Title
Comparing Two Approaches for Cloning Trace Amount of DNA

Authors
Chow, Julianna
Dalin, Eileen
Woyke, Tanja
et al.

Publication Date
2008-03-11

Comparing Two Approaches for Cloning Trace Amount of DNA

Julianna Chow[1], Eileen Dalin[1], Tanja Woyke[1], Susan Lucas[2], Jan-Fang Cheng[1]

[1]Lawrence Berkeley National Laboratory, [2] Lawrence Livermore National Laboratory

US Department of Energy Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, California 94598 USA

As a user facility, The US Department of Energy's Joint Genome Institute, in collaboration with scientists around the world, are able to generate DNA sequences for a diversity of organisms. Often times, the amount of DNA provided for library construction is limited. It is important to develop a protocol to minimize the amount of DNA required for library construction. In an attempt to test the minimum amount of DNA necessary for library construction, we decided to use two approaches to clone chloroplast DNA. The first approach was AMPure bead purification, using solid-phase paramagnetic bead technology to purify DNA fragments from contaminates and enzymes, with minimal loss of DNA. DNA samples used for this approach ranged from 100ng to 1ug. The second approach used was, Multiple Displacement Amplification (MDA), requiring as little as 10pg of DNA template to amplify up to micrograms of DNA. The AMPure bead purified and MDA samples were cloned into pUC18 to determine the quality of the libraries. Several metrics were used to measure the quality of the libraries which include: cloning efficiency, chimera rate, and coverage biases amongst the two techniques. We will present the data generated and the pros and cons from these two different approaches.

This work was performed under the auspices of the US Department of Energy's Office of Science, Biological and Environmental Research Program, and by the University of California, Lawrence Berkeley National Laboratory under contract No. DE-AC02-05CH11231, Lawrence Livermore National Laboratory under Contract No. DE-AC52-07NA27344, and Los Alamos National Laboratory under contract No. DE-AC02-06NA25396.

LLNL-ABS-402285