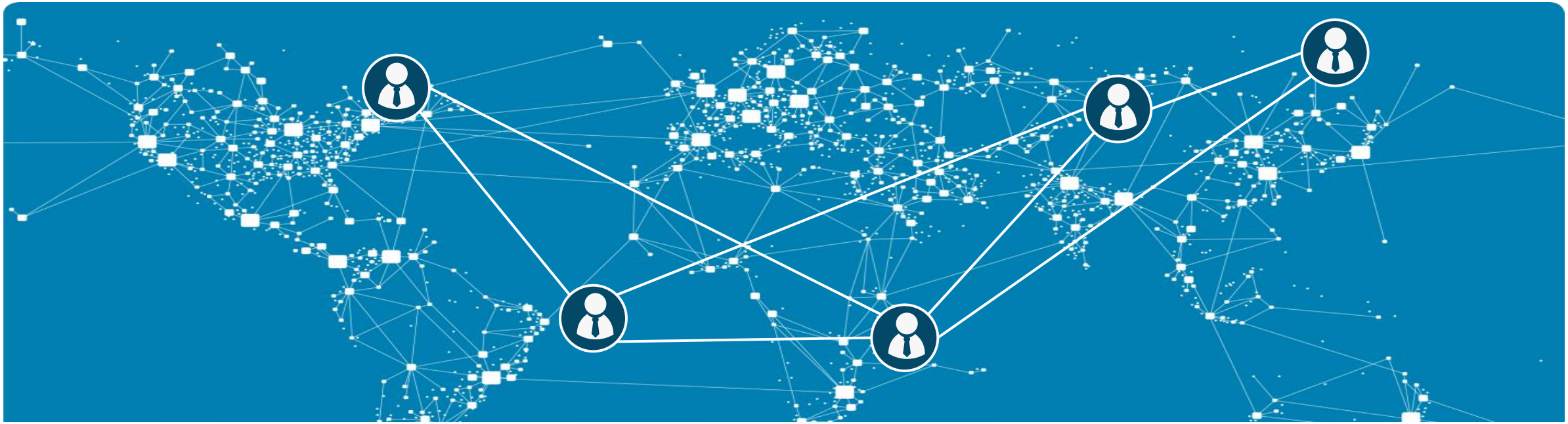
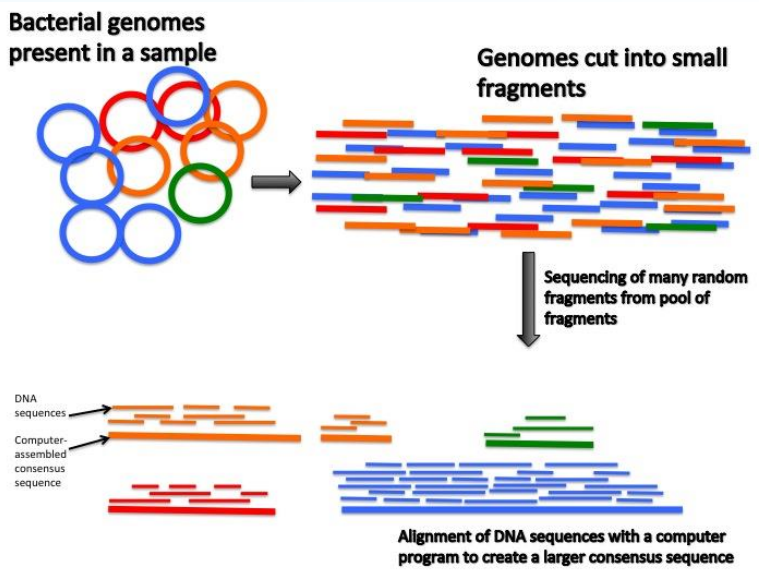
微生物组—宏基因组分析专题研讨会第22期



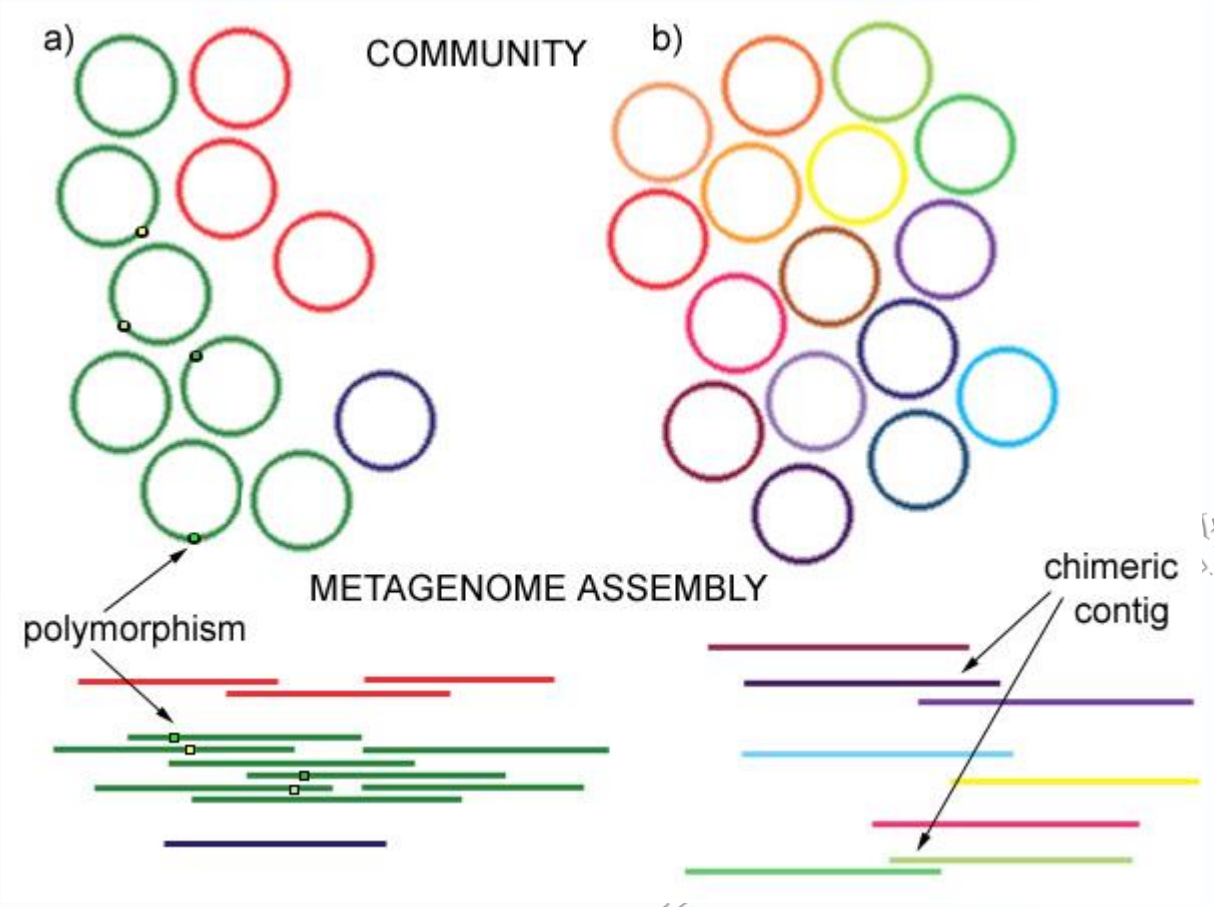
31 拼接/组装Assembly

易生信

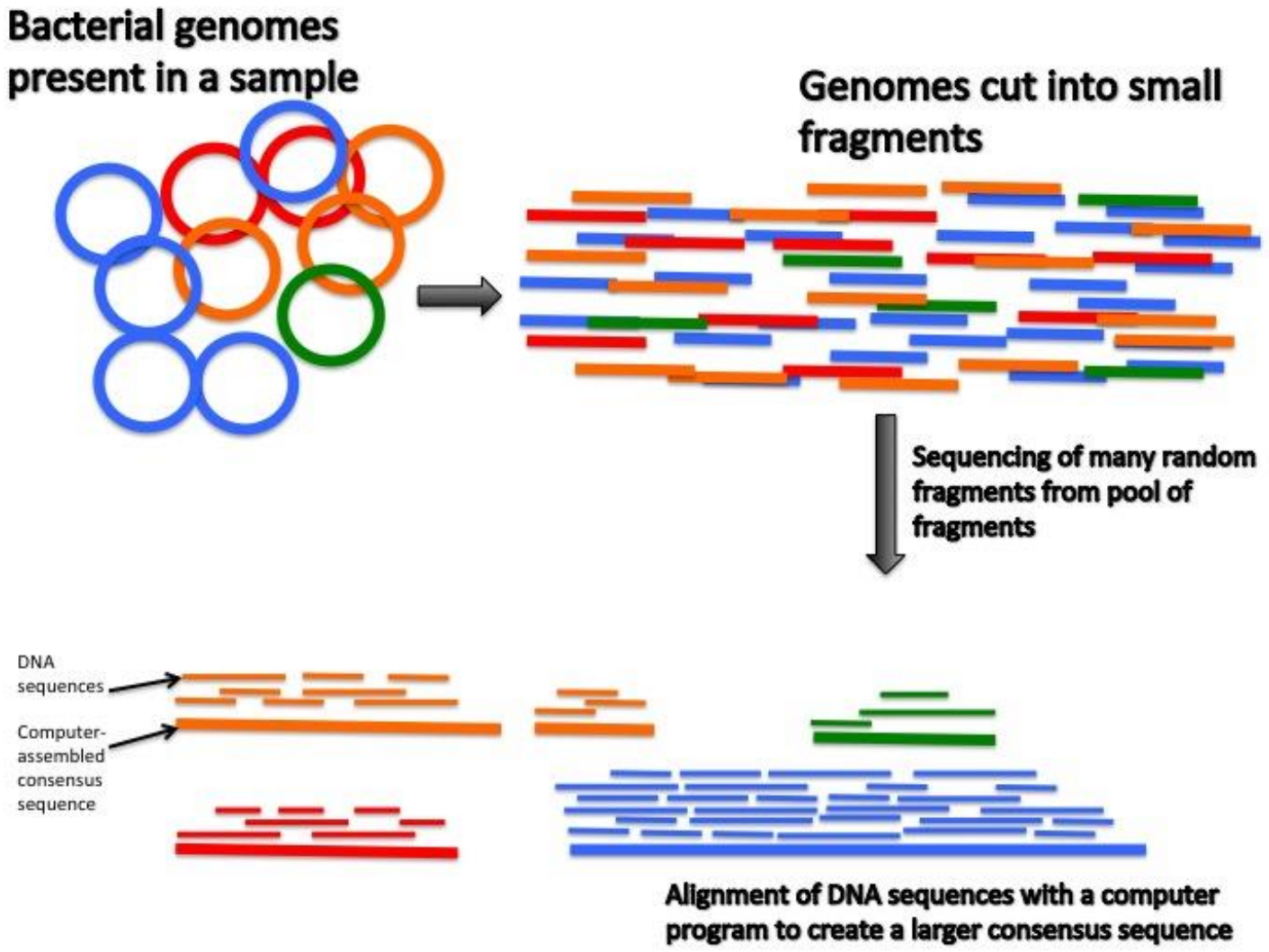
2024年11月10日



目录



组装/拼接 (Assemble)的基本原理



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
- Coverage：覆盖度，测序获得的序列占整个基因组的比例，如97%即3%没测到。

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

Yu Peng, Henry C. M. Leung, S. M. Yiu, *et al.* 2012. IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. ***Bioinformatics*** 28: 1420-1428. https://doi.org/10.1093/bioinformatics/bts174

IDBA-UD：组装非均匀覆盖度的宏基因组和单细胞数据

Sergey Nurk, Dmitry Meleshko, Anton Korobeynikov, *et al.* 2017. metaSPAdes: a new versatile metagenomic assembler. ***Genome Research*** 27: 824-834. https://doi.org/10.1101/gr.213959.116

metaSPAdes：新型多功能宏基因组拼接工具

Dinghua Li, Chi-Man Liu, Ruibang Luo, *et al.* 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. ***Bioinformatics*** 31: 1674-1676. https://doi.org/10.1093/bioinformatics/btv033

MEGAHIT：复杂宏基因组拼接的超快速解决方案

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\/hr\//;s/$/_1.fastq/'| tr '\n' ','|sed 's/,$//'` \
    -2 `tail -n+2 result/metadata.txt|cut -f 1|sed 's/^/temp\/hr\//;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
```

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\/hr\//;s/$/_1.fastq/'|sed 's/^/-1 /'|
tr '\n' ' '` \
  `tail -n+2 result/metadata.txt|cut -f 1|sed 's/^/temp\/hr\//;s/$/_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/hr/C1_1.fastq -1 temp/hr/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 ~/soft/OPERA-MS/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
```

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
```

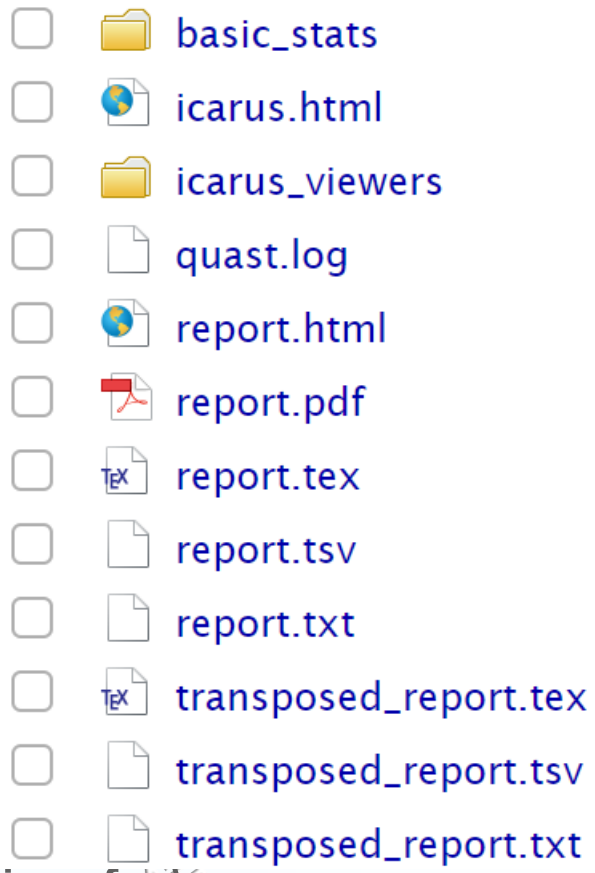
3.1.3 QUAST评估

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 …

☆ 99 Cited by 3049 Related articles All 18 versions




```
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
```

评估结果：megahit vs metaspades

Worst Median Best

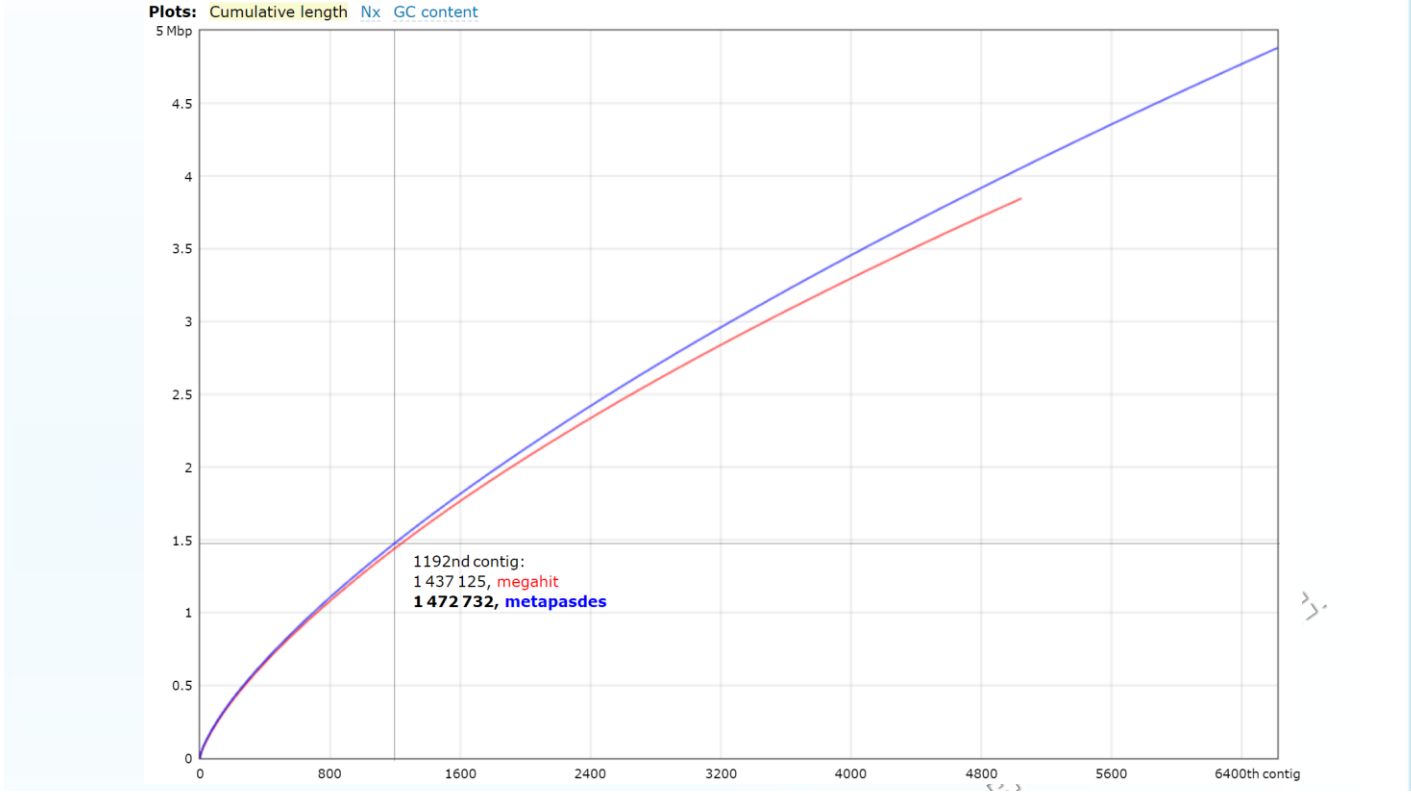
☑ Show heatmap

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
# contigs (>= 50000 bp)	0	0
Largest contig	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

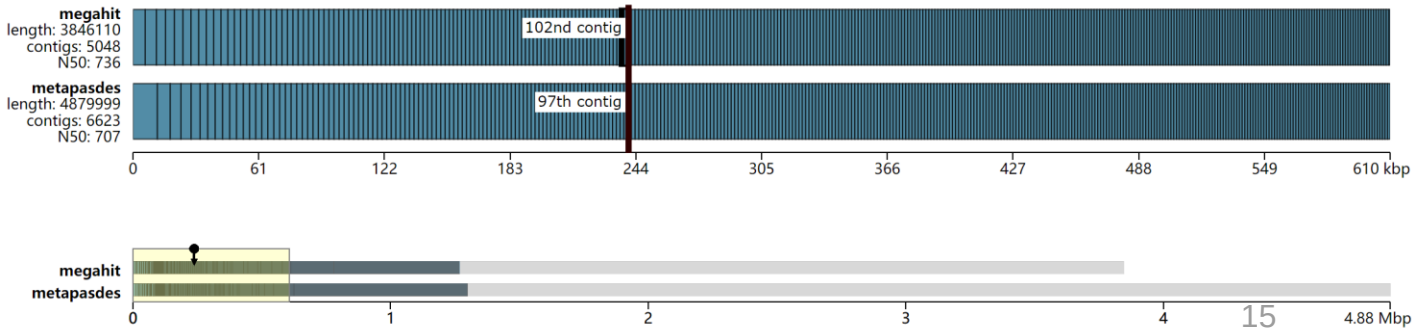
is the total number of assembly.

Mismatches

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



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



(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) …

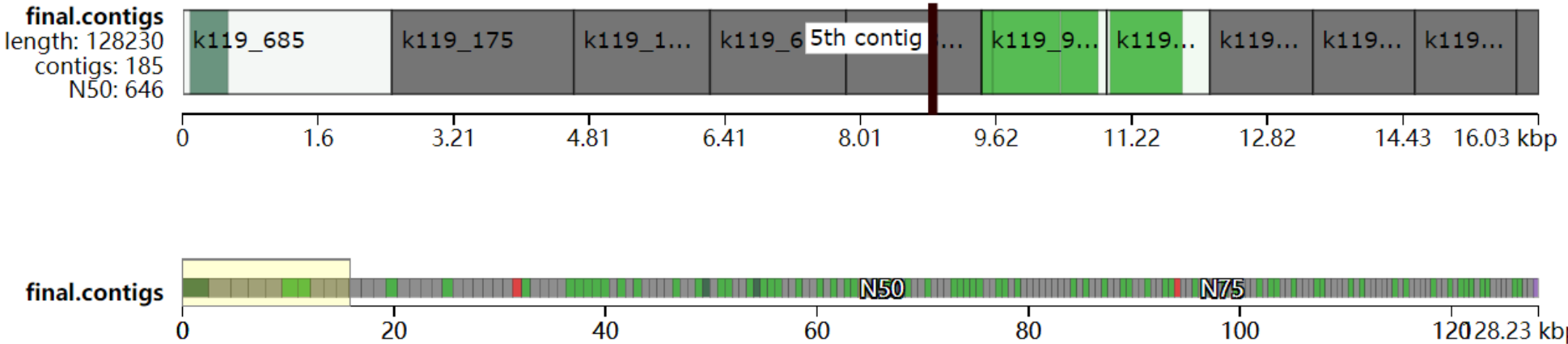
☆ 99 Cited by 205 Related articles All 13 versions

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

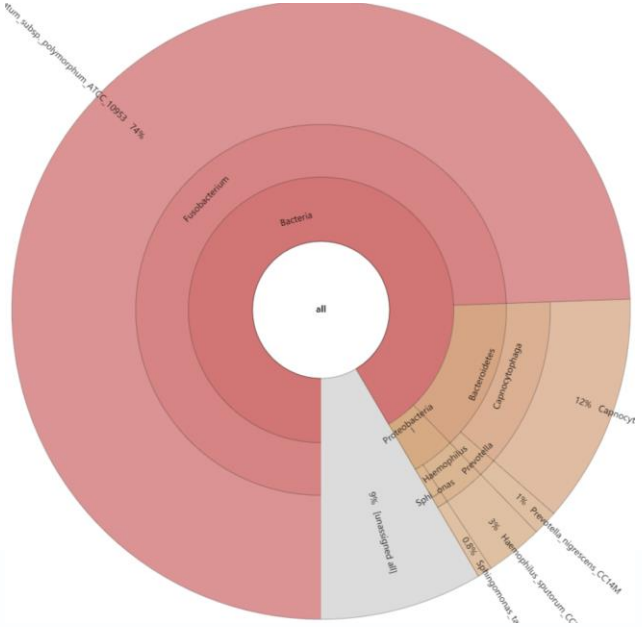


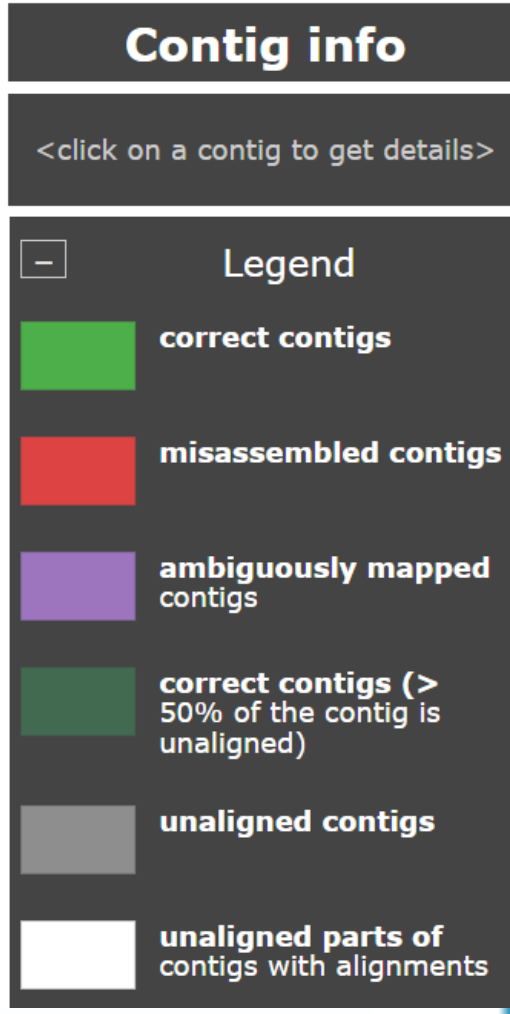
Misassemblies

# 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





Krona charts: final.contigs

评估错误组装、错配和插入缺失

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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