Applications

Note 094 | December 2007

Isolation of high quality BAC DNA using the Perfectprep® BAC 96 Kit from 5Prime on the epMotion® 5075 VAC from Eppendorf

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Abstract

Eppendorf's continuing efforts to provide complete systems for biological research have created a system for the automated isolation of BAC DNA. This system uses the 5Prime Perfectprep BAC 96 Kit on the Eppendorf ep*Motion* 5075 VAC allowing for the simultaneous, fully automated isolation of BAC DNA from 96 clones. The typical yield of a single BAC-clone from a 96-clone library plate was 0.5 – 1.0 µg, and the average Phred Q>20 score of one library plate was 676 bases with a passing rate of 100 % (scores >100 bases). Consistent results were obtained when isolating a single BAC clone from all 384 wells of four 96-well culture plates. The average Phred Q>20 score of 192 of these samples was 718 bases with a passing rate of 97.4 %. The "walk away" protocol requires approximately 75 minutes and results in highly pure BAC DNA with sufficient yields for five downstream applications such as four sequencing reactions and one fingerprinting analysis.

Introduction

Bacterial Artificial Chromosomes (BACs) are a very common and useful tool for genomic research due to their high cloning efficiency, clone stability, and easy handling. BACs can maintain DNA fragments as large as 300 kb while maintaining excellent stability. Fingerprinting and BAC-end sequencing are the major applications used by scientists to collect genomic sequence data and obtaining quality sequences with long read lengths expedites this research. It is also advantageous, especially in whole genome sequencing efforts, to generate sequence data in a high-throughput manner on fully automated workstations.

The 5Prime Perfectprep BAC 96 Kit and the Eppendorf ep*Motion* 5075 VAC are integrated to provide a complete system for obtaining highly pure BAC DNA using a fully automated approach. The kit uses a modified alkaline lysis technology with a vacuum driven protocol to isolate BAC DNA from bacterial cultures. The protocol on the ep*Motion* 5075 VAC is optimized to obtain yields of 0.5 – 1.0 µg of highly pure BAC DNA and requires approximately 75 minutes. The DNA is ready for immediate use in multiple downstream applications (at least 5 sequencing reactions, or 4 sequencing reactions and 1 fingerprinting analysis, or several PCR reactions), and the purity of the DNA results in accurate and long sequencing read lengths.

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Materials and Methods

- 5Prime Perfectprep BAC 96 Kit
- Eppendorf ep*Motion* 5075 VAC
- Human BAC Library plate

Growth of bacterial culture

Library Plate

1.5 ml of 2x YT medium containing 12.5 µg/ml of chloramphenicol was aliquoted into each well of a 96-deep well culture plate. Using a 96-pin replicator, the medium was inoculated from frozen glycerol stocks of one quadrant of a 384-clone BAC library. The cultures were grown at 37 °C overnight for 24 hours in a shaker at 325 rpm. The plate was centrifuged for 10 minutes at 1900 x g to pellet the cells, and the supernatant was poured off. The plate was blotted to remove residual medium.

Single Clone

15 µl of a single clone BAC gylcerol stock was inoculated into 5 ml 2x YT medium containing 5 µl 12.5 mg/ml chloramphenicol. This small culture was grown at 37 °C at 325 rpm for 6 hours. 600 ml 2x YT medium containing 600 µl 12.5 mg/ml chloramphen-icol was inoculated with 600 µl of the 5 ml starter culture. The culture was grown overnight (approximately 18 hours) in 37 °C incubator shaking at 325 rpm. 1.5 ml of the culture was aliquoted into four 96-deep well culture plates so that each well contained equivalent culture medium. The plates were centrifuged at 1900 x g for 10 minutes. The supernatant was decanted and the excess medium was removed by blotting. The pellets were stored at -20 °C until the plates were processed.

Processing

The pelleted cells were processed using the 5Prime BAC 96 protocol on the ep*Motion* 5075 VAC.

96 clones from a library plate were processed. Also, all wells of four plates containing the same clone (384 samples) were processed.

Table 1: Details of the worktable used in the 5Prime BAC 96 protocol

Position	Labware	Comment
A2	epT.I.P.S Motion 1000 µl	96 tips for 96 samples
A3	epT.I.P.S Motion 1000 µl	96 tips for 96 samples
A4	Collection Plate	Collects eluates
	85 mm Adapter	Height adapter for Collection Plate
B1	Position 1: Solution 1 Position 2: Solution 2 Position 3: Solution 3 Position 4: Trapping Buffer Position 5: Diluted Wash Solution Position 6: Elution Buffer	30 ml reservoir 30 ml reservoir 30 ml reservoir 30 ml reservoir 100 ml reservoir 30 ml reservoir
B2	epT.I.P.S Motion 1000 µl	24 tips for 96 samples
B3	epT.I.P.S. Motion 300 µl	8 tips for 96 samples
VACUUM	Filter Plate A	Filters lysate
	Vacuum Frame 1	Collar for vacuum chamber
	Filter Plate BAC	Traps BAC DNA
C1	Culture Plate	Contains bacterial pellets
C2	55 mm Adapter	Height adapter for Filter Plate BAC
C3	55 mm Adapter	Height adapter for Filter Plate A
C4	Vacuum Frame Holder	Height adapter for Vacuum Frame 1
T0	Gripper	Tool to move labware
T1	TM 1000-8	1000 µl 8-channel pipetting tool
T2	TM 300-8	300 µl 8-channel pipetting tool

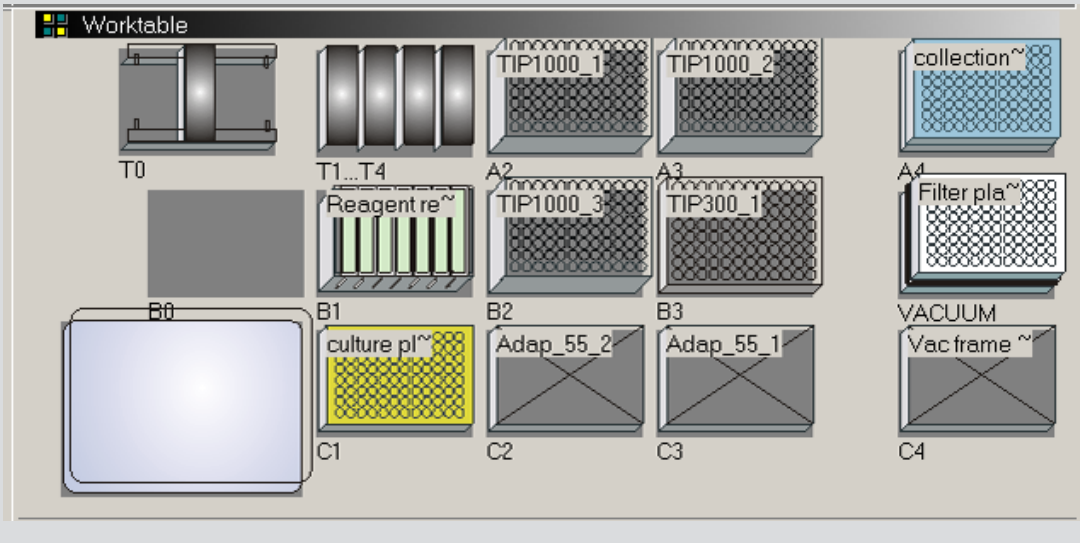

Figure 1: Screenshot from the ep*Motion* Editor showing the setup of the ep*Motion* 5075 VAC worktable for the 5Prime BAC 96 protocol.

Automated Fluorescent Sequencing

One half of the BAC DNA samples from each plate were sequenced using ABI BigDye™ V 3.1 chemistry on an ABI PRISM® 3700 DNA Analyzer. The reaction conditions and the cycling parameters are listed in Table 2 and Table 3.

Table 2: Sequencing Reaction Conditions (BigDye™ version 3.1; reactions)

Template DNA	10 µl
5x Sequencing Buffer (ABI)	3 µl
BigDye version 3.1 Ready Reaction Premix	2 µl
Primer T7 (10 µM)	1 µl
MgCl2 (25 mM)	0.6 µl
Molecular Biology Grade H2O	3.4 µl
Total Reaction Volume	20 µl

Table 3: Cycle Sequencing Parameters

Step 1	95°C for 4 minutes
Step 2	95°C for 15 seconds
Step 3	51°C for 15 seconds
Step 4	60°C for 4 minutes
Step 5	Go to step 2 and repeat 99 times
Step 6	10°C hold

To purify the cycle sequencing products, 5 µl of 125 mM EDTA was added directly to the samples followed by 60 µl of 95% highly pure ethanol. The plates were sealed and vortexed for 10 seconds. The samples were incubated for 25 minutes at room temperature in the dark and centrifuged for 30 minutes at 3000 x g. The ethanol was poured off and the plates were spun for 1 minute at 50 x g. 150 µl of ice-cold 70 % ethanol was added to wash the samples. The plates were centrifuged at 1900 x g for 15 minutes. The ethanol was poured off and the plates were spun for 1 minute at 50 x g. The samples were air dried for at least 15 minutes and resuspended in 8 µl of 0.1x TE prior to loading onto the 3700 DNA Analyzer.

Results

Agarose Gel Analysis

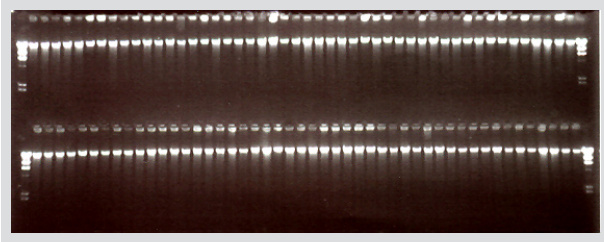

Figure 2: BAC DNA from one quadrant of a 384-clone library (96 samples) was isolated using the Perfectprep BAC 96 Kit on epMotion 5075 VAC. 10 µl of each sample were run on a 1% agarose gel and stained with ethidium bromide. The size marker is Lambda DNA digested with Hind III.

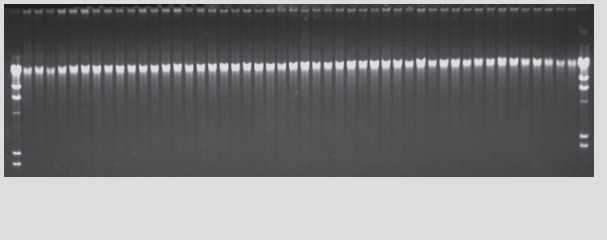

Figure 3: AC DNA samples from a plate containing the same single clone in all 96 wells were isolated using the Perfectprep BAC 96 Kit on the ep*Motion* 5075 VAC. 10 µl of each sample were analyzed on a 1% agarose gel. Four plates containing this single clone were processed, and this gel photo represents one of those plates. The size marker is Lambda DNA digested with Hind III.

Automated Fluorescent Sequencing

Library Plate:

The BAC DNA was sequenced using ABI BigDye™ V 3.1 chemistry on an ABI PRISM® 3700 DNA Analyzer. The average Phred Q>20 score is 676 bases, and the passing rate (scores > 100 bases) is 100 %. 72 % of the scores are over 700 bases.

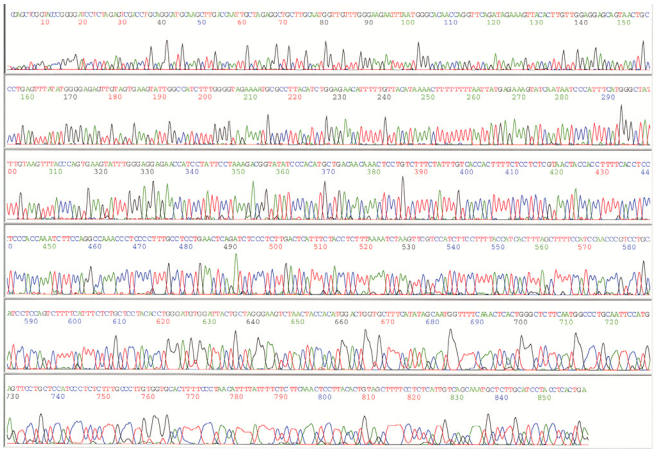

Figure 4: An example of a sequencing trace from this BAC library. The Phred Q>20 score of this electropherogram is 823 bases, and there are zero "N" no calls through 859 bases.

Single Clone

One-half of each of the four plates processed (48 samples for each plate) was sequenced using ABI BigDye™ V 3.1 chemistry on an ABI PRISM® 3700 DNA Analyzer. The average Phred Q>20 score for these samples is 718 bases and the passing rate (scores > 100 bases) is 97.4 %.

Discussion

BACs continue to be an important molecular biology tool in genomic research and isolation of this DNA is critical to a research project. Moreover, the yield of BAC DNA must be sufficient and the purity must be high for optimal performance in downstream applications. The Perfectprep BAC 96 Kit provides excellent yield of high quality BAC DNA in a 96-well format.

This kit is integrated onto the ep*Motion* 5075 VAC system, and the protocol is optimized to give high yields and high quality BAC DNA. Using the Perfectprep BAC 96 Kit, the typical yield of a BAC clone is 0.5 – 1.0 µg. The average Phred Q>20 sequencing score for the 96 different clones from library plate processed in this paper was 676 bases. This system for purification of BAC DNA also provides consistent results across a 96-well plate. To demonstrate this, all wells of four 96-well culture plates containing an aliquot of the same flask-grown single clone were processed with the 5Prime kit on the Eppendorf system. The agarose gel in Figure 3 illustrates that the yield across all wells of a plate are consistent. The average Phred Q>20 score for one half of the each of these four plates was 718 with a passing rate of 100%.

Conclusion

The Perfectprep BAC 96 Kit from 5Prime used on the ep*Motion* 5075 VAC from Eppendorf is a complete system for the isolation of BAC DNA in a 96-well, fully automated format. The process requires approximately 75 minutes and gives consistent results across the plate. The DNA yield is sufficient for several downstream applications, and the DNA purity ensures optimal results from these applications. High throughput genomic sequencing projects as well as studies concentrating on specific genes can benefit from this complete system for the isolation of BAC DNA.

References

Eppendorf
- Manual ep*Motion*® 5075
- Manual ep*Motion*® 5075 with integrated PC and epBlue
- Guidelines for processing the Perfectprep® BAC 96 Kit on the ep*Motion*® 5075 VAC workstation

5Prime
- Perfectprep® BAC 96 Kit Manual on www.5prime.com

Eppendorf Ordering Information

Product	Order no. international	Order no. North America
ep*Motion*® 5075 VAC 120 V (vacuum chamber included)	5075 000.164	960020014
ep*Motion*® 5075 VAC 230 V (vacuum chamber included)	5075 000.016	n/a

5PRIME Ordering Information

Product	Order no.
5Prime Perfectprep® BAC 96, 2 plates	2300300
5Prime Perfectprep® BAC 96, 10 plates	2300310
5Prime Perfectprep® BAC 96 Base Kit - 50 plates	2300320
Collection Plates - 50	2300230
Culture Plates - 50	2300240
For the 5Prime ordering information please visit www.5Prime.com	

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