Applications

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

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Abstract

Eppendorf continues to provide complete systems for biological research. One system involves processing the 5Prime Perfectprep® Plasmid 96 VAC Kit on the Eppendorf ep*Motion*® 5075 VAC. This system allows for the simultaneous, fully automated isolation of DNA from 96-plasmid clones. The average concentration of one 96-clone plasmid library was 168.1 µg/ml and the average Phred Q>20 score of these clones was 645 bases with a passing rate of 91.7 %. Consistent results were obtained when isolating a single high copy plasmid from all 384 wells of four 96-well plates. The average concentration of these 384 samples was 212.7 µg/ml and the average Phred Q>20 score of 192 of these samples was 743 bases with a passing rate of 99.0 %. The "walk away" protocol requires less than 60 minutes and results in highly pure plasmid DNA with sufficient yields for downstream applications such as sequencing, PCR, cloning, transformations, transfections and restriction enzyme analysis.

Introduction

As the genetic codes of more species are sought and as researchers focus on properties of specific genes, genomic DNA sequencing continues to be a fundamental laboratory technique. 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 highthroughput manner on fully automated workstations.

The 5Prime Perfectprep Plasmid 96 VAC Kit and the Eppendorf ep*Motion* 5075 VAC automated pipetting system are integrated to provide a complete system for obtaining highly pure plasmid DNA using a fully automated approach. The kit uses a modified alkaline lysis technology with a vacuum driven protocol to isolate highly pure plasmid DNA. The yield of DNA purified with the kit is sufficient for multiple downstream applications, and the purity of the DNA results in accurate and long sequencing read lengths.

The protocol on the ep*Motion* 5075 VAC is optimized for high yield and highly pure plasmid DNA. The procedure requires less than 60 minutes, and the DNA is ready for immediate use in applications such as sequencing, cloning, PCR, transformations, transfections, and restriction enzyme analysis.

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Materials and Methods

- 5Prime Perfectprep Plasmid 96 VAC Kit
- Eppendorf ep*Motion* 5075 VAC
- 96-clone Plasmid Library, IRAT-1 (Mammalian Gene Collection)
- Single plasmid clone – pSV-ß-galactosidase in TOP10 host cells.

Growth of bacterial culture

96-Clone Library Plate

1.2 ml of LB medium containing 50 µg/ml of ampicillin was aliquoted into each well of a 96-well culture plate. Using a 96-pin replicator, the medium was inoculated from frozen glycerol stocks of a 96-clone plasmid library. The cultures were grown at 37 °C overnight for 18 hours in a shaker at 300 rpm. The plate was centrifuged for 5 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

600 ml of LB medium containing 50 µg/ml ampicillin was inoculated with 300 µl of thawed glycerol stock of TOP10 cells containing the plasmid pSV-ß-galatosidase. The culture was grown overnight (approximately 16 hours) in a 37° C incubator shaking at 325 rpm. Cells were grown to an OD600 reading of 3.0. 1.2 ml of the culture was aliquoted into four 96-well culture plates so that all wells contain equivalent culture medium. The plates were spun at 1900 x g for 5 minutes, and the supernatant was poured off. The plate was blotted to remove residual medium.

Processing

The pelleted cells were processed using the 5Prime Plasmid 96 VAC DB 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. Plasmid DNA was eluted in 70 µl low-concentration TE buffer.

Table 1: Details of the worktable used in the 5Prime Plasmid 96 VAC DB 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	Reagent Reservoirs	6 reservoirs for kit solutions
	Position 1: Solution 1 Position 2: Solution 2 Position 3: Solution 3 Position 4: Binding Buffer Position 5: Diluted Purification 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	32 tips for 96 samples
B3	55 mm Adapter	Height adapter for Filter Plate DB
VACUUM	On frame: Filter Plate A	Filters lysate
	Vacuum Frame 1	Collar for vacuum chamber
	Inside manifold: Filter Plate DB	Binds plasmid DNA
C2	Culture Plate	Contains bacterial pellets
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

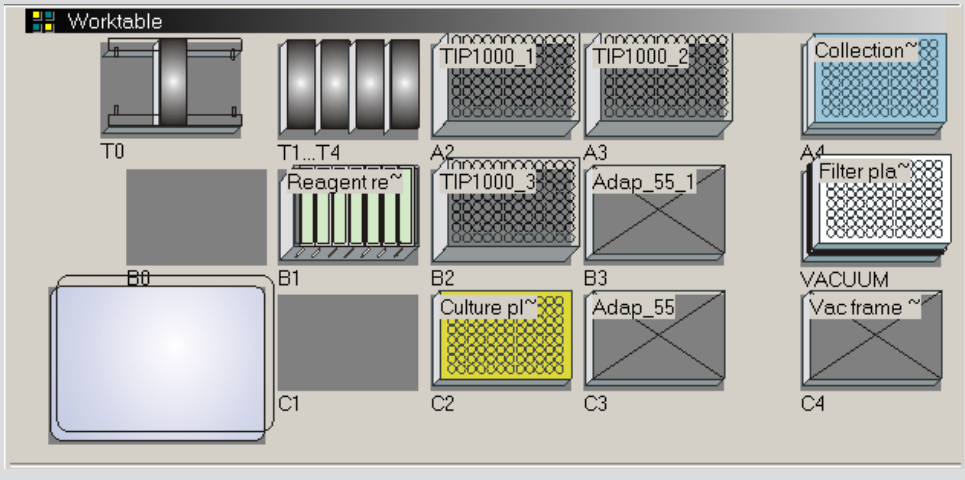

Figure 1: Screenshot from the ep*Motion* Editor showing the setup of the ep*Motion* 5075 VAC worktable for the 5Prime Plasmid 96 VAC DB protocol.

Automated Fluorescent Sequencing

All plasmid DNA samples were sequenced using ABI BigDye™ V 3.1 chemistry on an ABI PRISM® 3700 DNA Analyzer. The cycling conditions and reaction components are listed below.

Cycling conditions	Reaction Components
96 °C 2 minutes	2 µl 5x Reaction buffer
	2 µl 10 µM 496fwd primer
96 °C 10 seconds	2 µl BigDye v 3.1
50 °C 5 seconds	4 µl Plasmid DNA template
60 °C 4 minutes	10 µl MBGW
25 cycles	
	20 µl total reaction volume
10 °C forever hold	

To purify the cycle sequencing products, the samples were incubated in 15 µl of isopropanol for 15 minutes at room temperature and centrifuged for 30 minutes at 1900 x g. The isopropanol was poured off, the samples air dried and resuspended in 15 µl of 0.1x TE prior to loading onto the 3700 DNA Analyzer.

Results

Agarose Gel Analysis

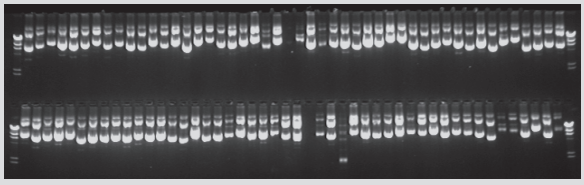

Figure 2: Plasmid DNA from the 96-clone library was isolated using the Perfectprep Plasmid 96 VAC Kit on the ep*Motion* 5075 VAC.
3 µl of each sample were run on a 1% agarose gel and stained with ethidium bromide. The size marker is Lambda DNA digested with Hind III.

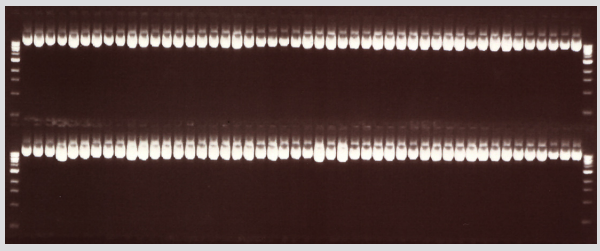

Figure 3: Plasmid DNA samples from a plate containing the same single clone in all 96 wells were isolated using the Perfectprep Plasmid 96 VAC Kit on the ep*Motion* 5075 VAC.
2 µl of each sample were analyzed on a 1% agarose gel. Four plates containing this single clone were processed, and this gel photo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

Table 3: The average concentration and A260/280 ratio of the 96-clone plasmid library plate.

Average Concentration (µg/ml)	Average A260/280 Ratio
168.9	1.93

Automated Fluorescent Sequencing

96-Clone Library Plate:
The plasmid DNA was sequenced using ABI BigDye™ V 3.1 chemistry on an ABI PRISM® 3700 DNA Analyzer.
The average Phred Q>20 score for the entire plasmid library is 645 bases and the passing rate (scores > 100 bases) is 91.7 %. 61 % of the scores are over 700 bases.

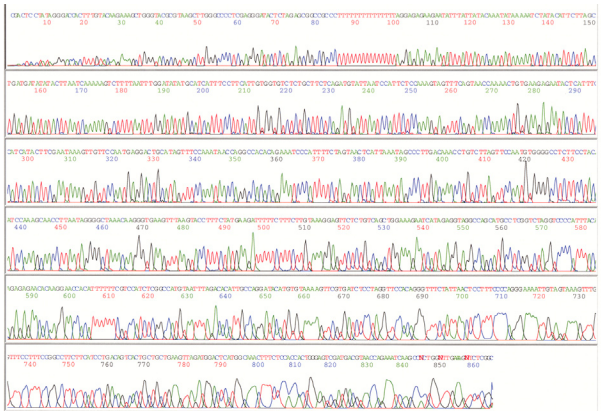

Figure 4: An example of a sequencing trace from this plasmid library. The Phred Q>20 score of this electropherogram is 795 bases and the first "N" no call is at 845 bases.

Single Clone

One-half of each of the four plates (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 743 bases and the passing rate (scores > 100 bases) is 99.0 %. 91.6 % of the passing scores are greater than 700 bases.

Discussion

Plasmids remain an important molecular biology tool used in many fields of biological research and isolation of this DNA is critical to a research project. Also, the yield of plasmid DNA must be sufficient and the purity must be high for optimal performance in downstream applications. The Perfectprep Plasmid 96 VAC Kit provides excellent yield of highly pure plasmid 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 highly pure plasmid DNA, free of genomic DNA, RNA and proteins. The average concentration of one 96-clone plasmid library plate processed with the Perfectprep Plasmid 96 VAC DB Kit on the ep*Motion* 5075 VAC was 168 µg/ml. The average Phred Q>20 sequencing score for this library plate was 645 bases.

This system for purification of plasmid DNA also gives 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 workstation. The average concentrations for each plate are 186.1, 200.3, 208.0 and 256.3 µg/ml and the standard deviations for these four plates are 20.29, 41.42, 31.58, and 48.33, respectively. The average A260/A280 ratios are 1.86, 1.79, 1.80 and 1.74 with standard deviations of 0.05, 0.10, 0.10 and 0.13, respectively, indicating that the DNA is free of proteins.

Conclusion

The Perfectprep Plasmid 96 VAC Kit from 5Prime used on the ep*Motion* 5075 VAC system from Eppendorf is a complete system for the isolation of plasmid DNA in a 96-well, fully automated format. The process requires less than 60 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 plasmid 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® Plasmid 96 VAC 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® Plasmid 96 VAC Kit, 2 plates	2300200
5Prime Perfectprep® Plasmid 96 VAC Kit, 10 plates	2300310
5Prime Perfectprep® Plasmid 96 VAC 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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