Title: The combined analysis as the best strategy for Dual RNA-Seq mapping

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Abstract:

In Dual RNA-Seq experiments, the simultaneous analysis of the data from both organisms' gene expression could be a challenge, especially when the interaction is between eukaryotic and prokaryotic organisms. One alternative is separating the reads during *in silico* data analysis. There are two main methods for the mapping step: sequential and combined. Here we present a combined approach in which the libraries were aligned against a concatenated genome to sort the reads before mapping them to the respective annotated genomes. This approach was compared with the sequential analysis. To perform the comparison, we used two independent libraries (one from maize and another from *Herbaspirillum seropedicae*). We mapped the individual libraries against the other reference genome and against a combined reference (formed by concatenating the reference genomes of maize and *Herbaspirillum*) to determine the number of reads that could cross-map. After, a Chimera Library (formed by concatenating the maize and *Herbaspirillum* libraries) was constructed and we performed the sequential and combined analysis. Libraries from two real Dual RNA-Seq experiments were also used. The sequential analysis consistently attributed more reads to the first reference genome used in the analysis (due to cross-mapping) than to the combined approach. More importantly, the combined analysis resulted in lower numbers of cross-mapped reads. Our results highlight the necessity of combining the reference genomes to sort reads previously to the counting step to avoid losing information in Dual RNA-Seq experiments.

Keywords: Concatenated reference genomes; sequential analysis; Plant-Microbe interactions; Plant Growth Promoting Bacteria.

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