Assembly, assembly stats, virulotyping, serotyping

Valeria Michelacci

WGS course,
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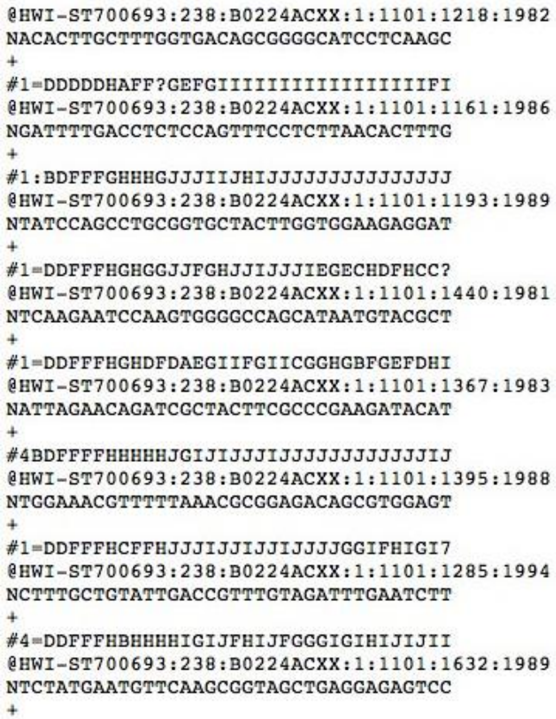
Assembly (e.g.: SPAdes)

Short sequencing reads

Partially assembled genome (contigs)

.fastq file



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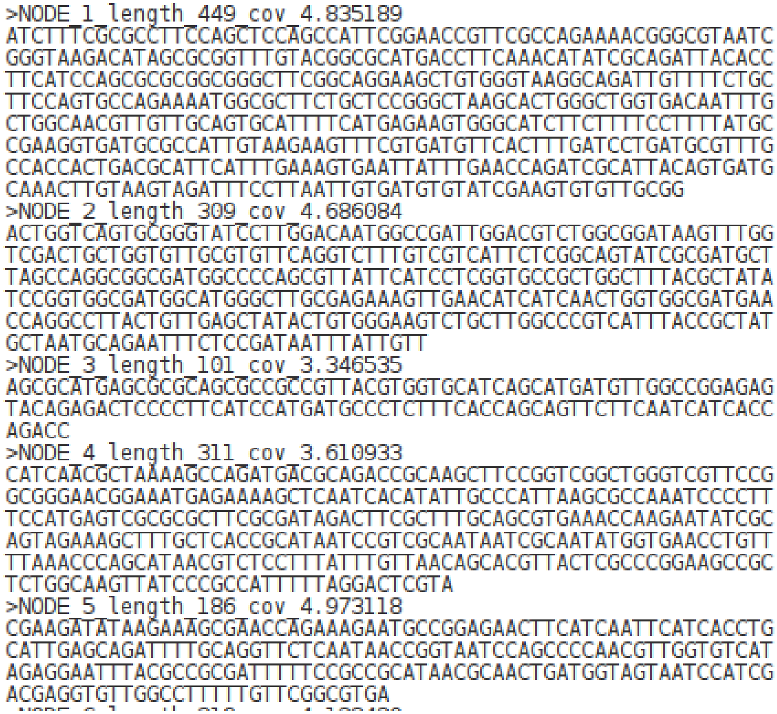
+
```

FastqSize ≈ GenomeSize x Coverage x 2

At least 0.5 GB per genome

.fasta file



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CGAAGGTGATGCGCCATTGTAAGAAGTTTCGTGATGTTCACTTTGATCCTGATGCGTTTG
CCACCACTGACGCATTCATTTGAAAGTGAATTATTTGAACCAGATCGCATTACAGTGATG
CAAACTTGTAAGTAGATTTCCTTAATTGTGATGTGTATCGAAGTGTGTTGCGG
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ACGAGGTGTTGGCCTTTTTGTTCGGCGTGA
```

FastaSize for *E. coli* contigs

~5.5 MB

Filter SPAdes repeats/1

Input: contigs file & file with contigs stats

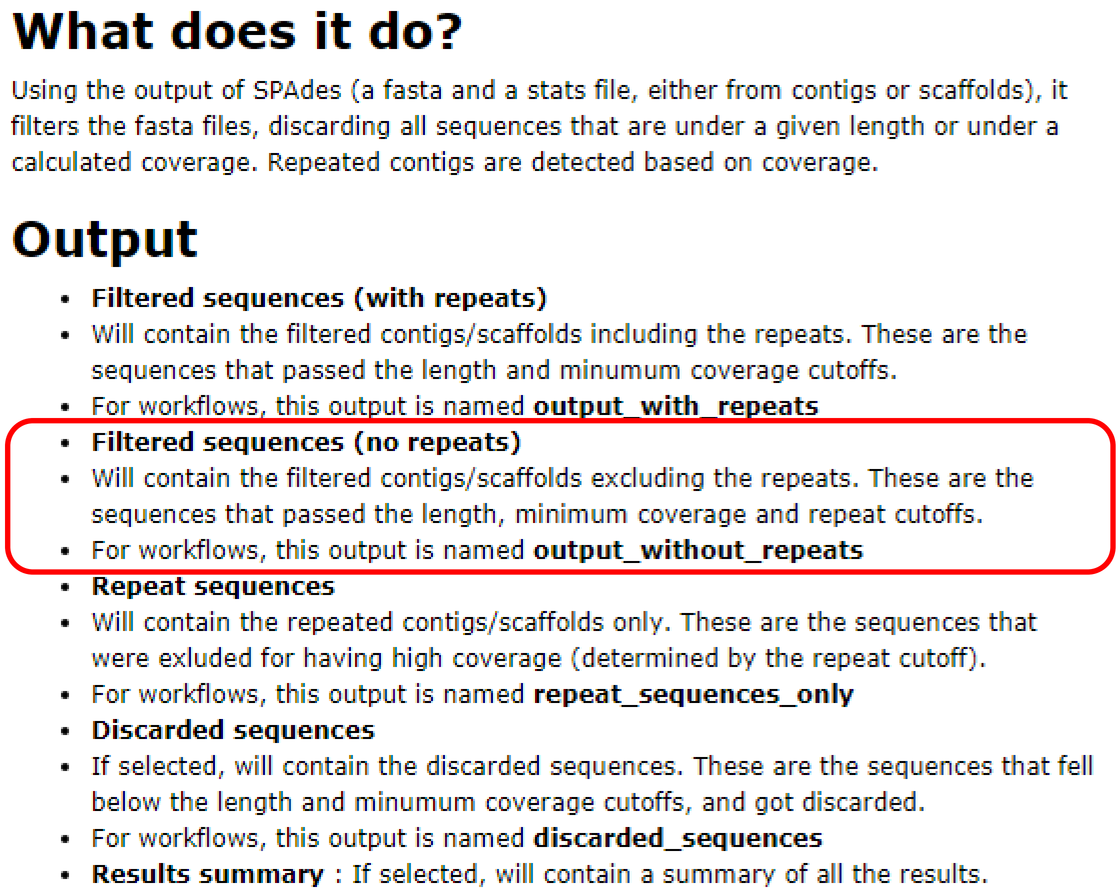
Coverage cut-off ratio:
This is the average coverage ratio cutoff. For example: if the average coverage is 100 and a coverage cut-off ratio of 0.5 is used, then any contigs with coverage lower than 50 will be eliminated.

Repeat cut-off ratio:
This is the coverage ratio cutoff to determine repeats in contigs. For exmaple: if the average coverage is 100 and a repeat cut-off ratio of 1.75 is used, then any contigs with coverage more than or equal to 175 will be marked as repeats.

Length cut-off

Length for average coverage calculation (default = 5000)

Filter SPAdes repeats/2



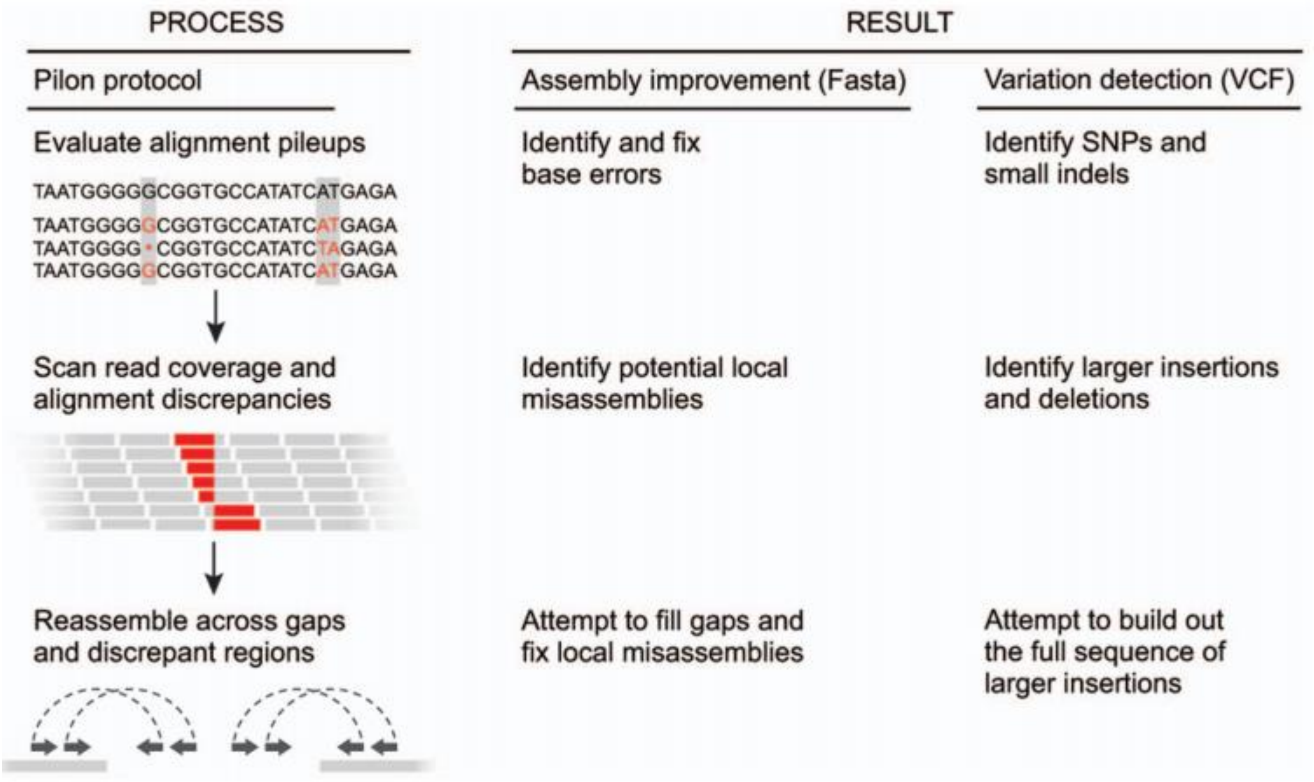
What does it do?

Using the output of SPAdes (a fasta and a stats file, either from contigs or scaffolds), it filters the fasta files, discarding all sequences that are under a given length or under a calculated coverage. Repeated contigs are detected based on coverage.

Output

- **Filtered sequences (with repeats)**
- Will contain the filtered contigs/scaffolds including the repeats. These are the sequences that passed the length and minumum coverage cutoffs.
- For workflows, this output is named **output_with_repeats**
- **Filtered sequences (no repeats)**
- Will contain the filtered contigs/scaffolds excluding the repeats. These are the sequences that passed the length, minimum coverage and repeat cutoffs.
- For workflows, this output is named **output_without_repeats**
- **Repeat sequences**
- Will contain the repeated contigs/scaffolds only. These are the sequences that were exluded for having high coverage (determined by the repeat cutoff).
- For workflows, this output is named **repeat_sequences_only**
- **Discarded sequences**
- If selected, will contain the discarded sequences. These are the sequences that fell below the length and minumum coverage cutoffs, and got discarded.
- For workflows, this output is named **discarded_sequences**
- **Results summary** : If selected, will contain a summary of all the results.

Pilon – contigs refinement



PROCESS	RESULT	
Pilon protocol	Assembly improvement (Fasta)	Variation detection (VCF)
Evaluate alignment pileups	Identify and fix base errors	Identify SNPs and small indels
Scan read coverage and alignment discrepancies	Identify potential local misassemblies	Identify larger insertions and deletions
Reassemble across gaps and discrepant regions	Attempt to fill gaps and fix local misassemblies	Attempt to build out the full sequence of larger insertions

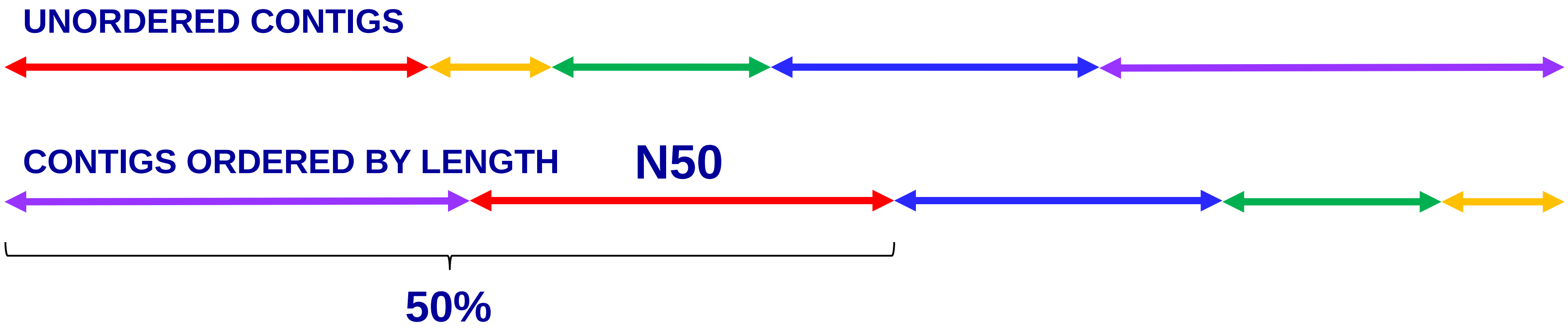
TAATGGGGGCGGTGCCATATCATGAGA
TAATGGGGGCGGTGCCATATCATGAGA
TAATGGGG•CGGTGCCATATCTAGAGA
TAATGGGGGCGGTGCCATATCATGAGA

Realignment of the reads on a «reference sequence»: we use Bowtie2 as alignment tool and the contigs as ref seq

Pilon uses the result of the alignment to improve the assembly: it outputs better assembled contigs

Assembly stats

N50 the **length** of the smallest contig among the set of the largest contigs that together cover at least 50% of the assembly



UNORDERED CONTIGS

CONTIGS ORDERED BY LENGTH

N50

50%

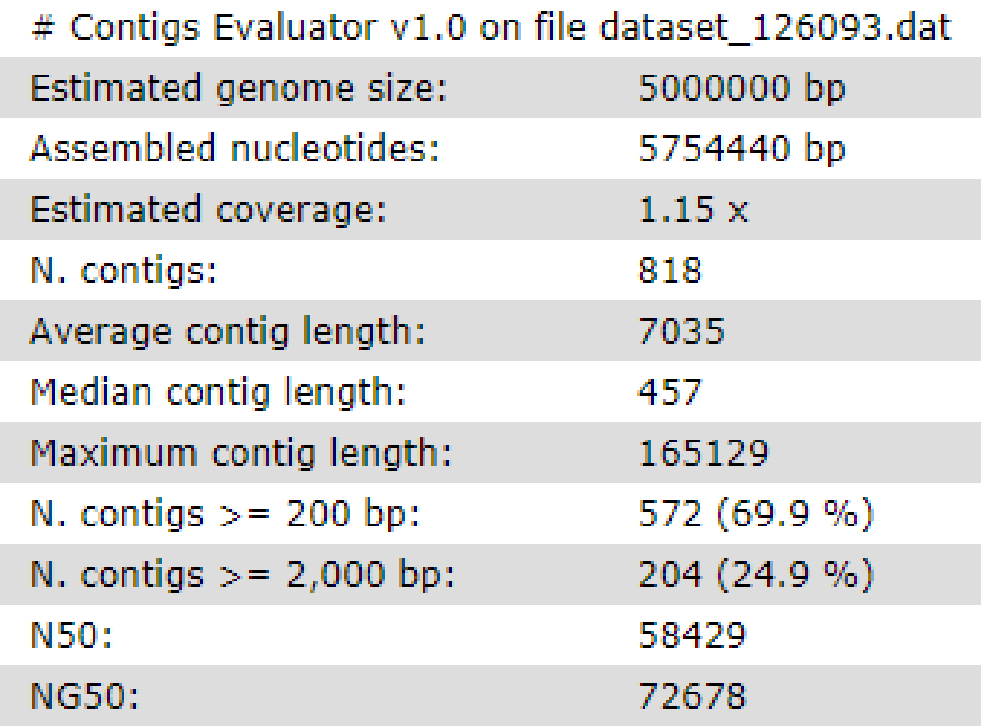
Other intuitive parameters to check:

Maximum contig length

Coverage of the contigs

Consensus length

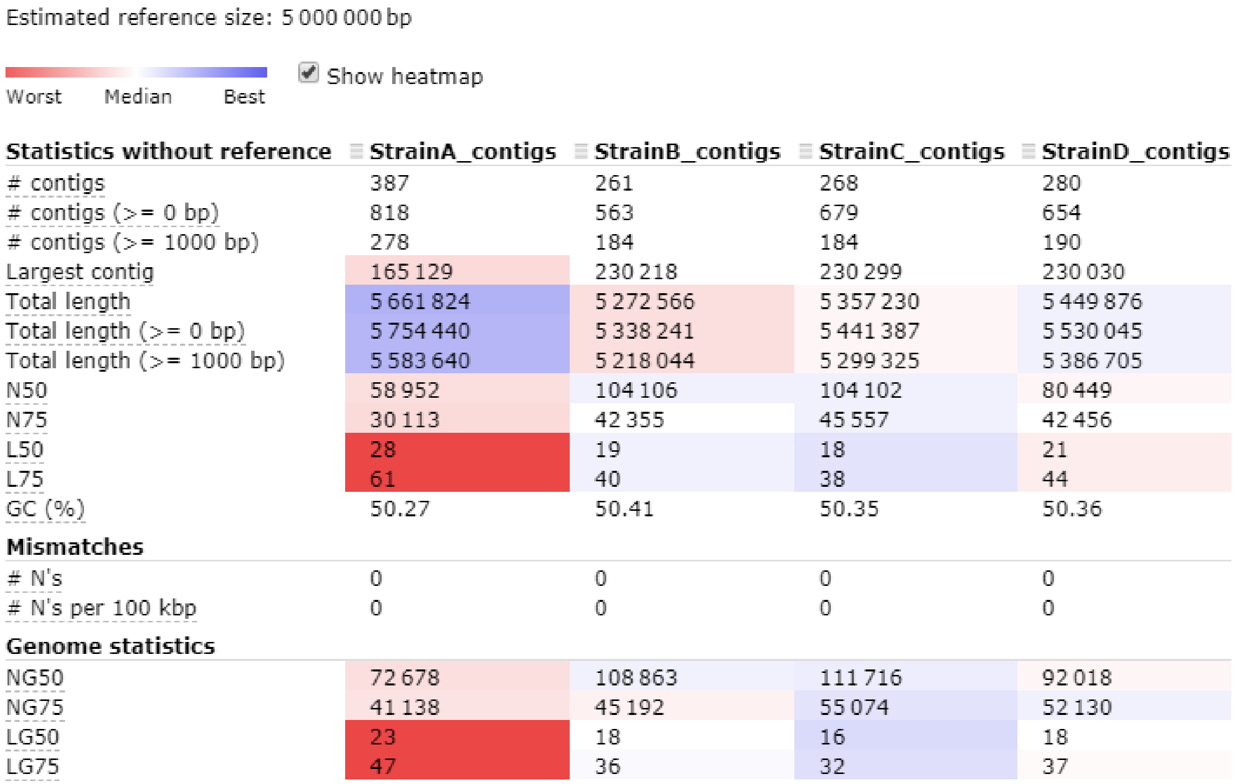
Assembly stats: check bacterial contigs



Contigs Evaluator v1.0 on file dataset_126093.dat

Estimated genome size:	5000000 bp
Assembled nucleotides:	5754440 bp
Estimated coverage:	1.15 x
N. contigs:	818
Average contig length:	7035
Median contig length:	457
Maximum contig length:	165129
N. contigs >= 200 bp:	572 (69.9 %)
N. contigs >= 2,000 bp:	204 (24.9 %)
N50:	58429
NG50:	72678

Assembly stats: Quast/1

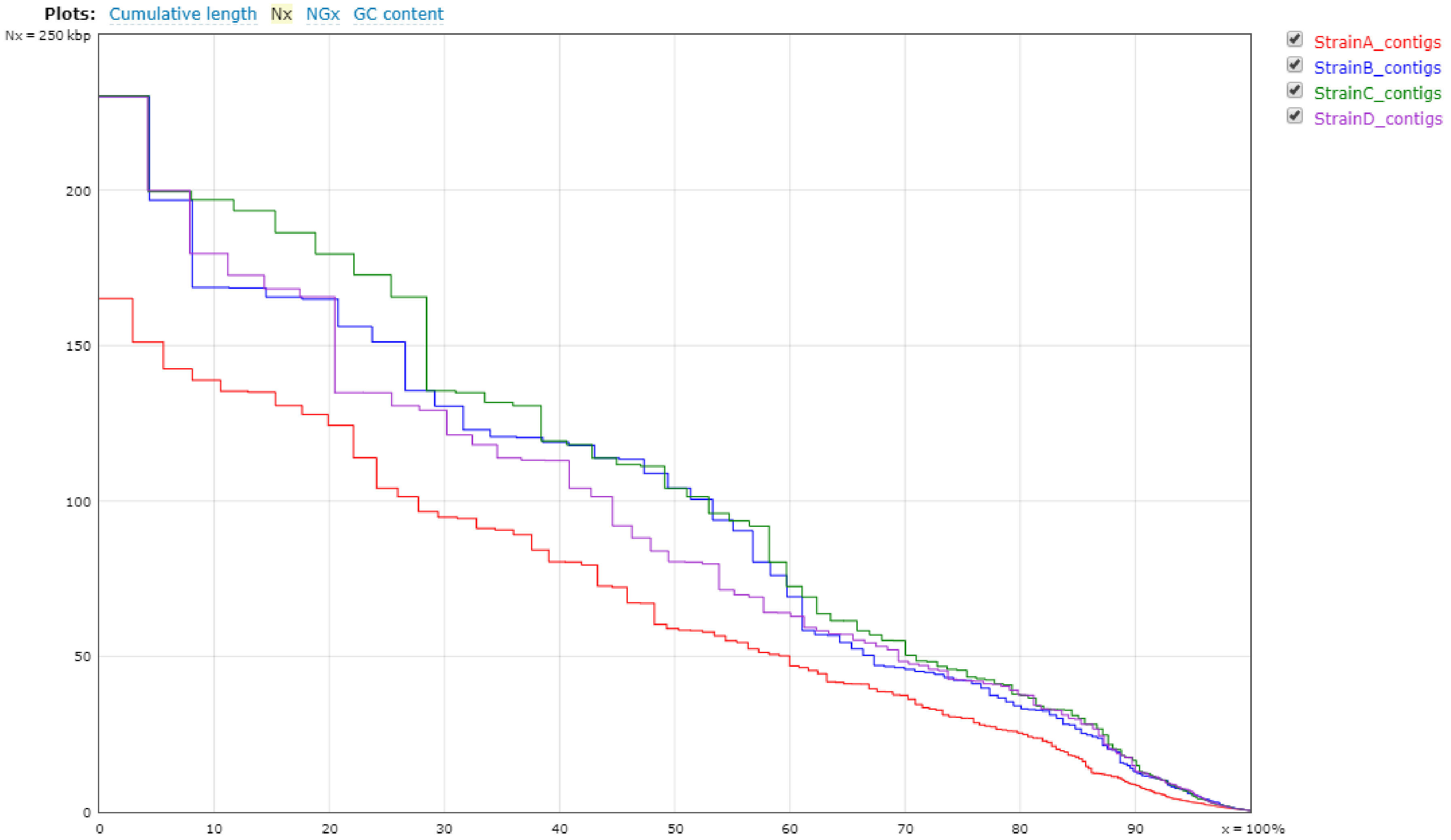


Estimated reference size: 5 000 000 bp

☑ Show heatmap

Statistics without reference	StrainA_contigs	StrainB_contigs	StrainC_contigs	StrainD_contigs
# contigs	387	261	268	280
# contigs (>= 0 bp)	818	563	679	654
# contigs (>= 1000 bp)	278	184	184	190
Largest contig	165 129	230 218	230 299	230 030
Total length	5 661 824	5 272 566	5 357 230	5 449 876
Total length (>= 0 bp)	5 754 440	5 338 241	5 441 387	5 530 045
Total length (>= 1000 bp)	5 583 640	5 218 044	5 299 325	5 386 705
N50	58 952	104 106	104 102	80 449
N75	30 113	42 355	45 557	42 456
L50	28	19	18	21
L75	61	40	38	44
GC (%)	50.27	50.41	50.35	50.36
Mismatches				
# N's	0	0	0	0
# N's per 100 kbp	0	0	0	0
Genome statistics				
NG50	72 678	108 863	111 716	92 018
NG75	41 138	45 192	55 074	52 130
LG50	23	18	16	18
LG75	47	36	32	37

Assembly stats: Quast/2


Plots: Cumulative length Nx NGx GC content
Nx = 250 kbp
x = 100%
☑ StrainA_contigs
☑ StrainB_contigs
☑ StrainC_contigs
☑ StrainD_contigs