Comparison of Protocols for Isolating Large Insert Clone DNA that is Suitable for High Throughput Library Construction

Karla Ikeda, Eileen Dalin, Miranda Harmon-Smith, Jan-Fang Cheng, Susan Lucas, & JGI Production Sequencing Department

DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

ABSTRACT

JGI has been generating sequences from numerous large insert size clones each year. These targeted sequences allow collaborators to study regions of interest without having to sequence the entire genomes. As part of the assembly QC process, JGI would also sequence a set of fosmids or BACs randomly selected from all eukaryotic genomes to aid in the assembly. Last year JGI has isolated DNA from about 1,200 large insert clones, constructed libraries from each clone, and sequenced them.

We have been using Qiagen® Plasmid Maxiprep protocol to isolate DNA from BAC/fosmid clones, which is laborious and time consuming. In the attempt to find a more efficient way of isolating DNA from these clones, we have compared several protocols including the GenElute™ HP Plasmid Maxiprep from Sigma, the BACMAX™ DNA Purification Kit from Epicentre, and the Edge BioSystems FosPrep™ 96 Fosmid Prep. An overview of each protocol will be presented. We will also provide the comparison of costs, amount of time, DNA yield, quality, and the suitability for library construction from using these DNA isolation protocols.

INOCULATIONS

Large Fosmid and BAC clones from streaked bioassays or glycerol stocks are inoculated one day prior to isolation. The inoculation procedure is similar for the Qiagen® Plasmid Maxiprep, the GenElute™ HP Plasmid Maxiprep, and the BACMAX™ DNA Purification Prep. The inoculations for the FosPrep™ 96 Fosmid Prep varies slightly from the other three preps.

Inoculations for the Qiagen®, GenElute™, and BACMAX™ preps:

- A single clone is inoculated into a flask containing LB media (250ml for the Qiagen® prep, 150ml for GenElute™, and 100ml for BACMAX™) and Chloramphenicol
- The flasks are grown for 16-18 hours in an Innova 4900 Upright Shaker at 37ºC.
- The culture is transferred to 250ml centrifuge bottles and the process of Isolations can begin.

Inoculations for the FosPrep™ 96 Fosmid Prep:

- A 96 well plate (resin lined) is filled with 275µl of media (prepared with Terrific Broth, Chloramphenicol, Phosphate buffer, and Arabinose) and 25µl of Fosmid glycerol stock.
- 8 wells were inoculated for each Fosmid clone.
- The plate is incubated for 18 hours at 37ºC with no shaking.

THE QIAGEN® PLASMID MAXIPREP

After 18 hours of incubation, the DNA is isolated from the cells.

- Cell culture is centrifuged to pellet the DNA.
- Cells are lysed open to extract the DNA.
- The supernatant undergoes 3 centrifugation events to remove unwanted cell debris.
- An anion exchange column is primed with an Isopropanol and Sodium Chloride buffer.
- DNA is added to the primed column and adheres to the resin.
- DNA is washed with a medium salt buffer to remove remaining cell debris.
- DNA is eluted from the columns with a high salt buffer then precipitated using Isopropanol
- The precipitated DNA is pelleted by centrifugation
- The DNA pellet is washed with 70% Ethanol, air dried, and resuspended overnight at 4ºC in 400µl of TE buffer.

GENELUTE™ HP PLASMID MAXIPREP KIT FROM SIGMA

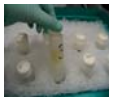
After 18 hours of incubation, the DNA is isolated from the cells.

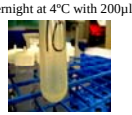
- Cell culture is centrifuged to pellet the DNA.
- The cells are lysed and a binding solution is added
- The mixture is filtered through manual syringes into columns with a silica membrane (primed with buffer)
- The columns are centrifuged and the DNA binds through high salt concentrations present.
- The columns are washed to remove remaining cell debris
- The DNA is eluted and precipitated with Isopropanol and Sodium Acetate buffer
- The precipitated DNA is pelleted by centrifugation
- The DNA pellet is washed with 70% Ethanol, air dried, and resuspended overnight at 4ºC in 200µl of TE buffer.

BACMAX™ DNA PURIFICATION KIT FROM EPICENTRE

After 16 hours of incubation, the DNA is isolated from the cells.

- Cell culture is centrifuged to pellet the DNA.
- Cells are lysed open to extract the DNA.
- Isopropanol is added to the supernatant and centrifuged to precipitate the DNA
- The pellet is air dried and reususpended in TE buffer
- RNase is added to the resuspended pellets and incubated at 37ºC for 30 minutes
- Additional TE buffer and BACMAX™ Solution 4 (containing ammonium acetate) is added and incubates on ice for 15 minutes.
- The mixture is centrifuged and the impurities are pelleted.
- The supernatant containing the DNA is precipitated with Ethanol
- The DNA pellet is air dried and resuspended overnight at 4ºC with 200µl TE buffer.

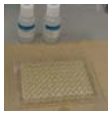




FOSPREP™ 96 FOSMIDPREP

After 18 hours of incubation, the DNA is isolated from the cells.

- The 96 well plate is centrifuged to pellet the DNA.
- The supernatant is decanted and the plate is placed in a plate shaker to resuspend the cells in any remaining media
- The cells are lysed and the DNA sticks to the resin on the bottom of the well
- The DNA is washed with a wash solution and the solution is decanted.
- The DNA is washed for a second time with Isopropanol and the solution is decanted.
- The plate is air dried for 30 minutes and then the DNA in the wells is resuspended in 50µl TE buffer.
- The 8 wells for each Fosmid clone were combined



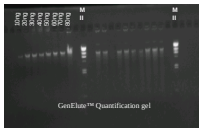
QUANTIFICATION

- A 1% agarose gel is used to quantify the isolated DNA samples. Marker II and 8 Lambda DNA concentration standards (10ng/µl, 20ng/µl,…, 80ng/µl) are loaded onto the gel.
- The gel is imaged and quantification software creates a standard curve by utilizing the concentration standards
- The concentration of the isolated DNA is then determined by comparison to the standard curve.

ISOLATION AND QUANTIFICATION RESULTS

GenElute™ HP Plasmid Maxiprep

- Isolation took 5 hours
- The DNA yield was less than half the concentration of samples isolated with the Qiagen® Maxiprep

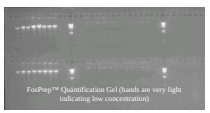


BACMAX™ DNA Purification kit

- Isolation took 5 hours
- First 2 tests yielded no DNA. There were however, some noticeable complications with this prep. Two visible layers were present when there should have only been one. DNA may have been lost during this part of the protocol for the first two tests as only the bottom layer was used.
- In the 3rd test the two layers were pipetted and an extra spin was done to remove more E. coli debris. This seemed to help and the DNA bands were observed.

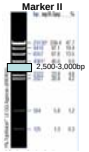
FosPrep™ 96 Fosmid Prep

- Isolation took 2 hours
- Isolated DNA samples were very bubbly and particulate matter was observed.
- The quantity of DNA obtained was very low
- Due to low yield troubleshooting involved decreasing reuspension time, freezing the cell culture overnight and extra washes were performed. None of these methods yielded clean enough DNA for subcloning.



SUBCLONING ISOLATED DNA

- DNA is randomly sheared to ~3,000bp using GeneMachine's Hydroshear
- DNA is blunt-end repaired
- The DNA is then size selected on an agarose gel.
- DNA 2,500-3,000bp is extracted and purified using Qiagen® gel extraction columns
- The DNA insert is blunt-end ligated into pUC18
- The plasmid is transformed into *E. coli* cells via Electroporation
- Glycerol is added to the transformation to create a plating stock
- The stock is plated out on a bioassay
- Colony PCR is performed to verify presence of insert.
- Library complexity is determined



Sheared Samples run on a gel:

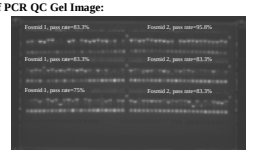
Qiagen® Samples | GenElute™ Samples | BACMAX™ Samples

Example of PCR QC Gel Image:

Row #1 – Qiagen® Samples

Row #2 – GenElute™ Samples

Row #3 – BACMAX™ Samples



SEQUENCING RESULTS

PREP USED	FOSMID CLONE	PASS RATE	READ LENGTH	*E. COLI* HITS	V80	PCR QC PASS RATE
Qiagen® Plasmid Maxiprep	Fosmid #1	99.13	660	18.3	1.125	83.3%
Qiagen® Plasmid Maxiprep	Fosmid #2	95.31	692	10	0.875	95.8%
GenElute™ HP Plasmid Maxiprep	Fosmid #1	96.22	722	40.5	3.125	83.3%
GenElute™ HP Plasmid Maxiprep	Fosmid #2	97.79	670	29.38	1.625	83.3%
Epicentre BACMAX™ DNA Purification	Fosmid #1	87.62	610	10.7	2.25	75%
Epicentre BACMAX™ DNA Purification	Fosmid #2	82.03	601	37	5.5	83.3%

PROTOCOL COMPARISIONS

Prep	Kit Cost ($)	Approx. Growth Media Cost ($)	Total cost for kit + media ($)	Cost per library for Isolations ($)	Prep time (Hr)	Average DNA yield (µg)	Ability to isolate Fosmid clones & BAC clones	Comments that can effect use of product
Qiagen® Plasmid Maxiprep	445	205.52	650.52	26.02	8	16.8	Fosmid and BAC	Takes 8 hours to complete 25 samples
GenElute™ HP Plasmid Maxiprep	412.5	117.44	529.94	21.1976	5	5.4	Fosmid and BAC	Use of syringes presents an ergonomic issue
Epicentre BACMAX™ DNA Purification	750	88.08	838.08	33.52	5	4	Fosmid and BAC	Problem with the two layers being present after centrifugation
FosPrep™ 96 Fosmid Prep	175	36.35	211.35	8.81	2	12.8	Fosmid only	Unclonable DNA due to dirty DNA

CONCLUSIONS

When performing the four isolations procedures, the Qiagen® Plasmid Maxiprep, GenElute™ HP Plasmid Maxiprep, BACMAX™ DNA Purification kit, and the FosPrep™ 96 Fosmid Prep, we found the FosPrep™ DNA to be unusable for further processing. The other three preps produced good quality DNA with high enough yield for subcloning. The sequencing results looked comparable between the GenElute™ and the Qiagen® preps, but the number of *E. coli* hits for the GenElute™ are almost double than that of the Qiagen®. Although the GenElute™ prep takes less time to complete, if the *E. coli* rate can't be decreased, this is not an ideal prep to use. Another factor to consider with the GenElute™ prep is the use of syringes which are a potential ergonomic concern. The BACMAX™ prep sequencing results appear to have a slightly higher fail rate and lower read length compared to the other two preps. It also produced inconsistent *E. coli* hits for the two libraries. Therefore, our results are inconclusive. This small scale experiment has eliminated the FosPrep™ as being a possible replacement, but a large scale test comparing the GenElute™ prep and the BACMAX™ prep to the current Qiagen® prep is needed before a decision can be made.

UCRL-POST-229321
LBNL-62528 Poster

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. DE-AC02-06NA25396.