ENVIRONMENTAL METAGENOMICS

Physalia course, online, 11-15 November 2024

Metagenome *de-novo* assembly and quality control

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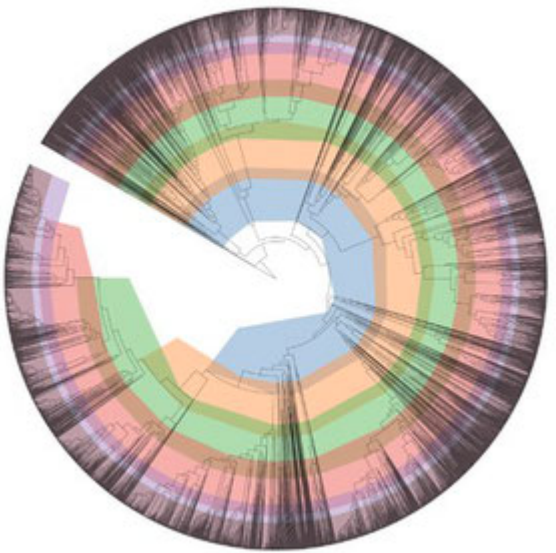

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NB: original course material courtesy:
Dr. Antti Karkman, University of Helsinki
Dr. Igor Pessi, Finnish Environment Institute (SYKE)

Typical analysis methods used in metagenomics

1) Alignment:

BOW TIE

BWA

stands for

Burrows Wheeler Aligner

Abbreviations.com

HOPS

2) Classification:

Centrifuge

MetaPhlan

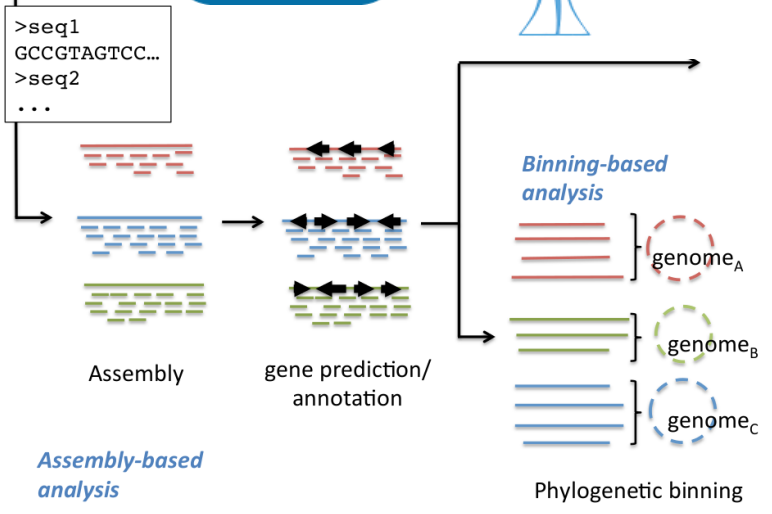
Clark

Reference based:
assume similarity to reference

3) De-novo assembly:

MEGA HIT

meta

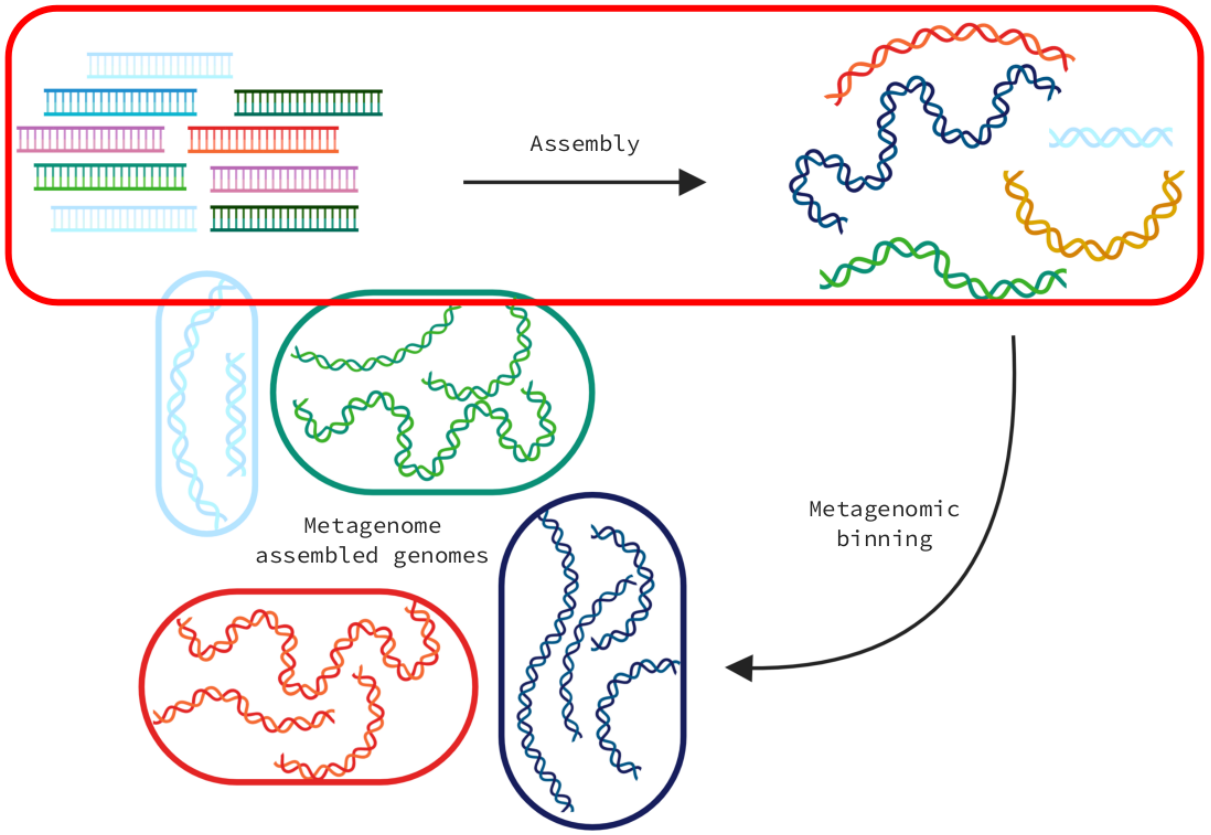


Reference free:
unbiased but challenging

De novo assembly

Assemble short nucleotide sequences into longer sequences by finding their overlap / consensus without reference genome

- No reference available
- Uneven and complex communities



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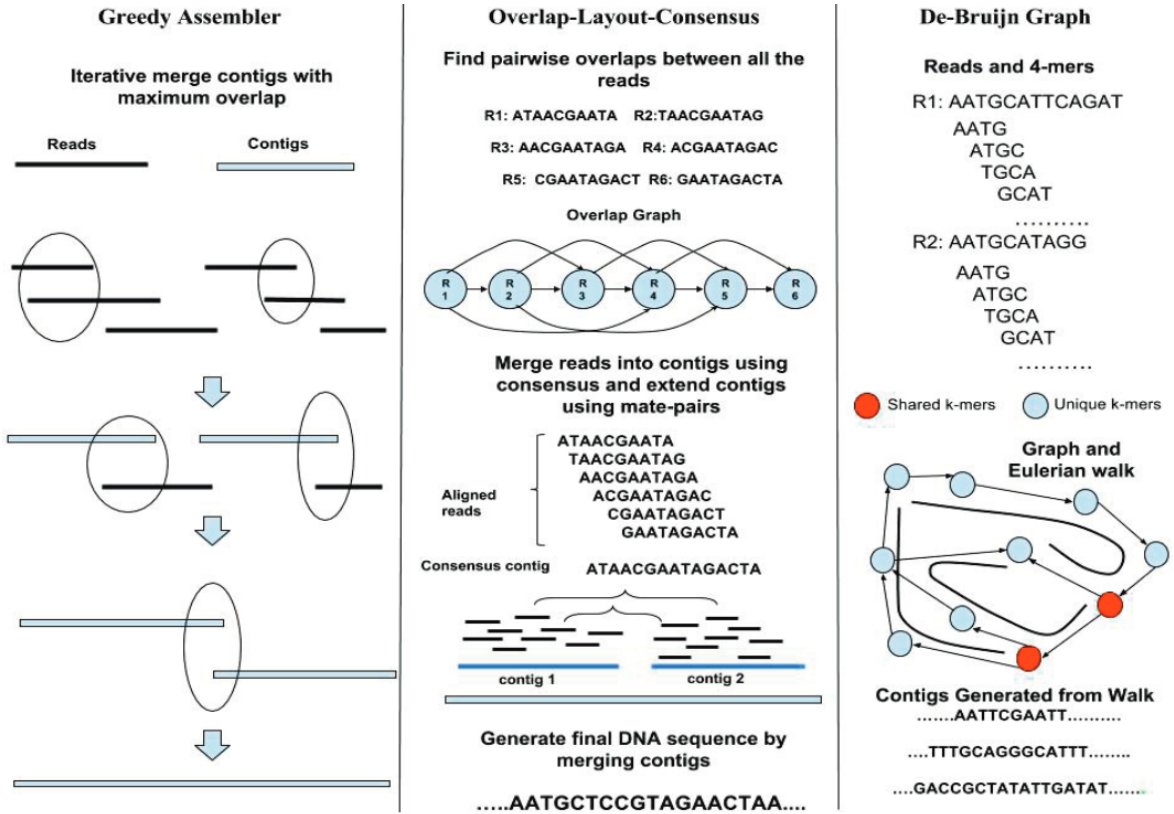

Figure 1: Overview of different de novo assembly paradigms. Schematic representation of the three main paradigms for genome assembly – Greedy, Overlap-Layout-Consensus, and de Bruijn. In Greedy assembler, reads with maximum overlaps are iteratively merged into contigs. In Overlap-Layout-Consensus approach, a graph is constructed by finding overlaps between all pairs of reads. This graph is further simplified and contigs are constructed by finding branch-less paths in the graph, and taking the consensus sequence of the overlapping reads implied by the corresponding paths. Contigs are further organized and extended using mate pair information. In de Bruijn graph assemblers, reads are chopped into short overlapping segments (k-mers) which are organized in a de Bruijn graph structure based on their co-occurrence across reads. The graph is simplified to remove artifacts due to sequencing errors, and branch-less paths are reported as contigs.

Ghurye et al., Yale Journal of Biology and Medicine, 2016

SOAPdenovo
Velvet
Spades

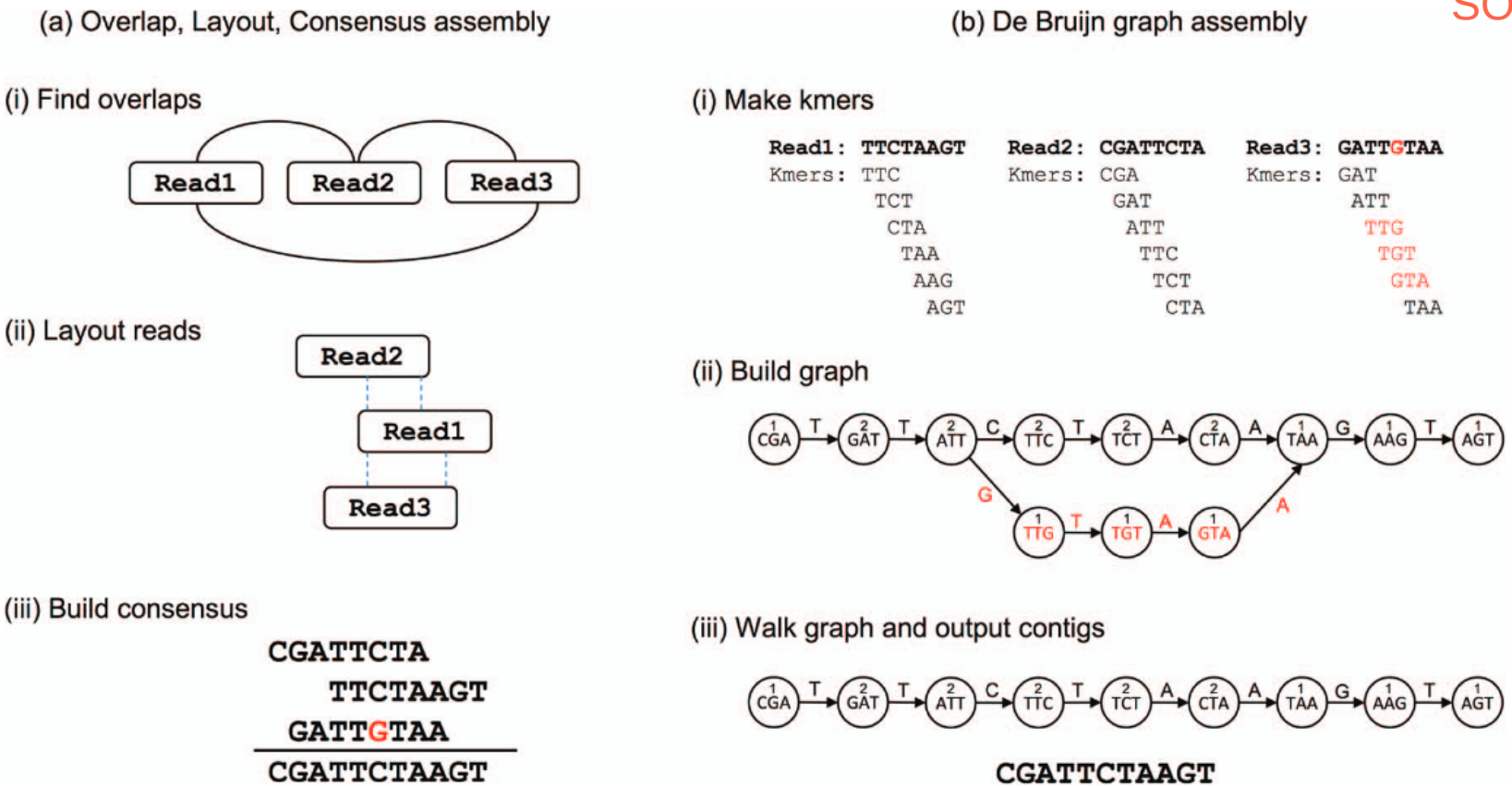

Figure 1. Two different approaches to genome assembly: (**a**) in Overlap, Layout, Consensus assembly, (i) overlaps are found between reads and an overlap graph constructed (edges indicate overlapping reads). (ii) Reads are laid out into contigs based on the overlaps (dashed lines indicate overlapping portions). (iii) The most likely sequence is chosen to construct consensus sequence. (**b**) In dBg assembly, (i) reads are decomposed into kmers by sliding a window of size *k* across the reads. (ii) The kmers become vertices in the dBg, with edges connecting overlapping kmers. Polymorphisms (red) form branches in the graph. A count is kept of how many times a kmer is seen, shown here as numbers above kmers. (iii) Contigs are built by walking the graph from edge nodes. A variety of heuristics handle branches in the graphs—for example, low coverage paths, as shown here, may be ignored.

Ayling et al., Briefings in Bioinformatics, 2020

Popular de Bruijn graph *de-novo* metagenomic assemblers for short Illumina reads

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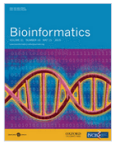

Volume 31, Issue 10
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Article Contents

< Previous Next >

JOURNAL ARTICLE

MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct *de Bruijn* graph

Dinghua Li, Chi-Man Liu, Ruibang Luo, Kunihiko Sadakane, Tak-Wah Lam
Author Notes

Bioinformatics, Volume 31, Issue 10, May 2015, Pages 1674–1676, https://doi.org/10.1093/bioinformatics/btv033
Published: 20 January 2015 **Article history**

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Abstract

Summary: MEGAHIT is a NGS *de novo* assembler for assembling large and complex metagenomics data in a time- and cost-efficient manner. It finished assembling a soil metagenomics dataset with 252 Gbps in 44.1 and 99.6 h on a single computing node with and without a graphics processing unit, respectively. MEGAHIT assembles the data as a whole, i.e. no pre-processing like partitioning and normalization was needed. When compared with previous methods on assembling the soil data, MEGAHIT generated a three-time larger assembly, with longer contig N50 and average contig length; furthermore, 55.8% of the reads were aligned to the assembly, giving a fourfold improvement.

Availability and implementation: The source code of MEGAHIT is freely available at https://github.com/voutcn/megahit under GPLv3 license.

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metaSPAdes: a new versatile metagenomic assembler

Sergey Nurk[1,4], **Dmitry Meleshko**[1,4], **Anton Korobeynikov**[1,2] and **Pavel A. Pevzner**[1,3]

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[4] These authors contributed equally to this work.

Abstract

While metagenomics has emerged as a technology of choice for analyzing bacterial populations, the assembly of metagenomic data remains challenging, thus stifling biological discoveries. Moreover, recent studies revealed that complex bacterial populations may be composed from dozens of related strains, thus further amplifying the challenge of metagenomic assembly. metaSPAdes addresses various challenges of metagenomic assembly by capitalizing on computational ideas that proved to be useful in assemblies of single cells and highly polymorphic diploid genomes. We benchmark metaSPAdes against other state-of-the-art metagenome assemblers and demonstrate that it results in high-quality assemblies across diverse data sets.

Metagenome sequencing has emerged as a technology of choice for analyzing bacterial populations and the discovery of novel organisms and genes (Tyson et al. 2004; Venter et al. 2004; Yooseph et al. 2007; Arumugam et al. 2011). In one of the early metagenomics studies, Venter et al. (2004) attempted to assemble the complex Sargasso Sea microbial community but, as the study stated, failed. On the other side of the spectrum of metagenomics studies, Tyson et al. (2004) succeeded in assembling a simple microbial community consisting of a few species.

These landmark studies (Tyson et al. 2004; Venter et al. 2004) used conventional assembly tools—namely, Celera (Myers et al. 2000) and JAZZ (Aparicio et al. 2002)—with minor modifications. Since they were published, many specialized metagenomic assemblers have been developed (Koren et al. 2011; Laserson et al. 2011; Peng et al. 2011, 2012; Boisvert et al. 2012; Namiki et al. 2012; Haider et al. 2014; Li et al. 2016). However, bioinformaticians are still struggling to bridge the gap between assembling simple and complex microbial communities (for a review, see Gevers et al. 2012). Meanwhile, many

« Previous | Next Article » Table of Contents

This Article

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Genome Res. 2017. 27: 824-834

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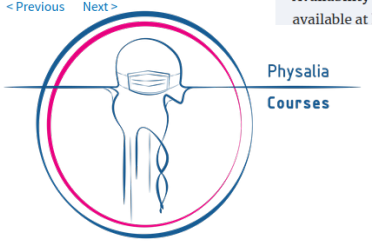

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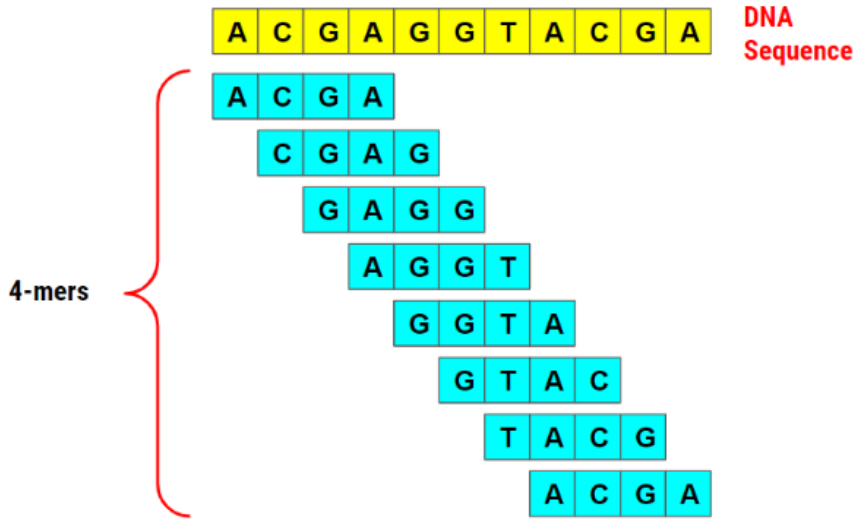
We are going to use Megahit in the exercises

De novo assembly using MEGAHIT

MEGAHIT: de Bruijn-graph assembler using a distribution of different k-mer lengths inferred from the length of the sequencing data



reasons for using MEGAHIT:

- **low-memory** footprint
- has little issues with the **presence of ancient DNA damage**
- works with **single-end data**

BUT: lower assembly quality than other assemblers for modern sequencing data (see CAMI II challenge; DOI: 10.1038/s41592-022-01431-4)

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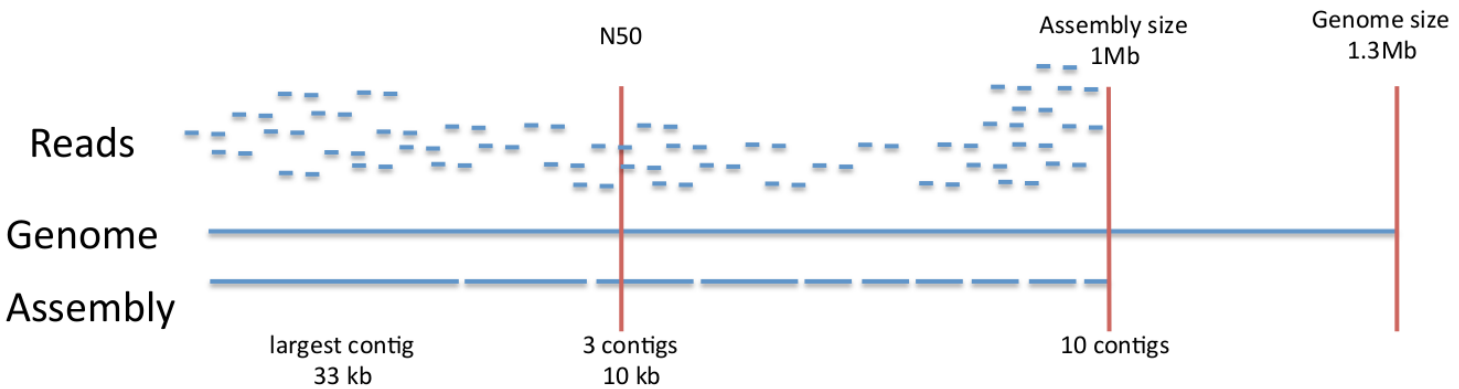
Assembly metrics

- assembly size
- number of contigs, largest contig
- N50



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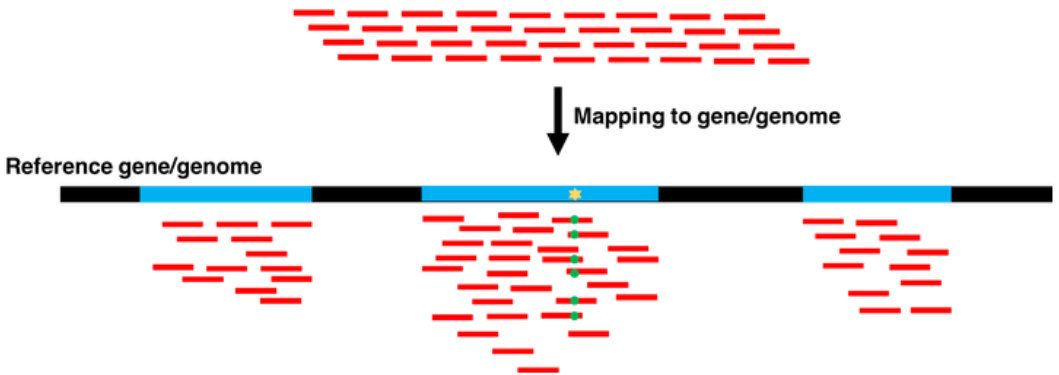
Alignment against the contigs

Many of the following steps require the alignment of the short-read data against the de novo assembled contigs, e.g.

- correction of the contig sequences
- binning of the contigs into MAGs (coverage along the contigs)
- quantification of the presence of ancient DNA damage



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Taxonomic classification - on contig level

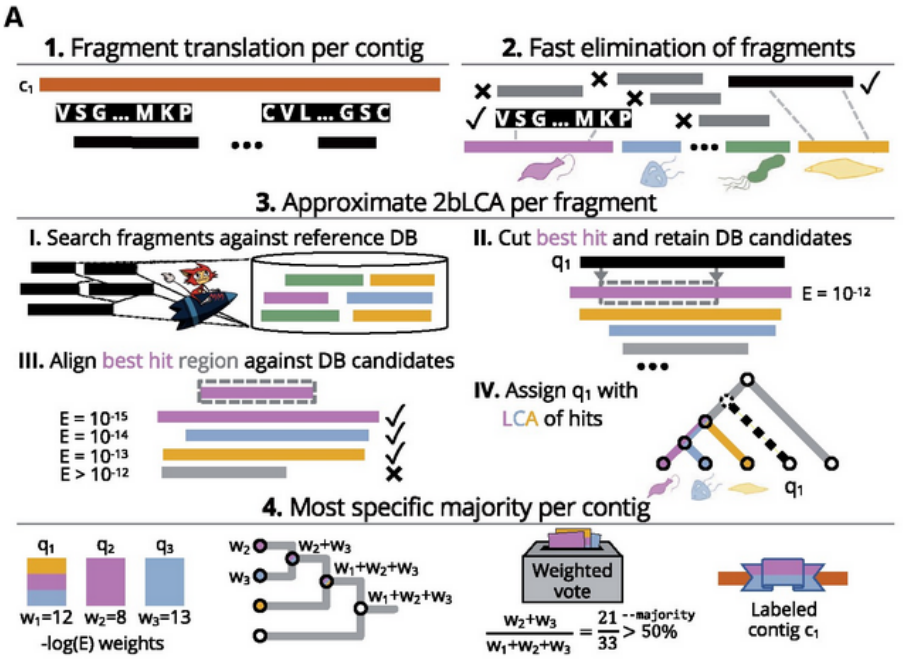
The likely taxonomic origin of contigs can be determined by aligning them against a reference database.

available aligners:

- BLAST/DIAMOND
- Kraken2
- Centrifuge
- MMSeqs2

available databases:

- NCBI NT/RefSeq
- GTDB



Mirdita *et al.* (Bioinformatics, 2021; doi: bioinformatics/btab184) Fig. 1

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