

Schedule

Saturday	2p – 5p	Rayan Chikhl	Metagenomics Assembly, then Open Lab
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Schedule

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Schedule

- 2 pm: metagenomics assembly lecture
- 3 pm: metagenomics assembly lab
or open lab

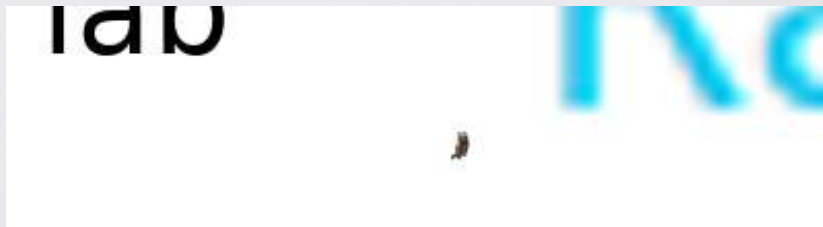
Rayar

Also at 4 pm: optional Metagenomics 'faculty lunch coffee'

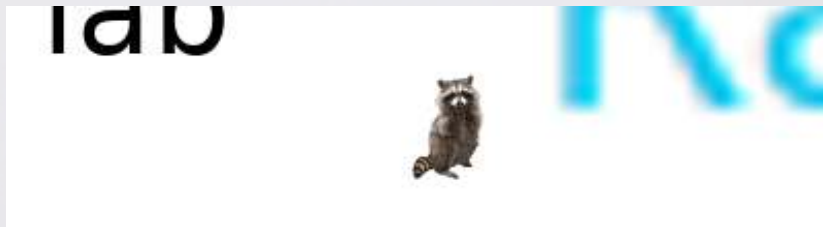
Schedule

- 2 pm: metagenomics assembly lecture
- 3 pm: metagenomics assembly lab
or open lab

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!



Congratulations to

1. **Forrest Walker**
2. **Alena di Primio**
3. ? *you?*

for completing the hidden *raccoon facts*
challenge

Metagenomics assembly

Rayan Chikhi

with some help from Dag Ahren and Sergey Nurk

Institut Pasteur

Workshop on Genomics 2020





!



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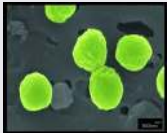


I wanted participants to know about..



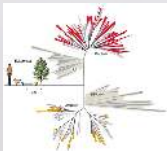
The discovery of Asgard archaea

[Takai and Horikoshi, 1999]



Analysis of single cells of a super-abundant ocean bacteria

[Kashtar *et al*, 2014]



Newfound groups of bacteria

[Brown *et al*, 2015]

Metagenomics

What?

- Term coined by Jo Emily Handelsman *et al* (1998)
- *the application of modern genomics technique without the need for isolation and lab cultivation of individual species* (Chen, Pachter 2005)

Why?

- Most microorganisms are not possible to culture and hence the only way to investigate their genome is to use metagenomics.

Metagenomics vs metataxonomics

Metataxonomics (will be on Microbiome day)

- 16S or 18S rRNA sequencing
- Fast and cost-effective
- Limited (no gene content, no viruses)
- Applications: taxonomic profiling, rRNA phylogeny, ..

Metagenomics

- Shotgun sequencing of DNA
- Versatile, enables assembly
- Applications: functional genome analyses, whole genome phylogeny, pathogen detection, ..

Source: Breitwieser et al, Briefings in Bioinformatics 2017

Metagenomics analysis scenarios

Assembly route

1. *de novo* assembly
2. contigs binning
3. taxonomic assignment

Species identification route

- Taxonomic assignment of reads
- Kraken2 (minimizers), Kaiju, Centrifuge, etc

Direct comparison route

- direct comparison of experiments (e.g. similarity matrix)
- Mash, Sourmash, Simka, etc
- (won't be covered here)

Elements of choice

Credit: H. Touzet, CNRS

Metagenome-Assembled Genomes (MAGs)

A MAG is **one bin** selected out of an assembled metagenome.

Advantages

- Well-established sequencing (Illumina)
- Cheap

Disadvantages

- In complex communities:
 - ▶ Only the most abundant taxa are likely to be "well" assembled
 - ▶ High computational requirements

SAGs (Single-Amplified Genomes)

Relies on recent techniques that allows for **isolation** of single cells followed by single cell **amplification**

Advantages

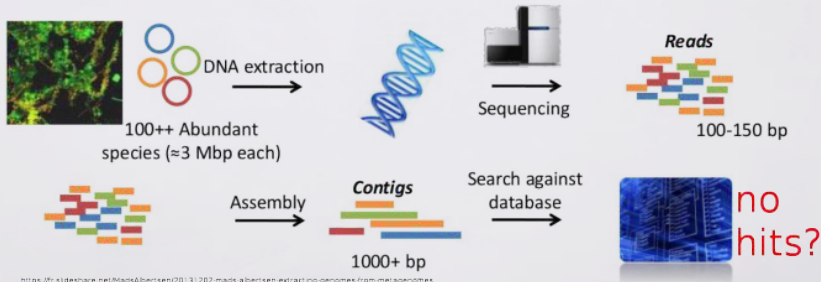
- Minimise the risk of false hybrid assembly
- It is possible to select which cells to sequence

Disadvantages

- Complex laboratory protocols
- Contamination (even from kits/reagents)
- Amplification is biased (new protocols are under development - spoiler alert: they're still biased)

Metagenomic assembly

Reconstruct **genomes of species**, possibly even **strains**, from short read sequencing data of an **environment**



Challenges

1. closely related strains
2. uneven depths, & low depths
3. inter-species repeats
4. size of datasets
5. lack of long reads

(adapted from A. Korobeynikov's talk)

A Intragenomic Repeats



B Intergenomic Repeats



Fig: Olsen *et al*, 2017

Credit: H. Touzet, CNRS

What comes after assembly

Contigs binning

- CONCOCT
- MetaBAT2
- MaxBin2

Taxonomic identification

- CAT/BAT
- ProPhyle
- PhyloPythiaS

anvi'o pipeline

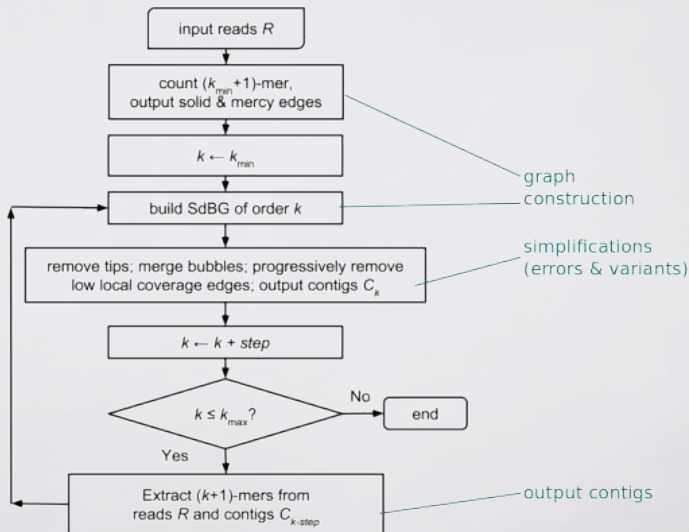
Metagenome assembly software

- **metaSPAdes** [Nurk *et al*, *Genome Res.*, 2017]
- **MEGAHIT** [Li *et al*, *Methods*, 2016]
- **metaFlye** [Kolmogorov *et al*, *bioRxiv*, 2019]
- **Minia-pipeline** [me!]
- IDBA-UD
- Ray-meta
- SOAPdenovo2
- metaVelvet/-SL
- Omega
- InteMAP
- Meraga
- Velour
- A*

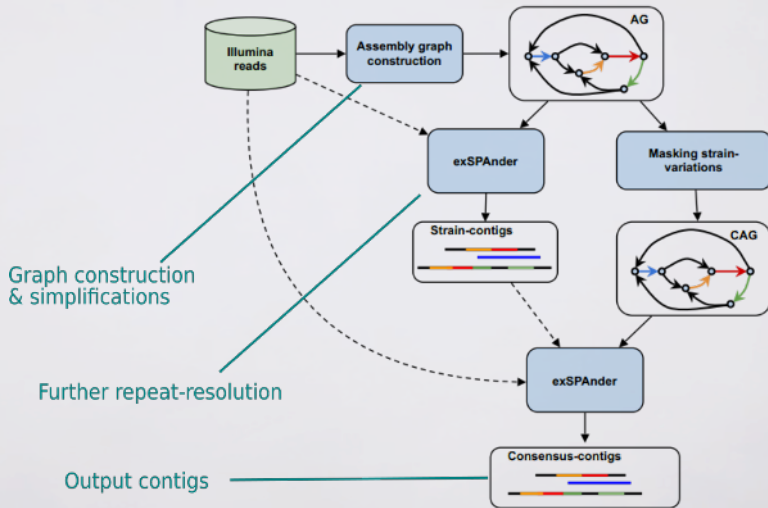
Under the hood of metagenome assemblers



MEGAHIT < v1.0



metaSPAdes



Multi-k

In principle, **better** than single-k assembly.

Visualization of multi-k graphs

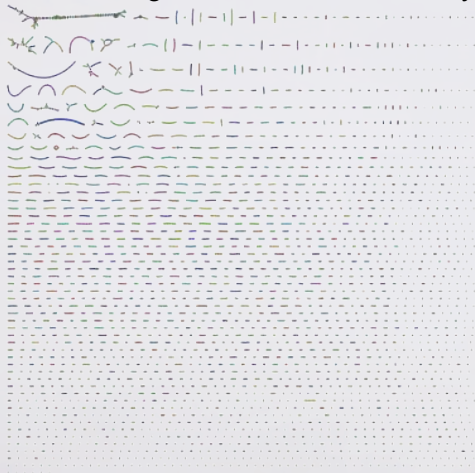
Salmonella genome, SPAdes assembly



$k = 99$

In contrast, with single-k

Salmonella genome, Velvet assembly



$k = 91$ (too high, but shown for comparison)

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

Metagenomics with long reads

1. metaFlye

[Kolmogorov *et al*, 2019]

2. wtdbg2

[Nicholls *et al*, *GigaScience*, 2019]

3. Canu

[see wtdbg2 article]

4. miniasm + Racon

Oxford Nanopore: **needs polishing**

Alternative route: HiC, linked reads

metaFlye

Too complex to describe its inner workings

metaFlye

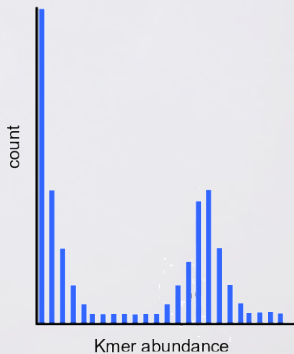
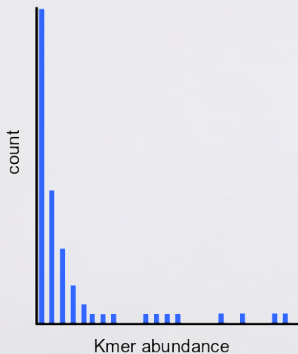
Too complex to describe its inner workings

metaFlye



When *can* you assemble

Look at k -mer histograms of the reads! (KMC, DSK tools)



Credit: www.cmbi.ru.nl/~dutilh/metagenomics/course_HAN_2014/Speth.pdf

Digital normalization

<https://github.com/dib-lab/khmer>

- Reduce dataset size
- Facilitates assembly

Potential drawbacks:

- assembly fragmentation
- low-coverage variant loss

Why you shouldn't use digital normalization

<http://ivory.idyll.org/blog/>

[why-you-shouldnt-use-diginorm.html](http://ivory.idyll.org/blog/why-you-shouldnt-use-diginorm.html)

Evaluation metrics

Same as regular assembly:

- N50, NG50
- Total size
- % of reads mapping correctly back to the assembly
- Number of predicted genes
- % of contigs matching some known references

Metagenome-specific:

- metaQUAST
- CheckM, marker genes, [Parks *et al*, *Genome Res.* 2015]
- VALET, internal consistency, [Olson *et al*, *BFB* 2017]

CAMI benchmark

- 3 artificial communities
 - ▶ low, medium, high complexity (600 genomes, 5x15 Gbp)
- 6 assemblers evaluated: MEGAHIT, Minia, Ray-meta, ..

Analysis | [OPEN](#)

Critical Assessment of Metagenome Interpretation—a benchmark of metagenomics software

Alexander der Sczyrba , Peter Hofmann [...] Alice C McHardy 

Nature Methods **14**, 1063–1071 (2017)

doi:10.1038/nmeth.4458

[Download Citation](#)

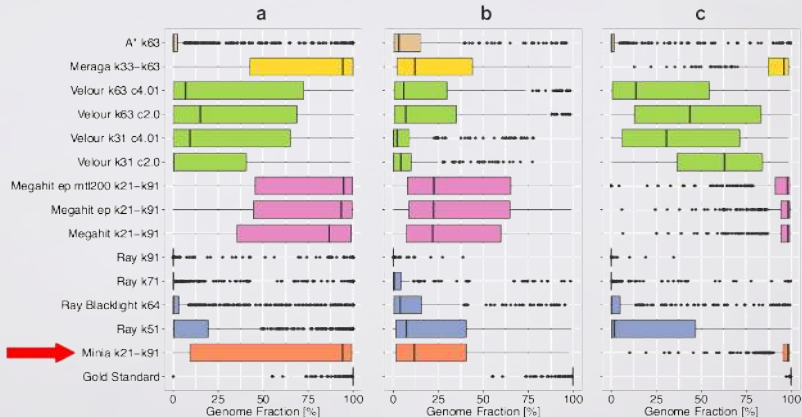
Received: 29 December 2016

Accepted: 25 August 2017

Published online: 02 October 2017

Quality of metagenome assembly

a: all genomes, b: genomes with ANI $\geq 95\%$, c: genomes with ANI $< 95\%$



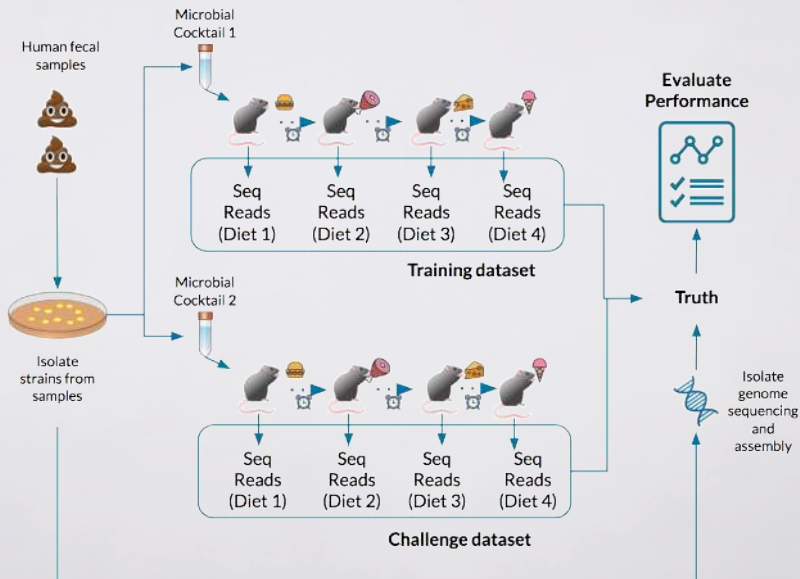
[Sczyrba, Nat Meth 2018]

No assembler could reconstruct **close strains**.

Metagenomics software is
still immature, story time..



Mosaic DNANexus Challenge 2018



Mosaic DNANexus Challenge 2018

Focus on **strains** assembly



mosaic

Evaluation metrics:

- Genome Fraction
- misassemblies

Mosaic DNANexus Challenge 2018

Focus on **strains** assembly



mosaic

Evaluation metrics:

- Genome Fraction
- misassemblies

Method	N50	Genome Fraction	# misassemblies
What a regular assembler would give	7.1 Kbp	84.1%	1998

Mosaic DNANexus Challenge 2018

Focus on **strains** assembly



mosaic

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Mosaic DNANexus Challenge 2018

Focus on **strains** assembly



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don't do it

Business

DNAnexus-Powered Mosaic Microbiome Platform Announces Winners of First Community Challenge

Business

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Business

DNAnexus-Powered Mosaic Microbiome Platform Announces Winners of First Community Challenge



→ **Evaluating** metagenome assemblies is hard

Conclusion

- Metagenome assembly is a hard problem
- Due to strains & low-abundance species, mostly
- Trade-off between **contiguity**, and **genome fraction/misassemblies**. Questions on assemblies ranking.
- So far, limited availability of: long reads, Hi-C, linked-reads

References:

- Ayling *et al*, New approaches for metagenome assembly with short reads, 2019
- metaFlye article
- out of RAM? <https://github.com/GATB/minia-pipeline>

Acknowledgments: Dag Ahren, Sergey Nurk, Camille Marchet, Antoine Limasset, the fantastic team of the Workshop on Genomics 2020, Chris Quince, Aaron Darling, Guillaume Rizk, Claire Lemaitre, Pierre Peterlongo, Charles Deltel, Paul Medvedev, Dominique Lavenier

Exercise

k-mers:

1. ACA
2. AGA
3. AGT
4. CAT
5. GTC
6. TAG
7. TCA
8. TTG

Two strains of a short genome are in this dataset, please assemble them. ignore reverse-complements

Exercise: solution



- Discard TTG (connected to nothing)
- Observe a *k*-mer was missing (GAC)
- Two strains: TAGTCAT, TAGACAT