I. AN OVERVIEW OF THE LIBRARY SUPPORT PROCESS USED AT THE JOINT GENOME INSTITUTE PRODUCTION GENOMICS FACILITY

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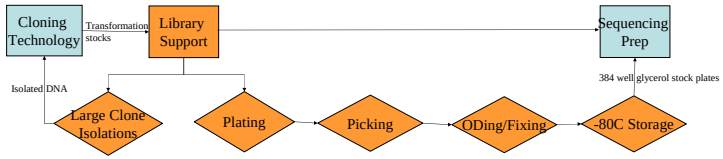
ABSTRACT

Library support is the first step in production sequencing at the Joint Genome Institute's (JGI) Production Genomics Facility (PGF). The goal of library support is to generate 384 well glycerol stock plates of each library for a given project. These plates are then passed on to the Sequencing Prep group for Rolling Circle Amplification. Transformation stocks of the 3, 8, and 40kb libraries for a given project are received into library support from cloning technology. The stocks are stored at -80 C until plated. Libraries are plated according to priority. The colonies produced from a plating event are then picked using robotic colony pickers and are inoculated into 384-well destination plates containing LB/glycerol + antibiotic. The destination plates are grown overnight and visualized on a Spectramax Optical Density reader to identify wells without growth. Wells containing no growth are aspirated and replaced with colony growth from a "fix source" plate generated from the same library. The destination plates are then sealed and stored at -80 C until sent to the Sequencing Prep area for Rolling Circle Amplification (RCA). This poster will elaborate on these steps in the library support process and detail further the project tracking and management, plating and picking procedures and the instruments that are utilized throughout these steps.

Workflow



PROJECT MANAGEMENT

For a given project, transformation stocks are created in Cloning Technology, and passed to Library Support for scheduled plating. Transformation stocks contain glycerol as a preservative for the E.coli cells and allow the stocks to be stored at -80 C until plated. Stocks received are for each library of insert size 3kb, 8kb, or 40kb. All libraries for a given project are scheduled through sequencing based on an initial 10 plate QC. The 10 plate QC is performed by the Quality Assurance (QA) team to determine the size of the organism, check for contamination, identify vector and insertless rates, and to request the final amount of plates needed for 8x coverage. If the 10 plate QC passes, the remaining plates are requested and the transformation stocks are again plated. If the 10 plate QC fails, then the library is failed and Cloning Technology must create a new library from the source DNA. Currently, management of projects is done using an Excel Spreadsheet. Dates are recorded for when the libraries are received, plated, sent for 10 QC, date of QC pass, final plating and final send for a project. Time intervals are calculated to provide information in reports as to the average time it takes projects to move through library support providing a basis for estimating the total time it takes to sequence a project.

Excel Spreadsheet for tracking projects through Library Support

Genus species	Project Type	Project	Project Year	Project I.D.	Library	Size	Stock Rcvd Date	Stock Rcvd From	10QC Date Plated	10QC Date Sent	10QC Date Passed	Time Rcvd to QC sent (days)	Time 10Qc sent to Pass Date	Number plates to Send	Final Plating Date	Final Send Date
Batrachochytrium dendrobatidis JAM81-0	microbe	CSP	2006	4001669	BIIW	3kb	1/3/2006	Hope	1/5/2006	1/10/2006	1/24/2006	7	14			
Batrachochytrium dendrobatidis JAM81-0	microbe	CSP	2006	4001669	BIIX	8kb	1/30/2006	Hope	1/31/2006	2/7/2006						
Batrachochytrium dendrobatidis JAM81-0	microbe	CSP	2006	4001669	BIIY	40kb	1/30/2006	Hope	1/31/2006	send						
Bifidobacterium longum infantis	misc	WFQ	2006	4002686	BTCF	8kb	1/30/2006	Hope	1/31/2006	2/6/2006				14		3/8/2006
Bifidobacterium longum infantis	misc	WFQ	2006	4002686	BTCC	3kb	1/30/2006	Hope	1/31/2006	2/6/2006	2/17/2006			14		3/8/2006
Bifidobacterium longum infantis	misc	WFQ	2006	4002686	BTCG	40kb	1/30/2006	Hope	1/31/2006					11		
Burkholderia phymatum STM815	microbe	DOE	2006	4002725	BTIT	3kb	3/7/2006	Hope	3/8/2006	3/15/2006						
Burkholderia phymatum STM815	microbe	DOE	2006	4002725	BTIU	8kb	3/7/2006	Hope	3/8/2006							
Burkholderia phymatum STM815	microbe	DOE	2006	4002725	BTIW	40kb	3/7/2006	Hope	3/8/2006							
Burkholderia phytofirmans PsJN	microbe	DOE	2006	4002732	BTOO	3kb	3/20/2006	Hope								
Burkholderia phytofirmans PsJN	microbe	DOE	2006	4002732	BTOP	8kb	3/20/2006	Hope								
Burkholderia phytofirmans PsJN	microbe	DOE	2006	4002732	BTOS	40kb	3/20/2006	Hope								

DETAILED WORK PROCESS IN LIBRARY SUPPORT

LARGE CLONE ISOLATIONS

Inoculation

Large fosmid, BAC and cosmid clones from streaked bioassays, glycerol stocks or agar stabs are inoculated one day prior to isolation and set up for overnight incubation.

A single clone is inoculated into a flask containing 250ml LB media and chloramphenicol.

After 18-20hrs incubation, the flasks are removed from the Innova 4900 Upright Shaker.

The isolation process is begun using Qiagen's Plasmid Maxi Kit.

Isolation

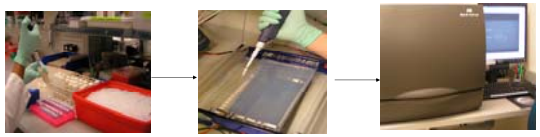
After 18 hours of incubation, samples are centrifuged to form a pellet of E.coli cells. The cells are lysed open with a lysis buffer to extract the DNA of interest. Once the cells are lysed, the samples are centrifuged using a Sorvall floor centrifuge, to separate out the DNA from the cell debris.

Isolation – cont'd

The DNA is then run through a column containing silicon beads and primed with a buffer composed of isopropanol and Sodium Chloride. This helps the DNA adhere to the columns so that the columns can be washed to remove any contaminants or cell debris. The DNA is then eluted from the columns and centrifuged with isopropanol to create a DNA pellet. This pellet is then washed with 70% Ethanol and centrifuged. The DNA pellet is dried by air and resuspended in TE buffer.

Quantification

Upon isolation of large clones, the DNA is quantified and diluted or concentrated to a target concentration of 40ng/ul. The DNA is then handed off to Cloning Technology for subcloning.



The DNA samples and standards are prepared for loading into 1% agarose gel.

Agarose gel electrophoresis is carried out to confirm the presence of plasmid DNA.

The gel is imaged using a Flour-s Multi-Imager and quantified using Multianalyst software.

PLATING

Library Support receives transformation stocks from the Library Creations group. The stocks are stored at -80 C until plated. Technicians are assigned multiple libraries to plate on a daily basis. One technician is assigned to plate 40 bioassays. On average, 120-160 bioassays are plated per day. Using the Production Data Base, the technician will determine which Teknova antibiotic bioassays to use with each library they are plating: Ampicillin, Chloramphenicol 20, Chloramphenicol 12.5, Kan 30, and Carbenicillin. Each bioassay will create approximately two and one-half 384 well plates. The bioassays are QC'd for contamination and irregularities.

Bioassays are dried in incubators.

Transformation stock stored at -80°c is thawed and mixed with S.O.C. media according to data base calculations.

Plating is done in a fume hood using 5mm glass beads.

Bioassays are incubated for 18 hours at 37 °c.

PICKING

Picking of individual colonies from the bioassays is an automated process using the Genetix Qpix and Qpix2 XT instruments. Our production line has 4 colony picking instruments that run for 16 hours a day. They produce on average 300 destination plates per day. We sterilize the environment with three 15min. UV irradiation events, at the beginning of the day, between shifts and at the end of the day, to eliminate possible contamination. We also perform routine sterility tests twice a day to ensure proper washing of the picking pins between each inoculation to eliminate the possibility of cross contamination.

Bioassays are pulled from incubators after 18-20 hours incubation and loaded onto a Qpix machine.

384 well destination plates are filled with LB/glycerol + antibiotic using a plate crane and microfill instrument.

Colonies are imaged by the attached LCD camera and selected based on preset criteria.

The colony picking instruments pick and inoculate the colonies into 384 well destination plates. These plates are then labeled with barcodes specific to the library that was picked and tracked in the database.

The destination plates created are then incubated for 18 hours at 37 °C

OPTICAL DENSITY & FIXING

After picking, each 384 well plate is QC'ed to ensure that adequate growth is obtained in every well. We use a SpectraMax instrument to obtain the optical densities of each well. Ranges and colors are set to indicate the growth pattern in the wells.

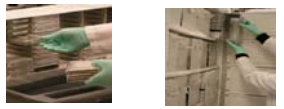
Wells with sufficient growth (0.11-0.70) appear green, minimal growth (0.071-0.1) appear yellow and no growth (<0.071) appear red. There is also an indication of overgrown (>0.70) that appears blue and may indicate contamination. These plates are not put through production.

Example of a 384 well map showing no growth and minimal growth

Wells indicated as having no growth are aspirated using a vacuum and replaced with a well that has sufficient growth from a "fix source" plate created from the same source library.

STORAGE

Plates are sealed and stored in -80 freezers until requested for further processing.



Plates being racked

Racks being stored at -80 C

On a daily basis, 252-294 plates are sent on to Sequencing Prep for Rolling Circle Amplification (RCA). Plates sent are based on priority and according to the availability. All plates must be frozen for at least 8 hours prior to sending for RCA. The plates being sent are batched according to the platforms they will get sequenced on, ABI or MegaBACE. Plates of individual libraries are equally distributed amongst the batches for randomization studies and to test various instruments used throughout the process.

CONCLUSION

Library Support receives several projects on a weekly basis from cloning technology. The projects are scheduled for plating to obtain enough plates for a 10 plate QC. The bioassays are picked and the 384 well plates generated are read on the Spectramax to ensure all wells have adequate colony growth. The plates are then stored until sent on for further processing in the Sequencing Prep line. If the 10 plate QC passes, the library is reassigned for final plating and all plates requested are sent on for final sequencing. All projects are tracked and managed thorugh the library support line to ensure timely sequencing.

LBNL-59904 Poster

UCRL-POST-220243

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 Livermore National Laboratory under Contract No. W-7405-Eng-48, Lawrence Berkeley National Laboratory under contract No. DE-AC02-05CH11231 and Los Alamos National Laboratory under contract No. W-7405-ENG-36.