Lawrence Berkeley National Laboratory

Recent Work

Title
454 Sequencing for Gap Closure in Microbial Genome Assemblies

Permalink
https://escholarship.org/uc/item/7b2581g1

Authors
Alessi, Joseph
Garcia, Hector
Warnecke, Falk
et al.

Publication Date
2006-03-13
454 Sequencing for Gap Closure in Microbial Genome Assemblies

Joseph Alessi, Hector Garcia, Falk Warnecke, Ed Kirton, Phil Hugenholtz, Paul Richardson and Feng Chen.

DOE Joint Genome Institute
2800 Mitchell Dr. B400
Walnut Creek, CA 94598

Most microbial genome finishing projects at the Joint Genome Institute require the use of a multitude of molecular techniques to achieve a finished genome. The majority of these techniques are applied to sequencing through gaps in the assembly. Traditional shotgun sequencing is known to have difficulty in both cloning of A/T rich regions and sequencing of G/C rich regions. To help alleviate this problem we have applied the 454 sequencing platform as another tool for gap closure. Although 454 Sequencing has been shown to have difficulty with homopolymer stretches of nucleotides, it does not have the same biases as shotgun sequencing. Therefore we feel these two approaches together can be complementary. We have developed a protocol in which gap-spanning fosmids are pooled together, from one or more projects. This DNA is sequenced with 454 and assembled using the Newbler Assembler and the resulting contigs are added into their respective projects. One 60x60 picotiter chip can yield as much as 32 Mb, allowing for the pooling of 20-28 fosmids with and average read depth of 20-28X. We chose fosmids that spanned gaps in a single microbial genome assembly as well as gap spanning fosmids within a given scaffold of a metagenomic sequencing project. We are also applying 454 technology to whole genome shotgun sequencing to assist with poorly assembled projects from Sanger sequencing alone.