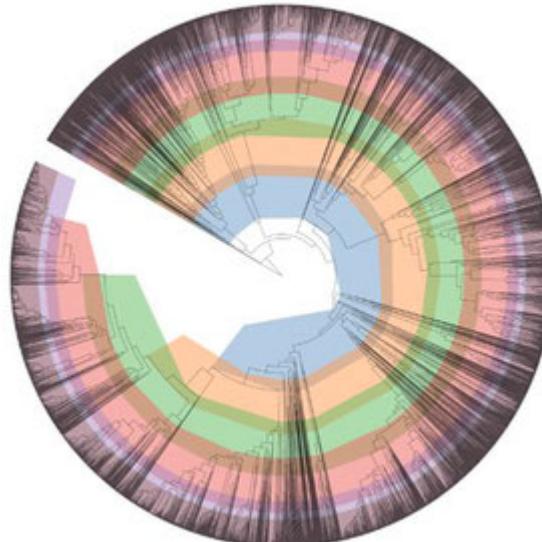


ENVIRONMENTAL METAGENOMICS

Physalia course, online, 11-15 November 2024

Data pre-processing: quality control and adapter removal

Nikolay Oskolkov, Lund University, NBIS SciLifeLab
Luis Pedro Coelho, Queensland University of Technology



NB: original course material courtesy:
Dr. Antti Karkman, University of Helsinki
Dr. Igor Pessi, Finnish Environment Institute (SYKE)

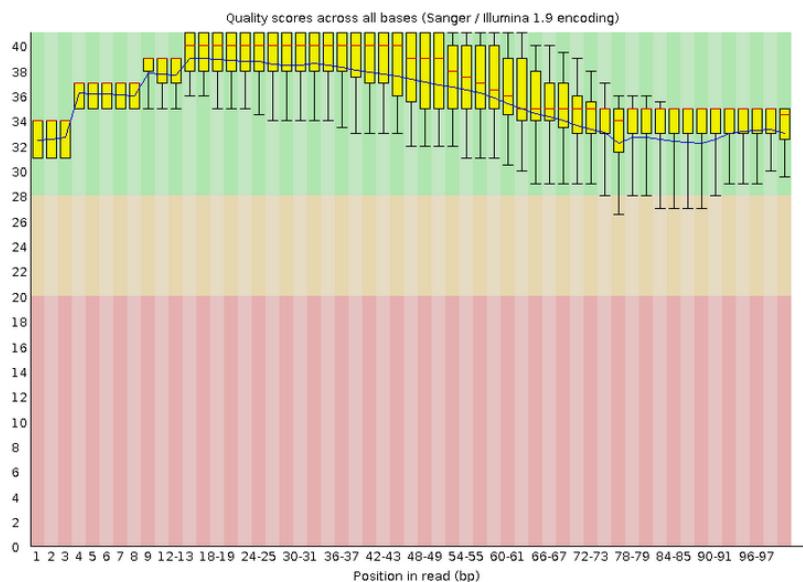
Summary

- ✓ Basic Statistics
- ✓ Per base sequence quality
- ✓ Per tile sequence quality
- ✓ Per sequence quality scores
- ! Per base sequence content
- ✓ Per sequence GC content
- ✓ Per base N content
- ! Sequence Length Distribution
- ✓ Sequence Duplication Levels
- ✓ Overrepresented sequences
- ✗ Adapter Content

Basic Statistics

Measure	Value
Filename	G69146_pe_1.fastq.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	8997912
Sequences flagged as poor quality	0
Sequence length	77-181
%GC	49

Per base sequence quality



Quality Scores

A quality score (or Q-score) expresses an error probability. In particular, it serves as a convenient and compact way to communicate very small error probabilities.

Given an assertion, A, the quality score, Q(A), expresses the probability that A is not true, P(~A), according to the relationship:

$$Q(A) = -10 \log_{10}(P(\sim A))$$

where $P(\sim A)$ is the estimated probability of an assertion A being wrong.

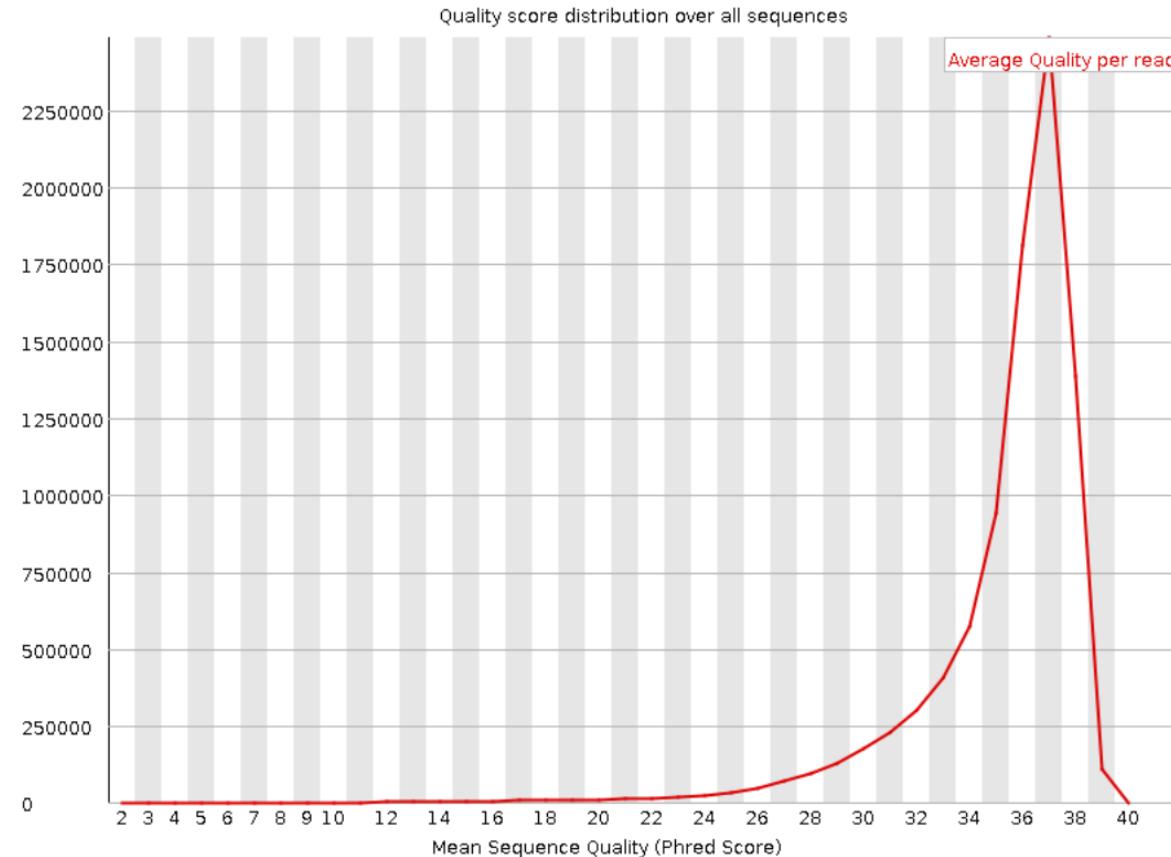
The relationship between the quality score and error probability is demonstrated with the following table:

Quality score, Q(A)	Error probability, P(~A)
10	0.1
20	0.01
30	0.001

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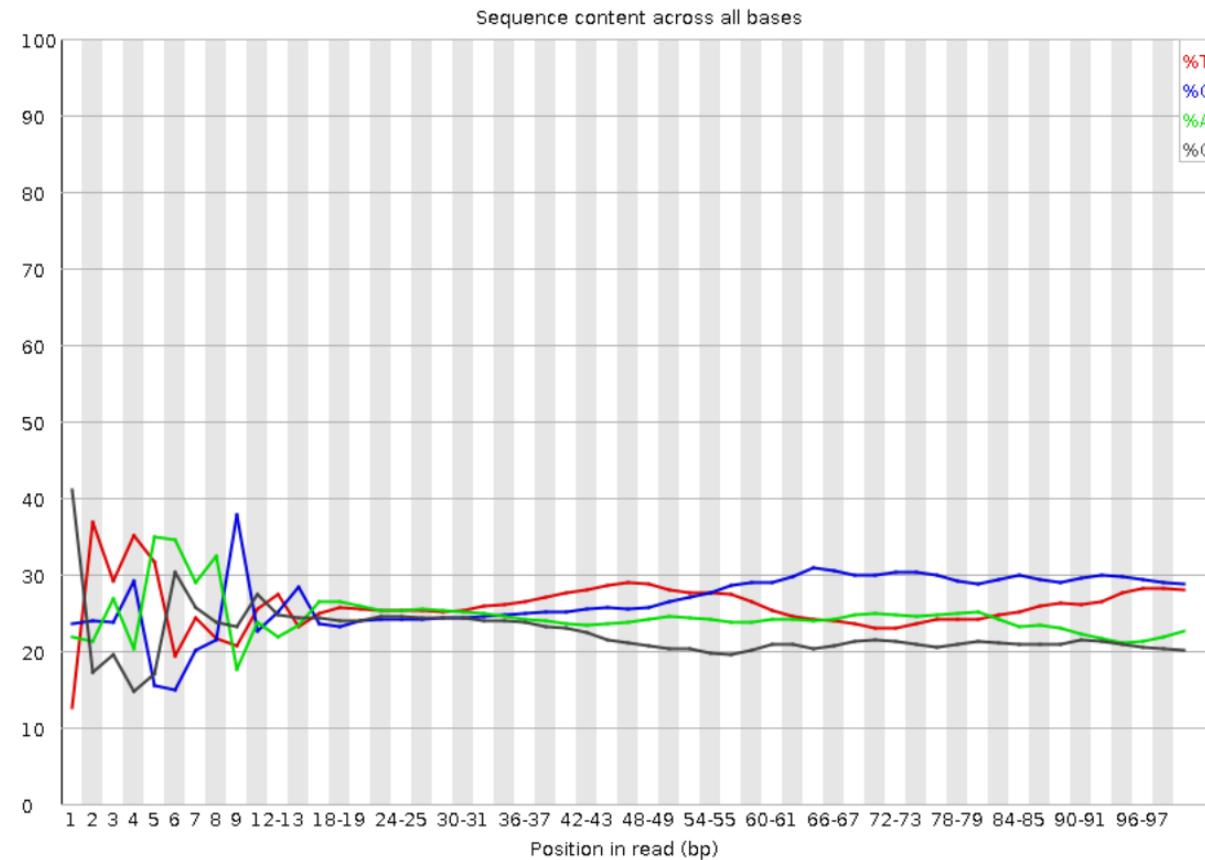
Per sequence quality scores



Summary

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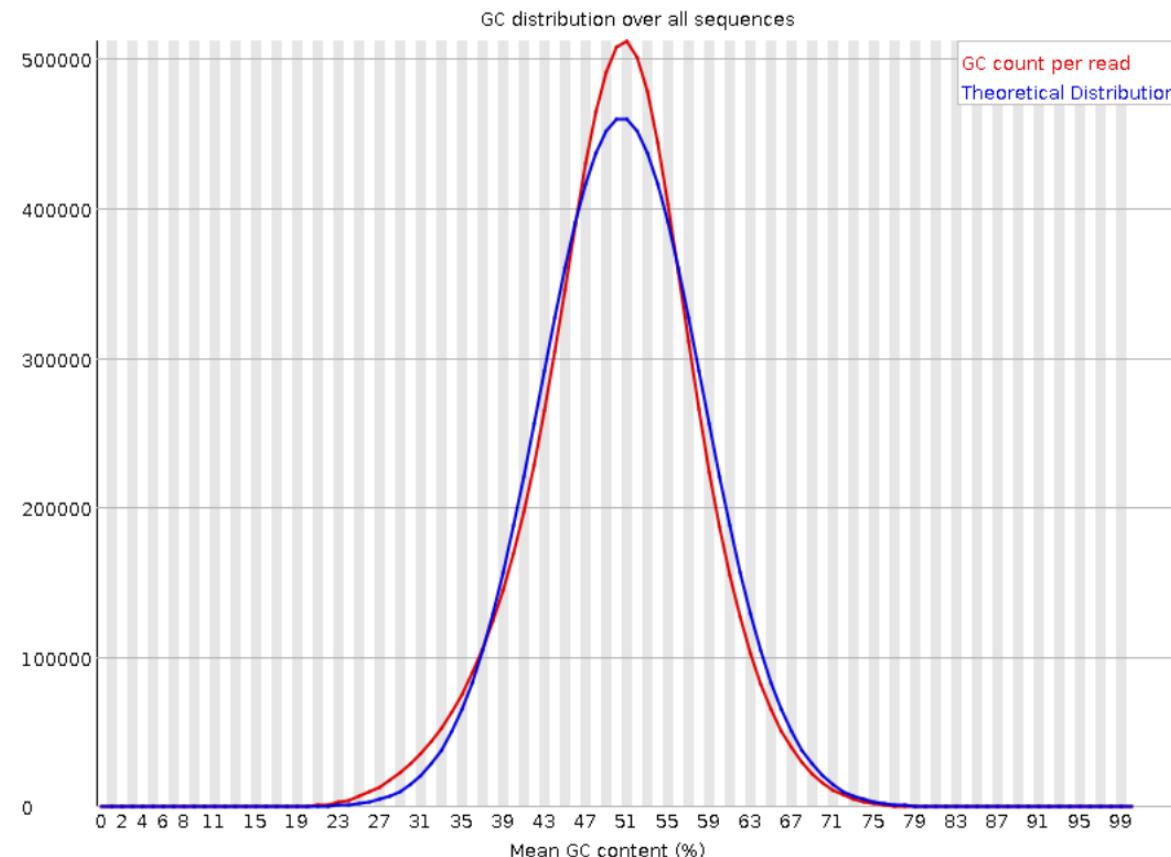
⚠ Per base sequence content



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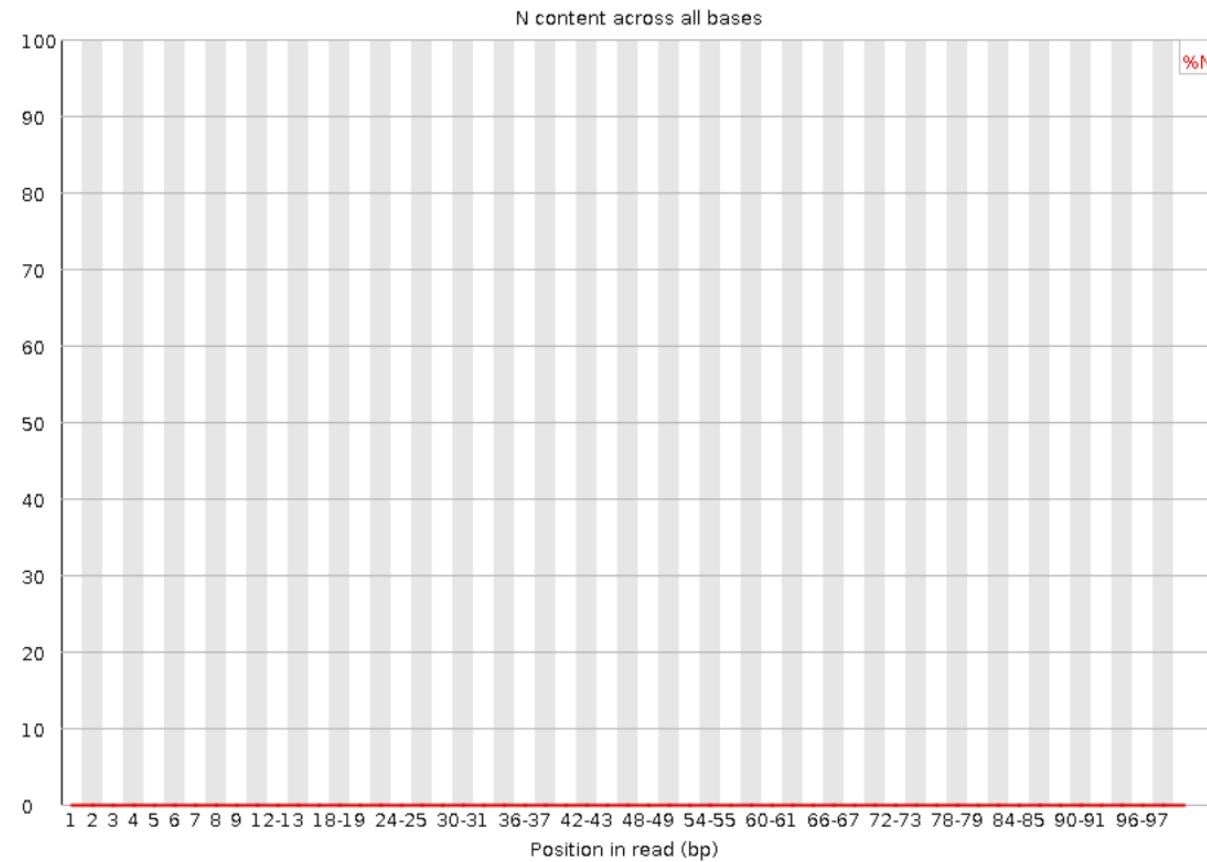
✓ Per sequence GC content



Summary

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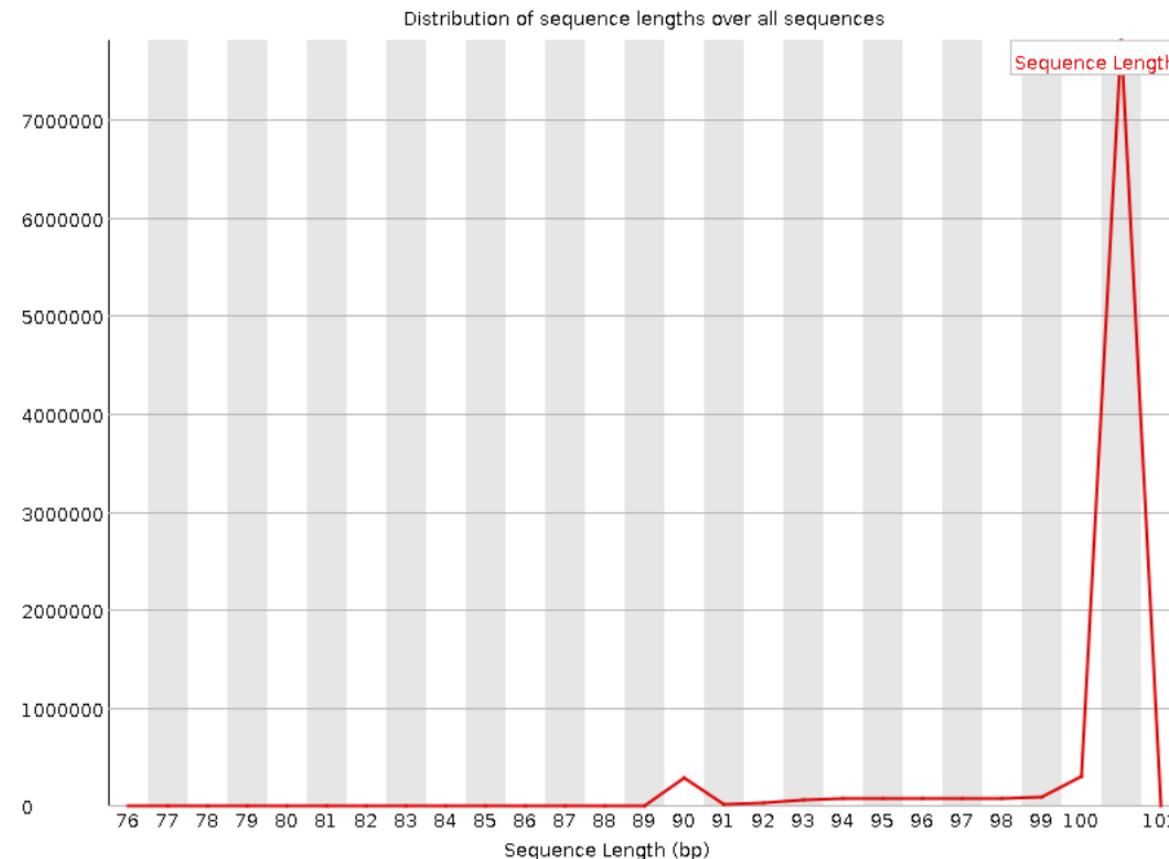
✓ Per base N content



Summary

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- ✓ [Overrepresented sequences](#)
- ✗ [Adapter Content](#)

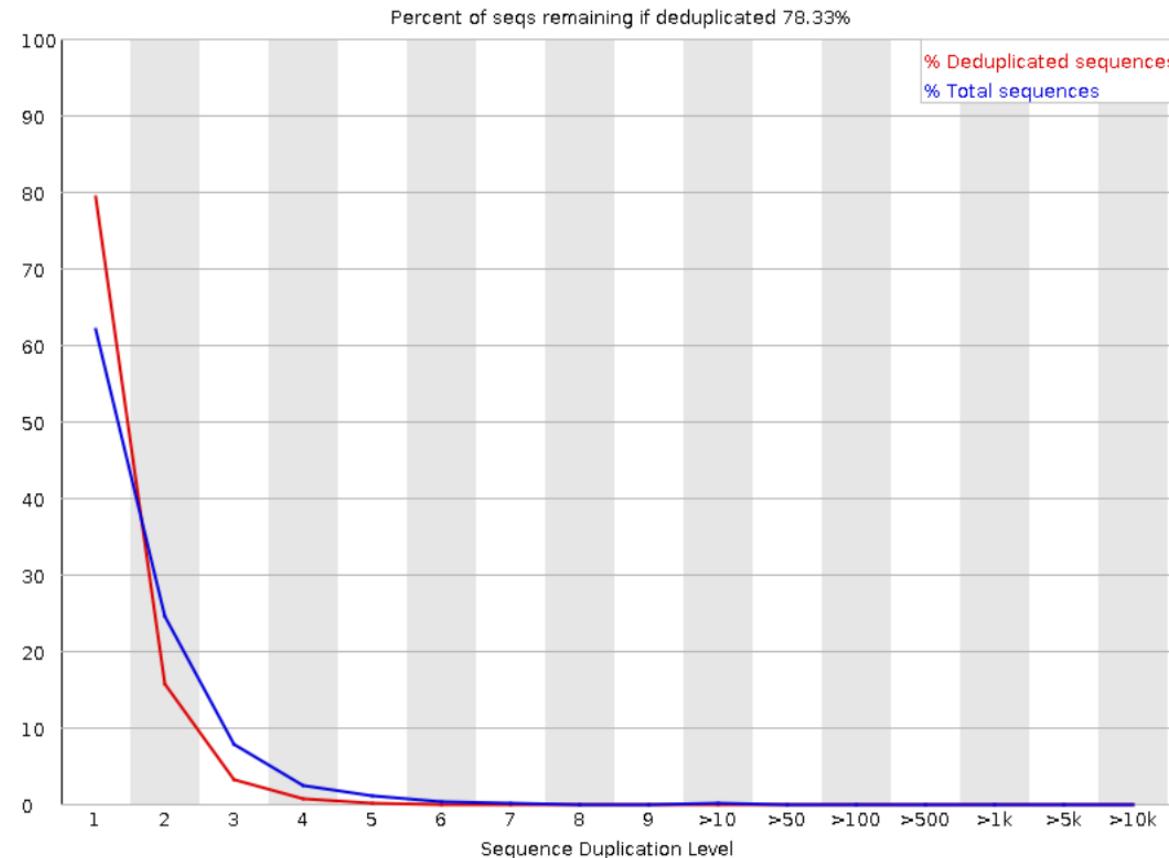
⚠ Sequence Length Distribution



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- ✓ [Overrepresented sequences](#)
- ✗ [Adapter Content](#)

Sequence Duplication Levels



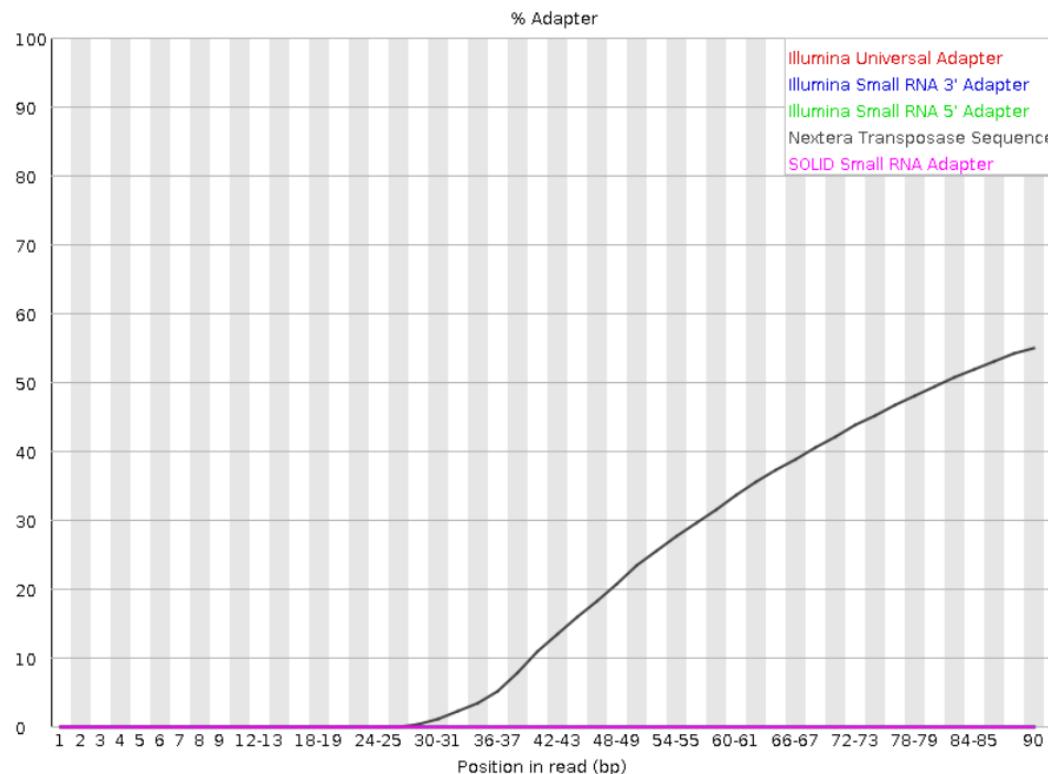
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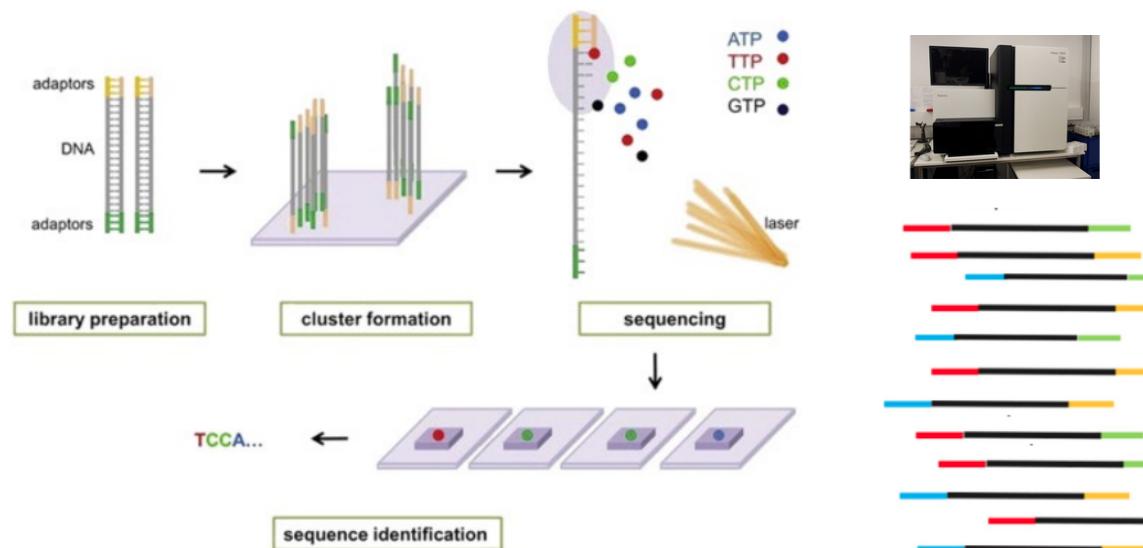
✓ Overrepresented sequences

No overrepresented sequences

✗ Adapter Content



Illumina DNA construct: indexes and adapters



Zhou et al. 2015. Atlas Oral Microbiol. <https://doi.org/10.1016/B978-0-12-802234-4.00002-1>

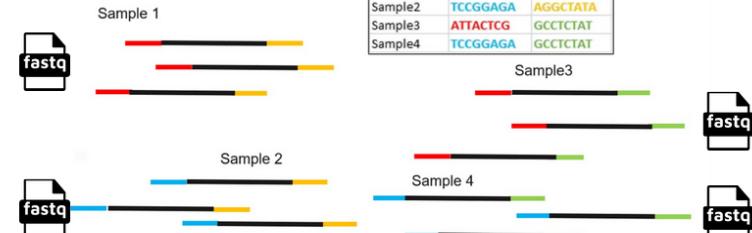
For Research Use Only. Not for use in diagnostic procedures.

illumina

Demultiplexing

- Assigns clusters to a sample, based on the cluster's index sequence which is provided in the sample sheet

[Data]		
Sample_ID	index	index2
Sample1	ATTACTCG	AGGCTATA
Sample2	TCCGGAGA	AGGCTATA
Sample3	ATTACTCG	GCTCTAT
Sample4	TCCGGAGA	GCTCTAT



GGTGTATACGGCGACCACCAcaccgacGGCCCTACACGACGCTCTTCCGATCTXXXXXXXXAGCACACGTCGGGCTCCAGTCACgacactaCCGTCTTCTGCTTG

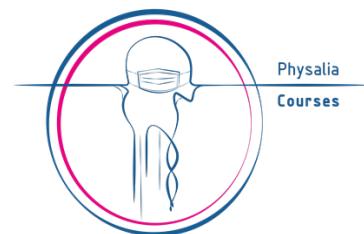
TTTACTATGCCGCTGGTGGTGTggctatttGGGATGTGCTGCAGGGGGCTAGAxxxxxxxxTCGTTGTGCAGACTTGAGGTCACTGtcgttgtatGGCAGGGGACGGGCG

[Adapter/Index Primer] [Index] [Target primer] [Target] [Target primer] [Index] [Adapter/Index Primer]

K00233;37:HGHLYBBXX;3:1101:2646:1121 1:N:0:NACGCATC+NGCTGGTG

NCGCATGAGCCGCTGTATCAGGCGCTGATCGGGCCGGCATTCAGTTGGGATAGATCGGGGGAGCACACGTC

#A7F<<GG<JFJEJJJJJJFEEJJJJJJJAFFEJFJJJJJJFJAFFEJAJFJJ<EJJJJFFF<FFA--FFFJJJJ



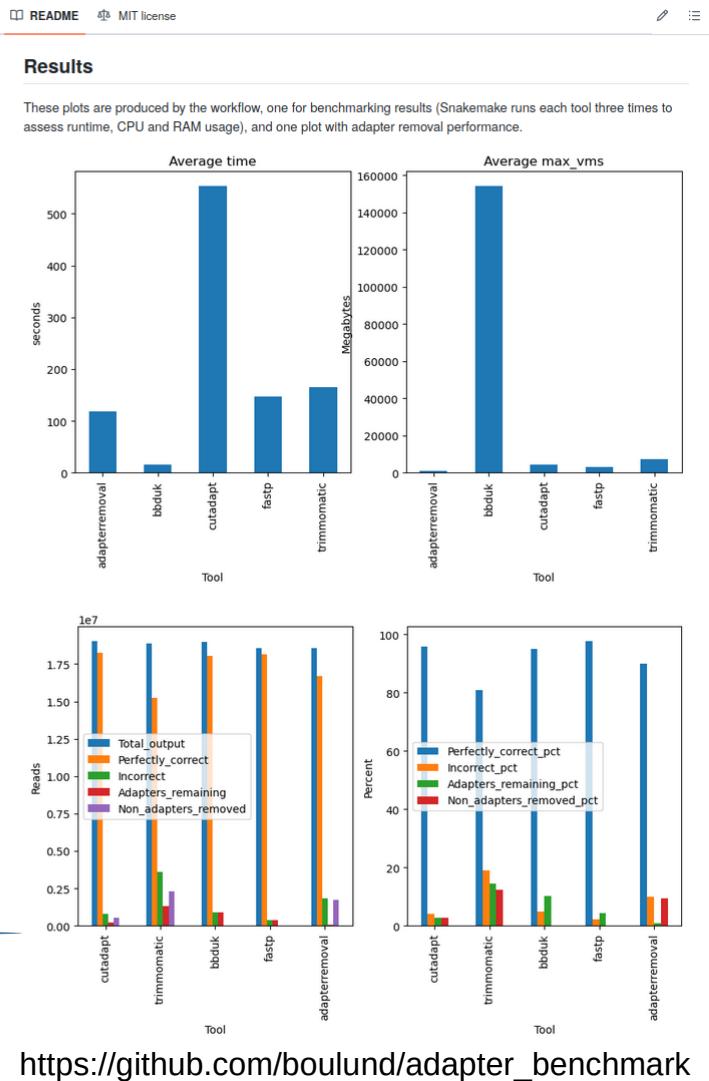
Popular adapter removal tools and benchmark results

Cutadapt

fastp

Trimmomatic

TrimGalore



https://github.com/boulund/adapter_benchmark

Benchmarking software tools for trimming adapters and merging next-generation sequencing data for ancient DNA

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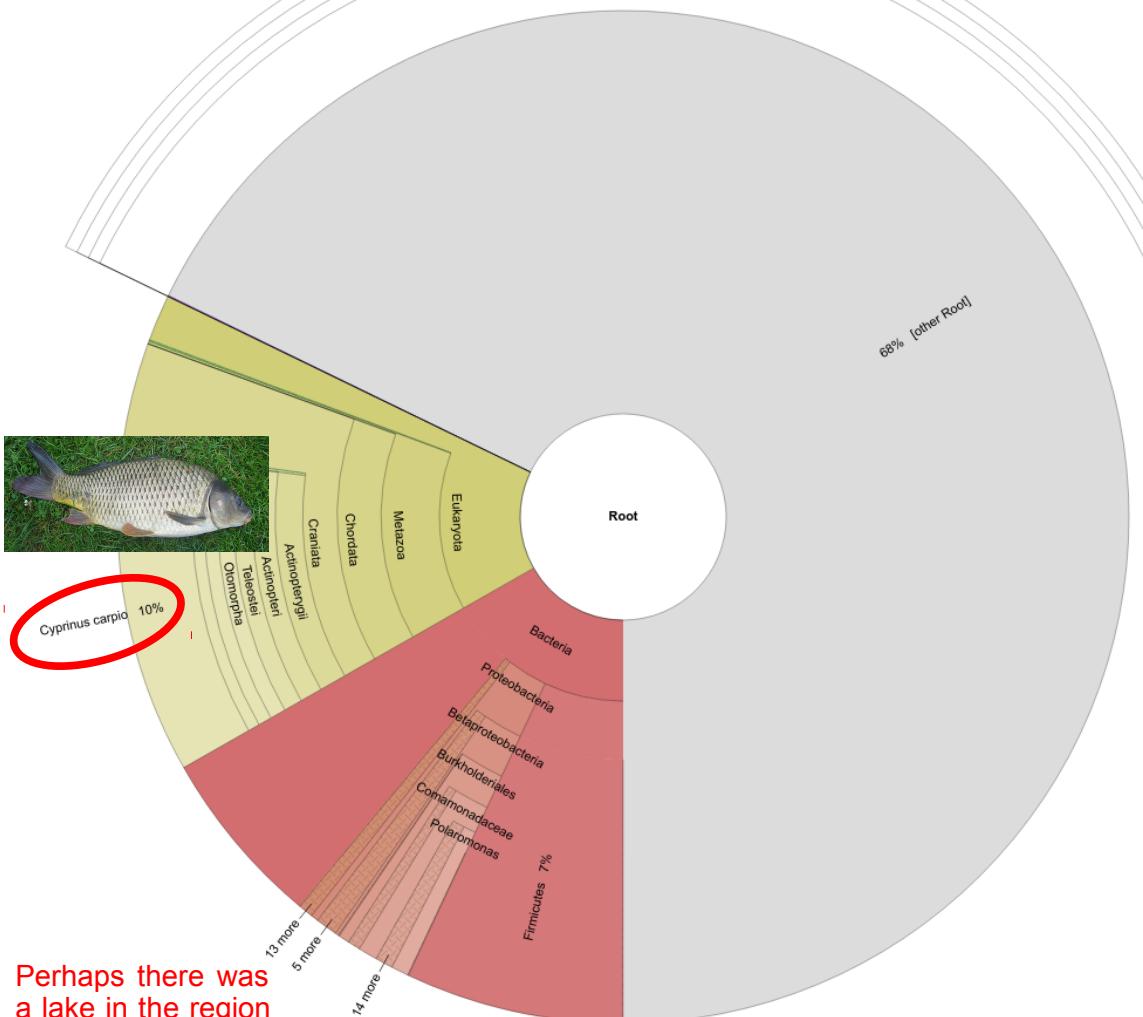
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Ancient DNA is highly degraded, resulting in very short sequences. Reads generated with modern high-throughput sequencing machines are generally longer than ancient DNA molecules, therefore the reads often contain some portion of the sequencing adaptors. It is crucial to remove those adaptors, as they can interfere with downstream analysis. Furthermore, overlapping portions when DNA has been read forward and backward (paired-end) can be merged to correct sequencing errors and improve read quality. Several tools have been developed for adapter trimming and read merging, however, no one has attempted to evaluate their accuracy and evaluate their potential impact on downstream analyses. Through the simulation of sequencing data, seven commonly used tools were analyzed in their ability to reconstruct ancient DNA sequences through read merging. The analyzed tools exhibit notable differences in their abilities to correct sequence errors and identify the correct read overlap, but the most substantial difference is observed in their ability to calculate quality scores for merged bases. Selecting the most appropriate tool for a given project depends on several factors, although some tools such as fastp have some shortcomings, whereas others like leeHom outperform the other tools in most aspects. While the choice of tool did not result in a measurable difference when analyzing population genetics using principal component analysis, it is important to note that downstream analyses that are sensitive to wrongly merged reads or that rely on quality scores can be significantly impacted by the choice of tool.

What if you forget to trim the adapters: soil sample



It turns out the Carp genome is full of Illumina adapters.

One of the first things we teach people in our [NGS courses](#) is how to remove adapters. It's not hard – we use [CutAdapt](#), but many other tools exist. It's simple, but really important – with De Bruijn graphs you will get paths through the graphs converging on kmers from adapters; and with OLC assemblers you will get spurious overlaps. With gap-filters, it's possible to fill the gaps with sequences ending in adapters, and this may be what happened in the Carp genome.

Why then are we finding such elementary mistakes in such important papers?

Why aren't reviewers picking up on this? It's frustrating.

This is a separate, but related issue, to genomic contamination – [the Wheat genome has PhiX in it; tons of bacterial genomes do too; and lots of bacterial genes were problematically included in the Tardigrade genome](#) and declared as horizontal gene transfer.

Bioinformatics Bits and Bobs

Rare and random blog posts about bioinformatics, genomics and evolution.

Monday, 29 September 2014

Why you should QC your reads AND your assembly

The genome sequence of the Common Carp *Cyprinus carpio* was [published in Nature last week](#). By coincidence, I was doing some QC on some domesticated Ferret (*Mustela putorius furo*) reads, which had thrown some kmer warnings in the [FastQC tool](#). I blasted the kmers in NCBI and was quite perplexed by the number of hits that I found in the carp genome. Nearly all of the first 150 hits were all from the carp genome. Anyway, I looked a bit further into my odd kmers and it turns out that they were the ends of some Illumina adapter sequences that had presumably been incorporated into the paired-reads on the shorter ends of the insert size. This then took me back to the Carp Genome - what had crept into that?