Reference guided genome assembly in metagenomic samples

Cervin Guyomar[1,2] , Wesley Delage[2], Fabrice Legeai[1,2], Christophe Mougel[1], Jean-Christophe Simon[1], Claire Lemaitre[2]

1 : INRA, UMR 1349 IGEPP, le Rheu, France
2 : INRIA/IRISA GenScale, Campus de Beaulieu, Rennes, France

Motivations

Metagenomics = A mixture of reads :

- from different genomes
- with polymorphism
  (SNPs and large structural polymorphism)
- with no close reference genome (most of the time)

Objectives :

Assemble an genome of interest
- from metagenomic reads,
- using a remote reference genome
- detecting stuctural polymorphism

Existing methods :

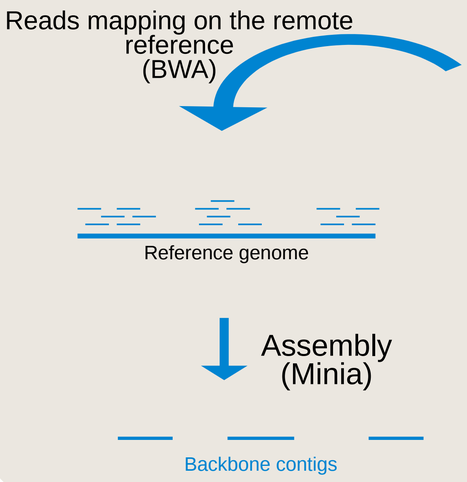
	Assembly first	Mapping first	Hybrid stategy
	De novo metagenomic assembly + contig taxonomic assignation *Ex : MegaHit[1] + Blast*	Assembly of reads Mapped on reference *Ex : BWA+Minia[2]*	Reference guided assembly Local assembly of the regions diverging from the reference *Ex : MitoBim[3]*
+	Assembly is reference-free	Assembly time reduced by read selection	- Conserved regions easily assembled using reference - Able to reconstruct diverging regions
-	- Time consuming and challenging assembly - Incomplete assembly if remote reference - Tricky contig filtering	- Requires a close reference genome - Incomplete assembly if remote reference	No tool suited for metagenomic data : - fail to detect structural polymorphism - No scale-up with metagenomic datasets

→ **Need for a tool dedicated to guided assembly in metagenomic context**

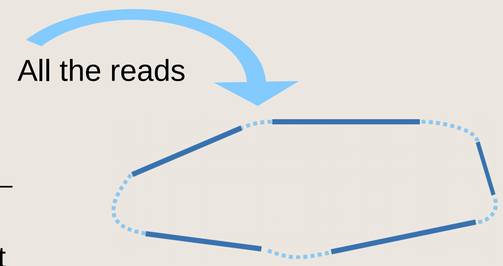
MindTheGap assembly workflow

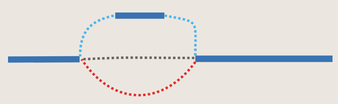
An hybrid approach :
- Mapping reads and assembly of conserved regions
- Gapfilling of diverging regions with all reads

Step 1 : Reference based read recruiting and backbone contig assembly



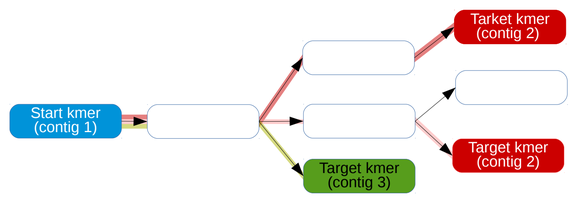
Step 2 : Reference free gapfilling between contigs using MindTheGap [4]





MindTheGap algorithm

- De Bruijn graph assembly starting from a contig end kmer
- Search target kmers in the contig graph



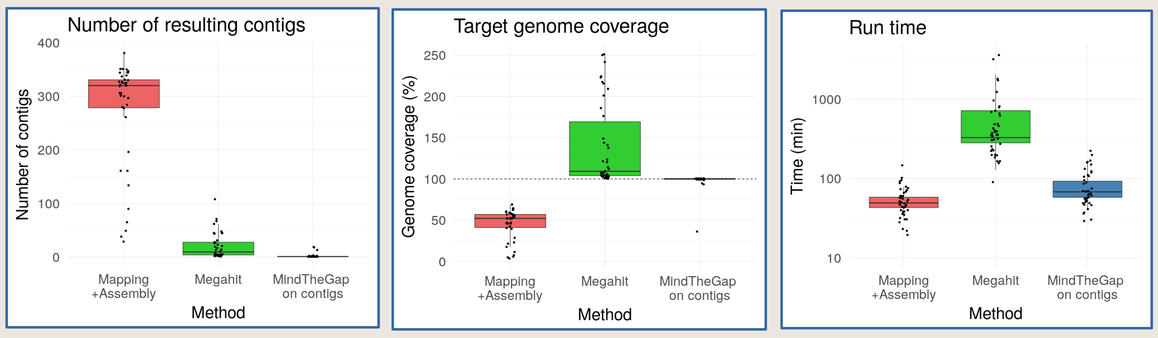
2 solutions between contig 1 and contig 2
1 solution between contig 1 and contig 3

Output :
super-contigs (fasta) + gfa format

Results in the pea aphid holobiont

Successful assembly of a bacterial genome in one circular contig

Assembly of *Buchnera aphidicola* from 42 pea aphid metagenomic samples [5]
using *Buchnera* genome from another species

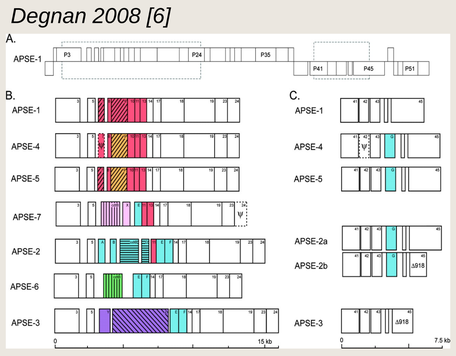


MindTheGap results
- One-contig assembly for 30 samples
- Genome length close to the real reference
- On average 7 times faster than Megahit

Discovery of unknown phage structural variants

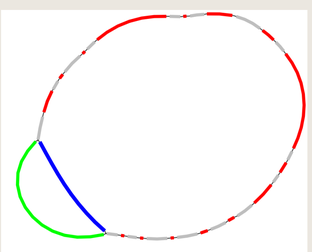
Assembly of the phage APSE from 42 pea aphid metagenomic samples

Degnan 2008 [6]



- 7 known variants known, differing by a ~5kb virulence cassette

Results :
- 3 new phage variants discovered in 5 samples
- Coabundant phage successfully assembled in 3 samples



In developpment on GitHub

https://github.com/GATB/MindTheGap/tree/contig_dev

References :

[1] : *Li et al (2015)* MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics*
[2] : Chikhi et al (2013) Space-efficient and exact de Bruijn graph representation based on a Bloom filter. *Algorithms for molecular biology : AMB*
[3] : Hahn et al (2013) Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads - A baiting and iterative mapping approach. *Nucleic Acids Research*
[4] : Rizk, G. et al., (2014) MindTheGap: integrated detection and assembly of short and long insertions. *Bioinformatics,*
[5] : Guyomar et al *(2018)* Multi-scale characterization of symbiont diversity in the pea aphid complex through metagenomic approaches (*in revision – Microbiome)*
[6] : Degnan and Moran (2008) Diverse Phage-Encoded Toxins in a Protective Insect Endosymbiont, *Applied and Environmental Microbiology*