Increasing throughput and quality of BAC-end sequencing by Automation of Montáge™ BAC$_{96}$ Miniprep technology on the Qiagen™ BioRobot® 9600

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ABSTRACT

Bacterial Artificial Chromosome (BAC) libraries are critical tools for physical mapping and the finishing process of genome sequencing. In BAC-end sequencing, the ends of thousands of BAC clones are sequenced and the data is assembled along with a number of other markers to generate a scaffold of the genome. BAC-ends are also useful for filling gaps and bridging contigs in the final stage of assembly and completion of the genome sequence. The ability to generate sufficient quantity and quality of BAC DNA for end-sequencing are the two biggest challenges in the process. In addition, genome sequencing facilities are looking for automated solutions in order to increase the throughput and speed of genome sequencing.

In this abstract we describe the automation of the Montáge™ BAC$_{96}$ miniprep kit from Millipore on the Qiagen™ BioRobot® 9600. The entire process except for an initial centrifugation step takes place on the instrument deck. The protocol was optimized to produce sufficient yield and quality of DNA for end-sequencing reactions. In comparison to other protocols, the Montáge kit shows improvements in overall sequence success rates, well-to-well reproducibility, and high Phred20 quality scores. The Montáge process is also faster than existing methods and a simpler protocol allows for easier automation.

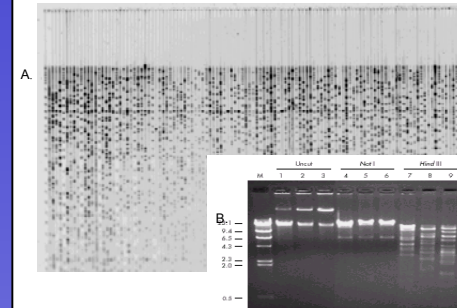## Montáge BAC$_{96}$ Miniprep Kit from Millipore

Kit components: BAC plate, Lysate Clearing plate, deep well block for cell culture, all buffers for alkaline lysis, washing and resuspending BAC DNA, and an optimized BAC sequencing protocol.

Millipore vacuum manifold integrated onto the Qiagen 9600 BioRobot Deck

Introduction

"BAC end sequence"

A BAC typically has an insert of about 100-150 kb with a vector size of about 10-15 kb. BAC-ends are short sequences of the BAC insert attached to vector sequence. They can be sequenced using standard PCR primers having sites in the 5'- and 3'-prime ends of vector sequence. Resulting sequences are 200-700 bp long and may be used for genome assembly, chromosome walking, ordering of existing contigs, and genome comparison by BLASTN and TBLASTX analyses.

One of the challenging but exciting uses of BAC-end sequences is the comparison of mammalian genomes which has recently become possible due to the near completion of the human genome sequencing project. Thousands of BAC-ends obtained from different mammalian libraries can be BLAST-analyzed against the human genome. Ends with significant hits can be used for direct genome-to-genome comparison and establishment of new genome markers. Information about conservative regions between genomes obtained from physical and RH mapping can be used for prediction of chromosome localization through BLAST-analyzed BAC-ends and BAC clones, containing these ends.

To perform large-scale cattle to human genome comparison we are using cattle BAC library CHORI 240. We are going to sequence about 60 000 BAC-ends and make a BLAST analysis against the human genome draft.

Comparison of Manual Methods

BAC$_{96}$ miniprep protocol on the Qiagen™ BioRobot® 9600

Resuspension
    100 µL/well of Solution 1 (1:15 min)
    shake 8:00 min at 800 rpm
Lysis
    100 µL/well of Solution 2 (1:15 min)
    shake 1:00 min at 550 rpm
    Incubate <5:00 min
Neutralization
    100 µL/well of Solution 3 (1:15 min)
    shake 2:00 at 700 rpm
Sample Transfer
    450 µL/well transferred to clearing plate (35:00 min)
    vacuum 12:00 min at 785 mbar (8 in Hg)
    discard clearing plate
    move BAC plate to manifold top
    vacuum 15:00 min at 785 mbar (8 in Hg)
Wash with Solution 4
    400 µL/well of Solution 4 (2:30 min)
    vacuum 5:00 min at 300 mbar (24 in Hg)
Resuspend DNA
    40 µL/well of Solution 5 (1:15 min)
    shake 10:00 min at 800 rpm

Comparison of quantity and quality of manually extracted BAC DNA using Competitor X and Millipore Montáge kits

BAC DNA purified using two competitive methods separated on an agarose gel. Lanes 1-4 from Company X kit. Lanes 5-8 from Millipore's Montáge kit.

DNA extracted with Montáge kit on the Qiagen BioRobot 9600

BAC DNA purified using Montáge BAC$_{96}$ kit on the Qiagen BioRobot 9600.

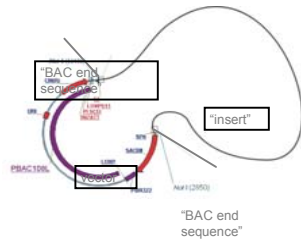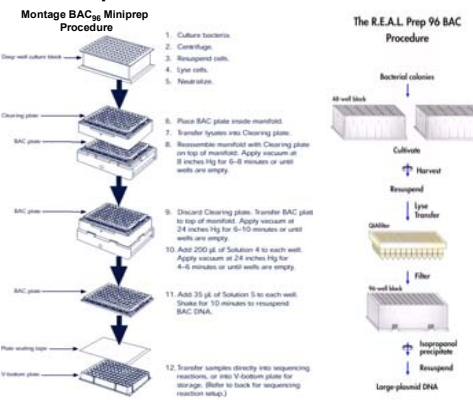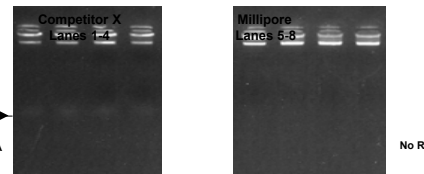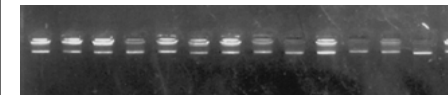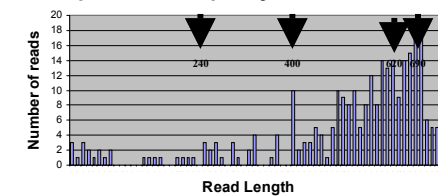## BAC DNA extracted with Montáge is suitable for fingerprinting

A. Fingerprinting: 96 BAC DNA samples prepared with Montage BAC$_{96}$ kit on the Qiagen BioRobot 9600 were digested with *Hind*III and electrophoresed on a PFGE.
B. Agarose gel electrophoresis of a sample of 3 BAC DNA samples that were left uncut (lanes 1-3), digested with *Not*I (lanes 4-6) or with *Hind*III (lanes 7-9)

Electropherogram of BAC end Sequence

Sequence read quality distribution

• PHRED20 Sequence Read Distribution of BAC DNA templates.
• Average Pass Rate was 92% for BAC end-sequences generated with the Montage BAC96 kit, which is 10% greater than the pass rate for the same BAC clones processed with Company X's kit. Data shown is for N = 288 samples.Pass Rate is defined as the fraction of BAC clones processed from cell culture and generating a PHRED20 read greater than 250 bp.

Process Comparison

Parameters of Comparison	Montage BAC 96 Miniprep Kit	TIGR Protocol
Process time for 2 plates	1 hour 40 min	2 hours 20 min
Number of manual interventions	1	2
Ease of automation	good	good
Sensitivity to small variations in the process	somewhat	less
Average DNA yield	2.5 µg	2.0 µg
Consistency of DNA yield	uniform	variable
RNA Contamination	no	yes
Average sequence quality (phred 20 bases)	510	430
Average success rate/plate	80%	75%

CONCLUSIONS

• The new Montage BAC$_{96}$ miniprep kit from Millipore is uniquely designed for BACs.

• The Montage kit is readily adaptable to the Qiagen 9600 BioRobot with:

    • Faster processing time

    • Less manual intervention

• The BAC DNA generated with the Montage BAC$_{96}$ kit provides

    • Significantly better sequence reads and greater yields than with the tested competitor

    • Suitable template for fingerprinting

Information

Please Visit the Millipore Booth (#800) for ordering information on the Montage BAC$_{96}$ Miniprep kit

For a copy of this poster, please drop your business card or mailing information below or request it at the Millipore booth