

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. At the same time, however, the close interdependence in such communities confounds genomic studies. The separate sequencing of the participating organisms is not feasible in many cases, leaving metagenomics approaches as the method of choice. Here we address how and to what extent larger and more complex eukaryotic genomes can be reconstructed from metagenomic data.

1. Assembly Evaluation with Simulated Twin Sets

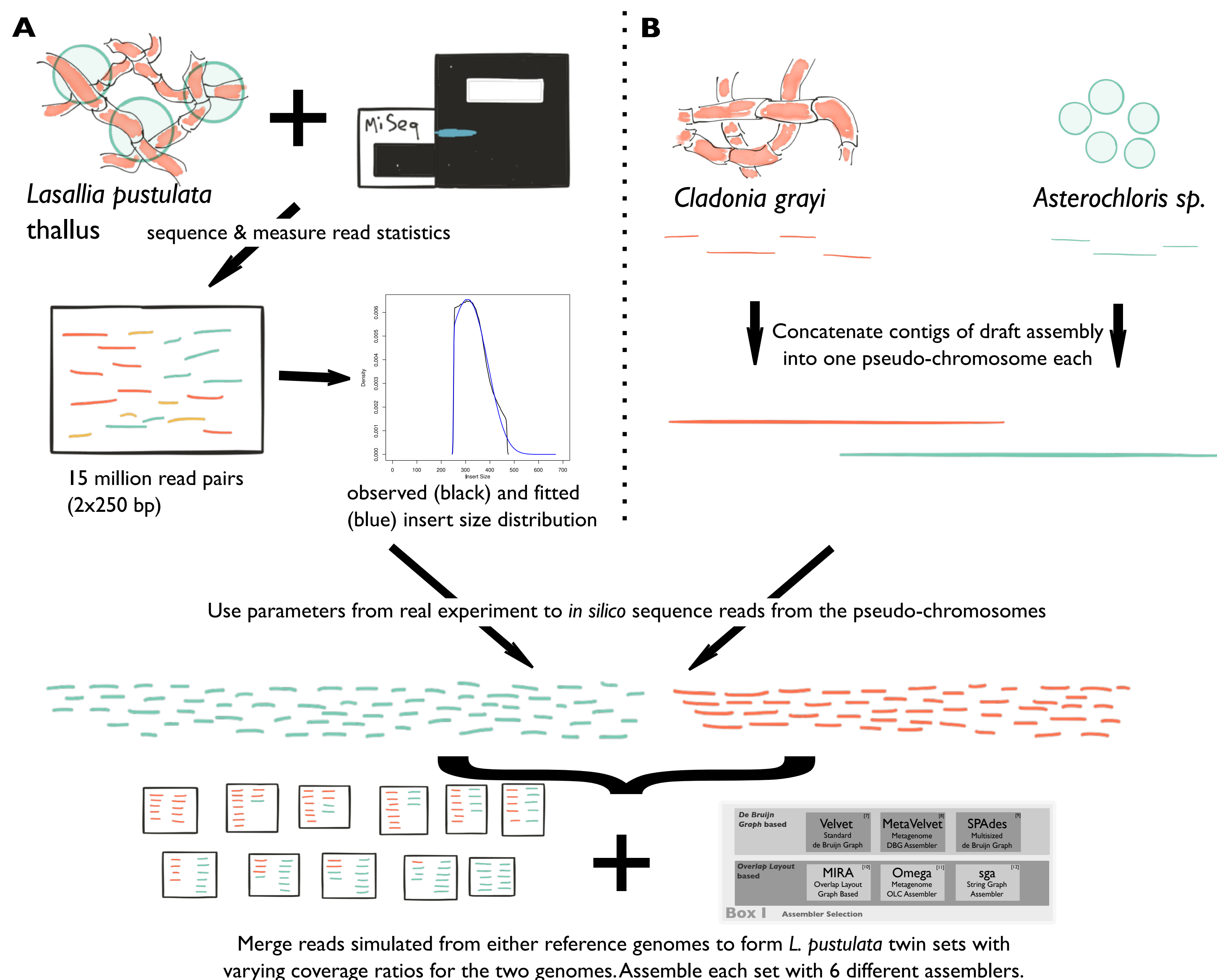


Figure 1: Workflow for generating twin sets. Twin sets resemble a real sequencing experiment with respect to insert size distribution, read number and read length. Each twin set is assembled using different assemblers and results are compared to the true genomic sequence.

Sequencing & Parameter Estimation DNA from *L. pustulata* was sequenced using Illumina MiSeq. The insert size distribution was estimated by overlapping read pairs using FLASH [1] (Fig. 1, A).

Simulation Using pseudo-chromosomes from *Cladonia grayi* [2] and *Asterochloris sp.* [3] as templates (Fig. 1, B), we simulated whole genome shotgun (WGS) reads with ART [6]. We compiled 11 twin sets by mixing simulated fungal & algal reads at varying ratios.

Assembly Twin sets were *de novo* assembled using six different programs (Box I). Assemblies were compared to the respective reference genomes for contiguity and correctness of assembly.

2. Assembly Results of the Twin Sets

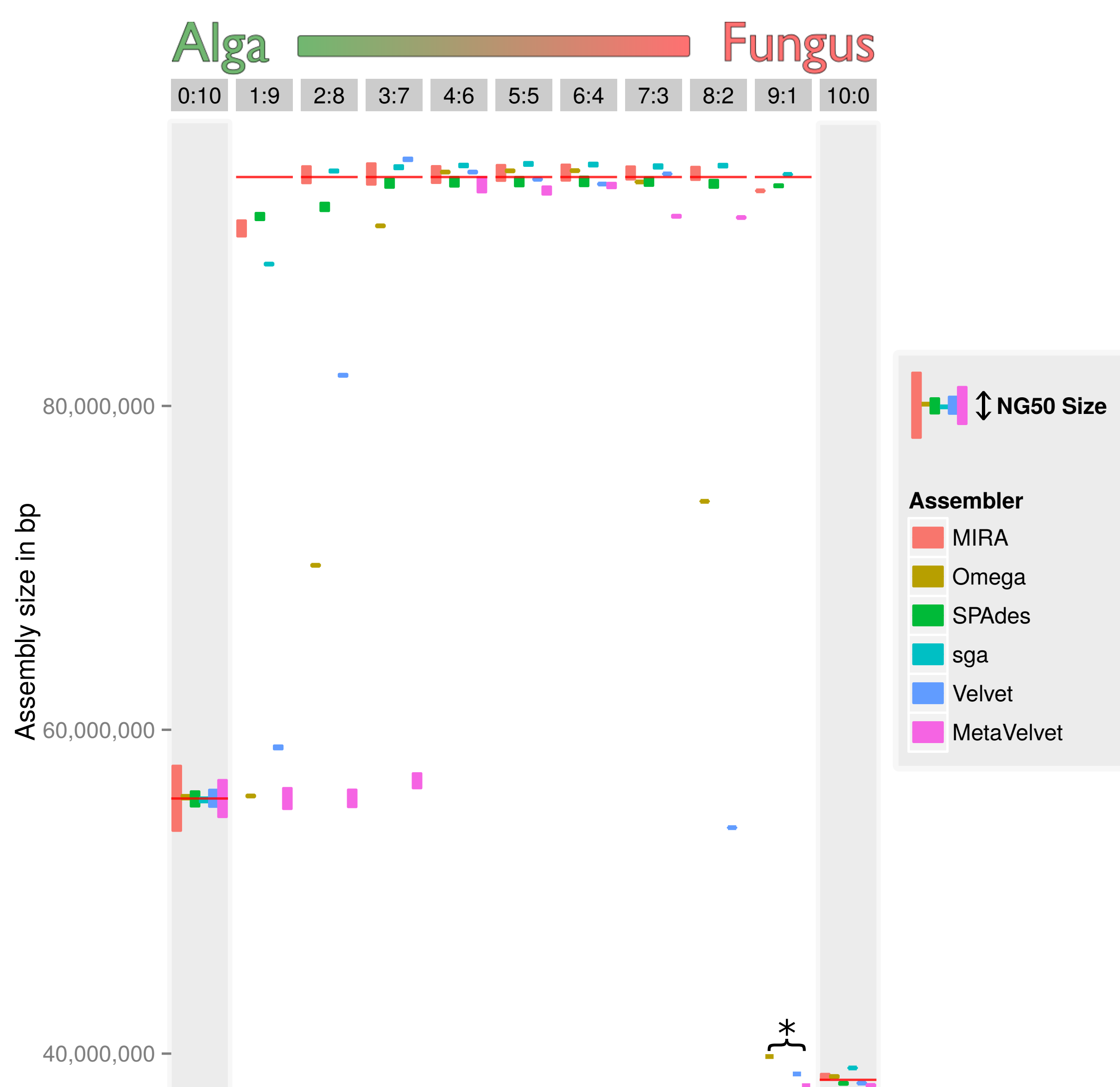


Figure 2: Assembly results for the 11 twin sets. Bars are centered at total assembly length, red lines indicate reference lengths. Height of bars shows the NG50 size. Assemblies marked with an asterisk cover less than 50% of the reference length. A default height was used in those instances.

Previously we use *in silico*-generated data sets to sound out the performance of different assembly paradigms on such eukaryotic species mixtures [0]. On this basis we have begun investigating the metagenome of *Lasallia pustulata* using a combined approach of Illumina short read and PacBio long read sequencing. From this data we have assembled the genome of the mycobiont and a major fraction of the algal genome. We integrate this data with genome sequences of closely related non-lichenized fungi providing the basis for analyzing how lichenization affects genome evolution.

3. Sequencing the *L. pustulata* metagenome

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

Box II Illumina Assembly Thallus

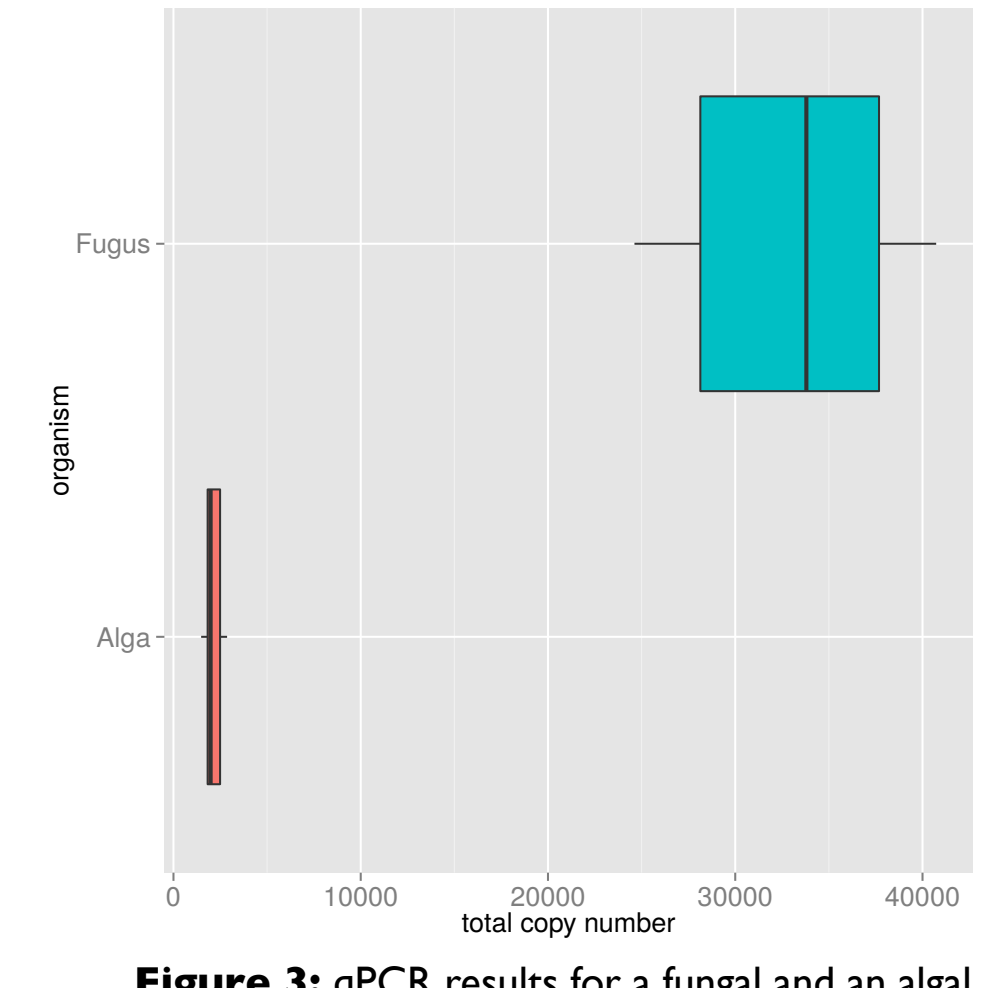


Figure 3: qPCR results for a fungal and an algal single copy. The fungal:algal ratio is around 15:1.

Short-Read Data The WGS data was assembled with MIRA and contigs were taxonomically assigned using MEGAN [13]. The algal assembly is much more fragmented than expected given the *in silico* study (Box II). This is a result of either a biased library preparation or the large difference in DNA content in the lichen thallus, as shown by quantitative PCR (Figure 3).

Long-Read Data PacBio sequencing was done to improve the assemblies. A total of 2,705,256 polymerase reads with a read N50 of 15kb were sequenced. To cope with the coverage differences we performed two different assemblies, targeting the fungal and the algal genome respectively (Figure 4). For high coverage data PacBio-only assemblies are state of the art, low-coverage data require hybrid assemblies using Illumina and PacBio data [14].

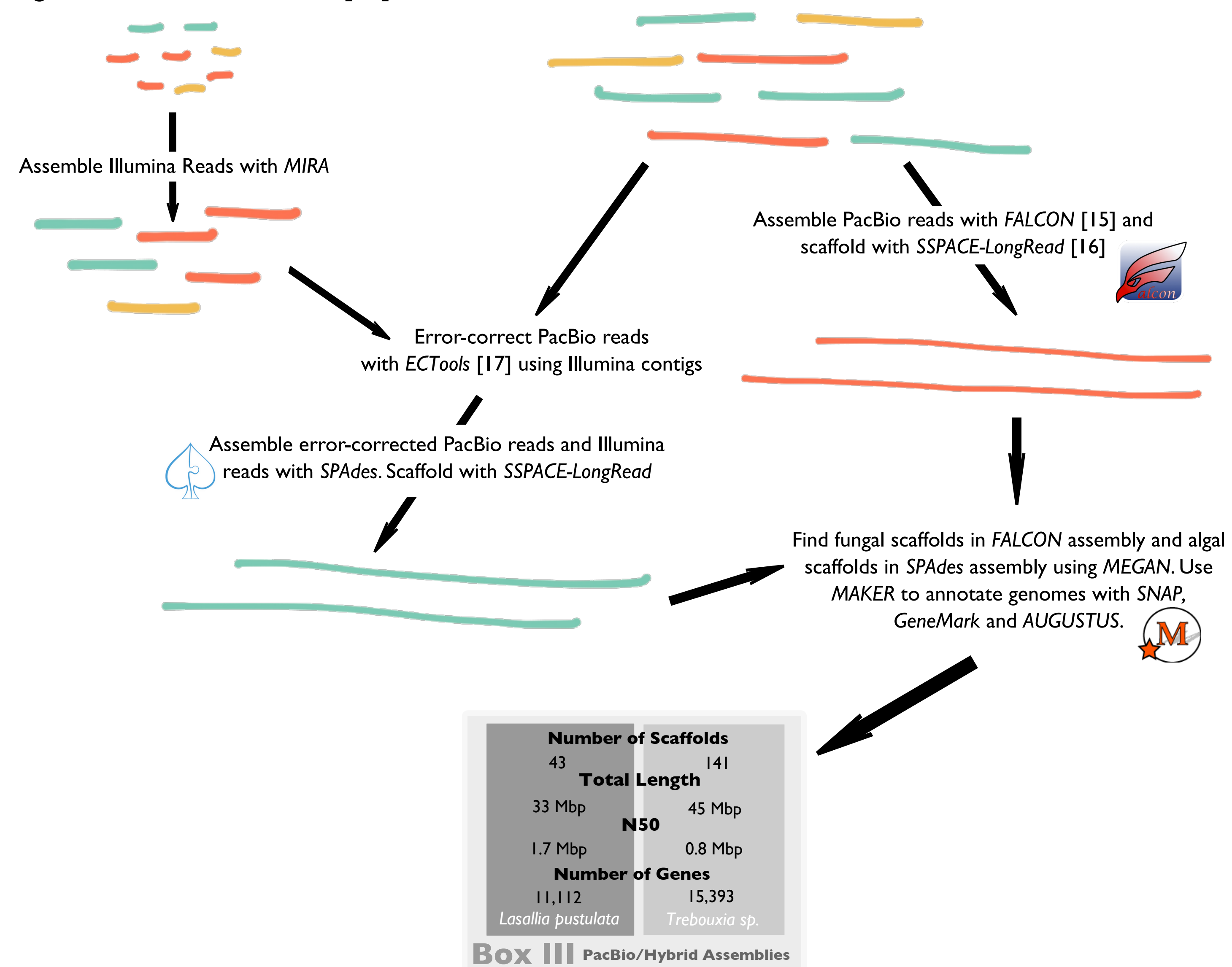


Figure 4: Outlining the assembly workflow for the genomes of the mycobiont and the photobiont. The mycobiont can be assembled directly from the PacBio data. The photobiont is assembled using a hybrid approach using PacBio and Illumina data.

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 [19] (Figure 5). In total 12,595 orthologous groups were formed (Figure 6).

Absence of LCA Genes For 1357 further groups genes were only found in 7 species. For these groups it is *L. pustulata* which is absent most often, hinting that these genes are lost over time in lichenization (Figure 7).

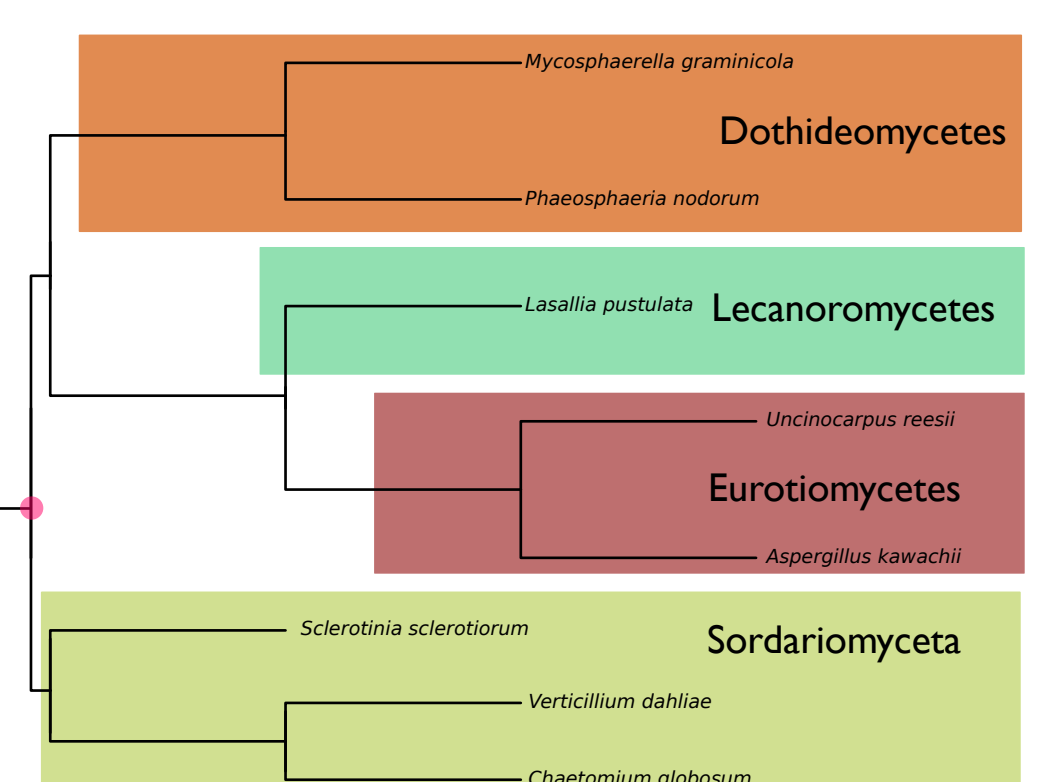


Figure 5: Tree of 8 species used for creating the LCA gene set.

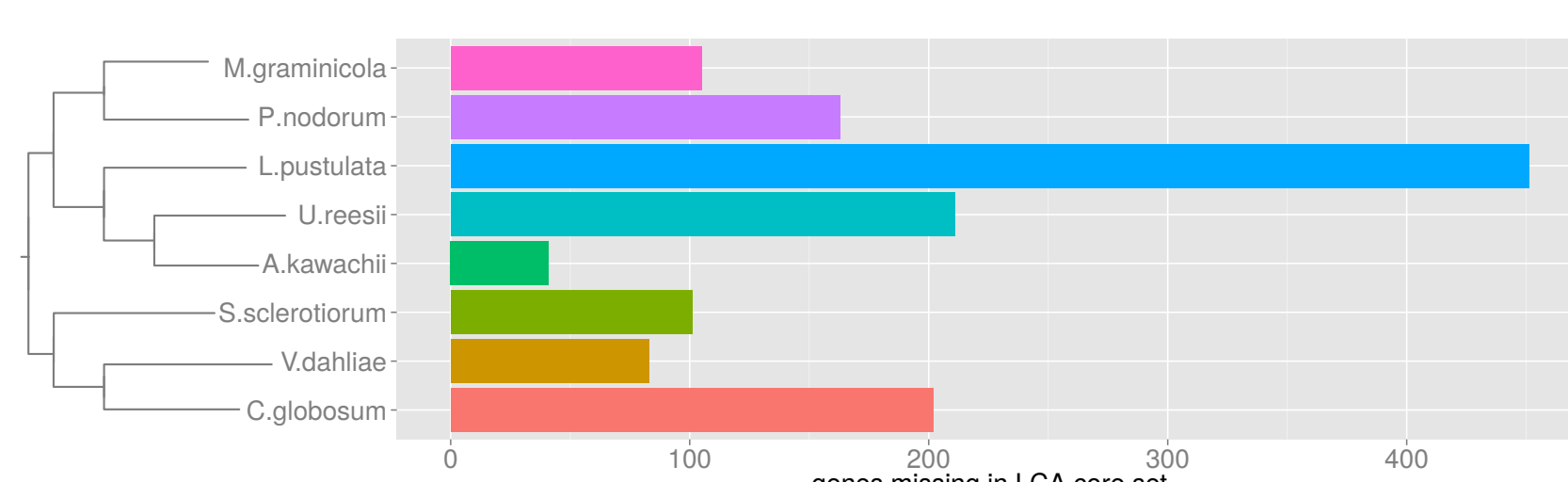


Figure 7: Distribution of genes missing in the LCA set. *Lasallia pustulata* is missing in twice as many orthologous groups as any other species.

Figure 6: Results of the orthology prediction with OMA. For 1153 groups all 8 species were found. For 1357 groups only 7 species were found.



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