BIOTECH SUPPORT GROUP

# ProPrep™ BAC Mini 100

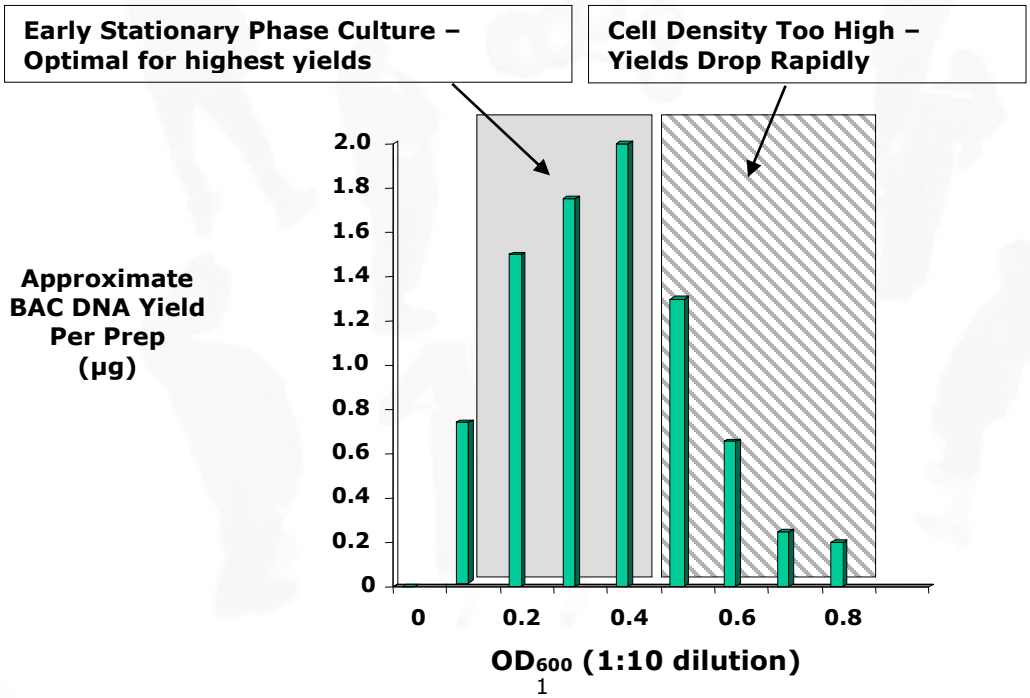
*Sequencing Quality Mini-Prep for for BACs & Large Plasmids*

| Product | Size | Item No. |
|---|---|---|
| **ProPrep™ BAC Mini 100** | 100, 2ml cultures | PMK-100 |

ProPrep™ BAC Mini 100 is a complete centrifuge tube based purification system based upon the proprietary reagent, ProCipitate™[1]. ProCipitate™ has been demonstrated to provide high quality DNA suitable for automated fluorescent sequencing of small to large insert DNA[2,3,4,5,6,7,8]. (Note: Some users report that adding an additional 4 mM $MgCl_2$ (final concentration) into the sequencing reaction mix improves sequencing.)

ProPrep™ BAC Mini 100 start with 2ml 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. The protocol is easily scaled up to larger cultures simply by increasing the reagent volumes proportional to the culture, i.e. 10ml cultures requires 5X reagent volumes.

**Effect of Cell Density on BAC DNA Yield**



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| Items Required | Quantity | ProPrep™ Mini BAC 100 | Storage |
|---|---|---|---|
| Alcohol | - | Not Supplied | --- |
| TE1 Resuspension Buffer | 12 ml | Supplied | Room Temp. (or 4°C after RNAse addition) |
| RNase Cocktail | 1 ml | Supplied | -20°C |
| AL2 Alkaline Lysis Buffer<br>If precipitate forms, solubilize by placing bottle in warm water | 12 ml | Supplied | Room Temp. |
| NB3 Neutralization Buffer | 12 ml | Supplied | Room Temp. |
| ProCipitate™ | 10 ml | Supplied | 4°C (best if used before date on label) |
| Wide Bore Pipette Tips | - | Not Supplied | --- |

**Proprep™ BAC Mini 100 can process 100 preps from 2ml cultures.**

## PROTOCOL

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

1. Grow 1.5 – 2.0 ml overnight LB or 2xYT culture (for BACs 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 microcentrifugation at [14,000 x g] for 2 minutes.

3. Decant and discard supernatant.

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.



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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 5-10 times with a wide bore pipette tip. Leave at room temperature for 2 minutes.

8. Microcentrifuge at [14,000 x g] for 15 minutes.

9. Carefully transfer the supernatant to a new tube. If solids are carried over, re-spin.

10. Add 200 µl of 100% Isopropanol (room temperature) into each supernatant, invert 3-4 times and leave for 20 minutes at room temperature.

11. Microcentrifuge at [14,000 x g] for 15 minutes to pellet the DNA.

12. Decant and discard supernatant.

13. Wash pellets with 200 µl of 70% Ethanol (room temperature) by carefully introducing at the side of the tube so as not to disturb pellet. If pellet is disturbed, microcentrifuge at [14,000 x g] for 10 minutes.

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

15. Resolubilize each of the samples in 30 - 50 µl of DI water. For small insert high copy plasmids, the sample is ready for use; for BACs, seal the tube and incubate for 30 – 60 minutes at 65°C to assist resolubilization. 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.]

This protocol is easily scaled up or down to accommodate different culture volumes. Simply adjust the reagent volumes proportional to the culture, i.e. 10ml cultures require 5x reagent volumes; 200 µl cultures, 10x less reagent volumes.

For high throughput 96 well formats, we can recommend 96 well filters which can be adapted to this protocol.



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## 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.


## CONTACT US

We welcome your questions and comments regarding our products.


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