ProPrep™ BAC 960

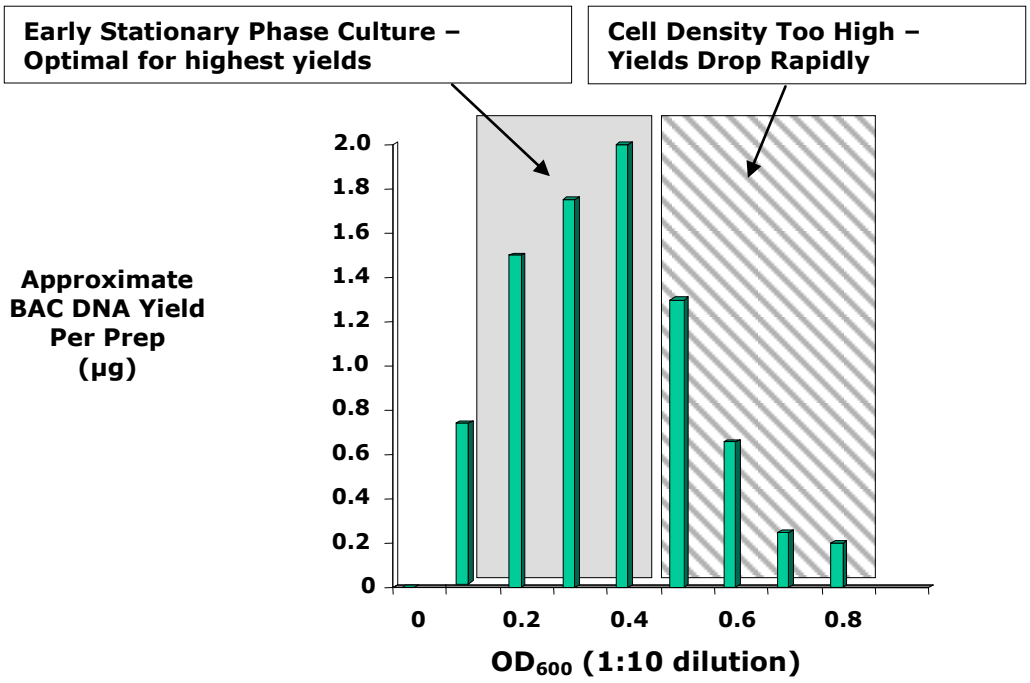
Sequencing Quality 96 Well BAC Prep

Product	Size	Item No.
ProPrep™ BAC 960	960, 2.0ml cultures	PLF-960

Complete purification system based upon the propietary reagent, ProCipitate™, that has been demonstrated to provide high quality DNA suitable for automated fluorescent sequencing of small to large insert DNA[1-8]. (Note: Some users report that adding an additional 4 mM $MgCl_2$ (final concentration) into the sequencing reaction mix improves sequencing.)

ProPrep™ BAC 960 systems start with 2.0 ml overnight cultures, and then utilize ProCipitate™ in a modified alkaline lysis protocol. Follow the protocol carefully, paying special attention to the growth and mixing recommendations. As an option, the ProPrep™ BAC 960-A protocol adapts easily to robots and automation; a simple change and there is no centrifugation required after initial cell concentration.

Effect of Cell Density on BAC DNA Yield



PROTOCOL

Items Required	Quantity	ProPrep™ BAC 960	Storage
Culture Items		Not Supplied	
Alcohol		Not Supplied	
TE1 Resuspension Buffer	120 ml	Supplied	Room Temp.
RNase Cocktail	10 ml	Supplied	-20°C (or 4°C after addition to TE1)
AL2 Alkaline Lysis Buffer If precipitate forms, solubilize by placing bottle in warm water	120 ml	Supplied	Room Temp.
NB3 Neutralization Buffer	120 ml	Supplied	Room Temp.
ProCipitate™	80 ml	Supplied	4°C (best if used before date on label)
96 well filter plate	10	Supplied	---
96 deep well filtrate collection plate	10	Supplied	---
Plate seal tape	10	Supplied	---
Wide Bore Pipette Tips		Not Supplied	

Proper mixing is imperative to insure consistent results. For optimum performance follow mixing instructions shown.

1. Grow 2.0 ml overnight LB or 2xYT culture (20 μg/ml chloramphenicol) to early stationary phase, a concentration range from $2 \rightarrow 4 \times 10^9$; corresponds to OD_{600} (1:10 dilution) = 0.2→0.4. 2xYT broth provides approximately 50% greater DNA yield than LB. TB broth is not recommended.

2. Concentrate cells by centrifugation at [2,000 x g] for 10 minutes.

3. Decant and discard supernatant. Blot dry the plate surface.

4. Add 120 μl of TE1 and 9 μl RNase cocktail to each well. Resuspend cells by vortexing. Insure that no cell clumps remain or yield will be reduced.

5. Add 120 μl alkaline lysis buffer AL2, shake briefly to mix, but do not vortex, and leave at room temperature for 2 minutes.

6. Add 120 μl neutralization buffer NB3, shake briefly to mix, but do not vortex, and leave at room temperature for 5 minutes.

7. Shake ProCipitate™ well to completely resuspend. Add 80μl to each sample using a wide bore pipette tip. VERY IMPORTANT - Mix by pipetting up and down 5 times with a wide bore pipette tip. Leave at room temperature for 2 minutes.

8. Transfer each sample to its corresponding well in the 96-well filter plate and vacuum filter at approximately 20" Hg. The filtrate contains the DNA. See "Tips on filter plate use" on next page. **Important Note:** If too much cell mass is employed, the reagents become saturated and yield and filtration can become inconsistent. If filtration is incomplete or inconsistent, consider reducing cell mass by lowering

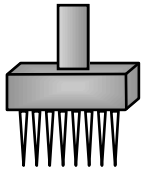
culture volume or incubation time. This will improve yield as reagent volumes are optimized for a maximum cell mass: 1.5 ml cultures at OD_{600} (1:10 dilution) = 0.2→0.4.

PROTOCOL, cont.

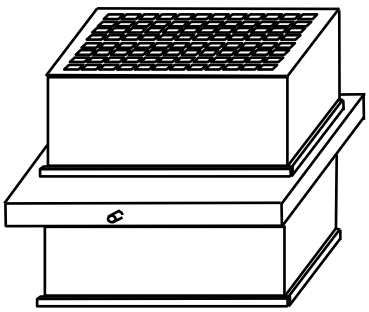
Tips on filter plate use

Inspect the gasket faces to insure that they are free of any foreign matter. Prior to adding samples, place filter plate on the vacuum manifold over receiver plate.

Transfer each sample to its corresponding filter plate well using wide bore pipette tips.



Apply full vacuum (18-20"Hg) until there is no no visible liquid in wells. A cake of solid material will become visible and may crack at the bottom of the wells. This is normal. Continue to apply vacuum for 10 - 25 minutes or until vacuum drops noticeably, greater than 1" Hg.



After filtration is completed, before removing the plates from the manifold, perform the following: Holding the plates together, raise and tap the assembly firmly down onto the bench top. Repeat. This dislodges any filtrate that may remain clinging to the bottom of the plate.

Alcohol Precipitation

NOTE - Alcohol precipitation adapted to 96 well filtration processes, in lieu of centrifugation, is available. Please contact technical services for details.

9. Add 200 µl of 100% Isopropanol (room temperature) into each filtrate, shake briefly to mix and leave for 20 minutes at room temperature.

10. Centrifuge at [2,000 x g] for 15 minutes to pellet the DNA.

11. Decant and discard supernatant.

12. Wash pellets with 200 µl of 70% Ethanol (room temperature), centrifuge at [2,000 x g] for 10 minutes.

13. Decant and discard the supernatant and air or vacuum-assist dry the pellets until no visible drops remain in each of the wells.

14. Resolubilize each of the samples in 30 - 50 μl of DI water, seal tape plate and incubate for 30 – 60 minutes at 65°C to assist resolubilization. Be sure the plate is well sealed or evaporation may occur. Shake once or twice during this period to assist resolubilization. [Note: Some users report that adding an additional 4 mM $MgCl_2$ (final concentration) into the sequencing reaction mix improves sequencing.]

REFERENCES

1. U.S. Patent Numbers 5,294,681, 5,453,493 and other patents pending.
2. Huang, G.M., et al, *A High-Throughput Plasmid DNA Preparation Method*, Analytical Biochem, 223:35-48, 1994.
3. Kelley, J.M., et al, *High-Throughput Direct End Sequencing of BAC Clones*, Nucleic Acids Reseach, Vol. 27, No. 6: 1539-1546, 1999.
4. http://www.hgmp.mrc.ac.uk/ISO9000/BIOLOGY/LIBRARIES/pig_BAC/procipitate.shtml
5. PE Biosystems User Bulletin. Subject: Sequencing Large DNA Templates.
6. Reddy, O.U.K., et al, *New Dinucleotide and Trinucleotide Microsatellite Marker Resources for Cotton Genome Research*, Journal of Cotton Science, 5:103-113 (2001).
7. Klein, R.K., et al, *High Throughput BAC DNA Isolation for Physical Map Construction of Sorghum*, Plant Molecular Biology Reporter, Kluwer Academic Publishers, 1998.
8. Bruce, D.C., et al, *BAC Library End Sequencing in Support of Whole Genome Assemblies*, poster DOE Joint Genome Institute and Center for Human Genome Studies, Los Alamos National Laboratory.