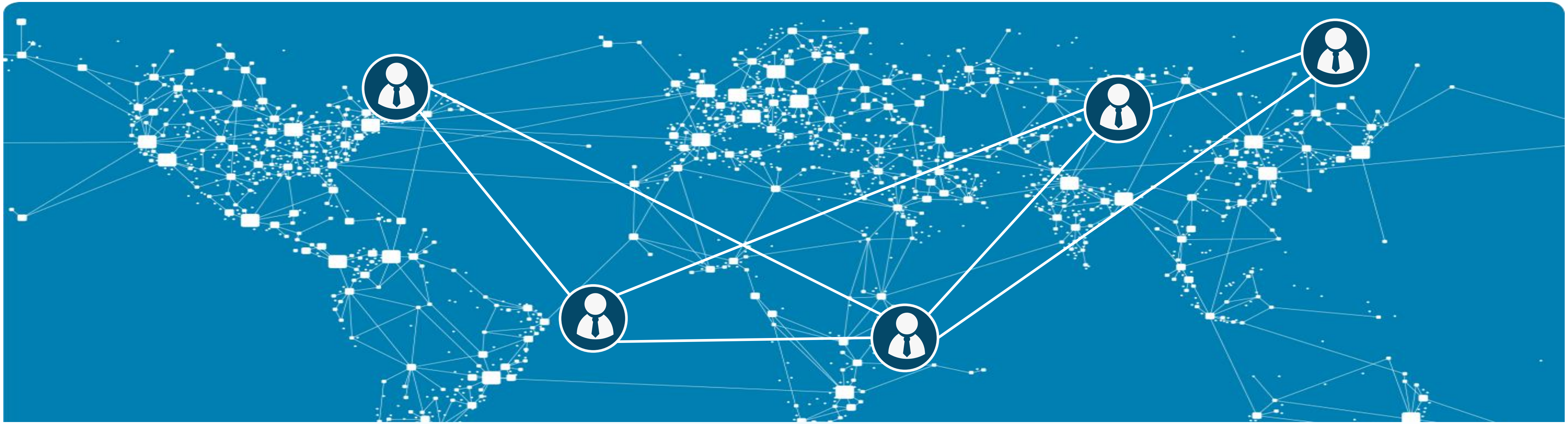
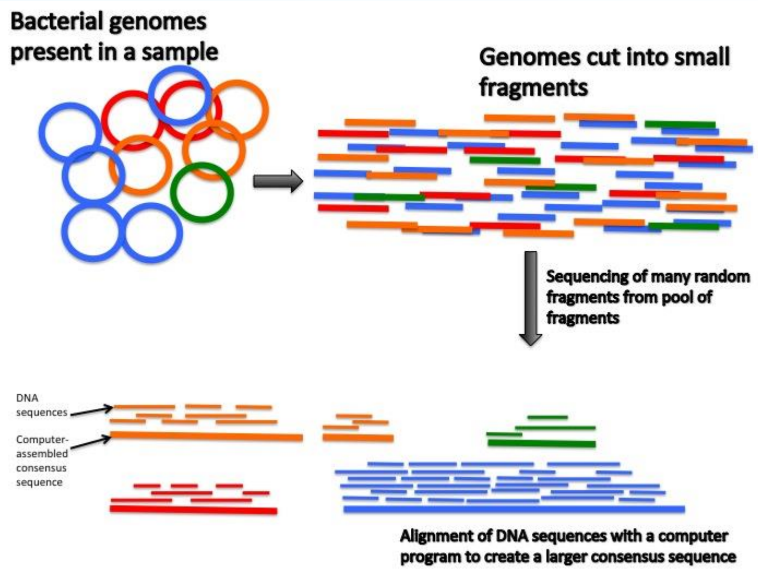
微生物组—宏基因组分析专题研讨会第14期



31 拼接/组装Assembly

易生信

2022年3月27日


Bacterial genomes present in a sample
Genomes cut into small fragments
Sequencing of many random fragments from pool of fragments
DNA sequences
Computer-assembled consensus sequence
Alignment of DNA sequences with a computer program to create a larger consensus sequence

目录

一. 质控KneadData

二. 物种分类kraken 2

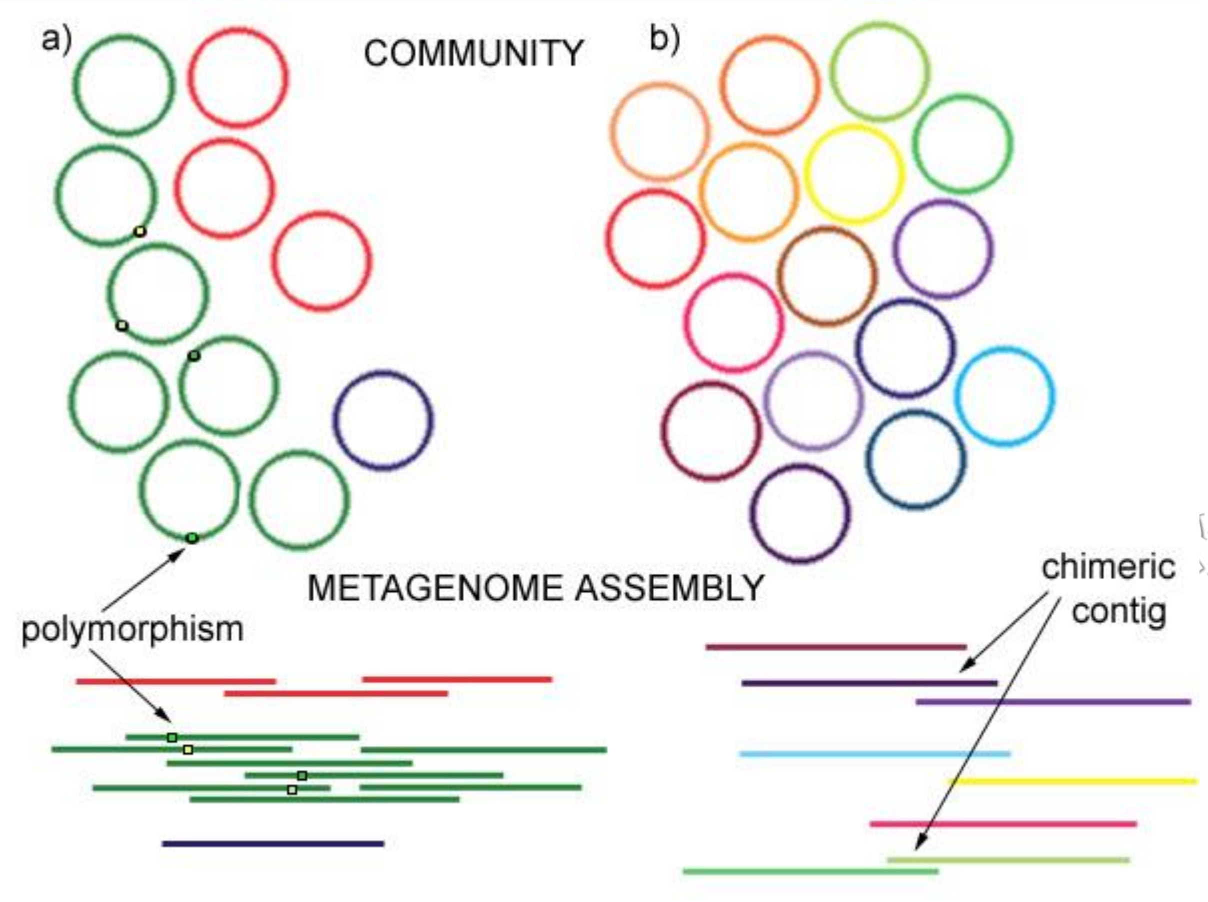
三. **序列组装/拼接**

四. 基因预测/注释

五. 基因聚类cd-hit

六. 基因定量salmon

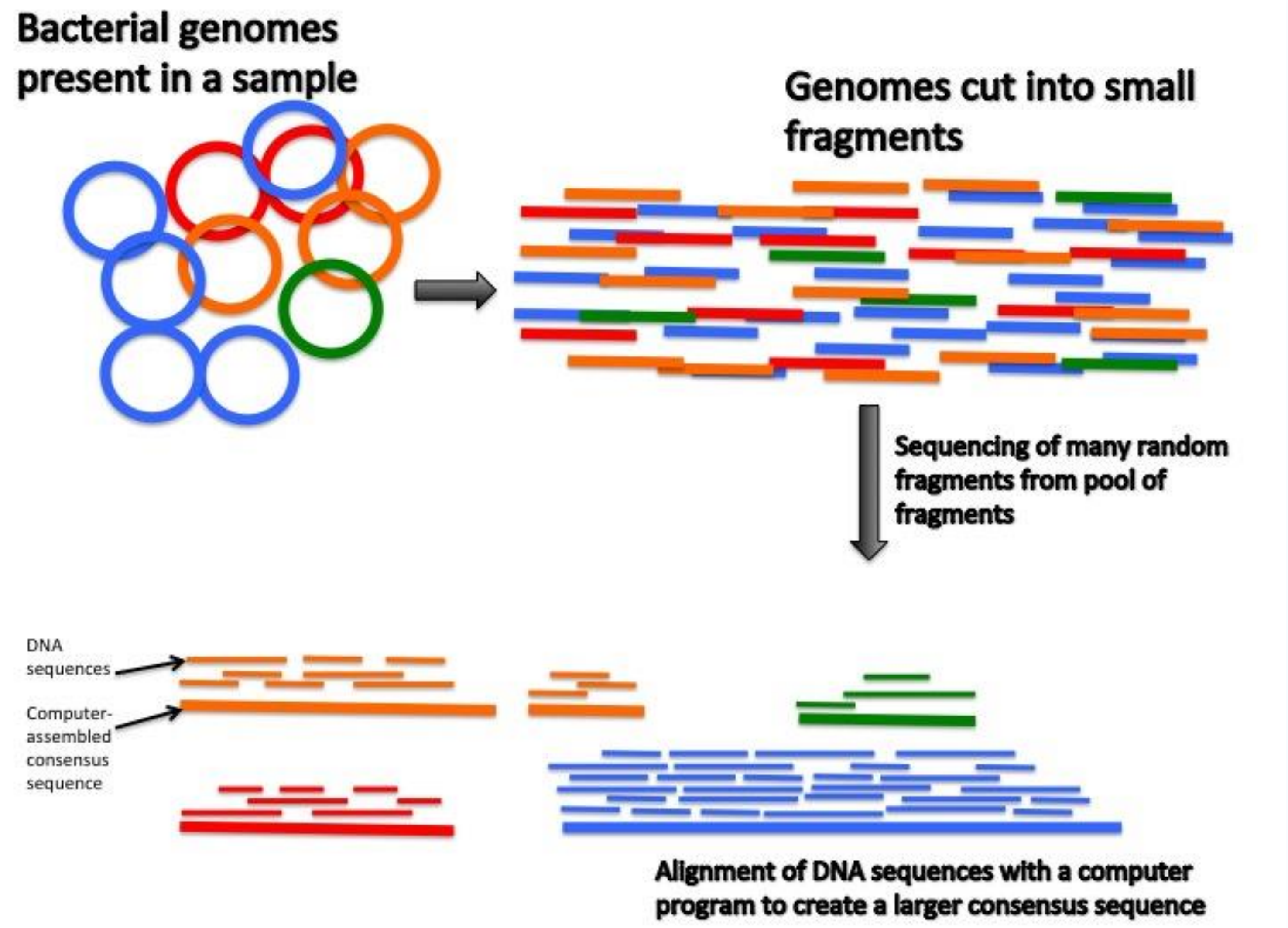
七. 基因功能注释



易汉博基因科技(北京)有限公司
EHBIO Gene Technology (Beijing) co., LTD

http://wiki.biomine.skelleftea.se/wiki/index.php/Metagenomics

组装/拼接 (Assemble)的基本原理


Bacterial genomes present in a sample
Genomes cut into small fragments
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Computer-assembled consensus sequence
Alignment of DNA sequences with a computer program to create a larger consensus sequence

https://teachthemicrobiome.weebly.com/sequencing-the-microbiome.html

拼接中常见名词

- Read: 读长，高通量测序平台产生的序列
- Contig：重叠群，基于读长之间的重叠区关系拼接获得的更长序列
- Scaffold：支架，双端测序时，同一条序列的两端读长分布于不同的重叠群上，可确定两个重叠群的方向和距离时，将重叠群中间用N连接后的更长序列
- N50: 将重叠群或支架按长度由大到小排列，累加总长度50%时，所在序列长度，用于表示拼接质量的重要参数
- Depth：测序深度，即测序总碱基与基因组大小的比值，如人类30x，即90G数据，宏基因组中要求较完整获得相对丰度1%的细菌基因组，测序量为：5 MB × 30x ÷ 1% = 15GB
- 覆盖度Coverage：测序获得的序列占整个基因组的比例，如97%即3%没测到。

https://wenku.baidu.com/view/e40659fe9e3143323968937a.html

组装软件评估

- 组装结果中存在大量错误
- 高复杂度的宏基因组推荐使用**MetaSPAdes** (Citated 1466)

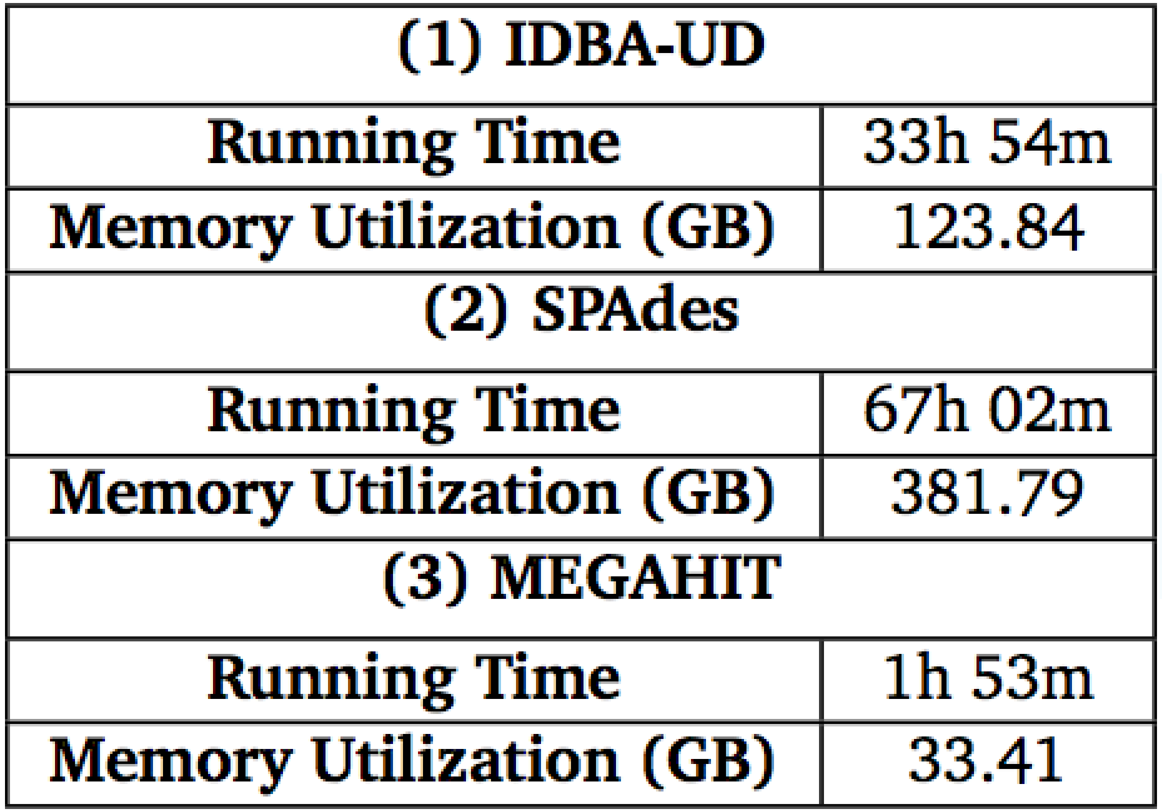
  Nurk, S., Meleshko, D., Korobeynikov, A. & Pevzner, P. A. metaSPAdes: a new versatile metagenomic assembler. ***Genome Research*** **27**, 824-834, doi:10.1101/gr.213959.116 (2017).
- 低复杂度的宏基因组推荐使用MaSuRCA (Citated 948)

  Zimin, A. V. *et al.* The MaSuRCA genome assembler. ***Bioinformatics*** **29**, 2669-2677, doi:10.1093/bioinformatics/btt476 (2013).
- **MEGAHIT是最保守的组装软件，拥有最小的N50和错误率** (Citated 2377)

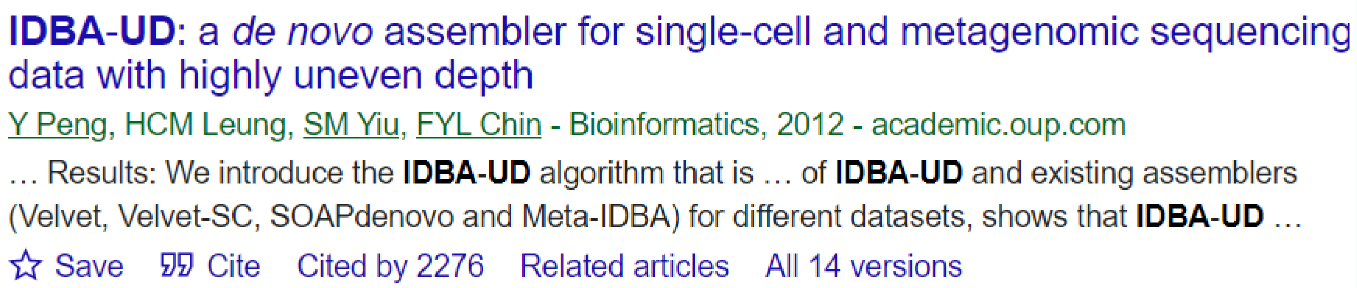
  Li, D., Liu, C.-M., Luo, R., Sadakane, K. & Lam, T.-W. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. ***Bioinformatics*** **31**, 1674-1676, doi:10.1093/bioinformatics/btv033 (2015).

Forouzan, E., Shariati, P., Mousavi Maleki, M. S., Karkhane, A. A. & Yakhchali, B. Practical evaluation of 11 de novo assemblers in metagenome assembly. *Journal of Microbiological Methods* **151**, 99-105, doi:https://doi.org/10.1016/j.mimet.2018.06.007 (2018).

拼接软件时间和内存比较



(1) IDBA-UD	
Running Time	33h 54m
Memory Utilization (GB)	123.84
(2) SPAdes	
Running Time	67h 02m
Memory Utilization (GB)	381.79
(3) MEGAHIT	
Running Time	1h 53m
Memory Utilization (GB)	33.41



IDBA-UD: a *de novo* assembler for single-cell and metagenomic sequencing data with highly uneven depth

Y Peng, HCM Leung, SM Yiu, FYL Chin - Bioinformatics, 2012 - academic.oup.com

… Results: We introduce the **IDBA-UD** algorithm that is … of **IDBA-UD** and existing assemblers (Velvet, Velvet-SC, SOAPdenovo and Meta-IDBA) for different datasets, shows that **IDBA-UD** …

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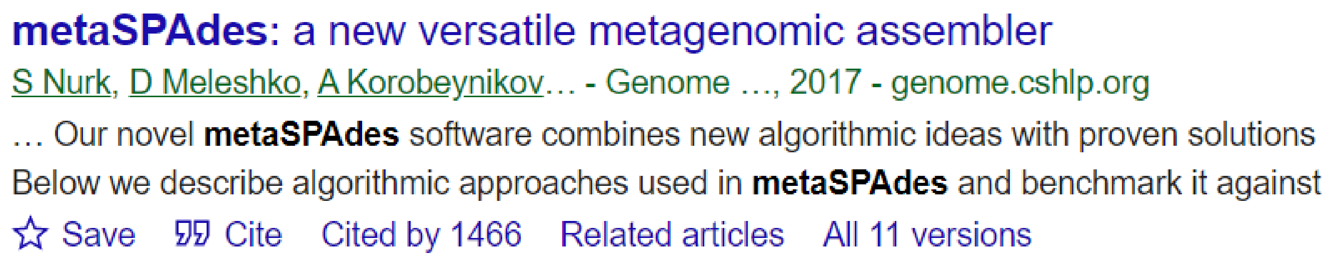
IDBA-UD：组装非均匀覆盖度的宏基因组和单细胞数据



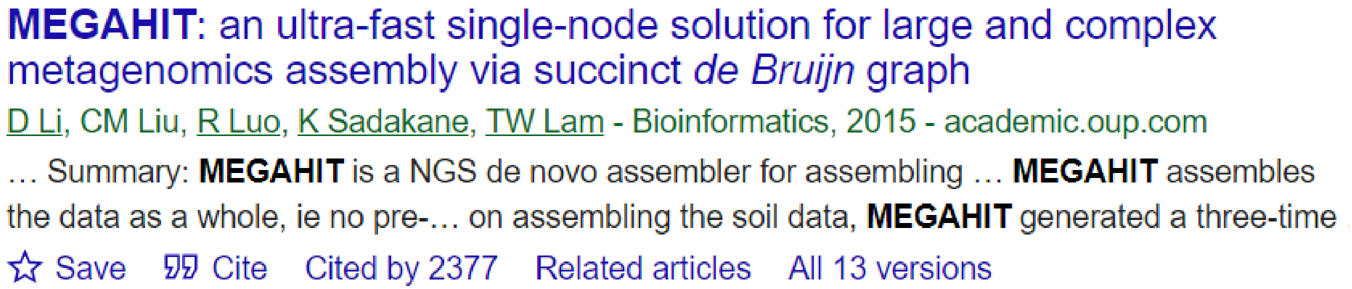
metaSPAdes: a new versatile metagenomic assembler

S Nurk, D Meleshko, A Korobeynikov… - Genome …, 2017 - genome.cshlp.org

… Our novel **metaSPAdes** software combines new algorithmic ideas with proven solutions Below we describe algorithmic approaches used in **metaSPAdes** and benchmark it against

☆ Save 99 Cite Cited by 1466 Related articles All 11 versions

metaSPAdes：新型多功能宏基因组拼接工具



MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct *de Bruijn* graph

D Li, CM Liu, R Luo, K Sadakane, TW Lam - Bioinformatics, 2015 - academic.oup.com

… Summary: **MEGAHIT** is a NGS de novo assembler for assembling … **MEGAHIT** assembles the data as a whole, ie no pre-… on assembling the soil data, **MEGAHIT** generated a three-time

☆ Save 99 Cite Cited by 2377 Related articles All 13 versions

MEGAHIT：复杂宏基因组拼接的超快速解决方案

综述metaSPAdes、IDBA-UD、MetaQuast、Prokka、metaProdigal

MEGAHIT——多快好省的组装神器

- 最快，最省内存，且在宏基因组拼接中质量可接受的软件
- -h显示参数详细
- -1/2左或右端文件，支持多文件；--12双端交替(interleave)的单文件；-r单端
- -t设置线程数，默认全用
- --use-gpu 支持GPU运算
- --continue 支持中断继续运行
- --k-min 27 --k-max 191 --k-step 20 手动设置kmer，**调整速度&精度**

组装拼接MEGAHIT(多快好省)和评估quast

MEGAHIT文章解读

Li, Dinghua, et al. "MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph." *Bioinformatics* 31.10 (2015): 1674-1676.
Li, Dinghua, et al. "MEGAHIT v1. 0: A fast and scalable metagenome assembler driven by advanced methodologies and community practices." *Methods* 102 (2016): 3-11.

3.1.1 MEGAHIT拼接

- **方法1. 混合组装(少量样本的推荐)**

  优点：简单快速获得一套参考序列，基因冗余度低，混合增加低丰度菌测序深度并且提高拼接长度和完整度
  缺点：需要更大内存，混样提高错误拼接、嵌合体风险，高丰度区域碎片化

- 方法2. 单样本组装(大量样本推荐)

  优点：内存资源消耗少，防止样本间污染和嵌合体组装，高丰度菌重叠群更长
  缺点：低丰度菌难组装较完整，样品间基因大量冗余，去冗余计算时间长

- 方法3. 混合+单样本组装(样本量可完成计算下推荐)

  优点：混合提高低丰度覆盖度，单样本防止样品间混淆，基因最完整
  缺点：计算资源和时间消耗大，下游基因注释、去冗余时间长

组装拼接MEGAHIT(多快好省)和评估quast   MEGAHIT文章解读

MEGAHIT拼接，混合快，单样本累计慢

```
# 组装，10~30m，TB级数据需几天至几周
time megahit -t 6 \
    -1 `tail -n+2 result/metadata.txt|cut -f 1|sed 's/^/temp\/qc\//;s/$/_1.fastq/'| tr '\n' ','|sed 's/,$//'` \
    -2 `tail -n+2 result/metadata.txt|cut -f 1|sed 's/^/temp\/qc\//;s/$/_2.fastq/'| tr '\n' ','|sed 's/,$//'` \
    -o temp/megahit
# -t设置线程数量，默认使用所有线程，可能会影响其他人工作
# -1/2输入文件：反引号(``)使用shell命令基于元数据获得输入文件列表
# -o 输出目录，必须不存在，否则需要删除再运行
# 超过300GB，k-mer尽量调大，如29+，否则会超软件上限
```

增加参数加速：--k-min 29 --k-max 141 --k-step 20

组装拼接MEGAHIT(多快好省)和评估quast MEGAHIT文章解读

3.1.2 metaSPAdes精细拼接

- 主页：http://cab.spbu.ru/software/spades/
- conda install spades # 安装软件
- metaspades.py -h # 查看帮助
- Meta帮助: http://cab.spbu.ru/files/release3.12.0/manual.html#meta
- metaspades.py --test # 运行测试数据
- 此软件 --iontorrent 支持PGM数据，甚至支持--pacbio和--nanopore三代测序数据
- 原文简介：metaSPAdes：新型多功能宏基因组拼接工具

Nurk, Sergey, et al. "metaSPAdes: a new versatile metagenomic assembler." *Genome research* (2017): gr-213959.

(可选) Metaspades组装，混合慢，单样本更快

```
# 混合组装：6线程 15分钟，内存100G
time metaspades.py -t 6 -m 100 \
  `tail -n+2 result/metadata.txt|cut -f 1|sed 's/^/temp\/qc\//;s/$/_1.fastq/'|sed 's/^/-1 /'|
tr '\n' ' '` \
  `tail -n+2 result/metadata.txt|cut -f 1|sed 's/^/temp\/qc\//;s/$/_2.fastq/'|sed 's/^/-2 /'|
tr '\n' ' '` \
  -o temp/metaspades
# t控制线程，m控制内存上限，反引号(``)使用shell命令基于元数据获得输入文件
-1 temp/qc/C1_1.fastq -1 temp/qc/C2_1.fastq ……

# 23M，contigs体积更大，megahit仅为8.3M
ls -sh temp/metaspades/contigs.fasta
```

90G土壤样本，2T内存，1个月没完成。相同数据量，不同数据复杂度消耗时间可差数十至数百倍。

Metaspades二、三代混合组装(提高片段长度)

- 以Illumina和Nanopore数据为例
- # 3G数据，耗时3h

```
i=SampleA
time metaspades.py -t 48 -m 500 \
  -1 seq/${i}_1.fastq -2 seq/${i}L_2.fastq \
  --nanopore seq/${i}.fastq \
  -o temp/metaspades_${i}
```

OPERA-MS二、三代混合拼接

- OPERA-MS是发表于Nature Biotechnology的专业二、三代混合组装工具，基于对短读长megahit/metaspades的组装结果，再进行组装以提高片段长度。

```
perl ../OPERA-MS.pl \
    --short-read1 R1.fastq.gz \
    --short-read2 R2.fastq.gz \
    --long-read long_read.fastq \
    --no-ref-clustering --num-processors 24 \
    --out-dir RESULTS
```

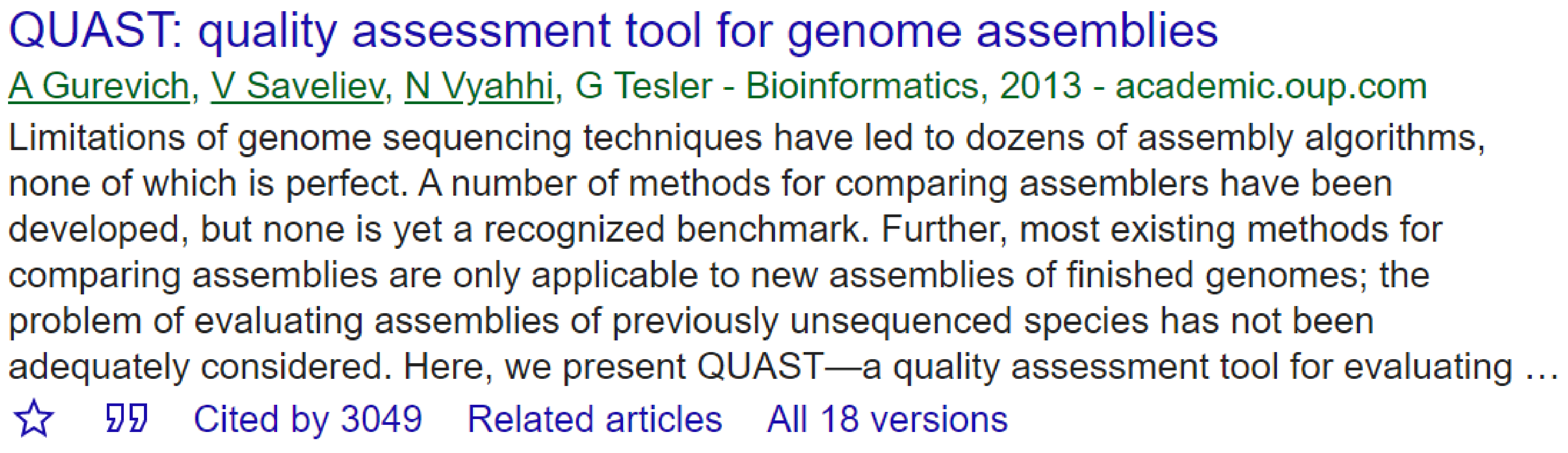
•NBT：宏基因组二、三代混合组装软件OPERA-MS文章解读 软件使用

OPERA-MS二代组装+三代优化

- 结果卡在第9步polishing，可添加--no-polishing参数跳过此步；短序列只支持成对文件，多个文件需要cat合并

```
perl ~/soft/OPERA-MS/OPERA-MS.pl \
    --contig-file temp/megahit/final.contigs.fa \
    --short-read1 R1.fastq.gz \
    --short-read2 R2.fastq.gz \
    --long-read long_read.fastq \
    --num-processors 32 \
    --no-ref-clustering \
    --no-strain-clustering \
    --no-polishing \
    --out-dir temp/opera
```

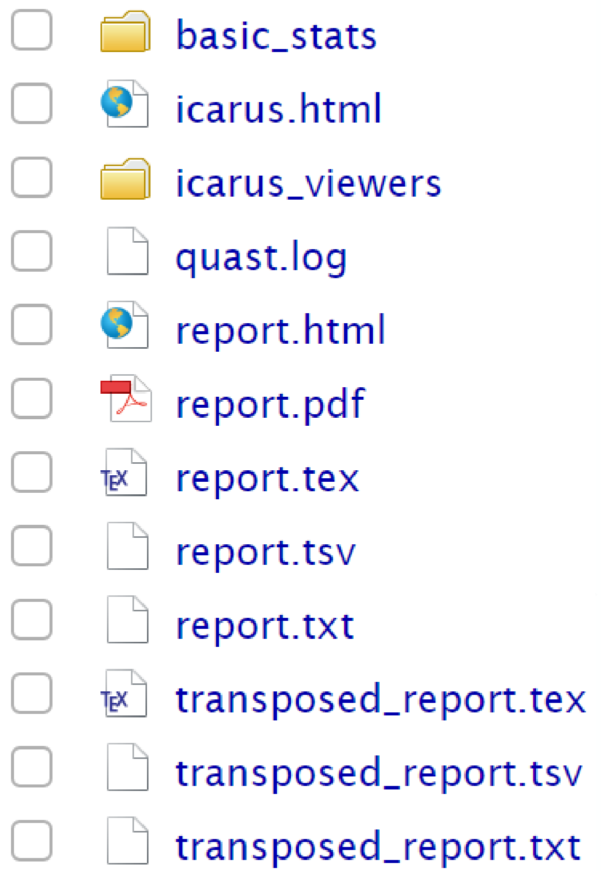
3.1.3 QUAST评估



QUAST: quality assessment tool for genome assemblies

A Gurevich, V Saveliev, N Vyahhi, G Tesler - Bioinformatics, 2013 - academic.oup.com

Limitations of genome sequencing techniques have led to dozens of assembly algorithms, none of which is perfect. A number of methods for comparing assemblers have been developed, but none is yet a recognized benchmark. Further, most existing methods for comparing assemblies are only applicable to new assemblies of finished genomes; the problem of evaluating assemblies of previously unsequenced species has not been adequately considered. Here, we present QUAST—a quality assessment tool for evaluating …

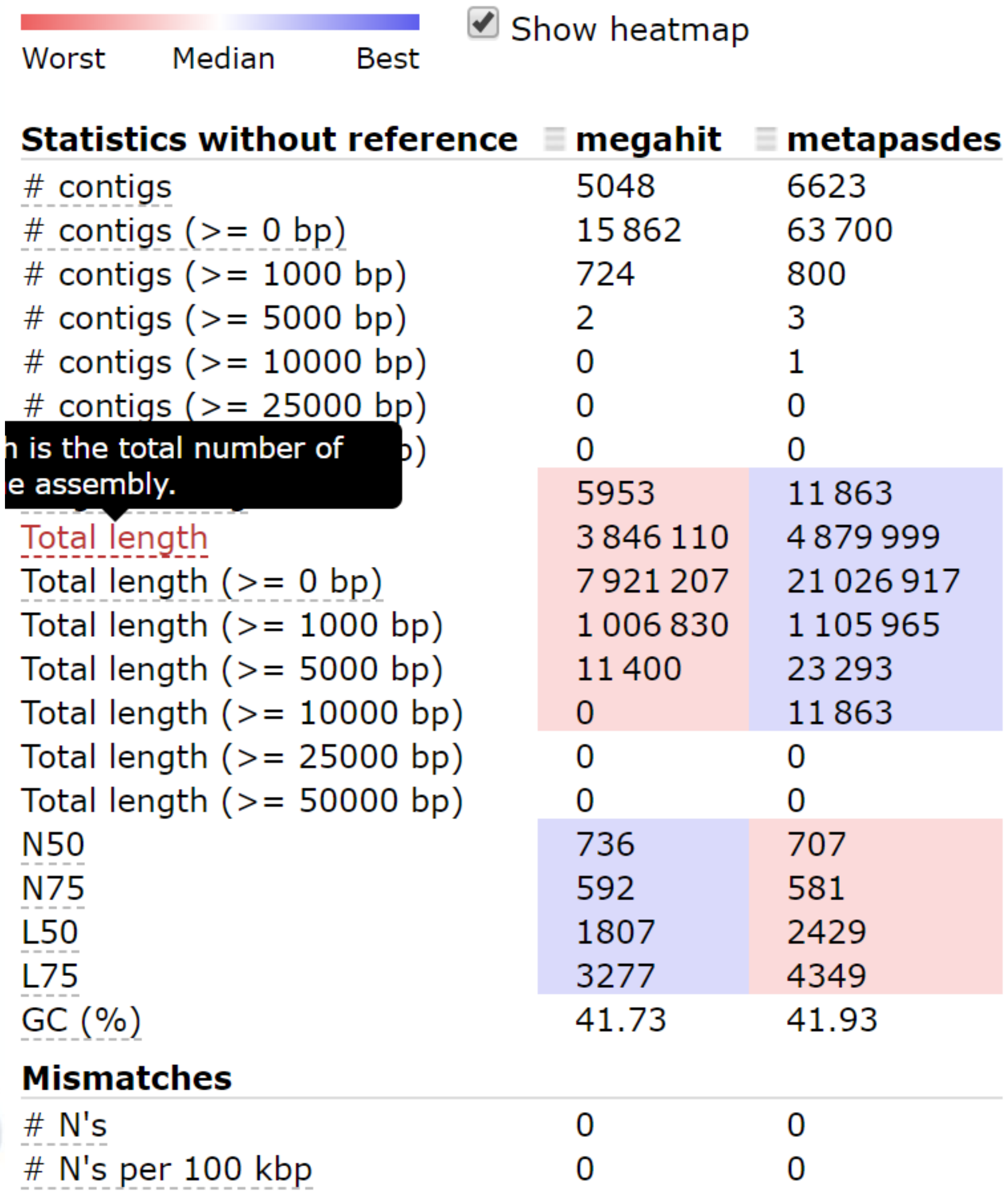


□ basic_stats
□ icarus.html
□ icarus_viewers
□ quast.log
□ report.html
□ report.pdf
□ report.tex
□ report.tsv
□ report.txt
□ transposed_report.tex
□ transposed_report.tsv
□ transposed_report.txt

```
quast.py -h # 显示帮助，评估单个组装结果，生成网页报告
quast.py temp/megahit/final.contigs.fa -o result/megahit/quast
# 评估多种组装结果
quast.py --label "megahit,metapasdes" temp/megahit/final.contigs.fa \
temp/metaspades/contigs.fasta -o temp/quast
```

软件安装和使用指南帮助 MetaQuast：评估宏基因组拼接

评估结果：megahit vs metaspades



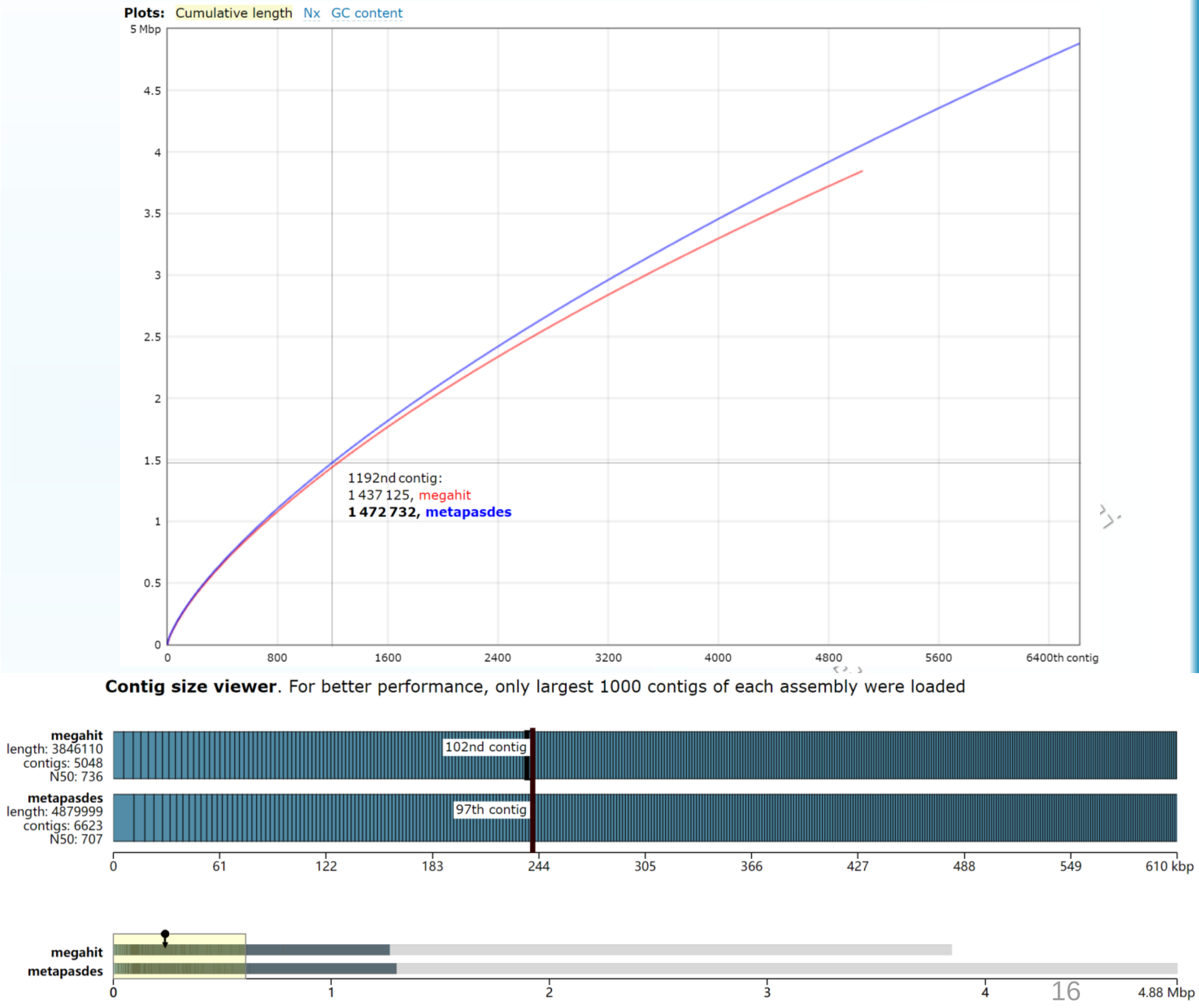
Worst Median Best

☑ Show heatmap

n is the total number of
e assembly.

Statistics without reference	megahit	metapasdes
# contigs	5048	6623
# contigs (>= 0 bp)	15 862	63 700
# contigs (>= 1000 bp)	724	800
# contigs (>= 5000 bp)	2	3
# contigs (>= 10000 bp)	0	1
# contigs (>= 25000 bp)	0	0
p)	0	0
	5953	11 863
Total length	3 846 110	4 879 999
Total length (>= 0 bp)	7 921 207	21 026 917
Total length (>= 1000 bp)	1 006 830	1 105 965
Total length (>= 5000 bp)	11 400	23 293
Total length (>= 10000 bp)	0	11 863
Total length (>= 25000 bp)	0	0
Total length (>= 50000 bp)	0	0
N50	736	707
N75	592	581
L50	1807	2429
L75	3277	4349
GC (%)	41.73	41.93

Mismatches		
# N's	0	0
# N's per 100 kbp	0	0



Plots: Cumulative length Nx GC content

Contig size viewer. For better performance, only largest 1000 contigs of each assembly were loaded

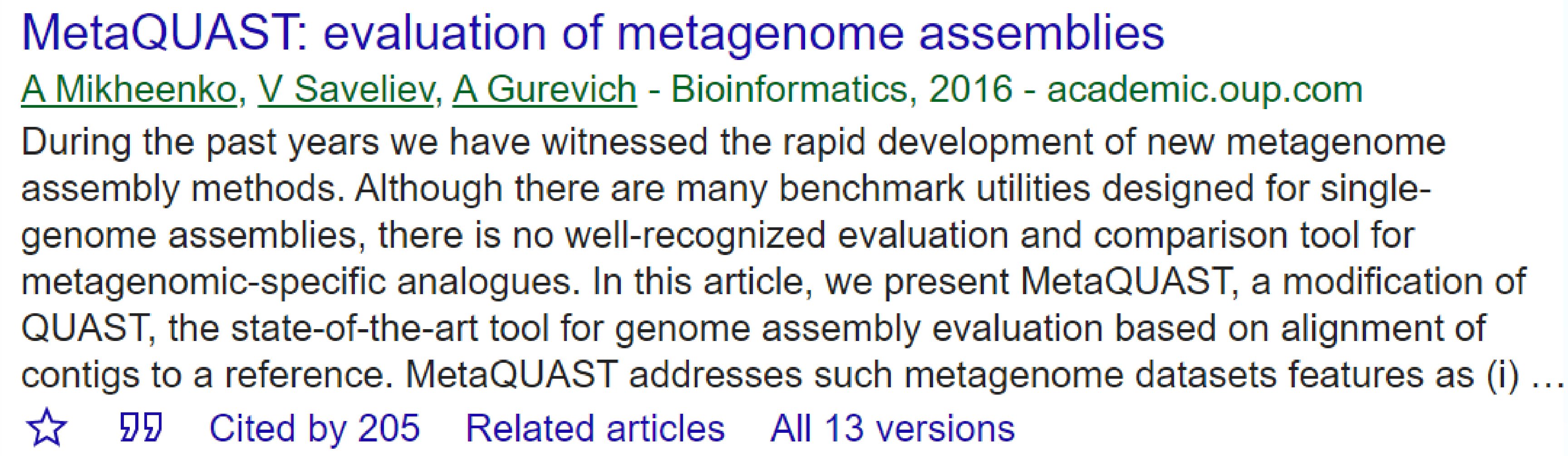
megahit
length: 3846110
contigs: 5048
N50: 736

metapasdes
length: 4879999
contigs: 6623
N50: 707

(可选) MetaQUAST评估基因组完整度

# 依赖数据库更全面评估，下载SILVA数据库确定细菌种类；然后在NCBI下载最高丰度的50个株的基因组，分析覆盖度(数据下载受网络限制，可能需很久，我测试下载极慢)

```
metaquast.py result/megahit/final.contigs.fa -o result/megahit/metaquast
```



MetaQUAST: evaluation of metagenome assemblies

A Mikheenko, V Saveliev, A Gurevich - Bioinformatics, 2016 - academic.oup.com

During the past years we have witnessed the rapid development of new metagenome assembly methods. Although there are many benchmark utilities designed for single-genome assemblies, there is no well-recognized evaluation and comparison tool for metagenomic-specific analogues. In this article, we present MetaQUAST, a modification of QUAST, the state-of-the-art tool for genome assembly evaluation based on alignment of contigs to a reference. MetaQUAST addresses such metagenome datasets features as (i) …

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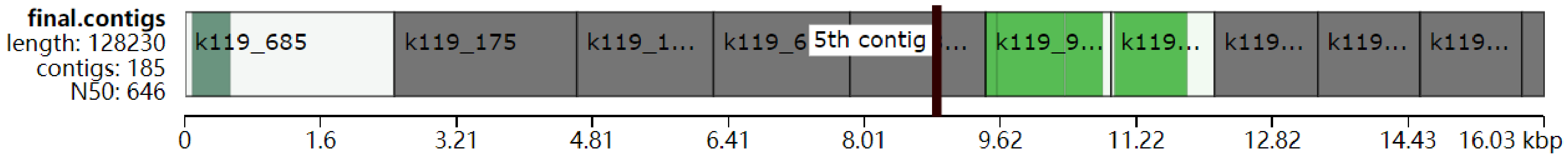
[MetaQuast：评估宏基因组拼接](#)

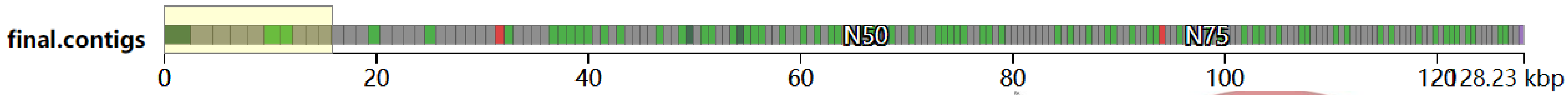
MetaQuast结果：评估错误组装、错配和插入缺失

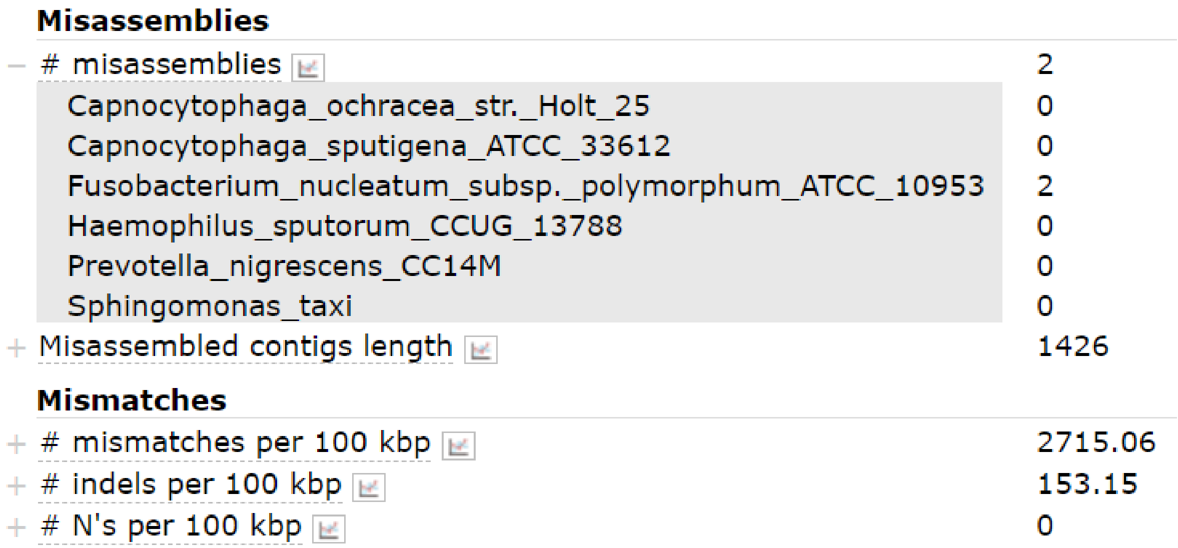
Contig size viewer

结果见：result/megahit/metaquast/report.html

View in Icarus contig browser —— Contig size viewer







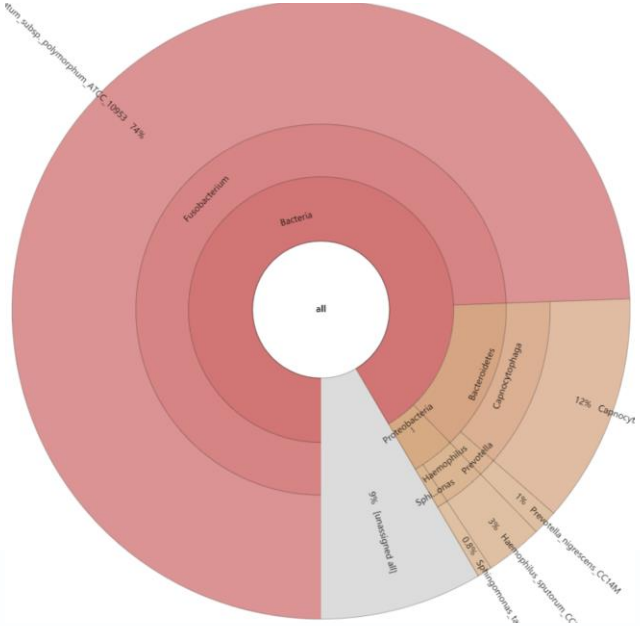
Misassemblies

− # misassemblies	2
Capnocytophaga_ochracea_str._Holt_25	0
Capnocytophaga_sputigena_ATCC_33612	0
Fusobacterium_nucleatum_subsp._polymorphum_ATCC_10953	2
Haemophilus_sputorum_CCUG_13788	0
Prevotella_nigrescens_CC14M	0
Sphingomonas_taxi	0
+ Misassembled contigs length	1426

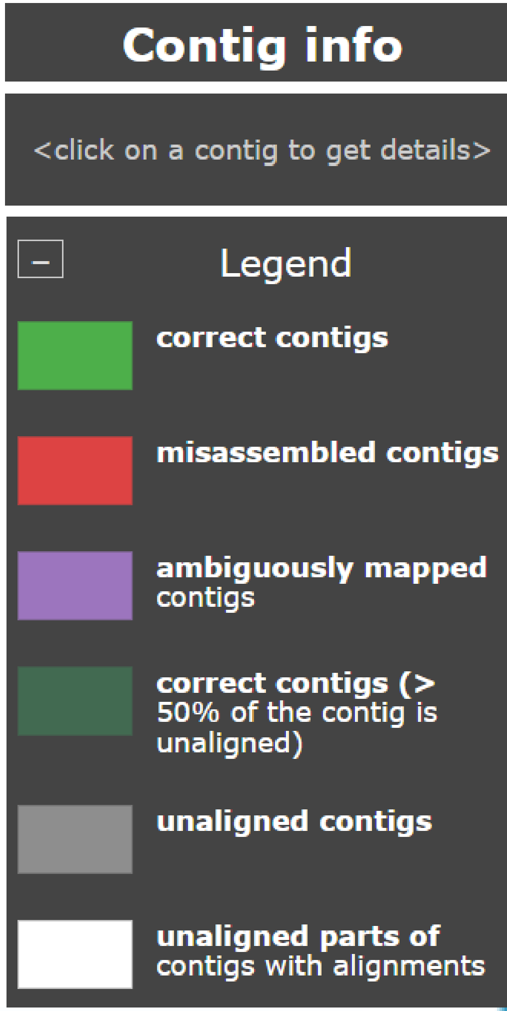
Mismatches

+ # mismatches per 100 kbp	2715.06
+ # indels per 100 kbp	153.15
+ # N's per 100 kbp	0

评估错误组装、错配和插入缺失



Krona charts: final.contigs



Contig info

<click on a contig to get details>

− Legend

🟩	correct contigs
🟥	misassembled contigs
🟪	ambiguously mapped contigs
🟩	correct contigs (> 50% of the contig is unaligned)
⬜	unaligned contigs
⬜	unaligned parts of contigs with alignments

总结

- MEGAHIT快速组装，适合30G~300G范围多样本混合组装，节省计算和内存资源； 默认按95%相似度种水平聚类，无法拼接株水平序列。
- metaSPAdes精细组装，但内存和时间消耗极大，适合单样本分别组装，可以拼接株水平重叠群，30G组装需上百线程1周，90G无法完成；
- 拼接长度和错误率也成正比，N50提高也伴随时嵌合体升高风险；
- 二、三代测序数据混合组装，首选metaSPAdes安装方便，显著提高片段长度；
- 二、三代测序数据混合组装OPERA-MS无Conda安装麻烦，但速度较快；
- QUAST快速评估常用组装指标，提供html/pdf报告，支持多个组装结果共同评估和比较；
- metaQUAST基于参考数据库进行更细致的评估，但下载成功率不高。

参考资源

- 宏基因组公众号文章目录　　生信宝典公众号文章目录
- 科学出版社《微生物组数据分析》——50+篇
- Bio-protocol《微生物组实验手册》——153篇
- Protein Cell：扩增子和宏基因组数据分析实用指南
- CMJ：人类微生物组研究设计、样本采集和生物信息分析指南
- 加拿大生信网 https://bioinformatics.ca/ 宏基因组课程中文版
- 美国高通量开源课程 https://github.com/ngs-docs
- Curtis Huttenhower http://huttenhower.sph.harvard.edu/
- Nicola Segata http://segatalab.cibio.unitn.it/

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