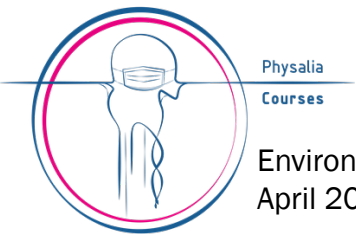


# Environmental metagenomics

Metagenome assembly

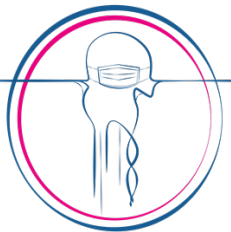
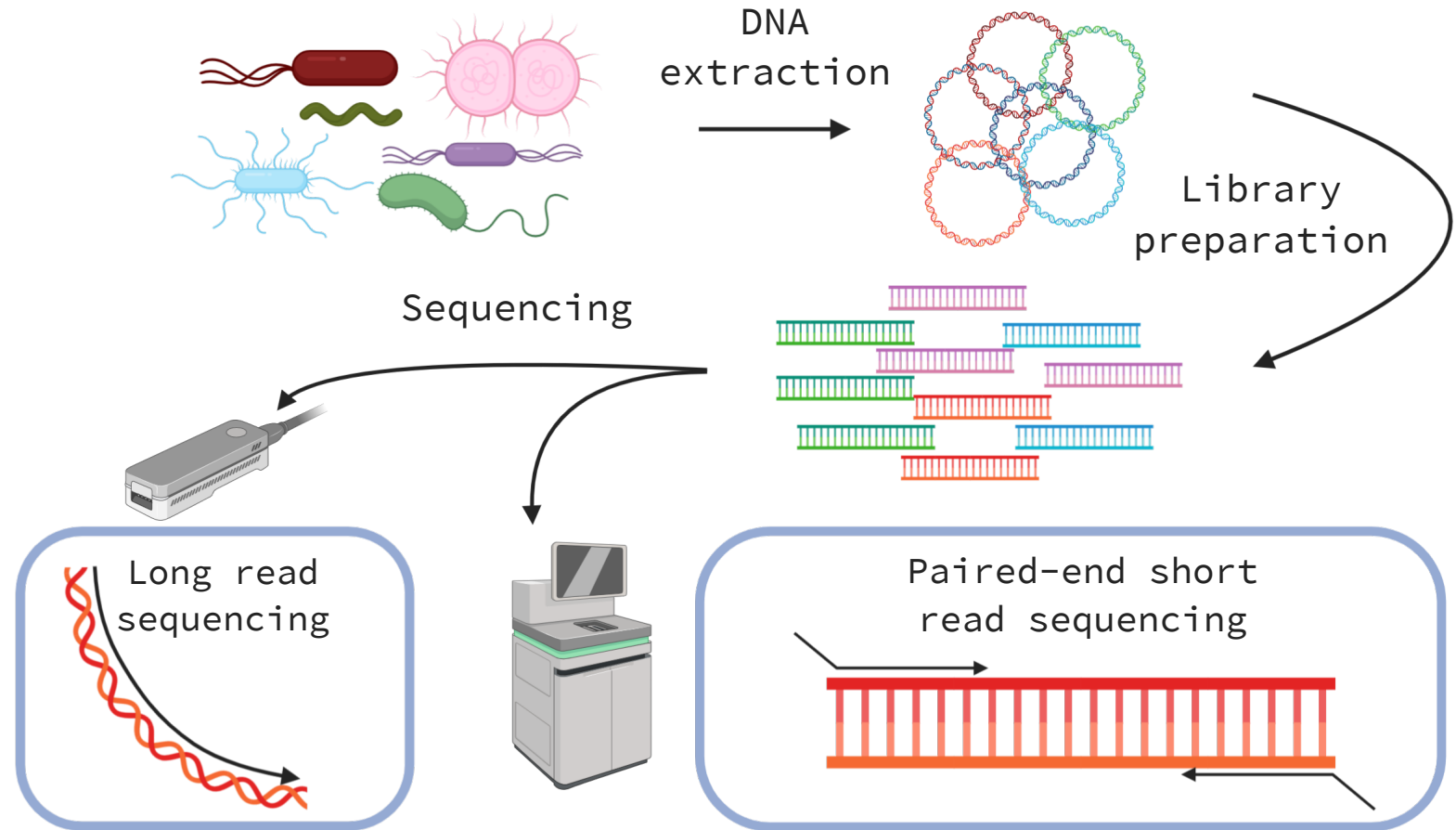


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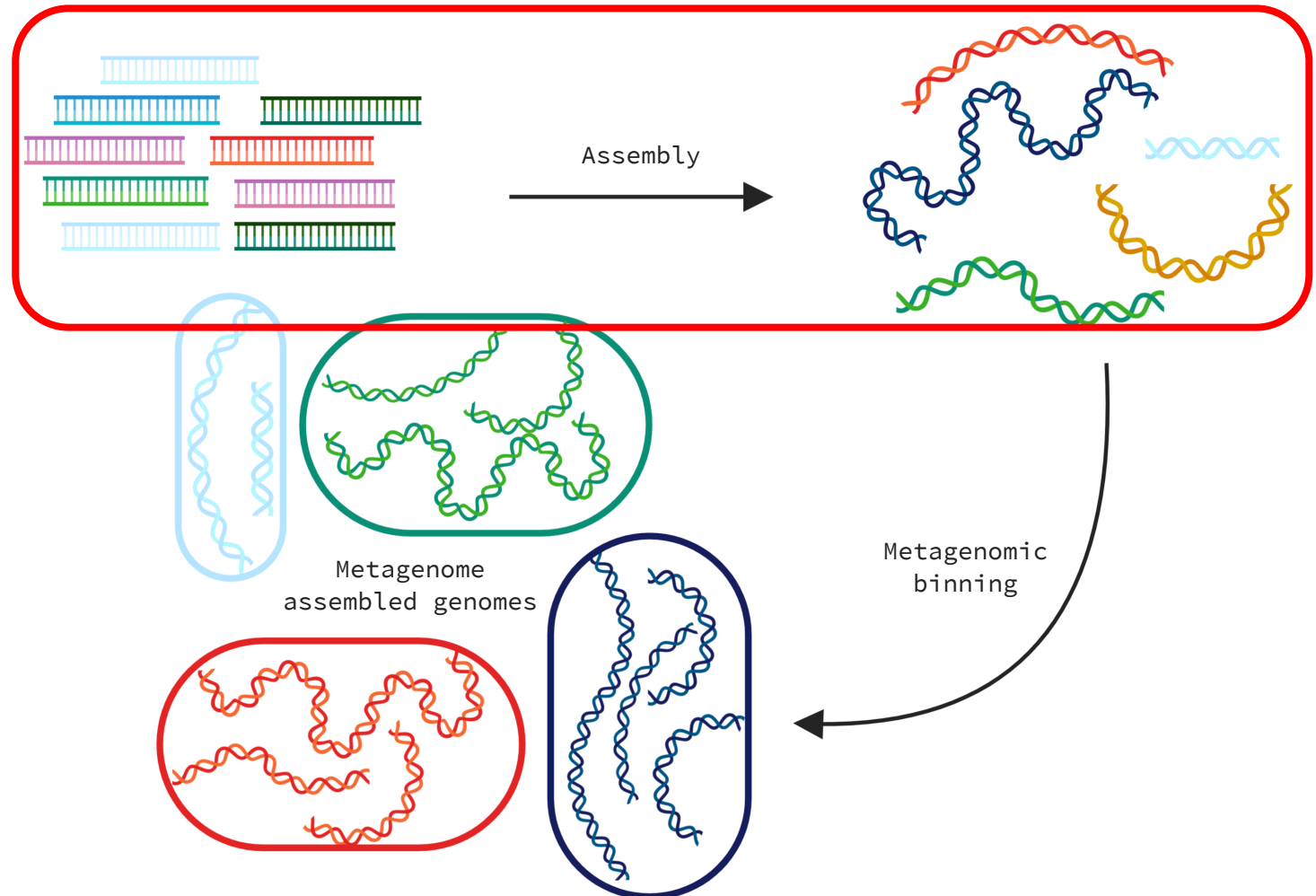
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# From samples to sequences



# De novo assembly

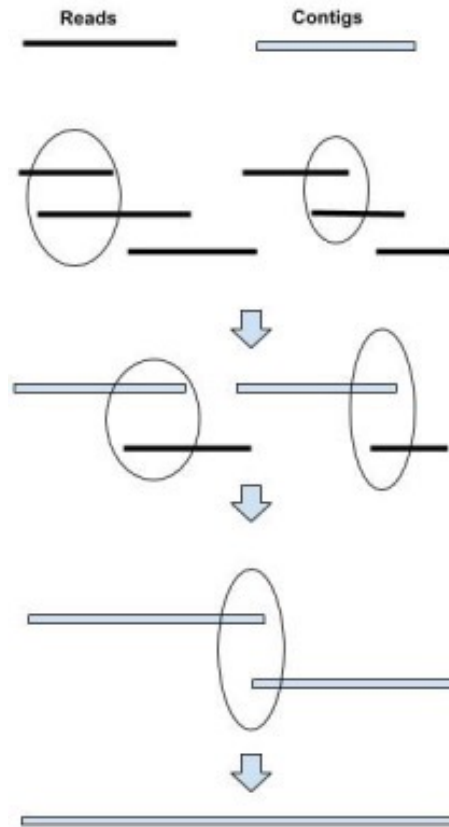
- Uneven and complex communities
- No reference available



# Assembly strategies

## Greedy Assembler

Iterative merge contigs with maximum overlap

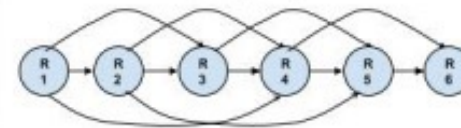


## Overlap-Layout-Consensus

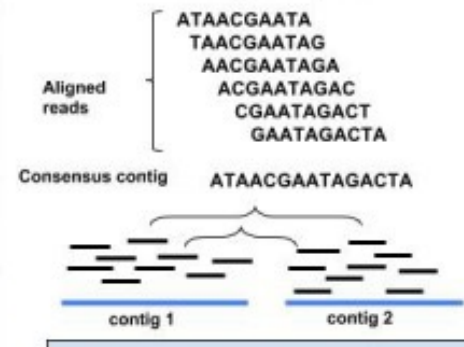
Find pairwise overlaps between all the reads

R1: ATAACGAATA R2: TAACGAATAG  
R3: AACGAATAGA R4: ACGAATAGAC  
R5: CGAATAGACT R6: GAATAGACTA

Overlap Graph



Merge reads into contigs using consensus and extend contigs using mate-pairs



Generate final DNA sequence by merging contigs

.....AATGCTCCGTAGAACTAA.....

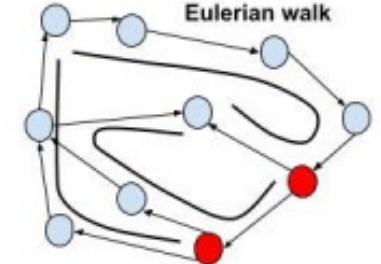
## De-Bruijn Graph

Reads and 4-mers

R1: AATGCATTCAGAT  
AATG  
ATGC  
TGCA  
GCAT  
.....  
R2: AATGCATAGG  
AATG  
ATGC  
TGCA  
GCAT  
.....

● Shared k-mers ● Unique k-mers

Graph and Eulerian walk



Contigs Generated from Walk

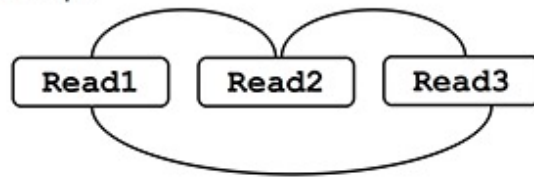
.....AATTCGAATT.....  
.....TTTGCAGGGCATT.....  
.....GACCGCTATATTGATAT.....

Ghurye et al. 2016. Yale J Biol Med.

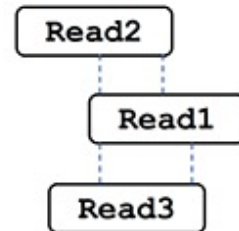
# Assembly strategies

(a) Overlap, Layout, Consensus assembly

(i) Find overlaps



(ii) Layout reads



(iii) Build consensus

```

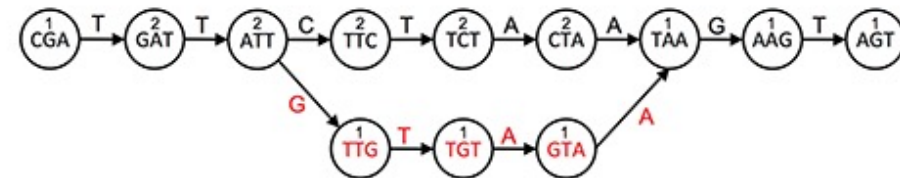
CGATTCTA
  TTCTAAGT
    GATTGTAA
-----
CGATTCTAAGT
    
```

(b) De Bruijn graph assembly

(i) Make kmers

<b>Read1: TTCTAAGT</b>	<b>Read2: CGATTCTA</b>	<b>Read3: GATTGTAA</b>
Kmers: TTC	Kmers: CGA	Kmers: GAT
TCT	GAT	ATT
CTA	ATT	TTG
TAA	TTC	TGT
AAG	TCT	GTA
AGT	CTA	TAA

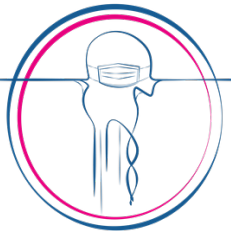
(ii) Build graph



(iii) Walk graph and output contigs



# Choice of kmer(s)?



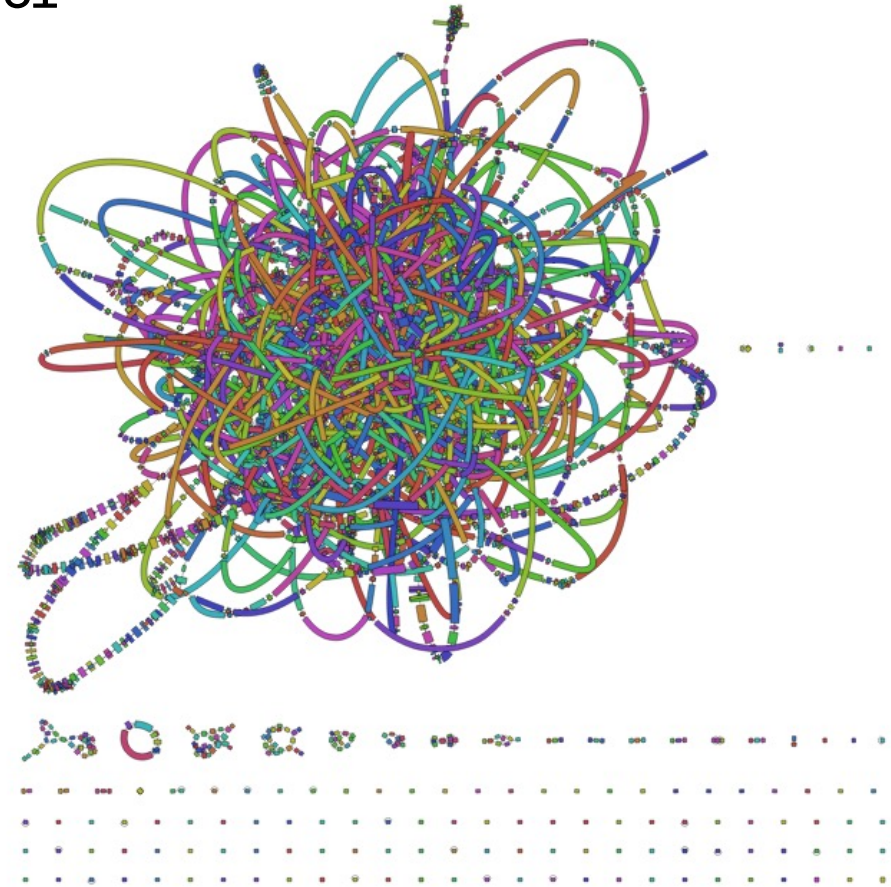
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# Choice of kmer(s)?

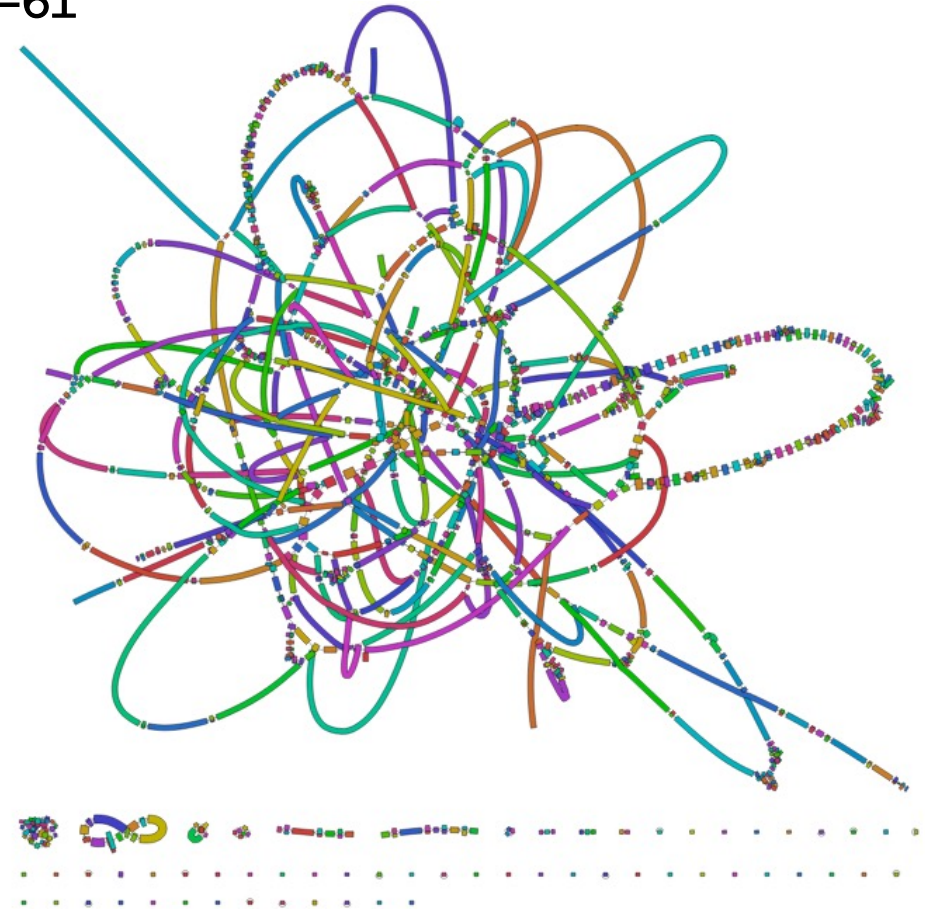
k=51





# Choice of kmer(s)?

k=61

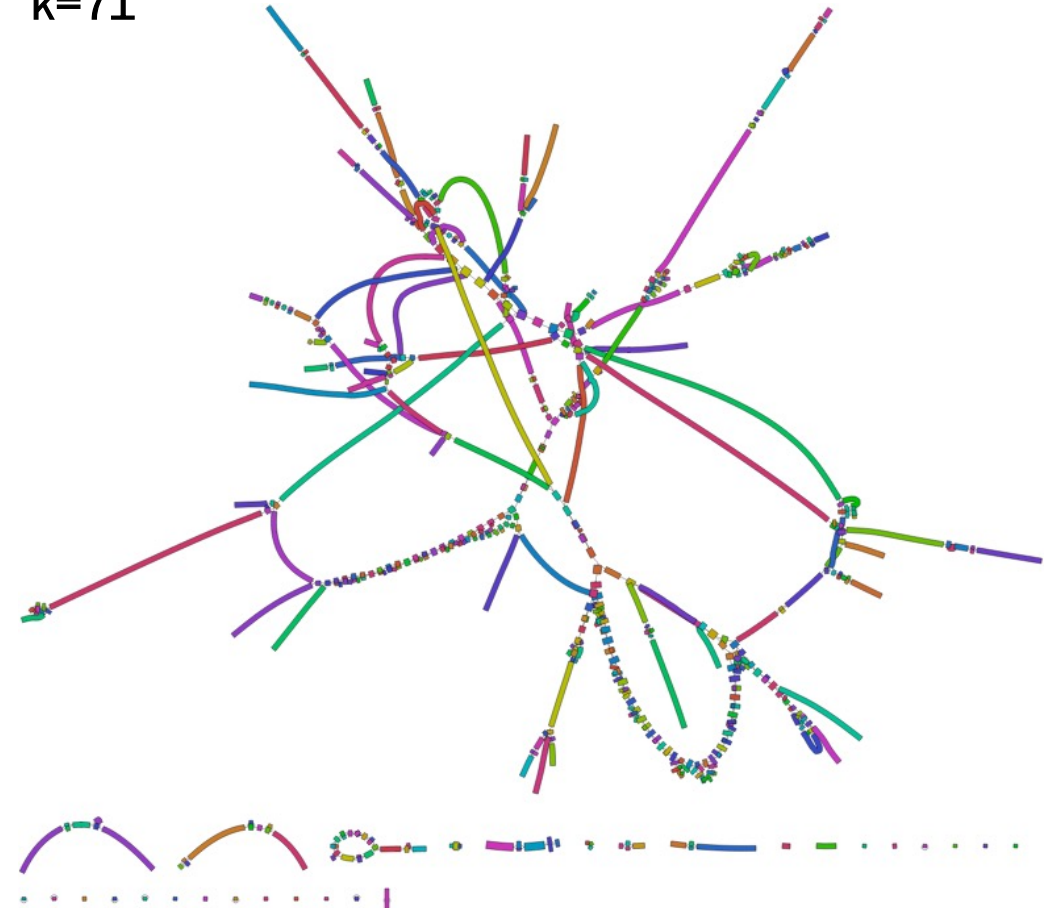


<https://github.com/rrwick/Bandage/wiki/Effect-of-kmer-size>



# Choice of kmer(s)?

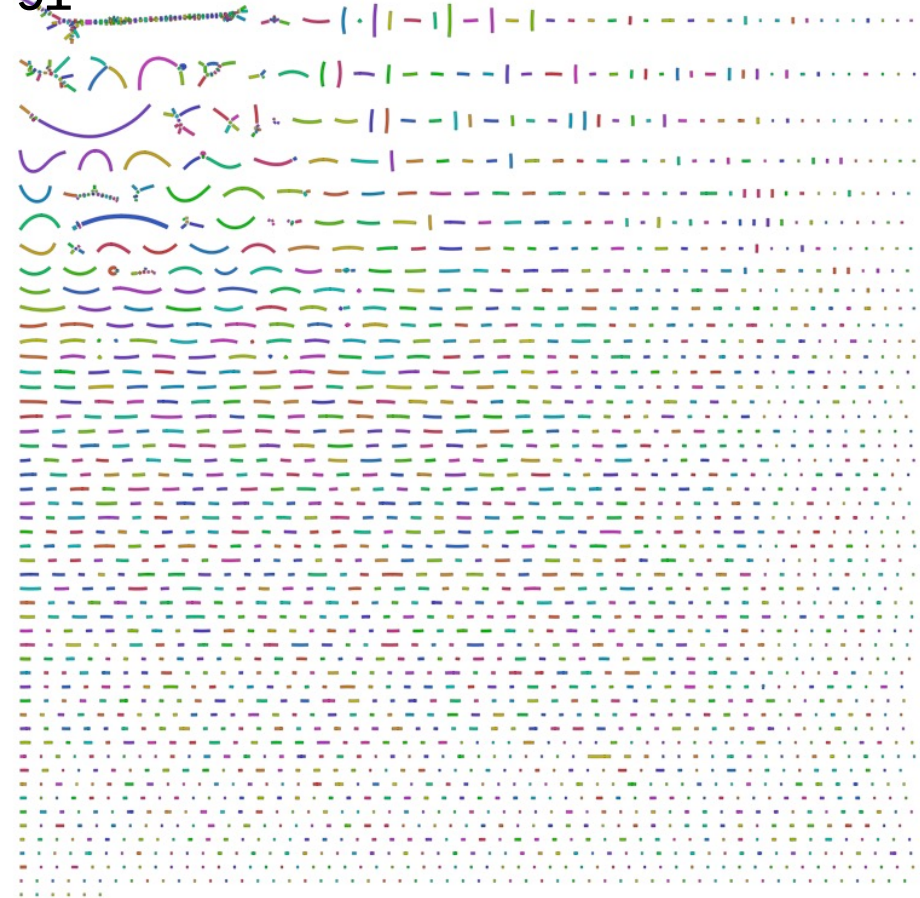
k=71



<https://github.com/rrwick/Bandage/wiki/Effect-of-kmer-size>

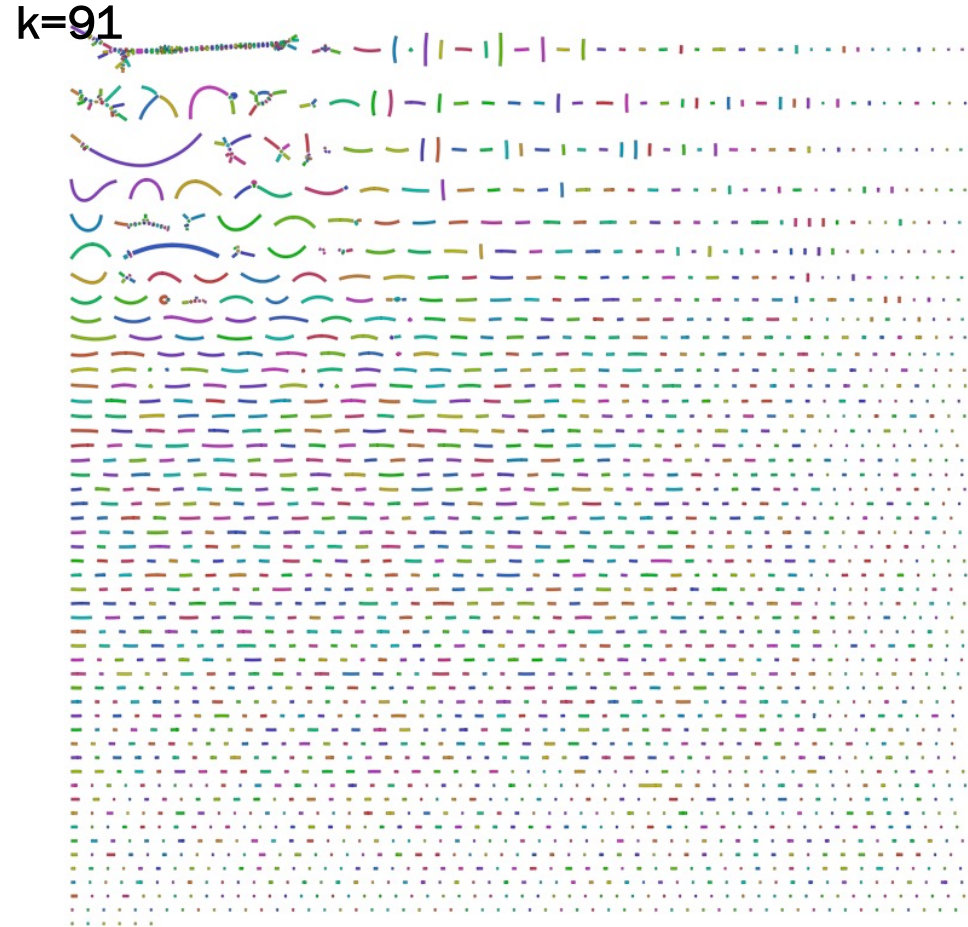
# Choice of kmer(s)?

k=91

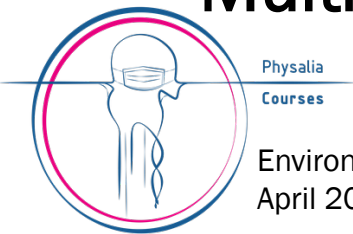


# Choice of kmer(s)?

- Short kmers → short contigs & many connections
- Longer kmers → longer contigs & fewer connections
- Things affecting optimal kmers:
  - Read length
  - Read depth
  - Sequence complexity
- **Multiple kmers**



<https://github.com/rrwick/Bandage/wiki/Effect-of-kmer-size>

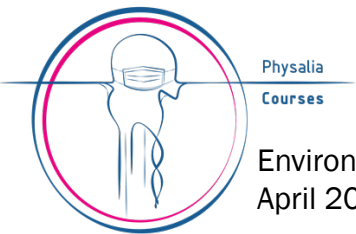


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# Which assembler to choose?



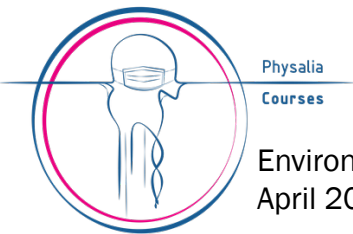
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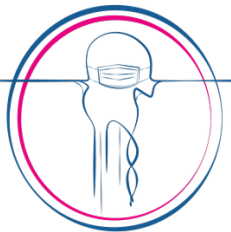
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# Which assembler to choose?

- **Availability:** The tool should be freely available either as download or webserver.
- **Usability:** The tool should have a proper manual, readme file or help function describing how to use it. In case of problems, the respective authors were contacted.
- **Adoption:** The tool should be widely used, or show potential of being widely adopted in the future.
- Reference: Lindgreen et al. 2016. Sci Rep.



# Different assemblers for short-reads



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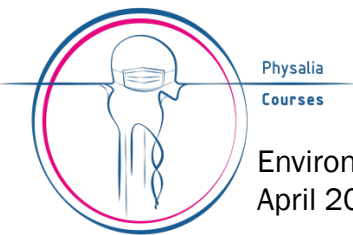


# Different assemblers for short-reads

**Table 1** Assembly statistics and computational requirements for assembly of the Tara Oceans metagenome. Time required is given in seconds, minutes and hours for illustrative purposes and memory in GB of RAM required

	Tara Ocean								
	CLC	IDBA-UD	MEGAHIT	metaSPAdes	MetaVelvet	Omega	Ray Meta	SPAdes	Velvet
Number of contigs ( $\geq 500$ bp)	50,716	163,815	216,938	185,419	67,161	15,982	6128	220,178	57,816
Total length	46,069,409	179,686,756	210,621,485	202,770,058	55,972,515	34,861,819	7,277,214	275,920,632	45,425,460
No. of long contigs ( $\geq 1$ kbp)	10,720	50,498	56,243	48,640	12,590	13,305	2179	70,711	8802
No. of ultra-long contigs ( $\geq 50$ kbp)	0	2	1	37	0	9	0	54	0
Largest contig	39,748	101,400	62,649	141,519	30,177	102,255	41,443	197,381	21,980
<i>N50</i>	880	1166	982	1124	805	2691	1329	1415	749
<i>L50</i>	14,113	38,236	58,246	39,033	21,544	2737	1345	39,617	19,631
Mapping rate (%)	38.98	52.24	55.92	64.03	4117	13.64	8.25	64.46	48.19
Time (seconds)	3527	69,782	10,455	125,862	2527	168,213	16,419	80,039	2342
Time (minutes)	58.78	1163.03	174.25	2097.70	42.12	2803.55	273.65	1333.98	39.03
Time (hours)	0.98	19.38	2.90	34.96	0.70	46.73	4.56	22.23	0.65
Memory required (GB)	16.23	42.84	10.58	66.53	109.37	30.7	42	157.75	109.37

Van der Walt et al., 2017. BMC Genomics



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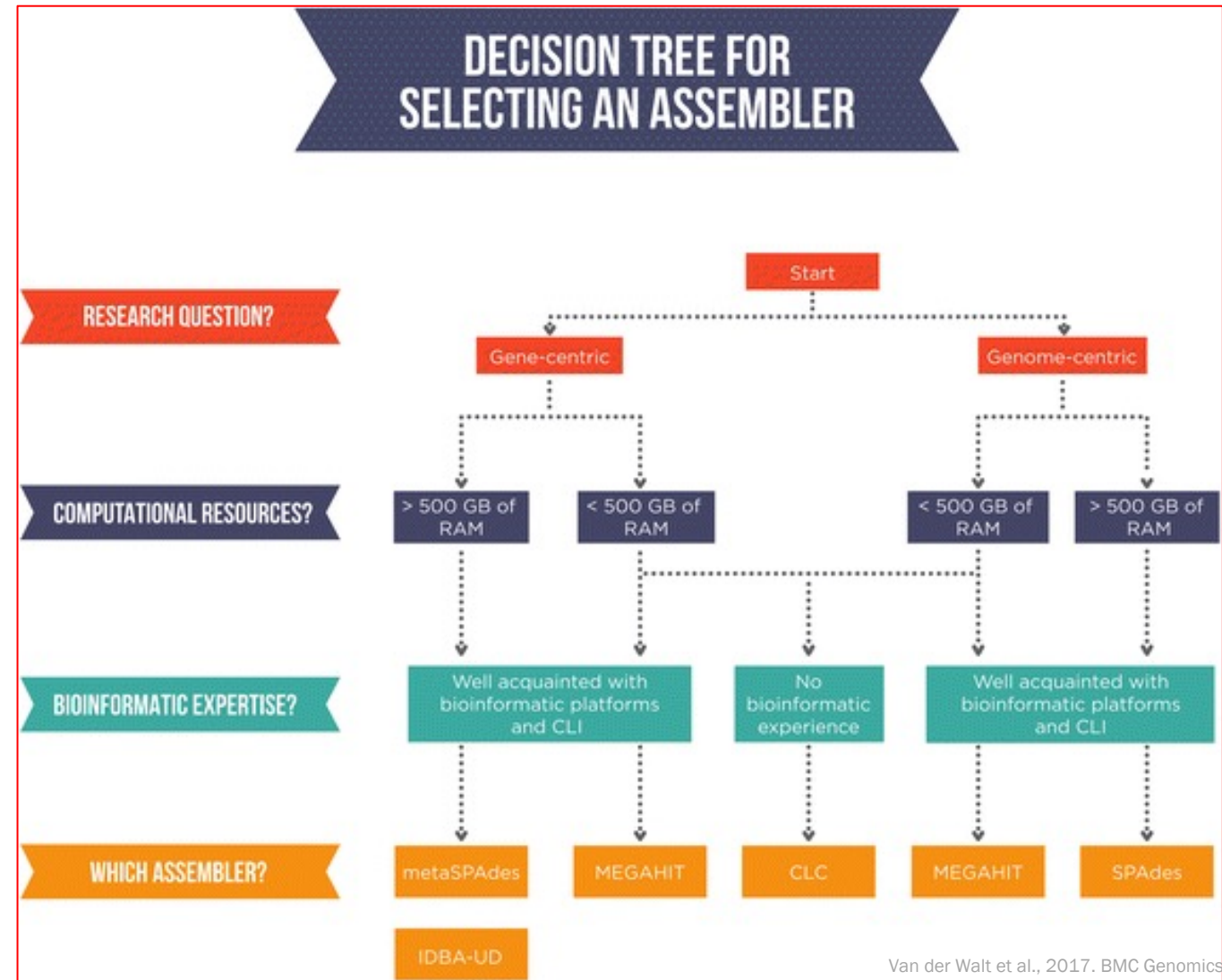
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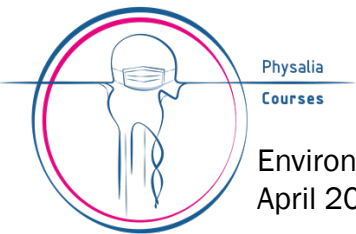
# Which assembler to choose?



Van der Walt et al., 2017. BMC Genomics



# Assembly QC



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# Assembly QC

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# Open questions in metagenomic assembly

- Choice of kmers
- To co-assemble or not?
- No reference – How to define a good assembly?
- Challenging elements for assembly
  - Repeat regions
  - Horizontally transferred genes
  - Extrachromosomal elements
- Others?

