De novo Assembly and Comparative Genomics on Eukaryotic Species Mixtures

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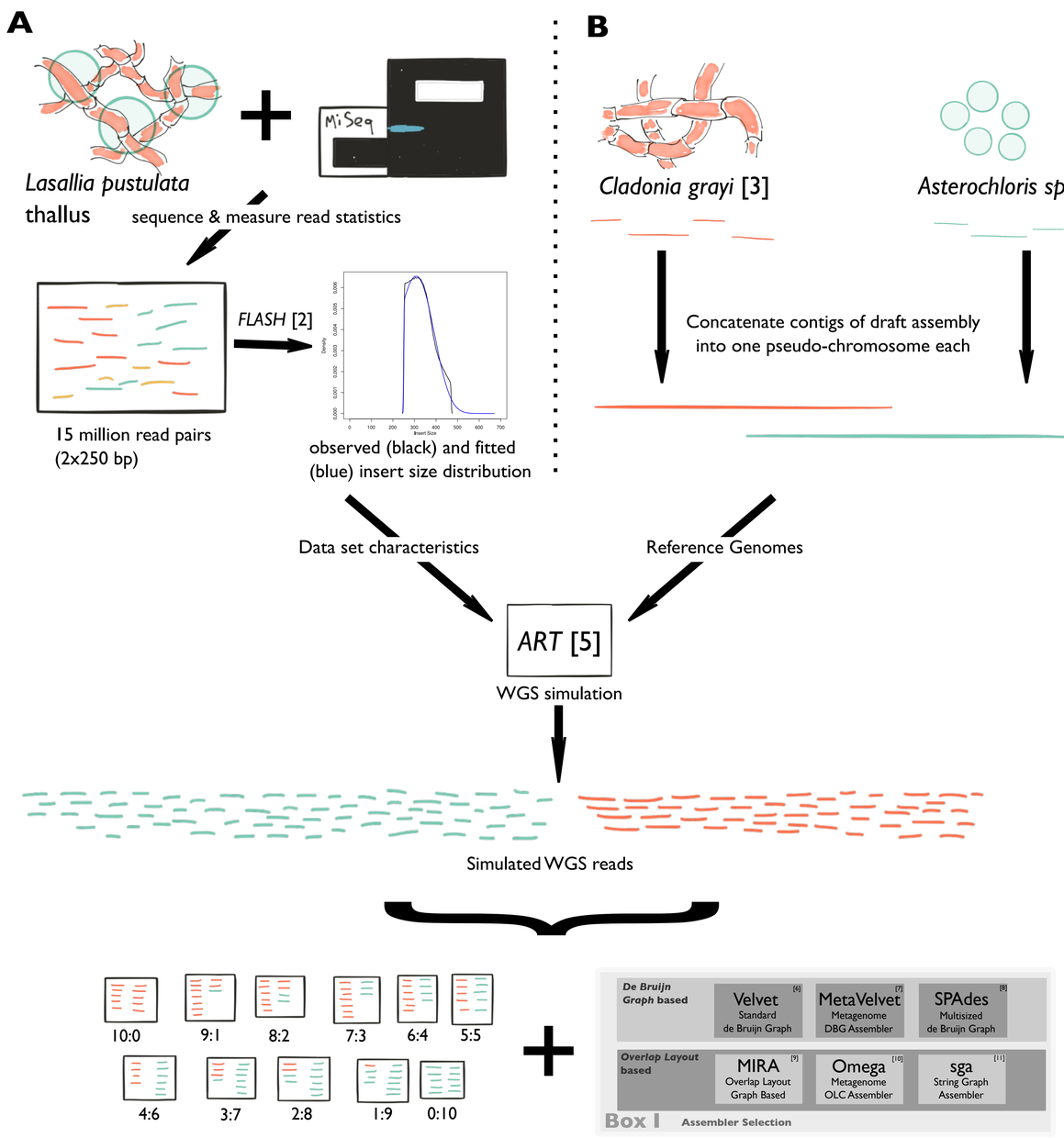
Summary

Mutualistic symbiotic relationships are found across organisms of all complexity. In extreme instances, as in some lichens, the interaction appears so close that the participating organisms grow only poorly – or even not at all – when cultivated in isolation. This renders mutualistic symbionts valuable objects to study the genomic basis of adaptation and co-evolution. The close interdependence in such communities, however, confounds genomic studies. In many cases separate sequencing of the participating organisms is not feasible, leaving metagenomics approaches as the method of choice. Here we address how and to what extent eukaryotic genomes can be reconstructed from such data.

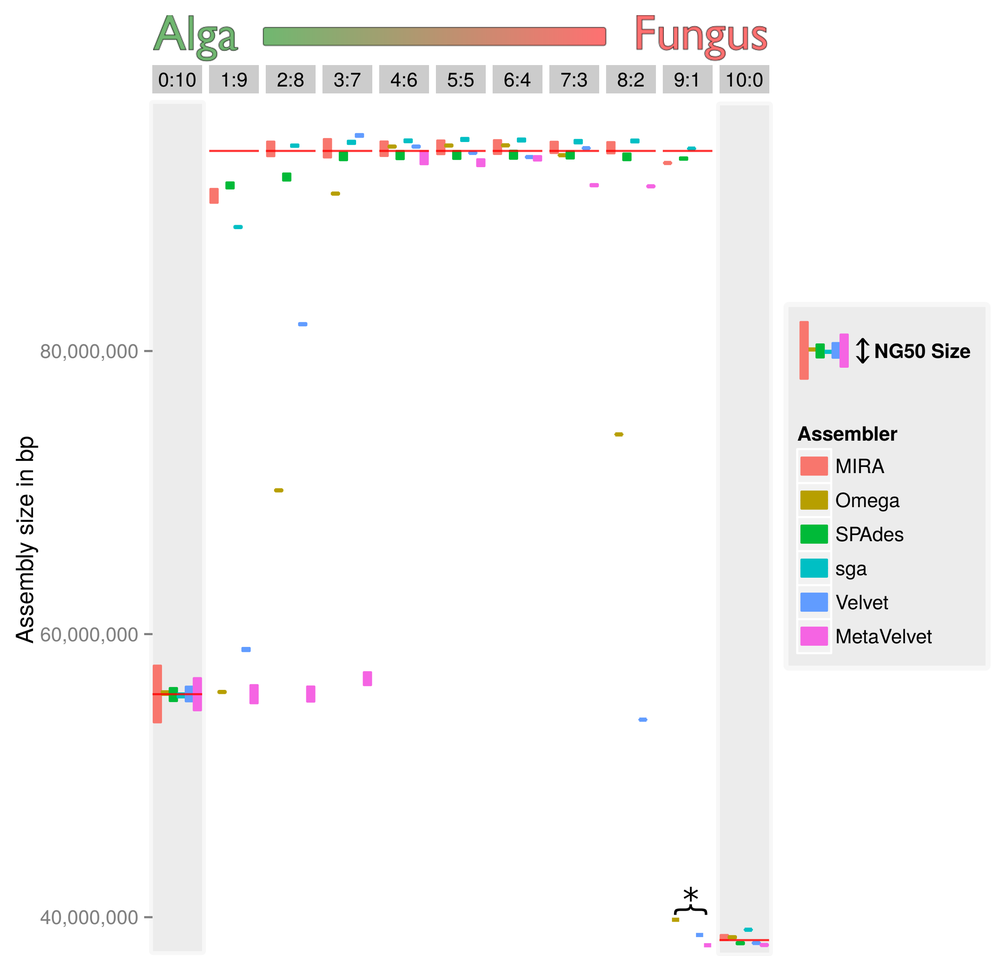
We use *in silico*-generated data sets to sound out the performance of different assembly paradigms on Whole Genome Shotgun (WGS) data from eukaryotic species mixtures. On this basis we have begun reconstructing the metagenome of the lichen *Lasallia pustulata*. Using a hybrid sequencing approach, that combines Illumina short read and PacBio long read data, we have assembled the genome of the mycobiont and a major fraction of the algal photobiont. We integrate this data with genome sequences of closely related non-lichenized fungi as a first step towards analyzing how lichenization affects genome evolution.

1. Assembler Evaluation with Simulated Twin Sets [1]



Merge reads simulated from either reference genomes to form *L. pustulata* twin sets with varying coverage ratios for the two genomes. Assemble each set with 6 different assemblers.

2. Assembly Results of the Twin Sets



Assembly results for the 11 twin sets. Bars are centered at total assembly length, red lines indicate reference lengths. Height of bars shows the NG50 size. Assemblies marked with an asterisk cover less than 50% of the reference length. A default height was used in those instances.

3. Sequencing the *L. pustulata* metagenome

Pilot Study

- Box II summarizes the assembly results of the metagenome skimming data (cf. 1A) with *MIRA*.
- A comparison to the twin set analysis (cf. 2) indicates issues with the reconstruction of the algal genome.
- A qPCR analysis of the lichen thallus reveals a substantially higher than expected fungal-to-algal genome ratio of 15:1 (Fig. 1).

Whole Assembly	Fungal	Algal	Bacteria
Number of Contigs: 64,180			
Total Length: 119 Mbp			
N50: 3.3 Kbp			
Number of Contigs	6977	8872	19,371
Total Length	37 Mbp	14 Mbp	34 Mbp
N50	19 kbp	2 kbp	3 kbp
Coverage	80x	10x	14x

Box II Illumina Assembly Thallus

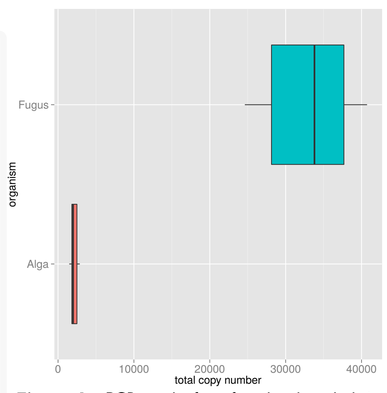
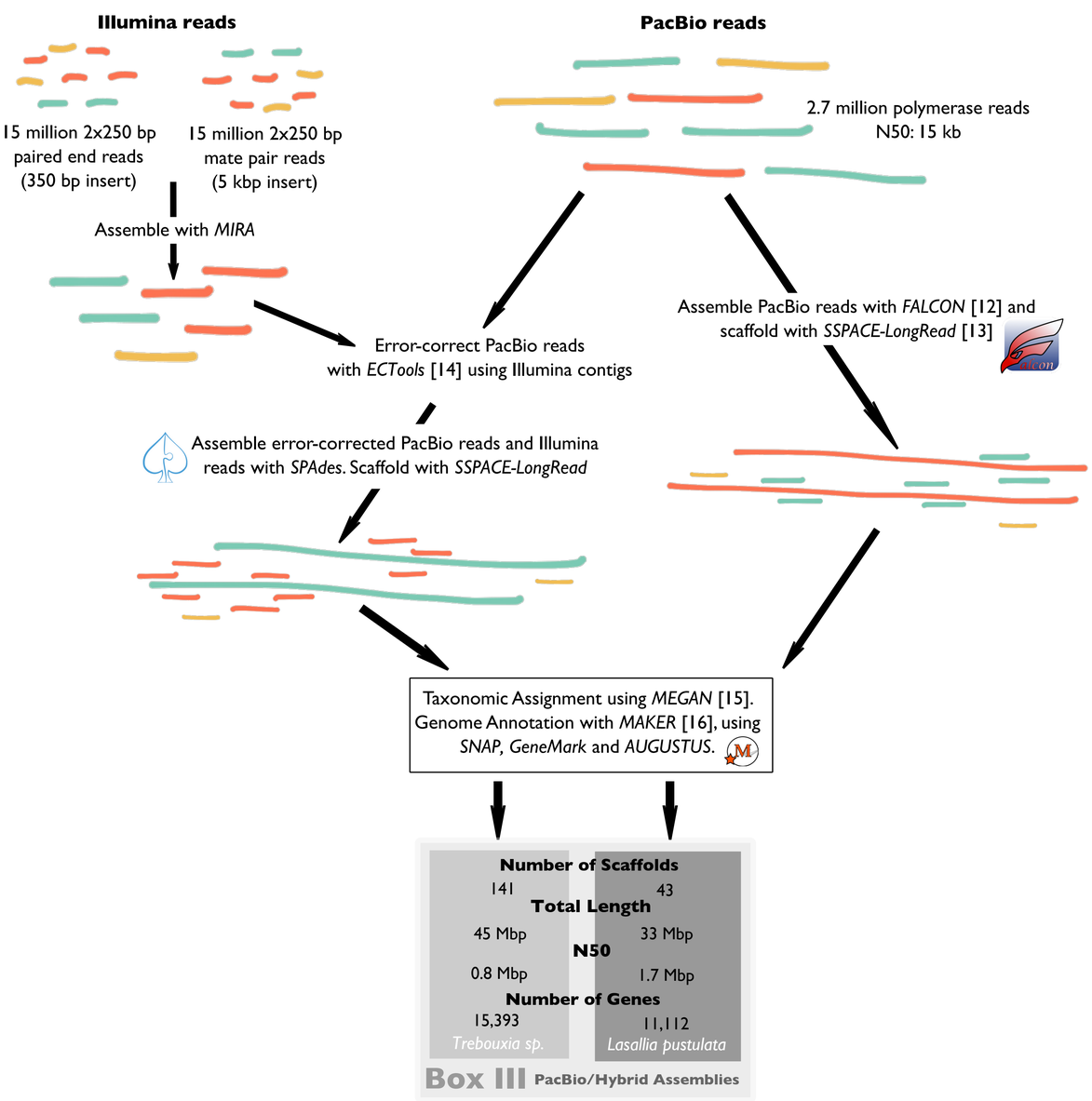


Figure 1: qPCR results for a fungal and an algal single copy gene. The fungal:algal ratio is around 15:1.

Final Assembly: Short-Read meets Long-Read



For the high coverage fungal genome, we follow the standard procedure of doing an assembly using only PacBio data. In case of the low-coverage algal genome, we use a hybrid assembly utilizing both PacBio and Illumina data [17].

4. Does Lichenization Facilitate Gene Loss?

Ancestral Gene Set To investigate lineage specific gene loss, the Last Common Ancestor (LCA) gene set of the Pezizomycotina was reconstructed using OMA [18] (Figure 2). In total 12,595 orthologous groups were formed (Figure 3).

Absence of LCA Genes For 1,357 groups, genes were only found in 7 species. In 1/3 or these groups the *L. pustulata* ortholog is missing, hinting that these genes are lost as a consequence of lichenization (Figure 4).

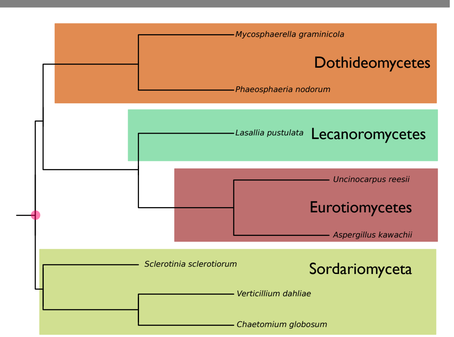


Figure 2: Tree of 8 species used for creating the LCA gene set.

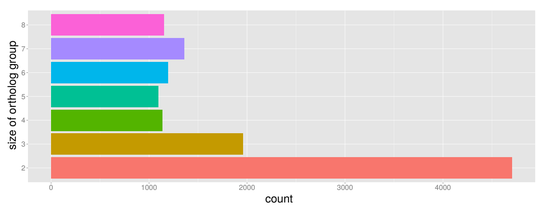


Figure 3: Results of the orthology prediction with OMA. For 1153 groups all 8 species were found. For 1357 groups only 7 species were found.

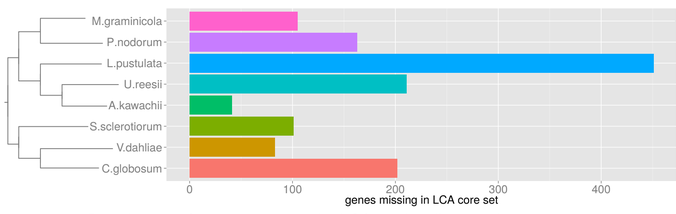


Figure 4: Distribution of genes missing in the LCA set. Lasallia pustulata is missing in twice as many orthologous groups as any other species.

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