**Doing *de novo* Assembly on Eukaryotic Species Mixtures – An *in silico* Evaluation**

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Mutualistic symbiotic relationships are found across organisms of all complexity. In extreme instances, as in some lichens, this symbiosis is so close that the participating organisms are no longer able to survive on their own. Such close interdependences make mutualistic symbionts valuable objects to study the genomic basis of co-evolution. At the same time this close interdependence confounds genomic studies, as the separate sequencing of the participating organisms is not feasible for many closely interacting symbionts. While there has been extensive work on prokaryotic metagenomics, it is still unclear how a eukaryotic species mixture influences the assembly outcomes. To investigate how the different assembly paradigms perform on such eukaryotic species mixtures, we performed an *in silico* study based on a lichen model. This approach offers the benefit of knowing the true genomes, making it feasible to not only compare summary statistics but also the correctness of the assembly results.

To emulate real data sets as closely as possible, we sequenced a eukaryotic species mixture, generated from the lichen *Lasallia pustulata*. Based on the observed parameters, such as the insert size distribution and read number, we generated 11 twin data sets based on the draft genomes of the lichenized fungus *Cladonia grayi* and its photobiont *Asterochloris sp.* The ratio of sequencing reads stemming from the two organisms was varied between the 11 twin data sets, to evaluate how the coverage distribution influences the assembly outcomes. We used 6 assemblers (*MIRA*, *Omega*, *sga*, *Velvet*, *MetaVelvet* & *SPAdes*), covering different 2 different *Overlap-Layout-Consensus* methods, 2 *de Bruijn Graph* based methods and 2 dedicated metagenome assemblers, for the evaluation.

Our results show that there are marked differences in the performance of the different assemblers. All assemblers depend on the absolute coverage, with a minimum coverage needed to yield good results. Furthermore the results are also dependent on the relative ratio with which the two organisms are present in the data set. This is especially true for assemblers based on *de Bruijjn Graph*s*,* where performance suffers most when coping with uneven coverages. We also see that the standard procedure of maximizing the N50 value is not advisable for eukaryotic species mixtures as it yields suboptimal assemblies. Of the six different assemblers we tested, *MIRA*, an all-purpose Overlap-Layout-Consensus assembler, consistently gives the best assemblies, regardless of the coverage ratio for the two organisms.