Title
Improvements to the Illumina Sequencing System and New Applications

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Improvements to the Illumina Sequencing System and New Applications

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Abstract

Next Generation sequencing products and procedures for the Illumina platform were evaluated, several of which immediately benefited JGI users. Paired-end reads of 35 bases each have become routine on our new GAii instruments with output in excess of 6 billion bases per run. Single reads have been used for polishing finished genomic sequence data for over 2 years, and collaborative work has progressed to include genomic sequencing. Paired-End Reads have been particularly useful for SNP detection. Our beta testing activities include positive results for barcode libraries (indexing) using Illumina’s 3

BASIC PRINCIPLE of the PROCESS

A. Make Clonal Single Molecule Arrays from shotgun DNA libraries

B. Run 4-color Sequencing-By Synthesis, imaging each cycle.

C. Call the bases by computer analysis of the series of photos.

Illumina GAii System: New Hardware Components

LONGER READS USING IMPROVED CHEMISTRY

FASTER AND BETTER DATA PROCESSING

NEW APPLICATIONS

Shotgun Libraries

The process begins with genomic DNA fragmentation, ligation to dsDNA adapters, and size selection of the library. The ends are added during a 12 cycle PCR amplification.

Paired-end libraries are made with a new PCR primer to add sequencing primer 2 to end 2.

INDEXING, which allows mixing several libraries in one lane and separating reads base on a barcode.

Large Gap Library Results

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.

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