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## Improved methods for genome sequencing of Liberibacters by BAC library-based metagenomics approach

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Liberibacters have not yet been successfully cultured; their minimal genomes carry multiple copies of several genes. Sequences identical to phage genomes have been found in many Liberibacters. Available evidences suggest that the Liberibacter genomes are adapting rapidly in different hosts and environments. Characterization of genomes of rapidly changing unculturable organisms can be challenging. We have used a model system based on *Candidatus* Liberibacter psyllaurous associated with tomato "psyllid yellows" (Hansen et al., 2008) to develop methodologies using alternate techniques for sequencing metagenomes. We have constructed a BAC library from infected tomato psyllids (*Bactericera cockerelli*). The library consists of 57,600 clones arrayed in 150 plates each with 384 wells. DNA from individual clones were pooled for screening purposes. Initial identification of clones with Liberibacter sequences were conducted based on 16s ribosomal sequences, and contiguous clones were characterized by end sequencing and identified as containing Liberibacter genome fragments. Screening of additional clones from the library was based on probes developed on such sequences. A total of 245 clones with Liberibacter genome fragments have been identified. A total of 63 bar-coded BAC clones were sequenced by using Roche 454 technology. BAC clones from this library contain large inserts (average size 70 kb). Similarities and differences with other well characterized genomes of Liberibacters (Duan et al., 2009, Lin et al., 2011) will be presented.
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