Optimization of the 454 Production Sequencing Workflow at the DOE Joint Genome Institute

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ABSTRACT

The U.S. Department of Energy (DOE) Joint Genome Institute's (JGI) Production Sequencing group is committed to the generation of high-quality genomic DNA sequence to support the mission areas of renewable energy generation, global carbon management, and environmental characterization and clean-up. Within the JGI's Production Sequencing group a robust Roche GS-FLX sequencer pipeline has been established. Optimization of the pipeline has been ongoing with the aim of continual process improvement of the laboratory workflow. These process improvement projects are being lead by the JGI's Process Optimization, Sequencing Technologies, Instrumentation & Engineering, and the core 454 Production groups. Primary focus has been on improving the procedural ergonomics and the technician's operating environment, reducing manually intensive technician operations with tools and robotic automation, reducing associated production costs, and improving the overall process and generated sequence quality.

Introduction

The DOE Joint Genome Institute (JGI) was established in 1997 to unite the expertise and resources in genome mapping, DNA sequencing, technology development, and information sciences pioneered at the DOE genome centers of Lawrence Berkeley National Laboratory (LBNL), Lawrence Livermore National Laboratory (LLNS), and Los Alamos National Laboratory (LANL). In January 1999, high-throughput DNA sequencing began at the Production Genomics Facility (PGF) in Walnut Creek, California.

The JGI Production Sequencing pipeline utilizes 8 Roche GS-FLX Sequencers, in addition to several capillary based and other new technology sequencing platforms. Although the GS-FLX platform has been fully integrated into the production pipeline, ongoing process optimization continues with several development projects being run by the JGI Process Optimization, Instrumentation & Engineering, and Sequencing Technology groups.

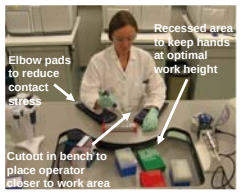
JGI 454 Laboratory

In July of 2008, the JGI opened its new technology sequencing laboratories to house next generation instruments and production pipelines. These new laboratories were designed with the technician in mind and feature advanced ergonomic workstations, equipment, and tools that allow the technicians to safely and efficiently prepare and sequence samples.

Many of the GS-FLX sample prep procedures are manually intensive and present potential ergonomic hazards when performed repetitively over time. The following is a selection of environmental laboratory improvements and tools implemented to mitigate these hazards.

Work Space

Lab bench workspace is set up with height adjustable tables and features cutouts for optimal placement of supplies and arm/hand working heights.



Pipettes

Multi-channel and electronic pipettes have replaced single channel manual ones. Multi-channel pipettes are used to dispense emulsions, and electronic repeaters like the Eppendorf XStream are used to dispense several different reagents throughout the process.

Tube & Bottle Openers

To assist in the uncapping of 1.5mL tubes, GeneMate's Microcentrifuge Tube Opener is used. For opening larger conical vials and 1L bottles, there are a variety of openers from which operators may choose.

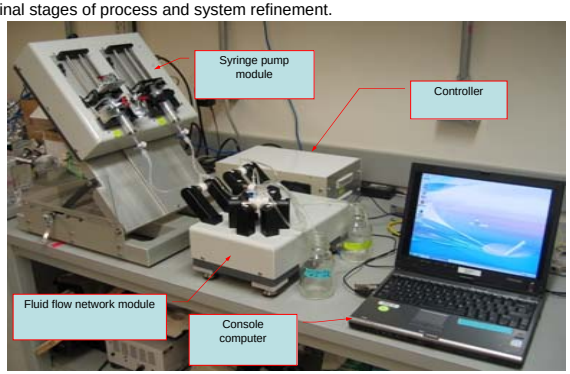
JGI 454 Process Optimization Projects

The following are a selection of process optimization projects that the 454 production group has implemented or is currently developing, with the aims of improving procedural ergonomics, reducing production costs, and improving the overall process and sequence quality.

Automated Emulsion Breaking & Sample Bead Recovery

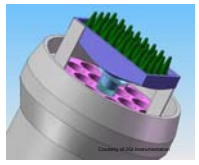
The JGI Instrumentation & Engineering and Process Optimization groups are currently developing an automated platform to break emulsions and to wash and recover the clonally amplified sample beads.

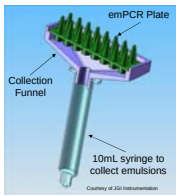
The system utilizes specially modified OEM syringe pumps in conjunction with a fluid flow network under computer control to separate the DNA beads from the emulsion and to condition them. It is designed to be operated under computer control in a continuous automatic fashion with minimal operator intervention. A console computer runs the control and graphical user interface software. The control software incorporates flexible, high-level protocol programming capability in a point and click interface. The phase 3 instrument (shown below) builds upon phase 1 & 2 manual prototypes that were used for proof of fluidic principles and process development. This instrument is in the final stages of process and system refinement.



Emulsion Collection using Centrifugation

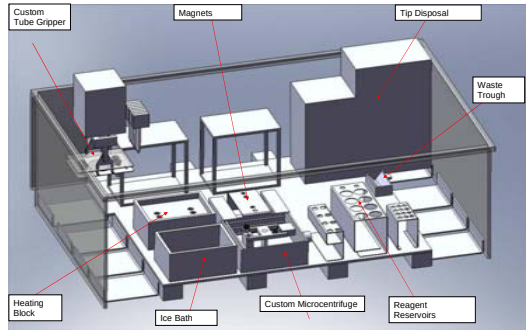
Collecting and pooling emulsions from PCR plates is a labor intensive process of manually aspirating via syringes. Vacuum manifolds have successfully been used to improve this process, but a simpler quicker method is centrifugation emulsion collection using a rectangular funnel placed into the top of a 10ml syringe. The syringe is plugged with a port cap at the other end, and the 96-well emulsion plate is cut into thirds by a special device and placed upside down into the funnel. The funnel and plate are then placed into a centrifuge and spun down to collect the sample in the syringe. This method was developed by the JGI Instrumentation and Process Optimization groups and is the current method used for emulsion collection in the Production group.





Automated Enrichment of Sample Beads

The JGI Instrumentation group is currently developing an automated platform to enrich clonally amplified sample beads recovered from emulsion PCR. Instrument design and proof of principle testing has begun with the Process Optimization group. This platform is being designed to automate the GS-Titanium enrichment process.



Pico Titer Plate (PTP) Reuse

The Pico Titer Plate (PTP) is a costly component used in the sequencing run. The JGI Process Optimization group has developed a procedure for allowing the reuse of PTPs for multiple runs. A brief 5 min sonication of the used PTPs is performed to remove the sequencing beads and then the PTPs are stored wet ready for reuse. The wet storage conditions has prevented surface spotting, which occurs when they are allowed to dry. The JGI Instrument group has developed interlocking plastic plate frames that serve to hold the PTPs as they are resting in the sonicator during cleaning. These frames also double as storage devices for keeping submerged PTPs organized in air tight plastic boxes. Validation of Titanium PTP reuse is currently underway.

Used PTP – post run

Used PTP – post sonication

Used PTPs – ready for reuse

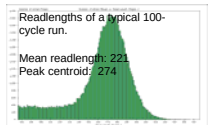
Interlocking PTP holder

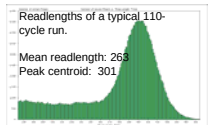
Increased Cycle Run Script

The GS LR70 run script provided by Roche dictates 100 cycles of reagents for each nucleotide. However, at the end of the run significant quantities of reagents are left unused. Furthermore, base-quality at the end of 100-cycles is high enough to suggest that there is sufficient reagent stability at the end of the run to perform additional cycles with the remaining reagents. A run script that incorporates an additional 10 reagent cycles was created by JGI's Technology Development group and has now replaced the standard 100 cycle runs. The 110 cycle script yields additional sequencing data at no additional reagent cost and minimal time cost.

Mean average readlengths for production projects at the JGI:

1-Oct-07 through 15-Nov-07 (100-cycles):	209 +/- 34 bases
16-Nov-07 through 21-Jan-08 (110-cycles):	234 +/- 36 bases
Net average readlength gain:	25 bases





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