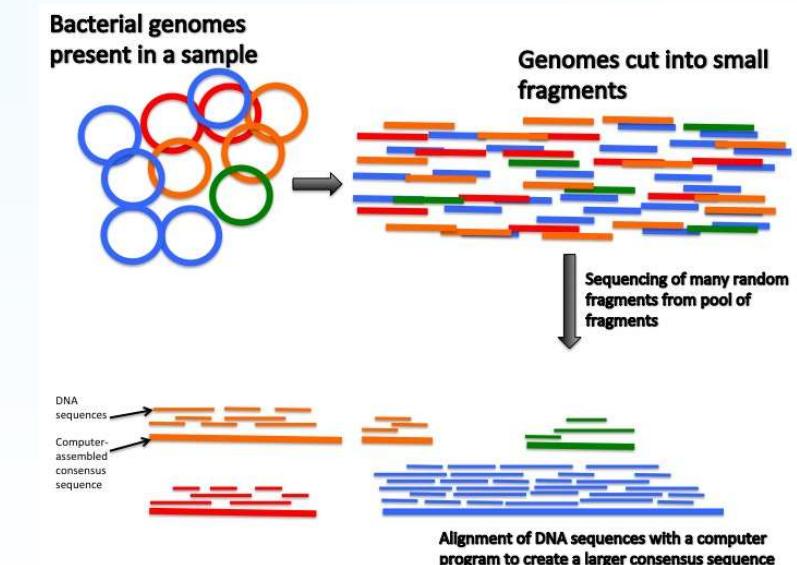




## 31 拼接/组装Assembly

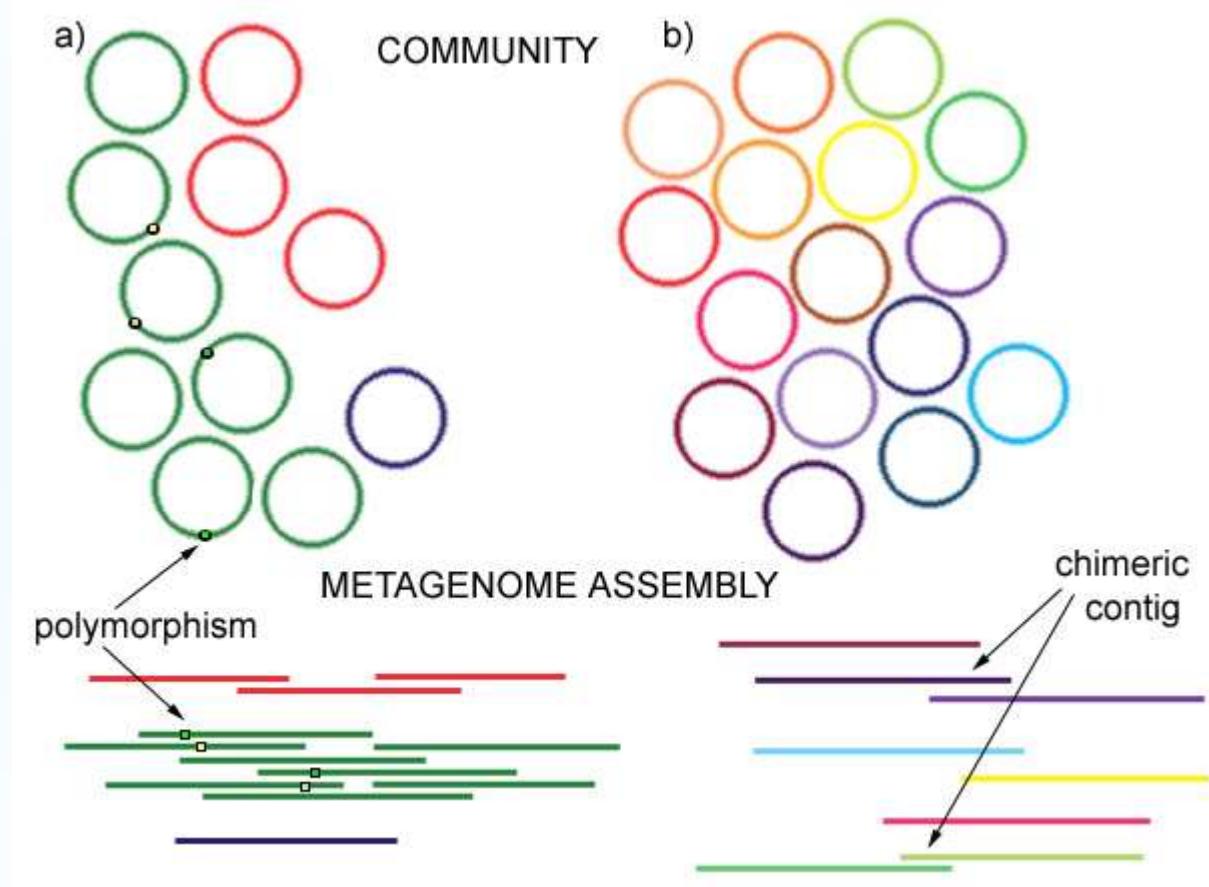
刘永鑫

2025年11月30日

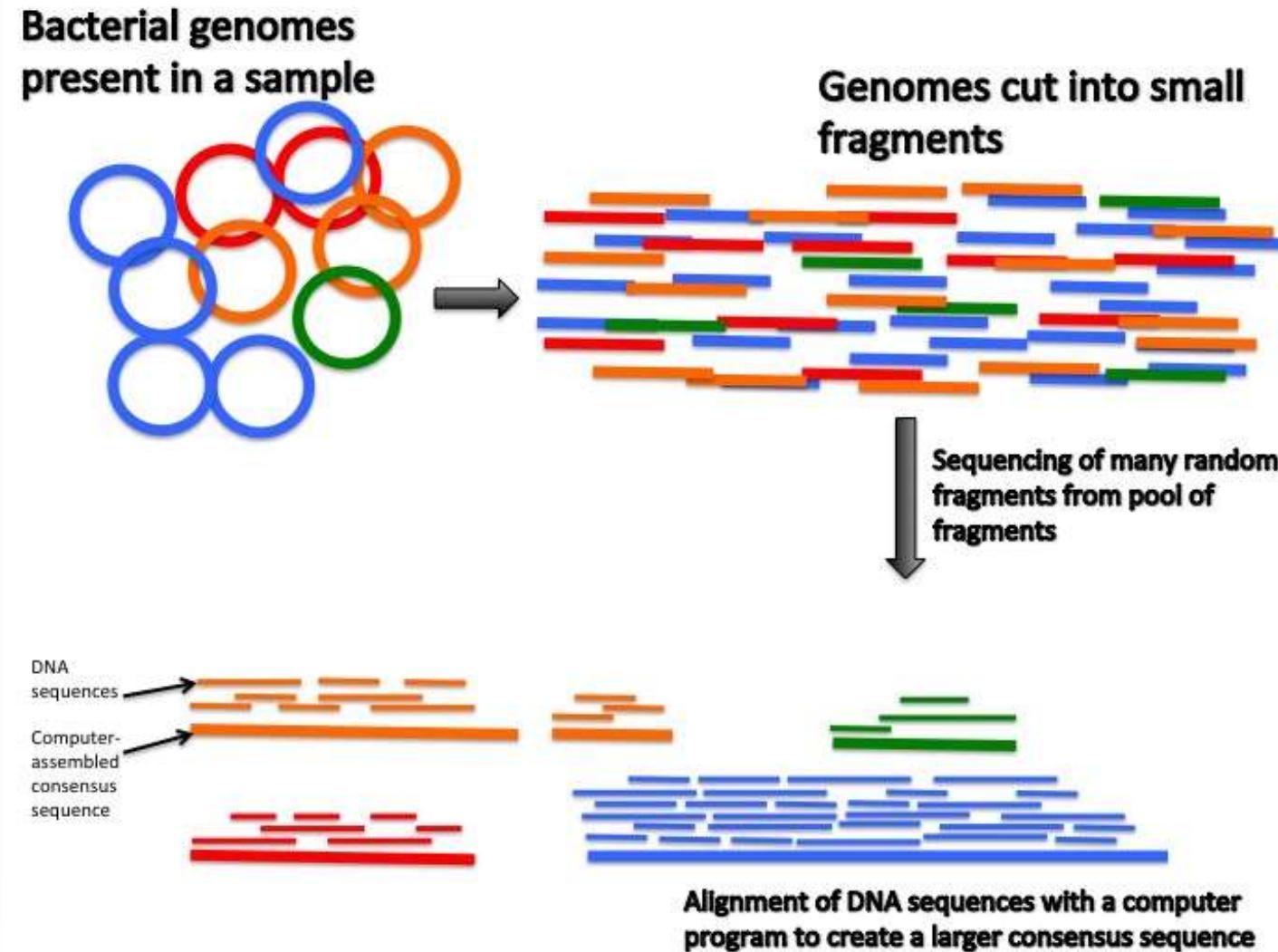


# 目录

- 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



# 组装/拼接 (Assemble) 的基本原理



# 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： $5 \text{ MB} \times 30x \div 1\% = 15\text{GB}$
- 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	
<b>Running Time</b>	33h 54m
<b>Memory Utilization (GB)</b>	123.84
(2) SPAdes	
<b>Running Time</b>	67h 02m
<b>Memory Utilization (GB)</b>	381.79
(3) MEGAHIT	
<b>Running Time</b>	1h 53m
<b>Memory Utilization (GB)</b>	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

# 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](#)

# (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}_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.fasta \
--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 ...

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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,metaspades" temp/megahit/final.contig

temp/metaspades/contigs.fasta -o temp/quast

<input type="checkbox"/>	basic_stats
<input type="checkbox"/>	icarus.html
<input type="checkbox"/>	icarus_viewers
<input type="checkbox"/>	quast.log
<input type="checkbox"/>	report.html
<input type="checkbox"/>	report.pdf
<input type="checkbox"/>	report.tex
<input type="checkbox"/>	report.tsv
<input type="checkbox"/>	report.txt
<input type="checkbox"/>	transposed_report.tex
<input type="checkbox"/>	transposed_report.tsv
<input type="checkbox"/>	transposed_report.txt

# 评估结果：megahit vs metaspades

Worst   Median   Best

Show heatmap

## Statistics without reference

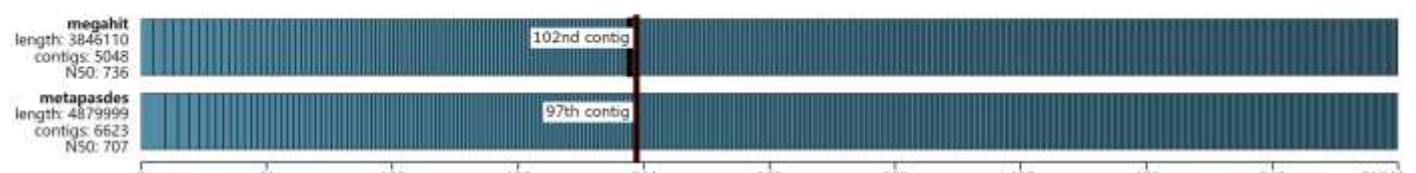
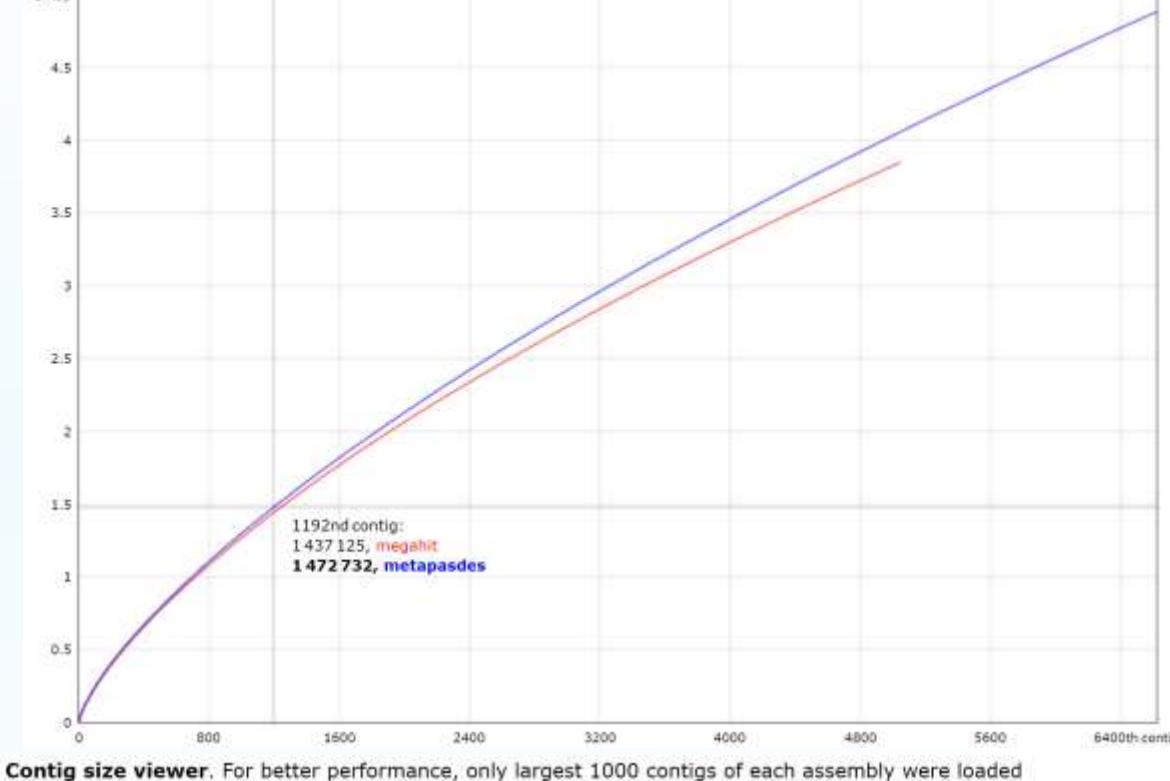
	megahit	metaspades
# contigs	5048	6623
# contigs (>= 0 bp)	15 862	63 700
# contigs (>= 1000 bp)	724	800
# contigs (>= 5000 bp)	2	3
# contigs (>= 10000 bp)	0	1
# contigs (>= 25000 bp)	0	0
n is the total number of contigs in the assembly.	5953	11 863
Total length	3 846 110	4 879 999
Total length (>= 0 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

5 Mbp



# 评估结果：megahit vs metaspades (new)

17 November 2025, Monday, 13:58:53

[View in Icarus contig browser](#)

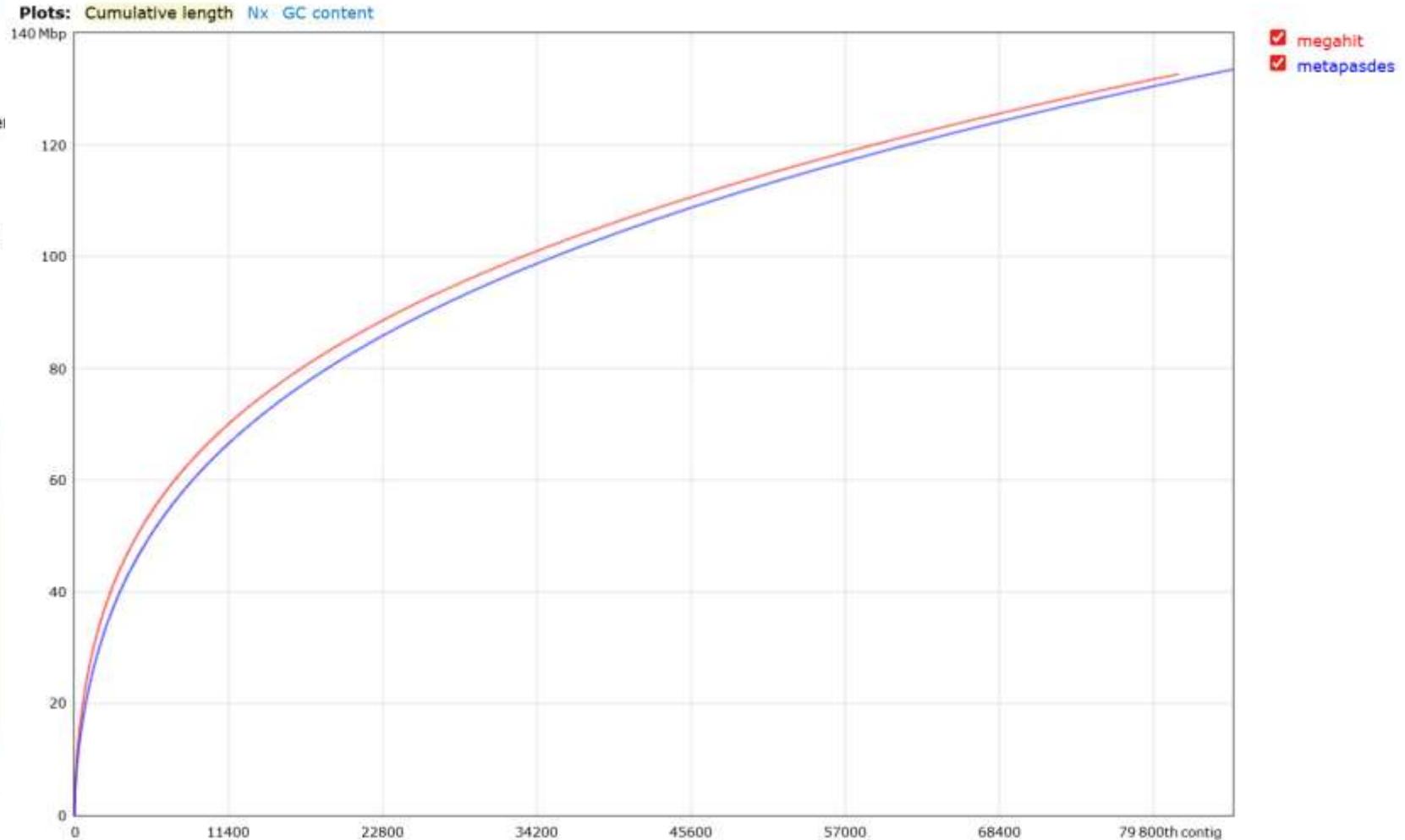
All statistics are based on contigs of size  $\geq 500$  bp, unless otherwise specified.

Worst Median Best  Show heatmap

Statistics without reference	megahit	metaspades
# contigs	81 626	85 694
# contigs ( $\geq 0$ bp)	152 762	361 671
# contigs ( $\geq 1000$ bp)	31 561	33 256
# contigs ( $\geq 5000$ bp)	3734	3759
# contigs ( $\geq 10000$ bp)	1403	1283
# contigs ( $\geq 25000$ bp)	277	212
# contigs ( $\geq 50000$ bp)	60	53
Largest contig	521 987	316 741
Total length	132 645 073	133 490 985
Total length ( $\geq 0$ bp)	159 264 049	211 721 186
Total length ( $\geq 1000$ bp)	98 429 348	97 820 424
Total length ( $\geq 5000$ bp)	46 001 025	42 068 095
Total length ( $\geq 10000$ bp)	30 035 642	25 052 847
Total length ( $\geq 25000$ bp)	13 070 919	9 556 454
Total length ( $\geq 50000$ bp)	5 647 870	4 439 945
N50	2430	2229
N90	641	631
auN	12 529	8822
L50	9769	11 489
L90	58 131	61 821
GC (%)	50.22	50.29

## Per base quality

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



有时几倍时间下metaspades很多指标还变的更差

# (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](#)

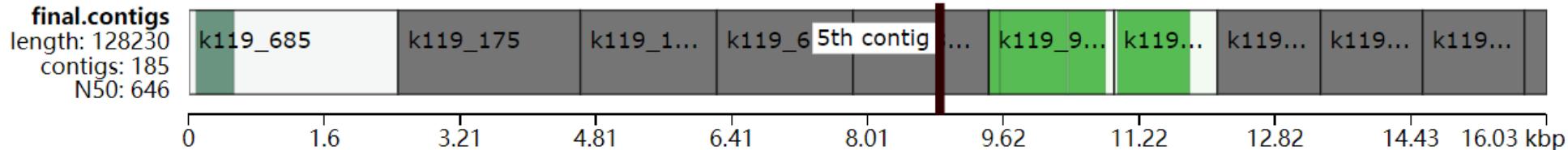
During the past years we have witnessed the rapid development of new metagenome assembly methods. Although there are many benchmark utilities designed for single-genome assemblies, there is no well-recognized evaluation and comparison tool for metagenomic-specific analogues. In this article, we present 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](#)



## Contig info

<click on a contig to get details>



### Legend

correct contigs

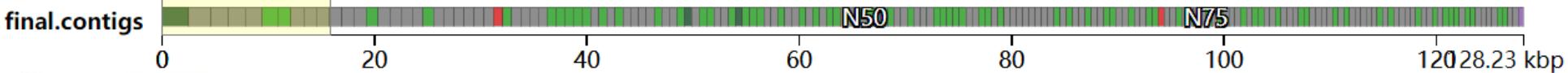
misassembled contigs

ambiguously mapped contigs

correct contigs (> 50% of the contig is unaligned)

unaligned contigs

unaligned parts of contigs with alignments



## Misassemblies

- # misassemblies

- Capnocytophaga\_ochracea\_str.\_Holt\_25
- Capnocytophaga\_sputigena\_ATCC\_33612
- Fusobacterium\_nucleatum\_subsp.\_polymorphum\_ATCC\_10953
- Haemophilus\_sputorum\_CCUG\_13788
- Prevotella\_nigrescens\_CC14M
- Sphingomonas\_taxi

+ Misassembled contigs length

2  
0  
0  
2  
0  
0  
0  
1426

## Mismatches

+ # mismatches per 100 kbp

2715.06

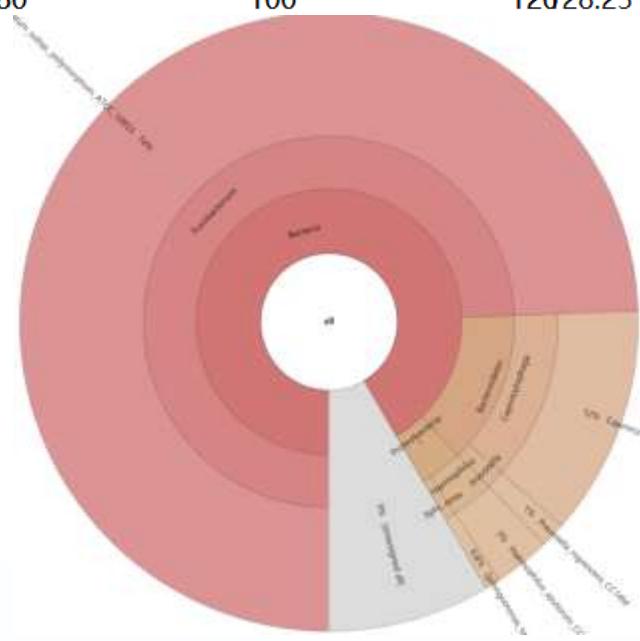
+ # indels per 100 kbp

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](#)

# 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提高也伴随着嵌合体升高风险；
- 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](#)
- [iMeta | 易宏基因组\(EasyMetagenome\): 用户友好且灵活的宏基因组测序数据分析流程](#)
- [iMetaOmics | 易扩增子\(EasyAmplicon\): 用户友好的扩增子测序数据分析指南](#)
- [iMeta | MicrobiomeStatPlot 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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