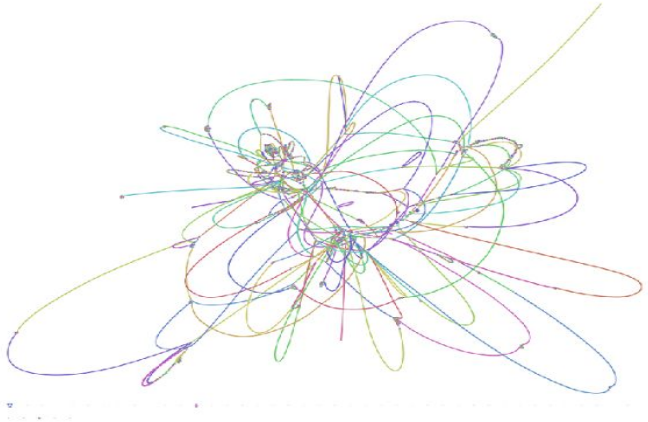


Genome Assembly Preliminary Results

Team 1: Vasanta, Qinwei, Tianze, Seonggeon, Kunal, Siarhei, Victoria, Nirav, Hunter

Presented by Nirav Shah, Hunter Seabolt, and SRR5666627

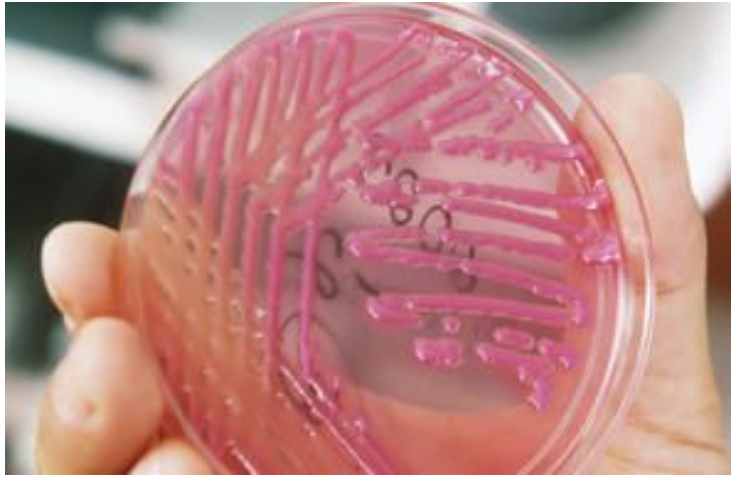
Presentation Outline

- Trim/QC
- Workflow
- Examples
- Comparison and selection of trimming software
- Clean Data

- Assembly and Preliminary Results
- Workflow
- Biological Considerations
- Reference Based genome assembly
- de novo genome assembly
- Comparison of de novo assemblers
- Post-processing
- QC
- Visualization

Data and Background

- Problem:
  - Antibiotic resistance in *Klebsiella*
- Data:
  - 260 isolates of *Klebsiella spp.*
  - 2 x 250 Illumina short reads (MiSeq platform)
- Background:
  - Generic biological characteristics
    - 1 chromosome, likely some plasmids
    - Genome size: ~ 5.3-5.5 Mbp
    - GC content: ~57.1 % GC



https://sciencesource.com/Doc/SCS/Media/TR7/f/3/6/f/SS2294165.jpg?d63641835809

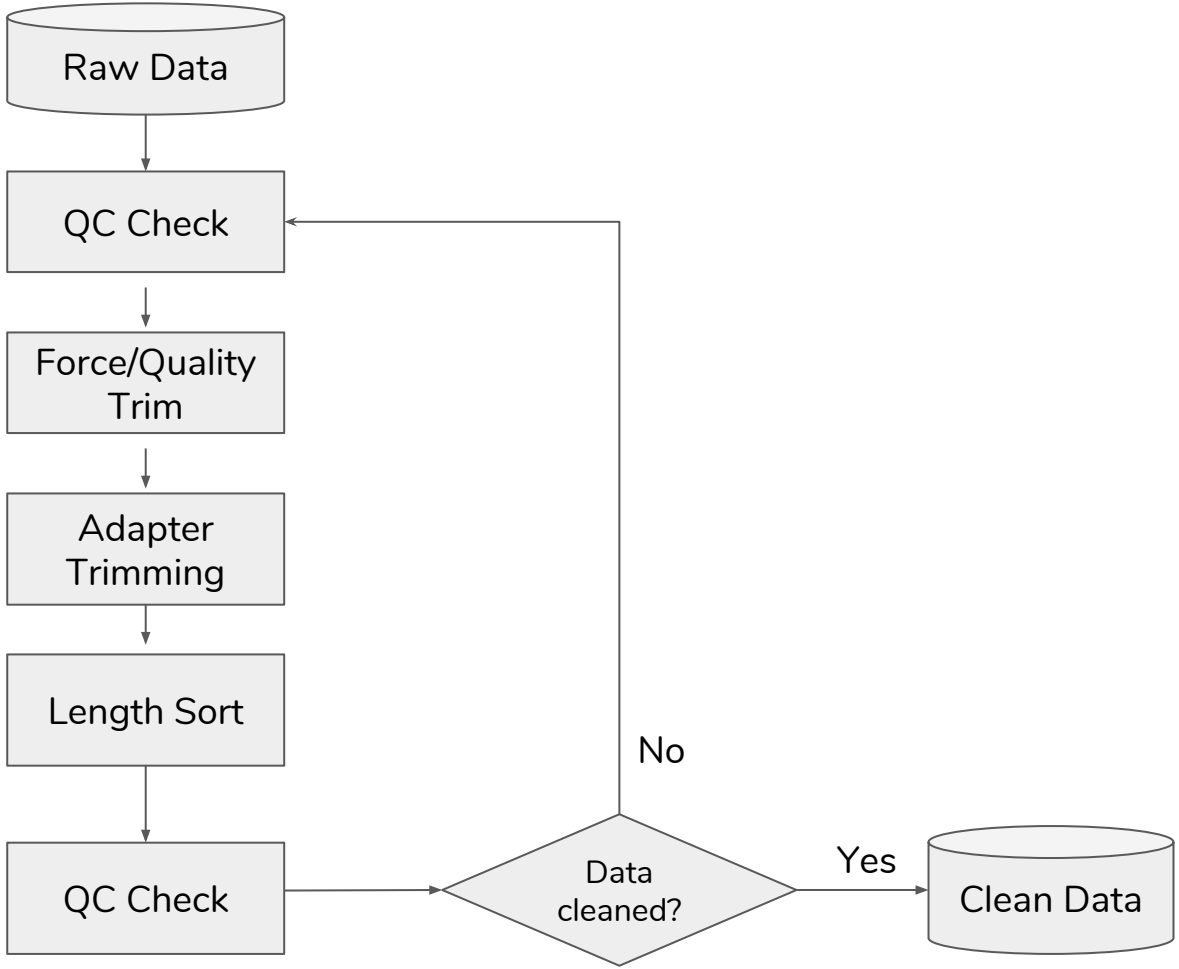
- Objective:

  Generate a pipeline to assemble and QC short read data, with respect to biological characteristics and downstream analyses.

- Remove sequencing bias
- Remove bases with low Q-score

- Remove sequencing adapters

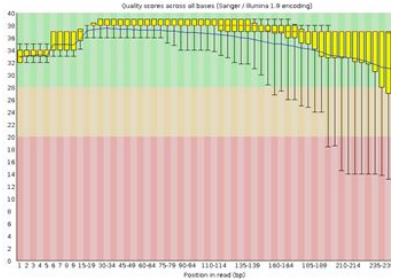
- Filter out short reads



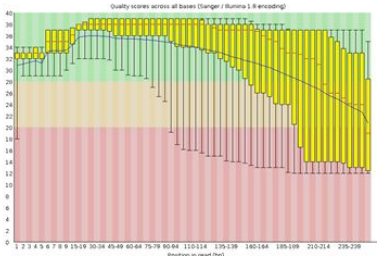
Raw, unloved sequence data

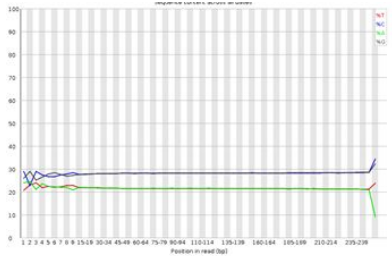
Forward Reads

Reverse Reads

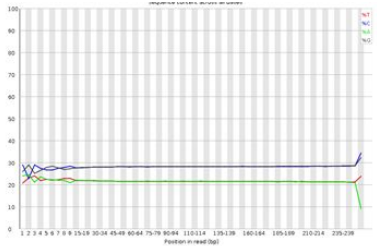


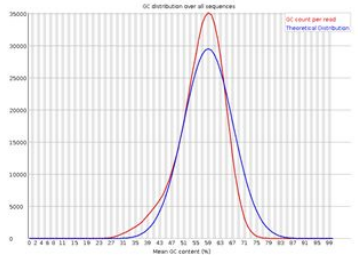
Avg. Phred Score



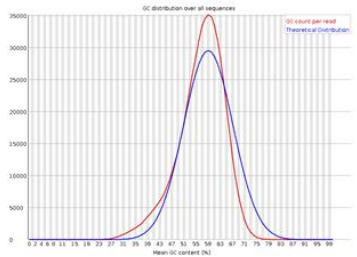


Avg Nucleotide %



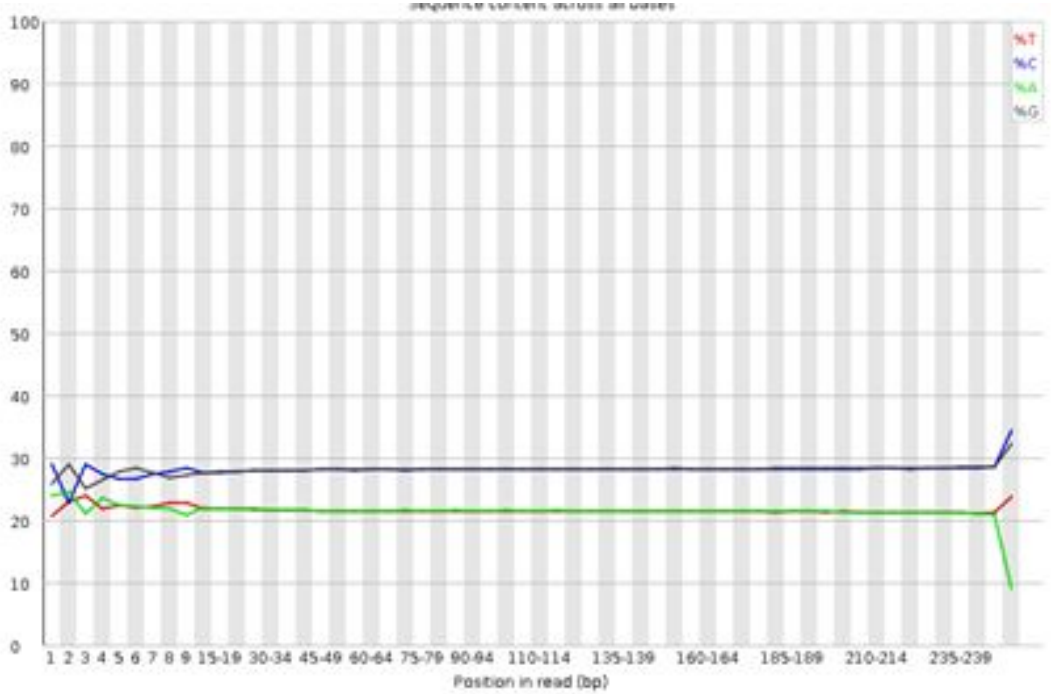


GC content



Andrews S. (2010). FastQC: a quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Trimming

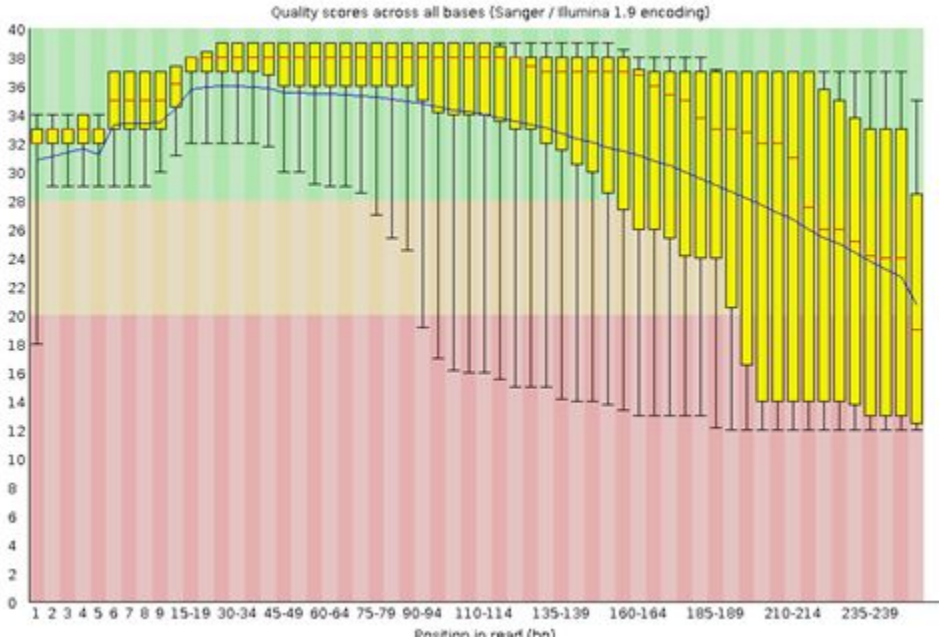


Per Base Sequence Content

Sequencing Bias

- Introduced during library preparation
- Non-random favoring of primer sequences during amplification
- To be removed or not?

Trimming Tools



Quality scores:

– Phred Q-score = $-10\log_{10}P$

* At 1x depth coverage:

Q-score	Incorrect Base Call (Probability)
10	1 in 10 (90%)
20	1 in 100 (99%)
30	1 in 1000 (99.9%)
40	1 in 10,000 (99.99%)

Trimming Software:

BBDuk

Trimmomatic

SolexaQA++

Sickle

Seqtk

TrimGalore

Trimming Software

BBDuk

Trimmomatic

SolexaQA++

Sickle

Seqtk

TrimGalore

What to look for?

Average Phred Score

Per Base Sequence Content

Adapter Content

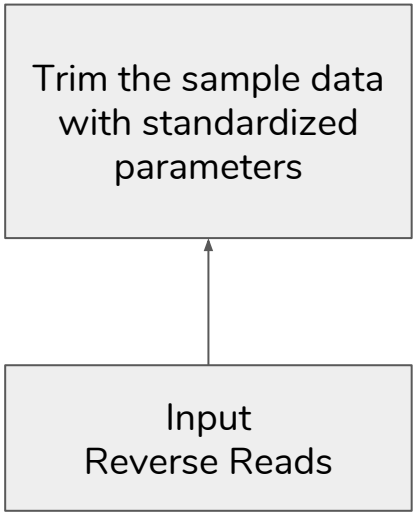
Sequence Length Distribution

Which trimming tool performs the best?

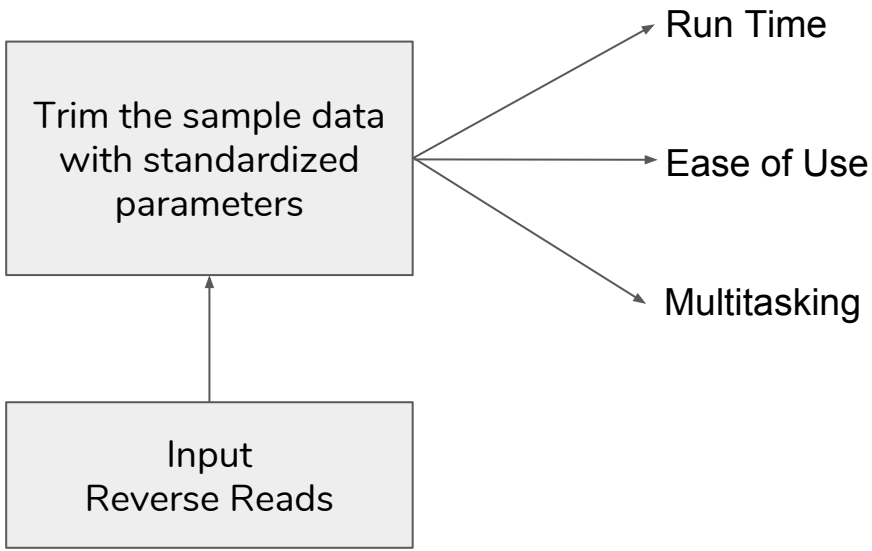
Which trimming tool performs the best?

Trim the sample data with standardized parameters

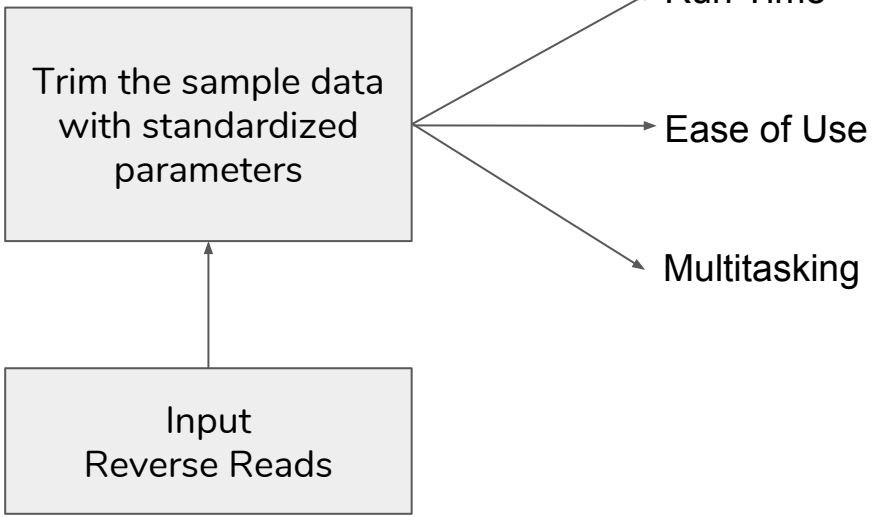
Which trimming tool performs the best?



Which trimming tool performs the best?



Which trimming tool performs the best?



Tools for Trimming:

BBDuk
Trimmomatic
SolexaQA++
Sickle
Seqtk
TrimGalore

Parameter:

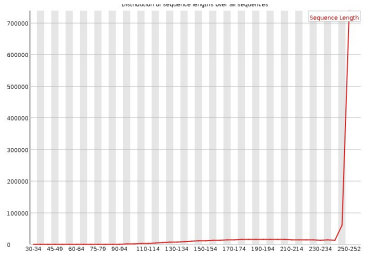
- Quality Trimming - Q20

Which tool performs the best?

Software	Run time (in seconds)	Output File (MB)	Multitask capacity
SolexaQA++	80.24	393.6	no
Sickle	5.19	508.3	no
TrimGalore	2.63	486.4	no
BBDuk	3.17	428.7	yes
Trimmomatic	3.09	383.9*	yes
Seqtk	3.41	433.5	yes

Which tool performs the best?

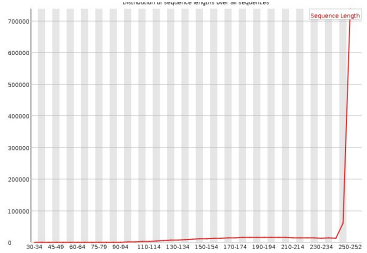
Raw Reads



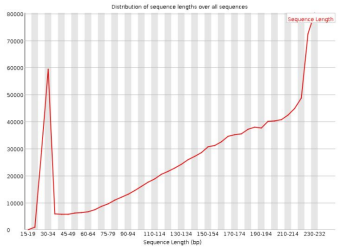
Software	Run time (in seconds)	Output File (MB)	Multitask capacity
SolexaQA++	80.24	393.6	no
Sickle	5.19	508.3	no
TrimGalore	17.7	486.4	no
BBDuk	3.17	428.7	yes
Trimmomatic	3.09	383.9*	yes
Seqtk	3.41	433.5	yes

Which tool performs the best?

Raw Reads

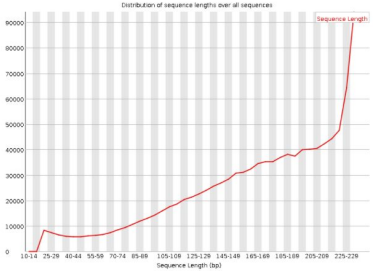


SeqTK



SolexaQA++

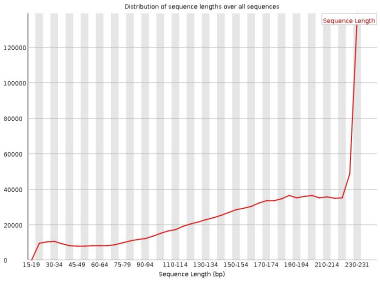
BBDuk



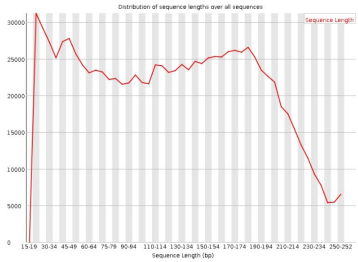
Software	Run time (in seconds)	Output File (MB)	Multitask capacity
SolexaQA++	80.24	393.6	no
Sickle	5.19	508.3	no
TrimGalore	17.77	486.4	no
BBDuk	3.17	428.7	yes
Trimmomatic	3.09	383.9*	yes
Seqtk	3.41	433.5	yes

Which tool performs the best?

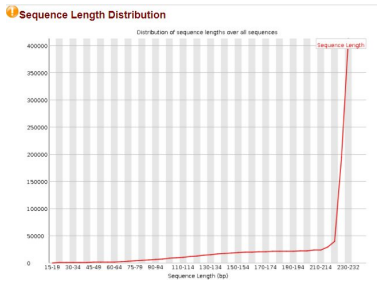
Software	Run time (s)	Output File (MB)	Multitask capacity
SolexaQA++	80.24	393.6	no
Sickle	5.19	508.3	no
TrimGalore	17.77	589.9	yes
BBDuk	3.17	428.7	yes
Trimmomatic	3.09	383.9*	yes
Seqtk	3.41	433.5	yes



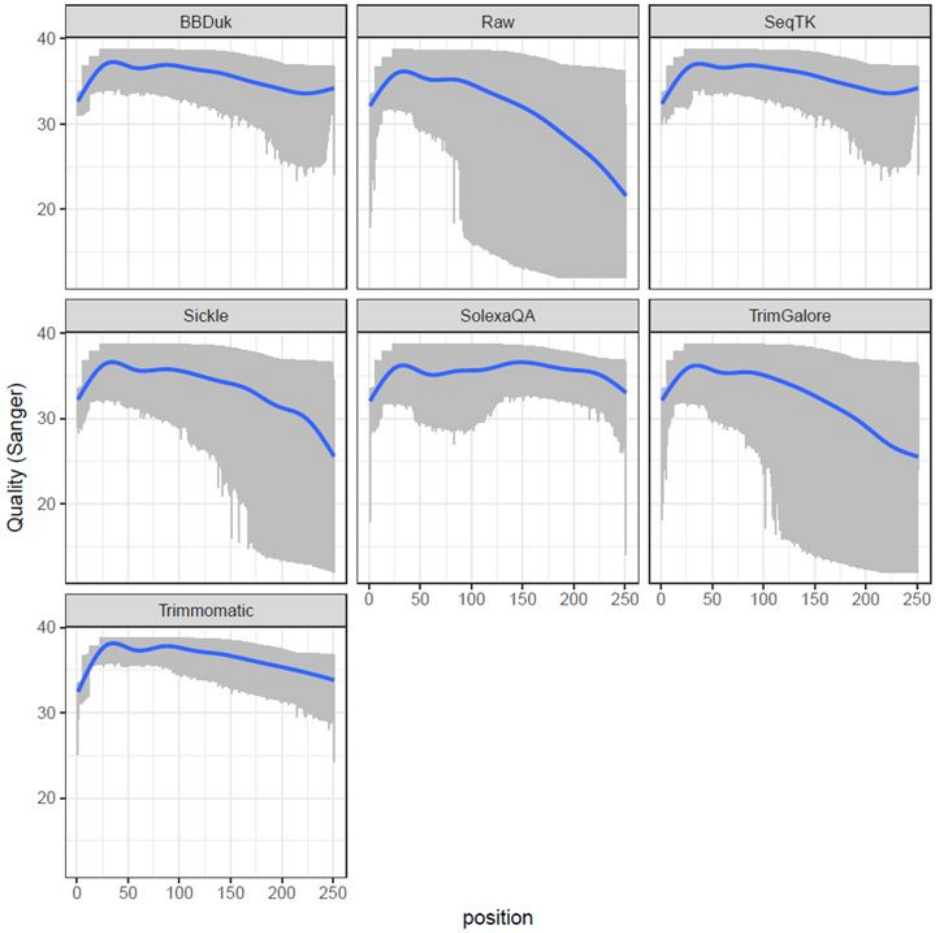
Trimmomatic

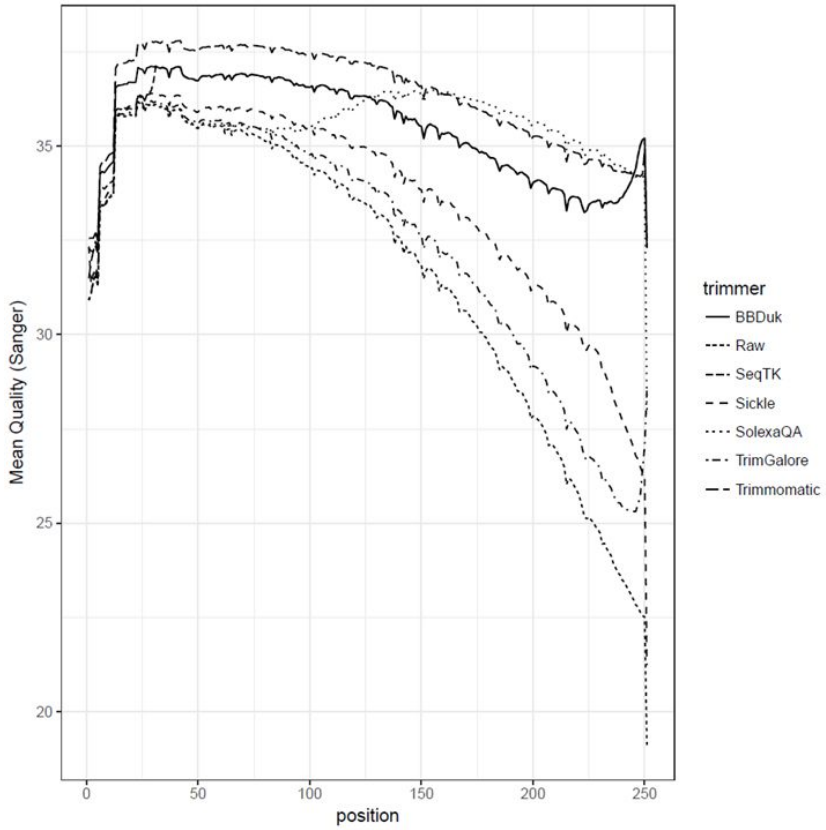


Sickle



TrimGalore



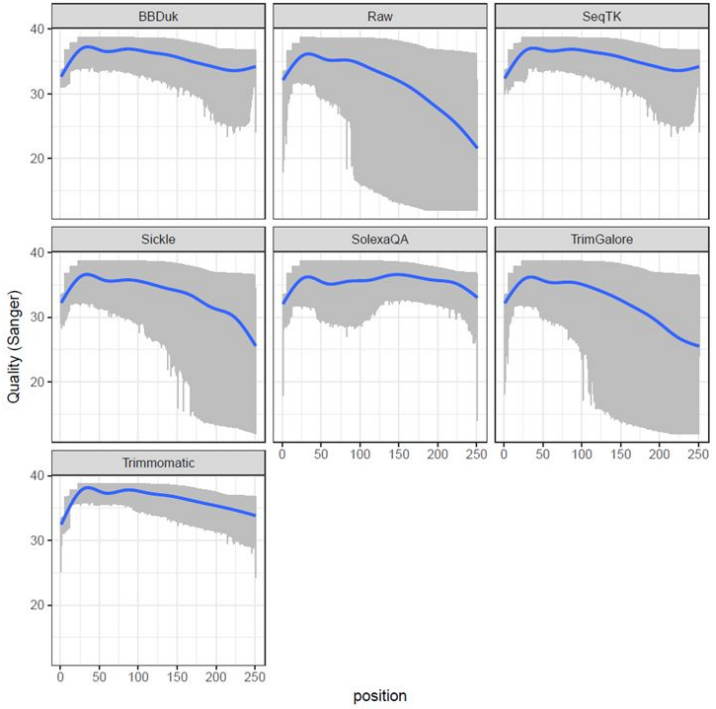


Graphs generated using QRQC(R)
Vince Buffalo (2012). qrqc: Quick Read Quality Control. R package version 1.32.0.
http://github.com/vsbuffalo/qrqc

Which tool performs the best?

Software	Run time (in seconds)	Output File (MB)	Multitask capacity
SolexaQA++	80.24	393.6	no
Sickle	5.19	508.3 ✓	no
TrimGalore	12.63	589.9	yes
BBDuk	3.17	428.7	yes
Trimmomatic	3.09 ✓	383.9*	yes ✓
Seqtk	3.41	433.5	yes

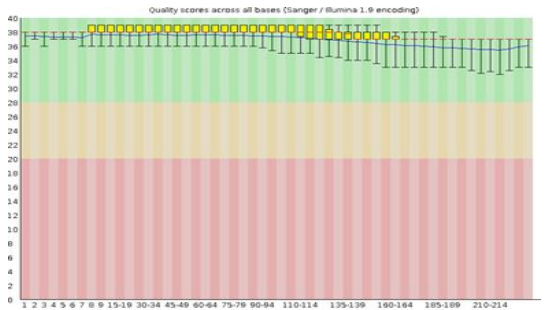
* Trimmomatic uses a sliding window trimming algorithm – was set to 1 here in order to be most comparable.



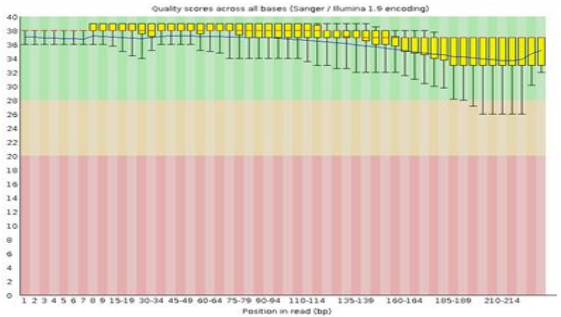
Winner: Trimmomatic

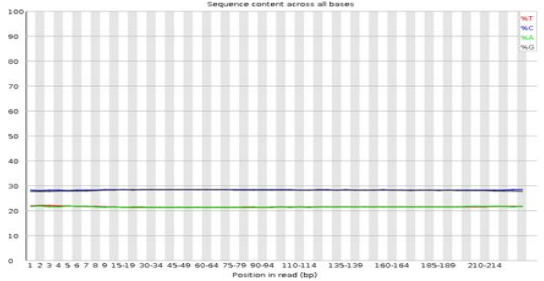
Clean Data, courtesy of Trimmomatic

Forward Reads

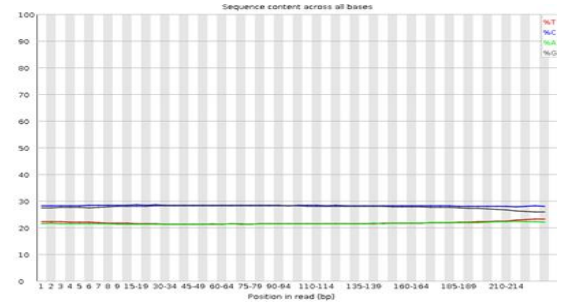


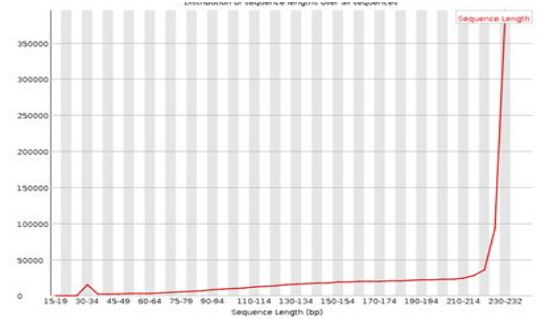
Avg. Phred Q-scores



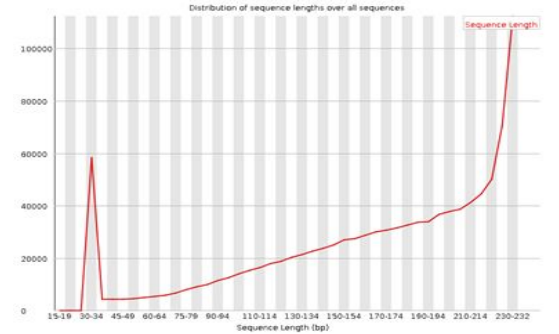


Avg nucleotide %

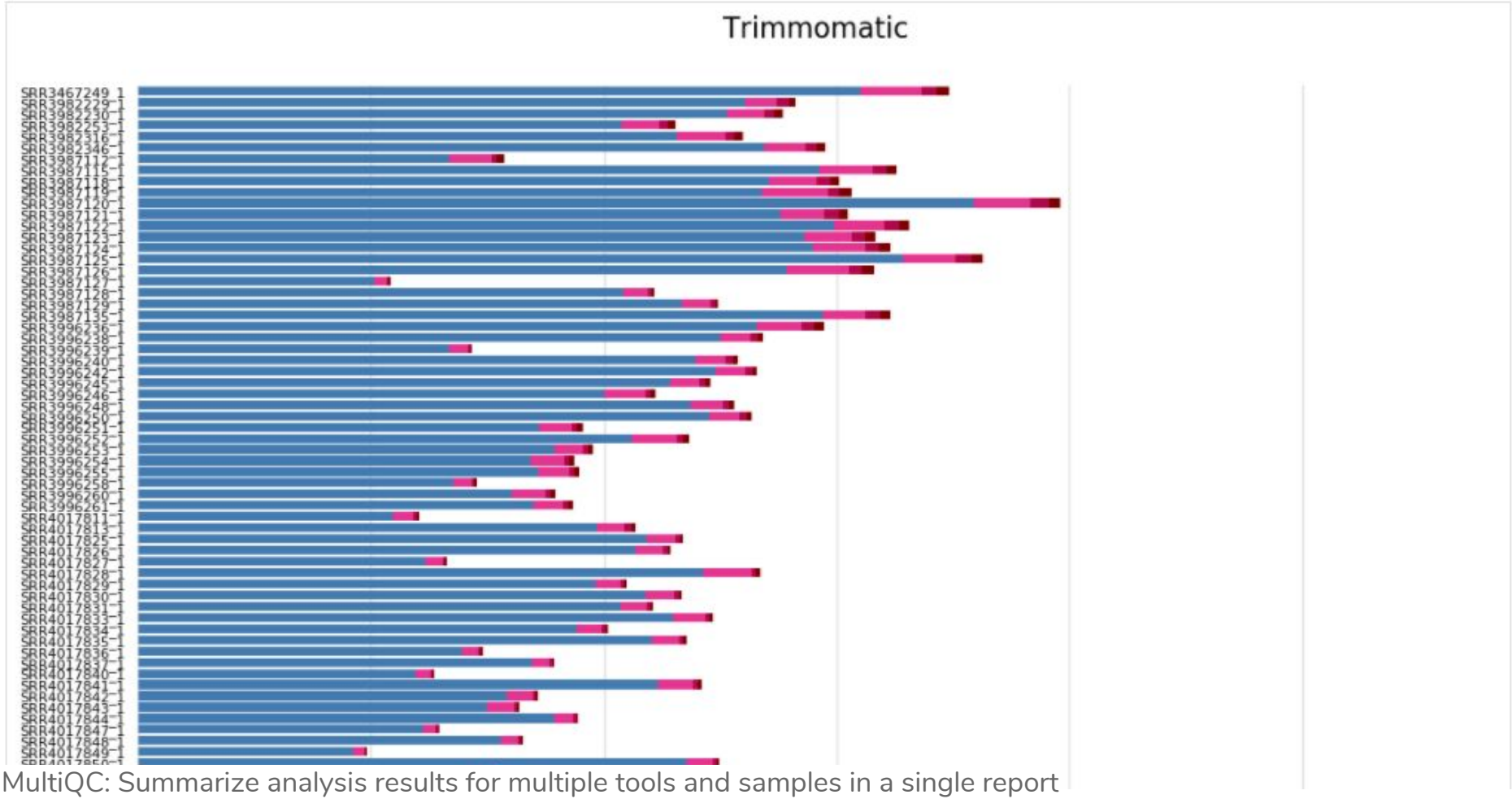




Length Distribution



Reverse Reads

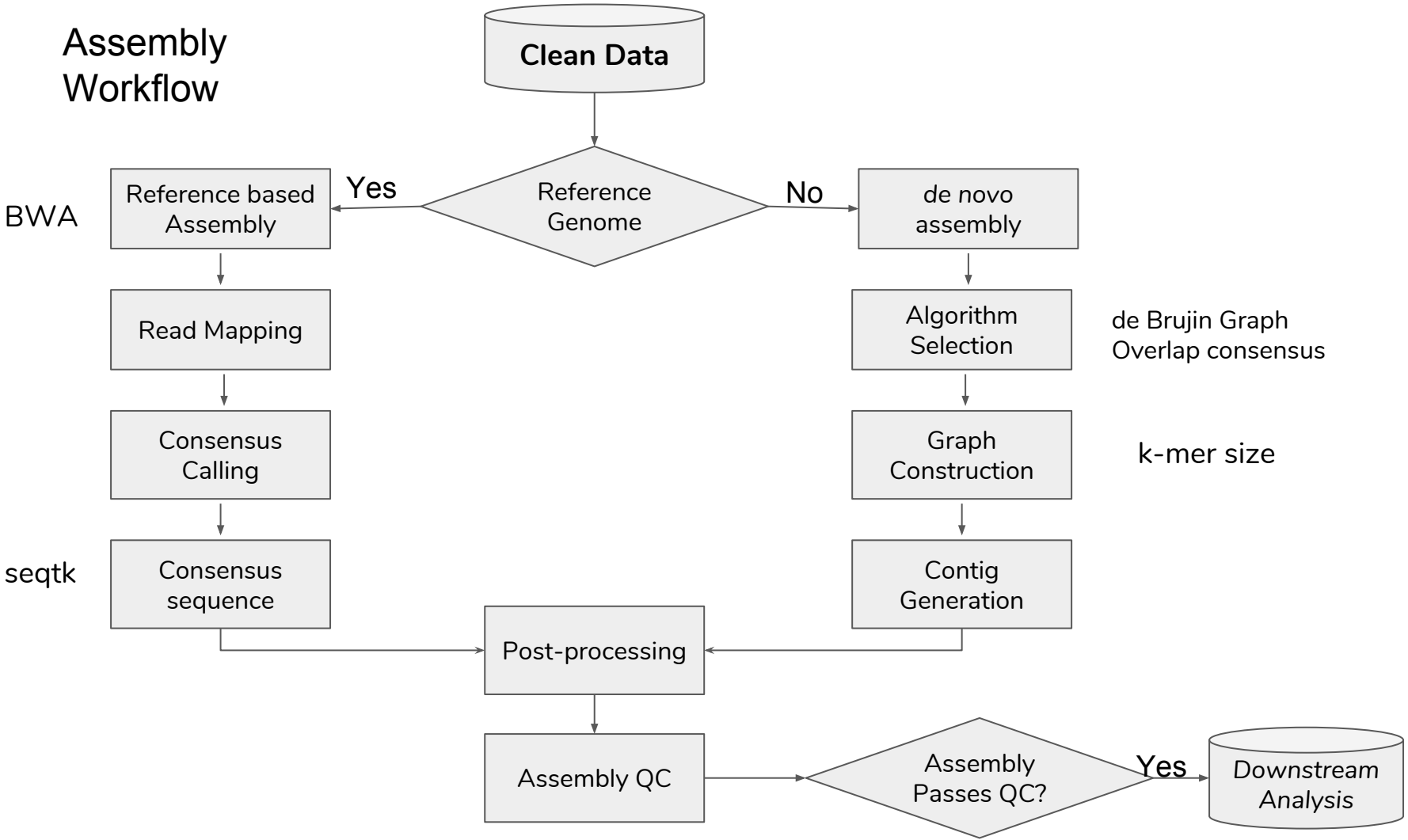


MultiQC: Summarize analysis results for multiple tools and samples in a single report

Philip Ewels, Måns Magnusson, Sverker Lundin and Max Käller

Bioinformatics (2016)

Assembly Workflow



Biological Considerations

Bacterial genomes are single, circular chromosomes

Ideal End Goal:
An assembly containing only 1 contig
(extra credit if it's circular)

How do we tell?

Which species of *Klebsiella* do we have?

What is a good reference genome to use?

When/how to check for contamination in our sequences?

How to detect and remove it?

What is contamination and how did it get there?

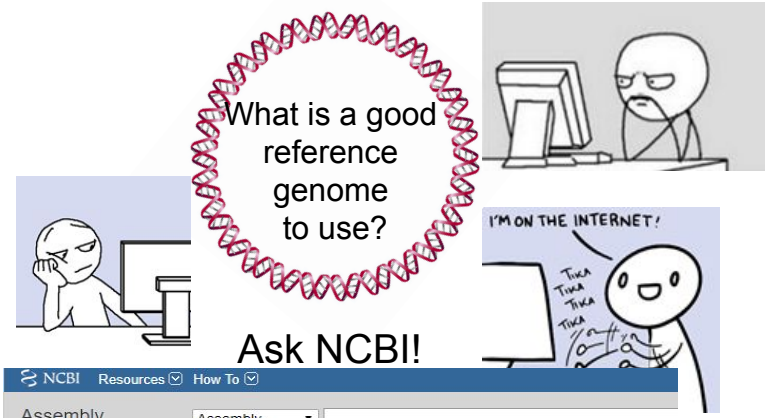
Reference Based Assembly

Which species of *Klebsiella* do we have?

- Mashtree
- Blast
- De novo assemble, then Blast, then re-assemble with reference
- Who cares? Just do de novo assembly

Ref-based Assembly pipeline

What is a good reference genome to use?



Ask NCBI!

NCBI Resources How To

Assembly

Assembly

Advanced Browse by organism

Full Report

ASM988v1

Organism name: Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044 (enterobacteria)
Infraspecific name: Strain: NTUH-K2044
BioSample: SAMD00060934
Submitter: National Health Research Institutes
Date: 2005/01/05
Assembly level: Complete Genome
Genome representation: full
GenBank assembly accession: GCA_000009885.1 (latest)
RefSeq assembly accession: GCF_000009885.1 (latest)
RefSeq assembly and GenBank assembly identical: yes

IDs: 31388 [UID] 10688 [GenBank] 31388 [RefSeq]

History (Show revision history)

Comment

This sequence was determined by the K. pneumoniae Genome Project at the Yang-Ming University VYM G study were from NRPGM of R.O.C.

Global statistics

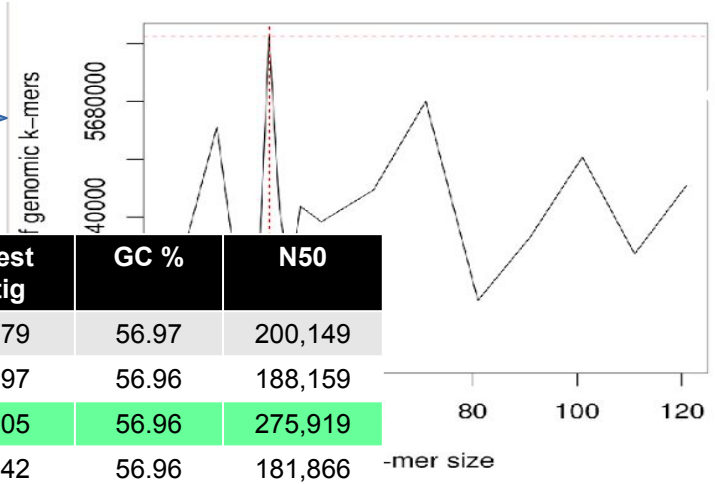
Total sequence length	5,472,672
Total assembly gap length	0
Total number of chromosomes and plasmids	2

De novo assembly: Kmer selection

- De Bruijn graph-based assemblers split reads into kmers for graph construction.
- Assembly outcome is heavily influenced by the choice of kmer values.
  - Problematic palindromes
  - Sweet spot between sensitivity and specificity
- **Where is this magical sweet spot?**
  - Short answer: it's different for every sample you assemble due to quality of seq data, genome complexity, etc. Garbage in, garbage out.

Tools for kmer estimation:

– Kmergenie
– Spades



kmer	Total length	# contigs	Largest contig	GC %	N50
41	5,743,700	82	469779	56.97	200,149
77	5,748,634	76	469897	56.96	188,159
99	5,745,519	68	471705	56.96	275,919
127	5,751,083	76	409842	56.96	181,866

Good kmer choice: fewer contigs, higher N50

Bad kmer choice: shorter total length, more contigs, lower N50

Preliminary results: comparison of assemblies

Using k=41, determined by kmergenie

Assembler	Run Time (s)	Kmer	# contigs	N50 (kbp)	Total length (Mbp)	GC %	# N's
Spades	403	41	82	200.1	5.74	56.97	370
Skesa	87	41	111	120.4	5.67	56.98	0
IDBA-UD	56	41	192	66.5	5.75	56.98	0
Tadpole	13.3	41	343	56.0	5.70	56.96	0
IDBA-Hybrid	81	41	190	62.5	5.63	56.98	0
Ref-based (Samtools)	395	--	2	5,248.5	5.47	58.08	521,755

Caveats to comparison:

– all assemblers compared here support multi-threading. This parameter left as default.

– Only Spades and Tadpole allow for add'n single end read input (not used here)

– Skesa does not include a built-in scaffolder.

Which assembly is best?

Overall, Spades and Skesa are pretty comparable with this kmer value.

Preliminary results: comparison of assemblies

Using k=99, determined by us using Spades

Assembler	Run Time (s)	Kmer	# contigs	N50 (kbp)	Total length (Mbp)	GC %	# N's
Spades	421	99	68	275.9	5.745	56.96	170
Skesa	89.5	99	195	60.7	5.668	56.96	0
IDBA-UD	50.8	99	121	119.7	5.748	56.96	0
Tadpole	15.3	99	244	44.5	5.745	56.97	0
IDBA-Hybrid	82.8	99	161	83.5	5.751	56.96	0
Ref-based (Samtools)	395	--	2	5,248.5	5.47	58.08	521,755

Caveats to comparison:

– all assemblers compared here support multi-threading. This parameter left as default.

– Only Spades and Tadpole allow for add'n single end read input (not used here)

– Skesa does not include a built-in scaffolder.

Which assembly is best?

Spades (k=99) has lowest # contigs, highest N50 of all de novo assemblies attempted.

Drawback: also takes the longest to run, has some N's

Preliminary results: Draft QC (Quast)

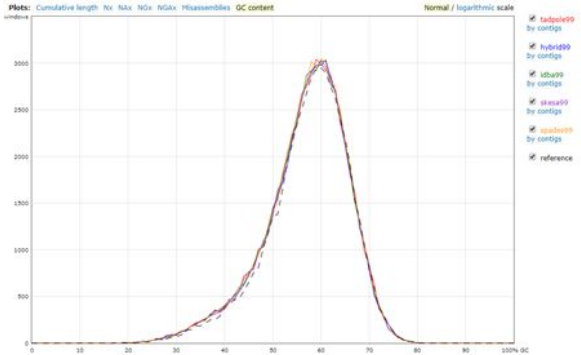
For draft assemblies where k=99

Genome statistics heatmap (m=500)

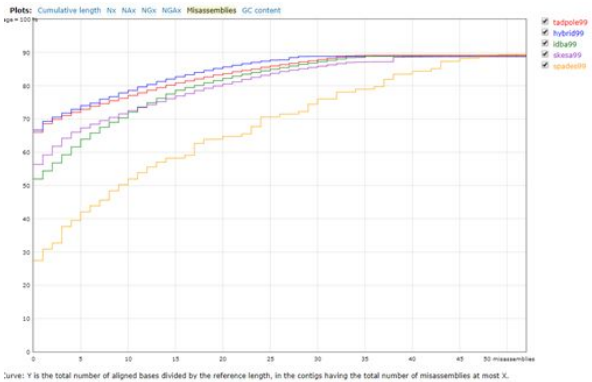
Worst Median Best — Show heatmap

Genome statistics	tadpole99	hybrid99	idba99	skesa99	spades99
Genome fraction (%)	88.971	88.82	88.823	88.699	89.301
Duplication ratio	1.004	1.001	1.002	1.001	1.003
Largest alignment	97 787	172 993	218 046	115 609	293 196
Total aligned length	4 879 022	4 862 260	4 864 172	4 856 472	4 894 465
NGA50	34 754	45 738	61 658	44 045	101 399
LGA50	49	34	28	38	15
Misassemblies					
# misassemblies	38	29	36	43	51
Misassembled contigs length	1 468 194	1 324 238	2 292 894	2 119 335	3 799 323
Mismatches					
# mismatches per 100 kbp	652.46	650.16	629.83	651.1	647.1
# indels per 100 kbp	11.6	10.72	10.84	11.93	12.48
# N's per 100 kbp	0	0	0	0	2.91
Statistics without reference					
# contigs	270	225	183	183	86
Largest contig	140 979	172 993	265 440	155 498	471 761
Total length	5 697 803	5 651 712	5 698 915	5 665 016	5 729 945
Total length (>= 1000 bp)	5 679 755	5 626 540	5 683 766	5 657 369	5 717 665
Total length (>= 10000 bp)	5 232 064	5 306 730	5 400 185	5 384 767	5 604 812
Total length (>= 50000 bp)	2 553 392	3 473 859	4 233 885	3 494 964	5 309 579

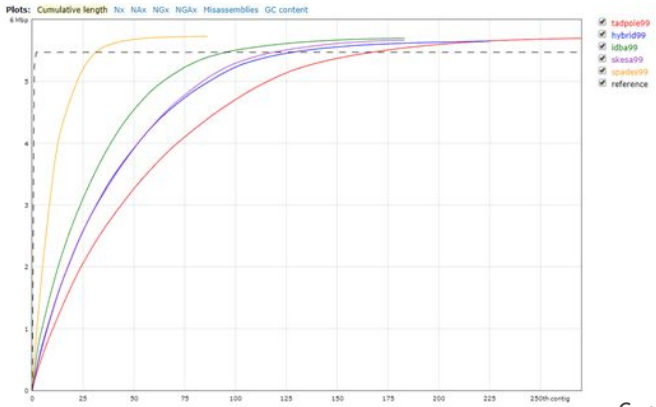
GC content



Misassemblies compared to reference



Cumulative length of draft assemblies

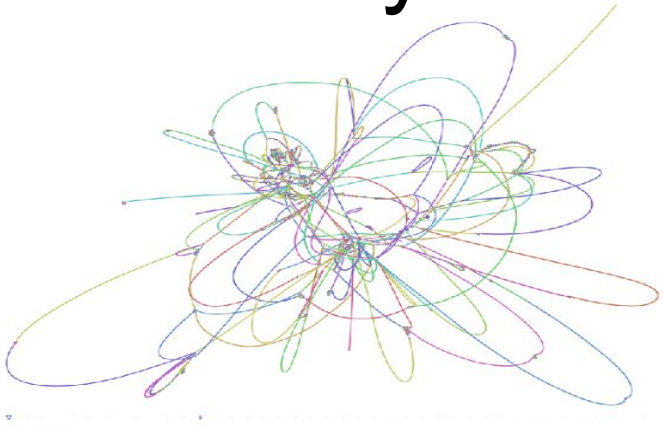


Indications of contamination

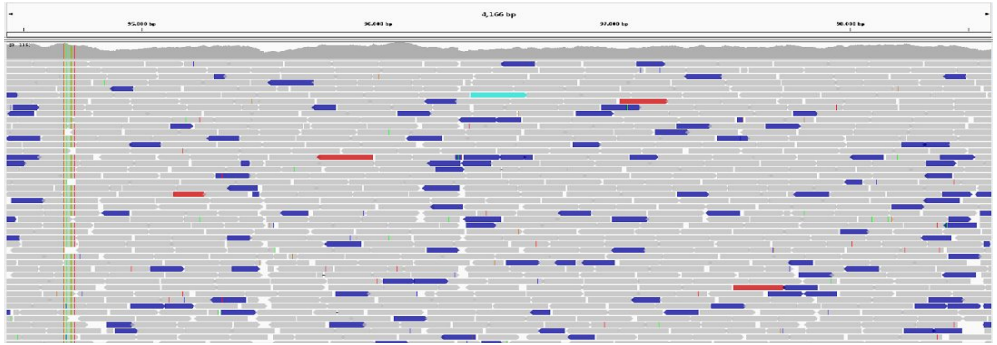
- GC content
- Many misassemblies compared to reference genome*
- Depth coverage anomalies
- Highly fragmented assemblies

Gurevich et al. QUAST: quality assessment tool for genome assemblies, *Bioinformatics* (2013) 29(8): 1072-1075.

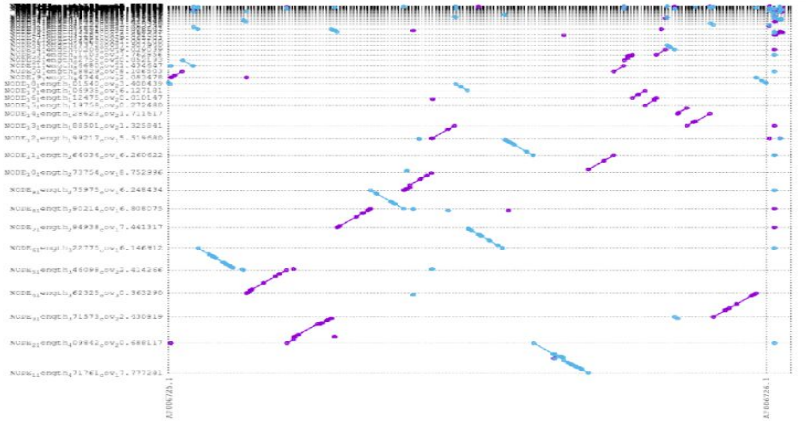
Preliminary results: Visualization



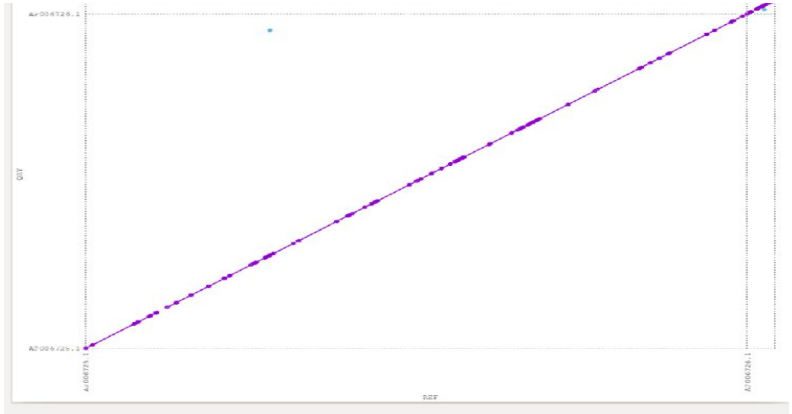
Visualize the de Bruijn graph with Bandage



Visually inspect alignment quality with IGV

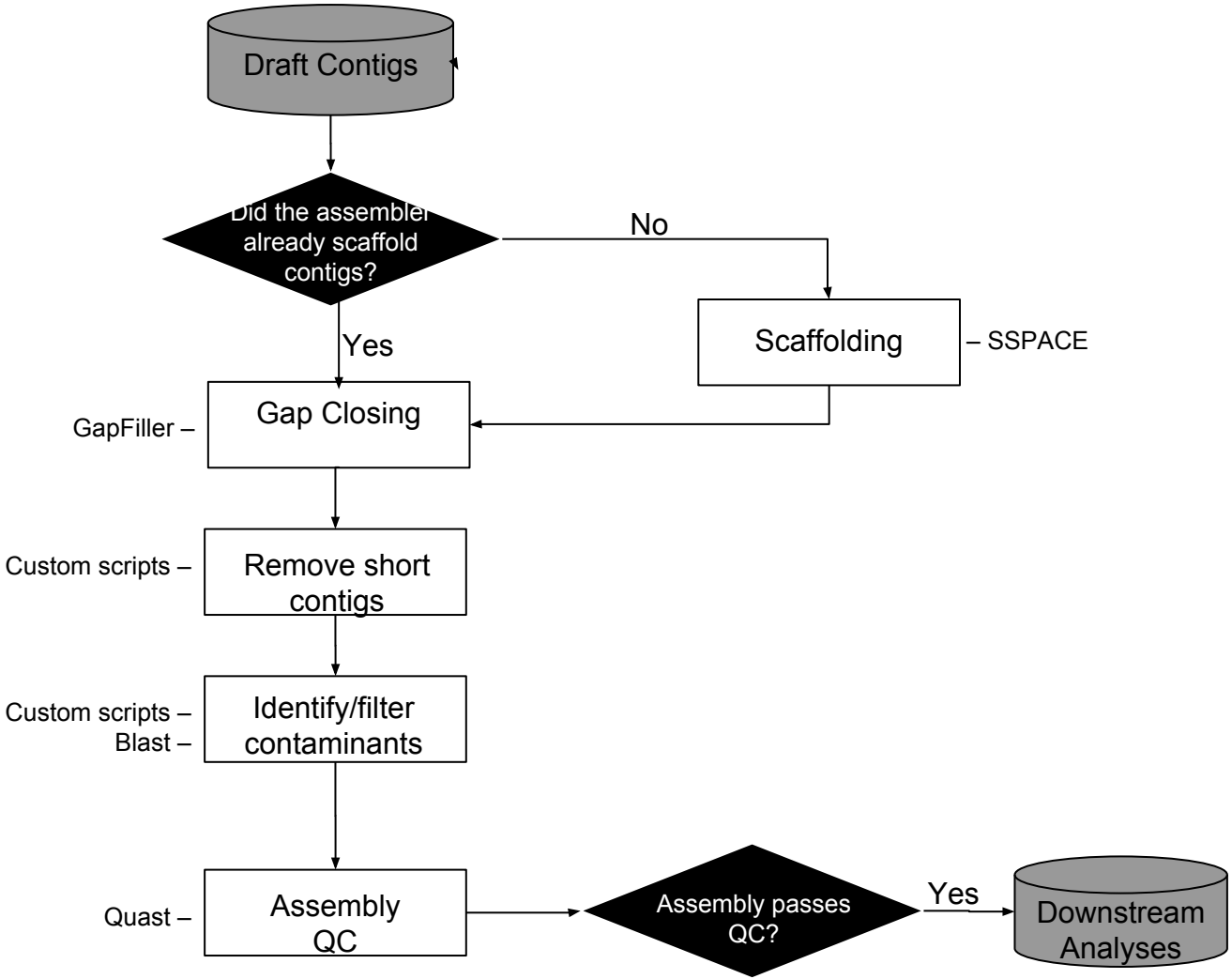


- Check for circular (ie. closed) chromosome



Identify indels, duplications, reversals, etc. using Mummer

Post-Assembly Finishing



Questions?

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Look for a homework announcement shortly!