II. An overview of the sequencing prep process at the Joint Genome Institute's Production Genomics Facility

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Abstract

The sequencing prep process is the second step of the production process at the Joint Genome Institute (JGI) Production Genomics Facility (PGF). The goal of the process is to prepare labeled fragments in 384-well plates for loading onto the capillary sequencers. The process begins by alliquoting 2ul of glycerol stock from the Library Support Process using Matrix PlateMate Plus. The 384-well plates, containing glycerol stock and buffer, are placed on the PE 9700 thermocyclers and heated to 95C for 5 minutes in order to lyse the cells and release the plasmids. The amplification of plasmid DNA is performed using Templiphi, a kit made by GE Healthcare in order to generate large quantities of template. TempliPhi is added with a multidrop micro instrument and samples are incubated at 30C for 20 hours to allow the amplification to occur. The amplified template is then heated to 95C to inactivate the enzyme. Small amounts of amplified DNA are transferred using a Robbins Hydra Twister robot into two 384-well plates to set up dual sequencing reactions. The Chemistry cocktail is added by the Cavro Dispensing instrument and then cycled. For high GC templates we utilize a 5% final concentration of DMSO that is added to our TempliPhi and chemistry reactions. Before DNA can be loaded onto the capillary sequencer, the leftover reagents, cell debris, buffers, and salts must be removed from the sample. The last step in the Sequencing prep process is the cleanup step. The process uses a modified magnetic bead protocol to purify DNA fragments from the sequencing reaction and get it ready for sequencing. This step is performed using the Beckman Coulter Biomek FX robots. The reactions are then ready to load on either the ABI 3730xl or the MegaBACE 4500 for capillary electrophoresis. This poster will present a detailed overview of the sequencing prep process reviewing each step and the instruments that are used.

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