An Overview of the Sequencing Prep Process at the Joint Genome Institute's Production Genomics Facility

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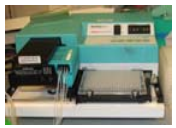
Abstract

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 generated by the Library Support Process using a Matrix PlateMate Plus. The 384-well plates, containing glycerol stock and buffer, are placed onto 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 for 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 into the capillary sequencer, the leftover reagents, cell debris, buffers, and salts must be removed from the sample. The post sequencing cleanup 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.

Step 1) Rolling Circle Amplification

The purpose of this step is to amplify circular DNA template for cycle sequencing.

1. 1ul of buffer is dispensed into 384-well axygen plates using a Multidrop Micro dispenser (Picture 1).



Picture 1. Multidrop Micro

2. Plates filled with buffer plates, along with the 384-well glycerol stock plates (containing *E. coli* with the DNA inserts) created in the Library Support Process, are loaded onto the Matrix PlateMate Plus robots. 2µL of glycerol stock contents are transferred to the buffer plate. A mixing step is performed and 2ul are aspirated off, leaving 1ul mixture of cells and DNA (Picture 2).

Picture 2. PlateMate Plus robot

3. The 384-well Axygen plates are placed onto the PE9700 Thermocyclers and heated to 95°C for 5 minutes where cells are lysed and the plasmids are released (Picture 3).

Picture 3. PE 9700 Thermocyclers

4. Once the cells are lysed, TempliPhi Premix, a kit made by GE Healthcare, is dispensed into each plate using the Multidrop Micro dispenser (Picture 1).

The TempliPhi Premix contains:
- •Phi29 Polymerase
- •Random hexamers
- •dNTP's

(Fig 1)

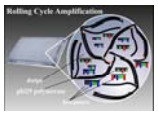


Fig 1

Picture 5. Incubator

5. Plates are then sealed with Velocity 11 heat sealer & incubated for 20 hours at 30°C (Picture 5).

6. During the incubation, Rolling Circle Amplification occurs producing up to 109 copies of each circular template (Fig.2).

Fig 2. Electron Micrograph of nondenatured TempliPhi amplified product. For size comparison, 2 copies of pUC DNA appear in the top right section of this image.

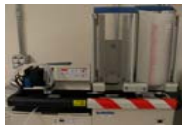
7. After 20 hrs the amplification process is halted by heat-inactivating the enzyme on the Thermocyclers for 10 minutes at 95°C.

The plates are now ready for Sequencing Chemistry step.

Step 2) Sequencing Chemistry (Sanger Reaction)

The Sanger reaction step is a modified PCR reaction that produces an enormous number of dye labeled DNA fragments.

1. 5ul of water is added to each RCA amplified sample plate using the Matrix Wellmate robot (Picture 6).



Picture 6. Matrix Wellmate robot

2. Microliter amounts of amplified DNA are transferred into two 384-well axygen plates to set up dual sequencing reactions using the Robbins Hydra Twisters (Picture 7).

Picture 7. Robbins Hydra Twister

3. A chemistry cocktail, comprised of specific primers, Taq Polymerase, dNTP's, ddNTP's, buffer, & water, is added to each plate using the Cavro Dispensing Instrument (Picture 8).

Picture 8. Cavro Dispenser

4. The plates are then sealed and placed on the PE 9700 Thermocyclers for 30 cycles where the Sanger Reaction occurs (Picture 9).

Picture 9. Thermocycler room

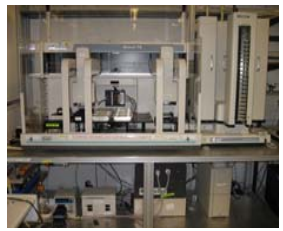
5. The new robot (CyBio Well Vario) that will combine the aliquot step of the Hydra Twister and the dispense step of the Cavro dispenser is currently in the implementation process. The goal is to make the process more efficient and increase the throughput and allow walk away time (Picture 10).

Picture 10. CyBio Well Vario

Step 3) Post Sequencing Clean Up

Before the plates can be loaded onto the capillary sequencers, the leftover reagents, cell debris, buffers, and salts must be removed from the sample. This is accomplished in Post Sequencing Cleanup step. This process uses a modified magnetic bead protocol to purify DNA fragments from the sequencing reaction and get it ready for capillary electrophoresis. This step is performed entirely on the Beckman Coulter Biomek FX robot (Picture 11 a & b).

Picture 11b. Beckman Coulter Biomek FX Deck layout



Picture 11a. Beckman Coulter BioMek FX

Process Steps

1. 384-well axygen plates obtained from sequencing chemistry (source plate) and empty 384-well axygen plates (destination plate) are loaded into the Biomek FX Stackers.

2. The Biomek places the source plate onto the deck and adds 10µL of BET solution (200 proof ethanol, water, Tetra ethylenglycol, and washed Seradyne Magnetic Carboxylate-Modified Beads) to the plate.

3. DNA is induced to attach to the beads but not the excess terminators. (Fig 3, step 1).

Fig 3.

4. The source plates are returned to the stackers and incubated for 15 minutes.

5. After the incubation, the source plates are placed onto the magnets where the DNA/beads are drawn down so waste solution can be aspirated (Fig 3, Step 2).

6. 15µL of 70% EtOH is then dispensed and aspirated from the plates (Fig 3, Step 3).

7. The plates are then returned to the stackers and incubated for 10 minutes to allow the plates to dry.

8. The source plates are placed onto the stage and 15 µL of water is dispensed into the plate. The plates are then transferred to the magnets. Because DNA has a higher affinity for water, the beads are drawn down while the DNA remains in solution (Fig 3, Step 4).

9. Lastly, 10µL of water + DNA is transferred to the destination plates and returned to the stackers (Fig 3, Step 5).

The plates have been successfully processed through Sequencing Prep and are now ready for capillary electrophoresis and are sent to either MegaBACE 4500 or ABI 3730xl sequencing platform where sequencing is performed.

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