³⁹, Enrico Lavezzo³⁸, Barbara Di Camillo⁴⁰, Stefano Toppo³⁸, Liang Lan⁴¹, Nemanja Djuric⁴¹, Yuhong Guo⁴¹, Slobodan Vucetic⁴¹, Amos Bairoch^{42,43}, Michal Linial⁴⁴, Patricia C Babbitt³, Steven E Brenner⁸, Christine Orengo²³, Burkhard Rost²⁵, Sean D Mooney² & Iddo Friedberg^{45,46}

Automated annotation of protein function is challenging. As the number of sequenced genomes rapidly grows, the overwhelming majority of protein products can only be annotated computationally. If computational predictions are to be relied upon, it is crucial that the accuracy of these methods be high. Here we report the results from the first large-scale community-based critical assessment of protein function annotation (CAFA) experiment. Fifty-four methods representing the state of the art for protein function prediction were evaluated on a target set of 866 proteins from 11 organisms. Two findings stand out: (i) today's best protein function prediction algorithms substantially outperform widely used first-generation methods, with large gains on all types of targets; and (ii) although the top methods perform well enough to guide experiments, there is considerable need for improvement of currently available tools.

available¹. The computational annotation of protein function has therefore emerged as a problem at the forefront of computational and molecular biology.

Many solutions have been proposed in the last four decades2-10, yet the task of computational functional inference in a laboratory often relies on traditional approaches such as identifying domains or finding Basic Local Alignment Search Tool (BLAST)11 hits among proteins with experimentally determined function. Recently, the availability of genomic-level sequence information for thousands of species, coupled with massive high-throughput experimental data, has created new opportunities for function prediction. A large number of methods have been proposed to exploit these data, including function prediction from amino acid sequence12-16, inferred evolutionary relationships and genomic context17-21, protein-protein interaction networks22-25, protein structure data26-28, microarrays29 or a combina

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First CAMI challenge

- Benchmark assembly, (tax.) binning and taxonomic profiling software
- Extensive, high-quality benchmark data sets from unpublished data
- Publication with participants and data contributors

Aims

- Overview of tools and use cases
- Standards
- Facilitate future benchmarking
- Indicate promising directions for tool development
- Suggestions for experimental design

Contest opened in early 2015



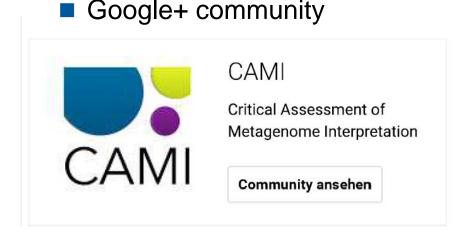


- All design decisions (data sets, evaluation measures and principles) should involve the community
- Data sets should be as realistic as possible
- Evaluation measures should be informative to developers and understandable also by applied community
- Reproducibility (data generation, tools, evaluation)
- Participants should not see any of the data before



Community Involvement

- ISME Roundtable, Hackathons & workshops
- Announcements in blogs & tweets
- www.cami-challenge.org with newsletter



NATURE METHODS | METHAGORA

The Critical Assessment of Metagenome Interpretation (CAMI) competition

27 Jun 2014 | 6:36 PM | Posted by Tal Nawy | Category: Bioinformatics, Computational, Guest Post, Metagenomics

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Thomas Rattei

ANJA VENIER

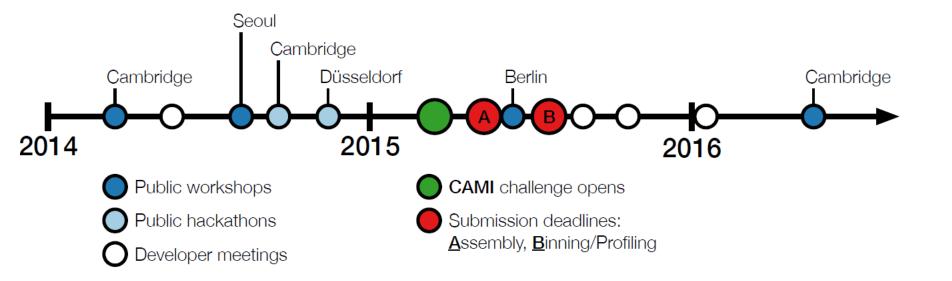
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Timeline first CAMI Challenge

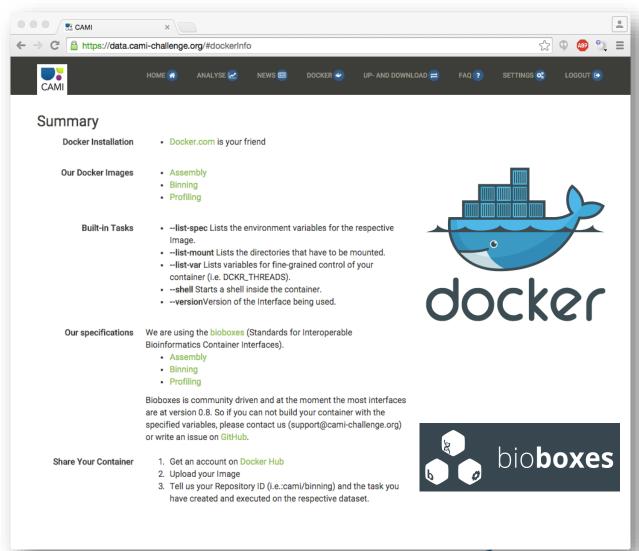






Reproducibility and Standardization

- Standard formats for binning and profiling
- Standard interfaces for tool execution
- Bioboxes (docker containers) for tools and metrics
- Currently 25 tools in bioboxes – semiautomatic benchmarking in future challenges



Barton et al., Gigascience 2015



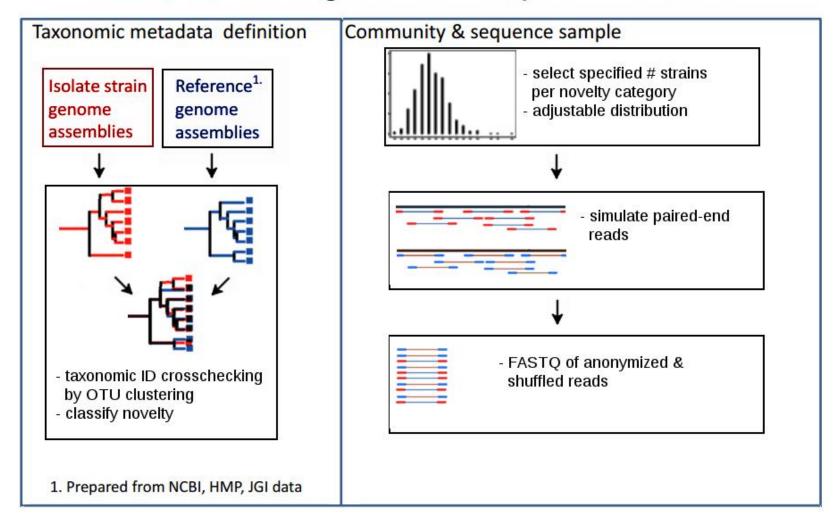
Challenge Data sets – Design principles

- As realistic as possible, challenging
- Common experimental setups and community types
- Unpublished data
- Strain-level variation
- Different taxonomic distances to sequenced genomes (deep branchers included)
- State-of-the-art sequencing technologies
- Non-bacterial sequences





Simulated Metagenome Sample Generation

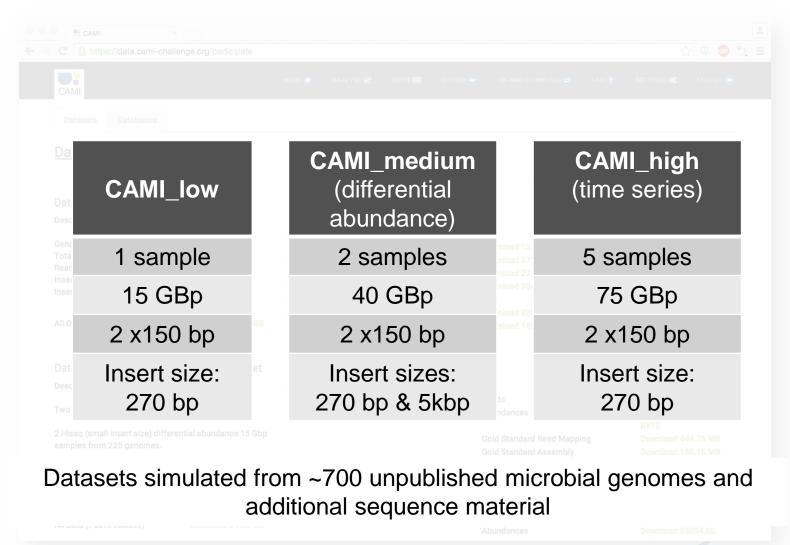






CAMI Datasets









1st Challenge Timeline



- Challenge opens
- Read data sets posted
- Assembly contest starts

May 20, 2015

- Assembly challenge closes
- Assembly gold standard posted
- Binning & profiling contest starts

8 weeks

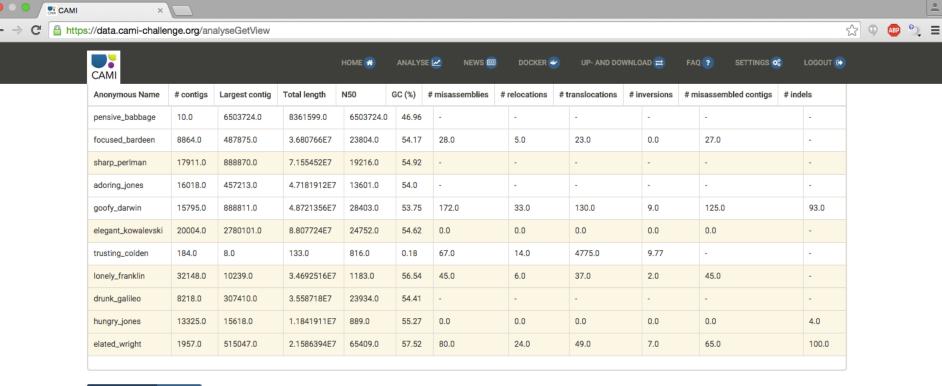
July 18, 2015

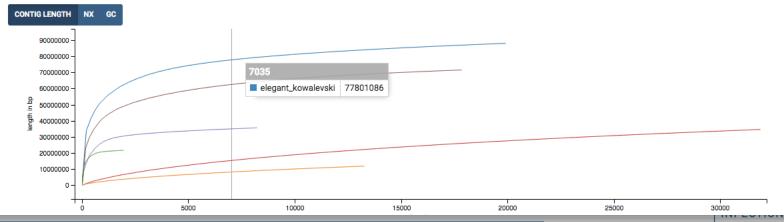
- Challenge closes
- Evaluation starts





CAMI Evaluation Metrics



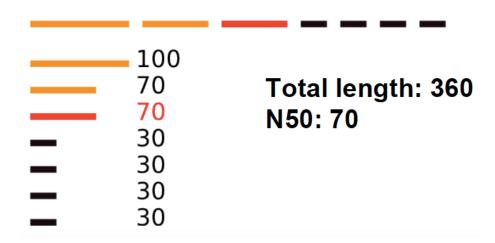


Assembly Evaluation Metrics

Basic Statistics:

- Number of contigs
- Number of large contigs (i.e. > 1000 bp)
- Largest contig length
- Total assembly length
- N50:

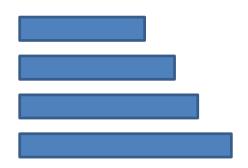
The length for which the collection of all contigs of that length or longer covers at least half an assembly (50%)



Assembly Evaluation Metrics

Reference-based statistics:

- Reference length
- Reference GC %
- Number of chromosomes
- Number of genes/operons
- NGx, LGx



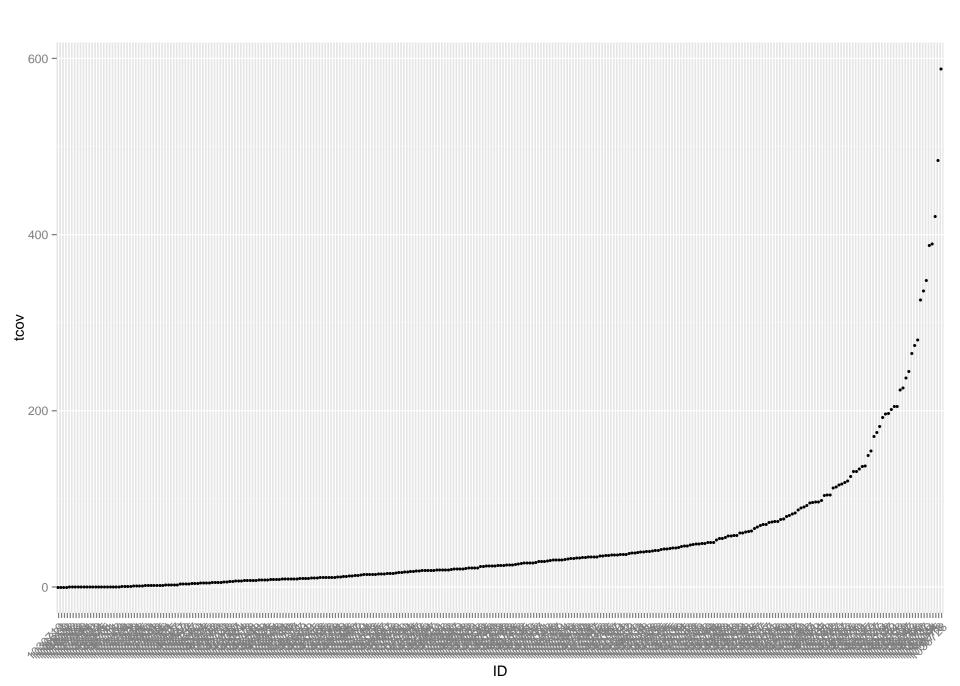
• Genome fraction %

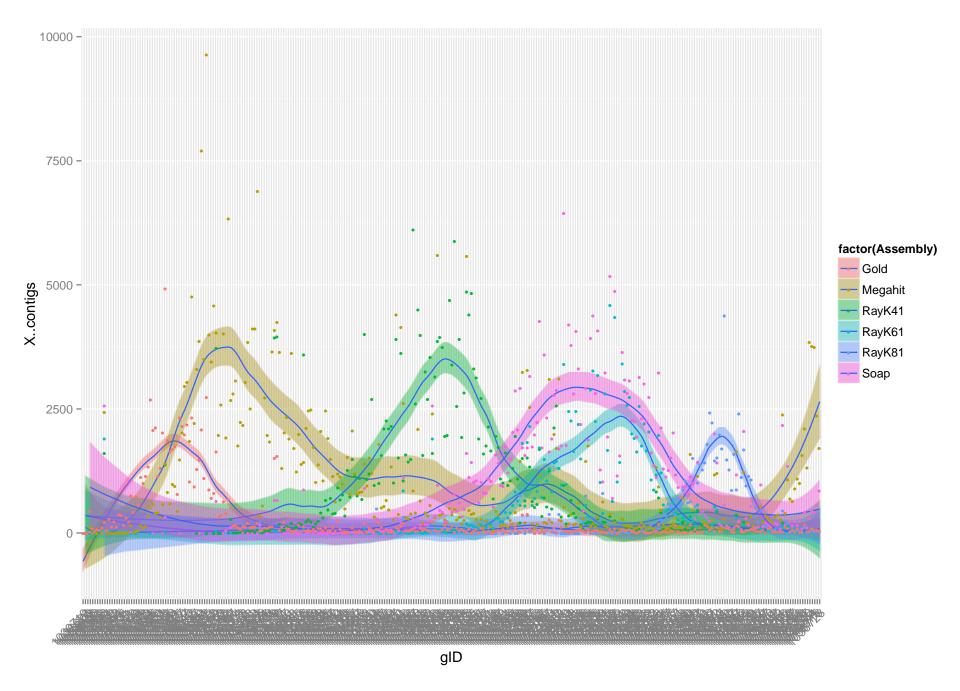
- Genome fraction %
- Duplication ratio

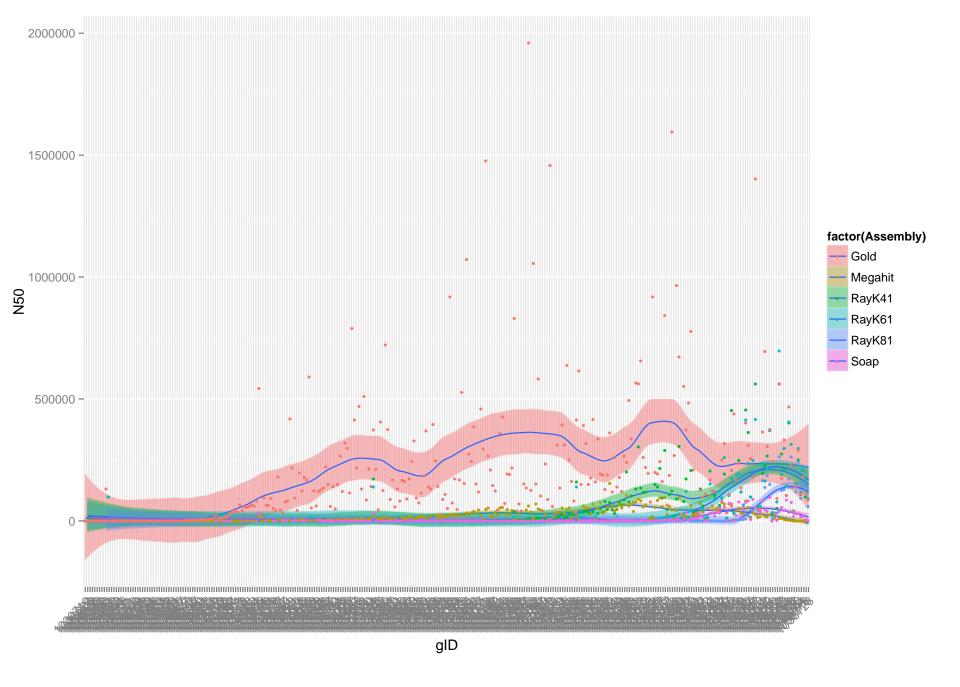
- Genome fraction %
- Duplication ratio
- Number of gaps

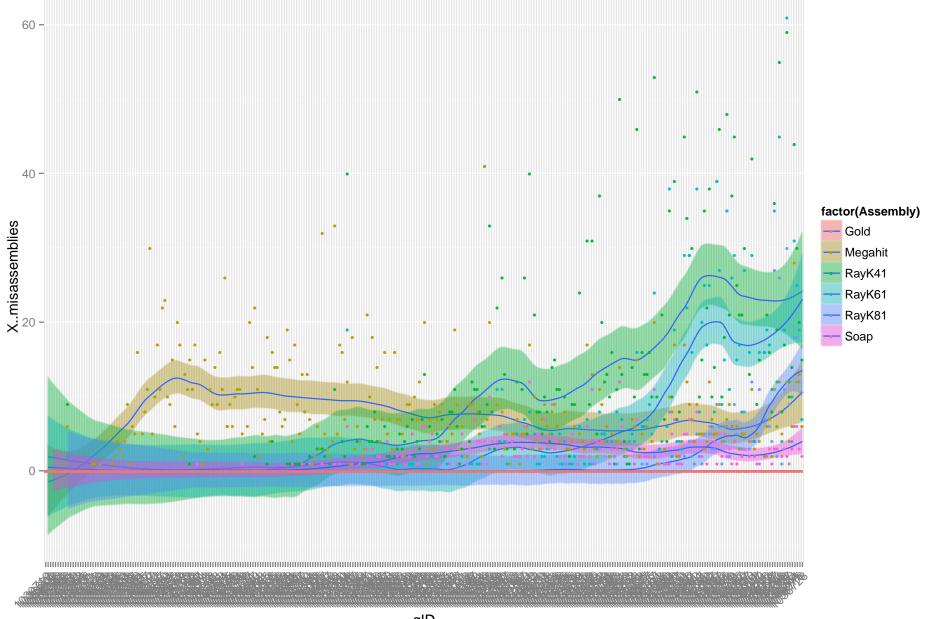
- Genome fraction %
- Duplication ratio
- Number of gaps
- Largest alignment length

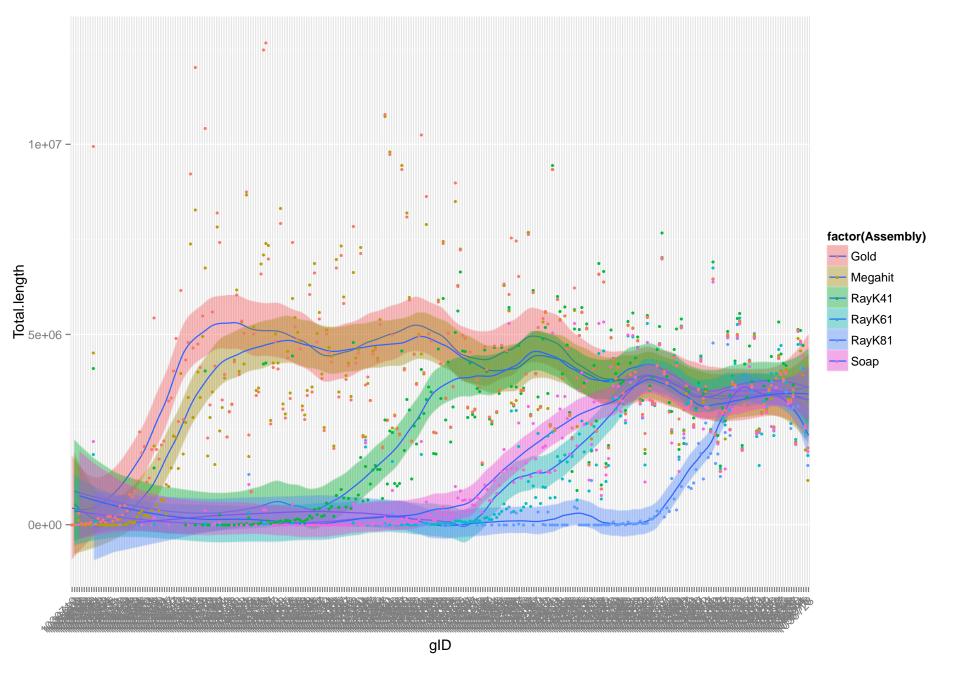
- Genome fraction %
- Duplication ratio
- Number of gaps
- Largest alignment length
- Number of unaligned contigs (full & partial)









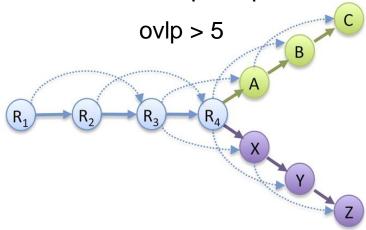


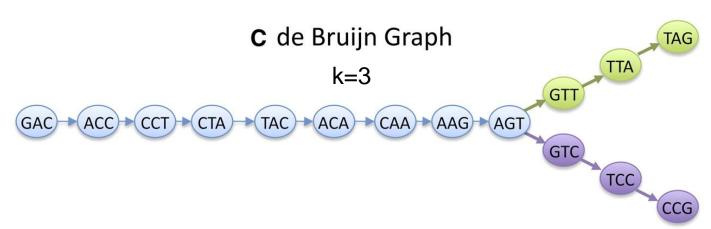
Overlap graph vs de Bruijn graph for assembly.

A Read Layout

GACCTACA R2: ACCTACAA R3: CCTACAAG R4: CTACAAGT **A**: TACAAGTT B: ACAAGTTA C: CAAGTTAG X: TACAAGTC Y: ACAAGTCC CAAGTCCG Z:

B Overlap Graph





Schatz M C et al. Genome Res. 2010;20:1165-1173



Effect of kmer size: 51-mer

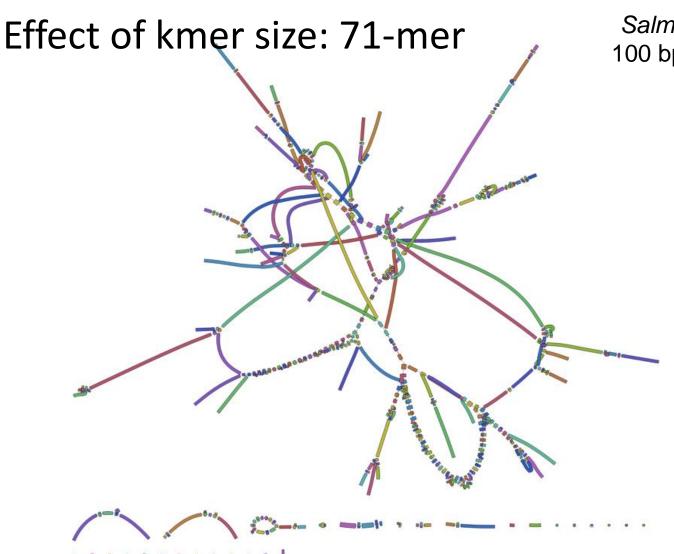




Effect of kmer size: 61-mer





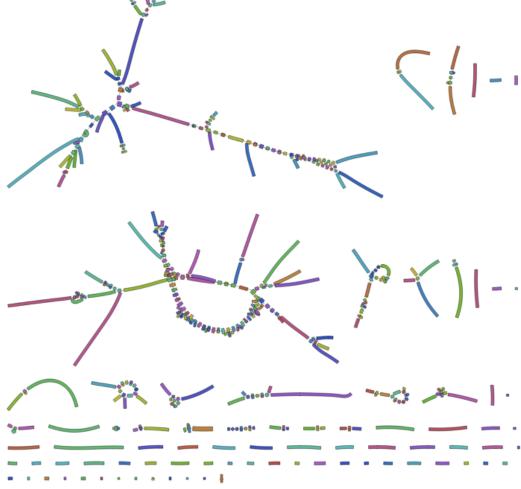






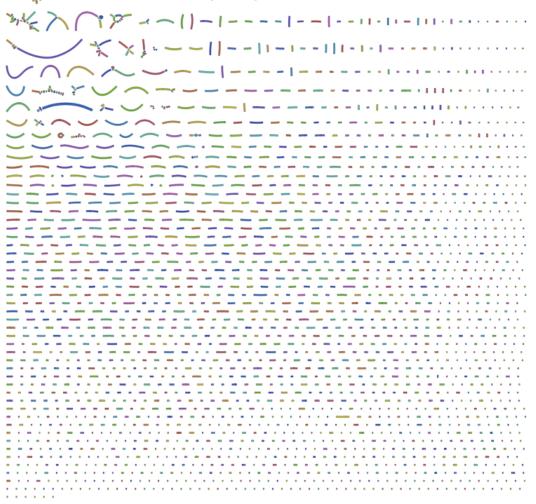
Effect of kmer size: 81-mer







Effect of kmer size: 91-mer











Universität elefeld

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Be part of It! Be part-challenge.org

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Extreme Science and Engineering **Discovery Environment**

Phil Blood









About



An offshoot of the Genome 10K project, and primarily organized by the

UC Davis Genome Center,

Assemblathons are contests to assess state-of-the-art methods in the field of genome assembly.

Assemblathon 1

This page will serve as an archive of all material relating to Assemblathon 1 (2010 – 2011). Existing Assemblathon 1 webpages will be converted to blog posts and linked to from this page. Also note that the UC Santa Cruz Assemblathon team, have their own webpage with code, documents and data from Assemblathon 1.

Data