Evaluation of High-Throughput BAC DNA Isolation Using the Aurum™ Plasmid 96 Kit

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Introduction

Isolation of bacterial artificial chromosome (BAC) DNA has become a routine procedure in genomic projects of various scales, such as map-based cloning of a gene of interest, construction of physical maps of individual chromosomes, and whole genome sequencing. Any of these projects could be significantly compromised by insufficient throughput.

High-throughput preparation of BAC DNA in 96- and 384-well formats and amenability of the process to automation have significantly accelerated large-genome sequencing projects for various organisms and have been used extensively for filling unsequenced gaps in large eukaryotic genomes. Another application where high-quality BAC DNA is needed is high-throughput fingerprinting of BACs for developing physical maps of chromosomes and contigs of entire genomes with overlapping BACs. Fingerprint maps have been generated for various complex genomes (Yu et al. 2002, Lander et al. 2001). Reliable preparations of high-quality BAC DNA are also crucial for generating genome scaffolds by sequencing from mapped BAC ends (that is, from sequences adjacent to the BAC vector cloning site).

This article presents data on high-throughput BAC DNA preparation using the Bio-Rad Aurum plasmid 96 kit. This kit has been shown to be very effective for regular plasmid DNA isolation (Day et al. 2002). To make the kit amenable to large-scale fingerprinting and sequencing projects, the experimental conditions were optimized for high-throughput isolation of BAC DNA in the 96-well format.

Methods

Tammar wallaby BACs were kindly donated by J Graves (Australian National University, Canberra). BACs in *Escherichia coli* cultures were taken from –80°C glycerol stocks. Starter cultures were prepared in 96-well blocks (1 ml of 2x YT medium per well) and grown for 7 hr at 37°C. Various culture volumes were tested to optimize the existing protocol for BAC isolation. The optimal protocol was as follows: 10 µl of each starter culture was used to inoculate 3 ml of 2x YT medium in 48-well plastic growth blocks. Clones were grown for at least 20 hr at 250 rpm, then DNA extracted from them using the Aurum plasmid 96 kit. Random clones were checked on agarose gels. Prepared BACs were then subjected to fingerprinting or end sequencing.

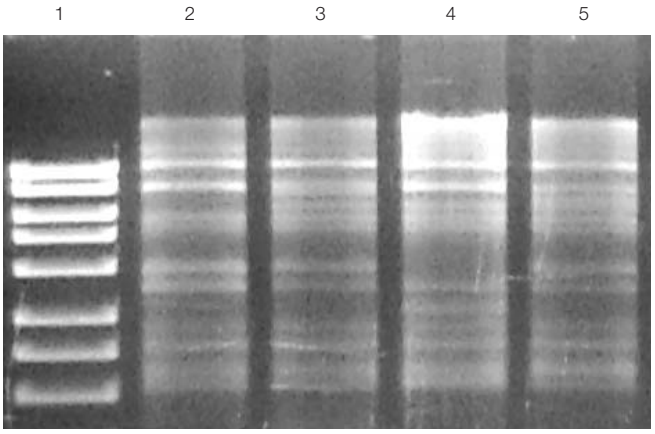

Fig. 1. Restriction digestion of four BAC clones with *Xba*I endonuclease (lanes 2–5). Half of the sample isolated from 3.0 ml was digested for 3 hr at 37°C and separated on a 1% agarose gel. The kit provided consistent amounts of BAC DNA in the range 0.7–1.2 mg. Lane 1 contained 1 kb ladder.

For cycle sequencing, half of each prep was used along with 5 pmol of either T7 or M13R sequencing primer and 3–4 µl of BigDye Terminator 3.1 sequencing mix (Applied Biosystems, Foster City, CA) in a total volume of 16 µl. Dilution buffer was not used. The cycling conditions were as follows: initial denaturation step at 96°C for 4 min followed by 100 cycles of 96°C for 10 sec, 50°C for 10 sec, and 60°C for 4 min. Extended products were separated on the ABI PRISM 3730xl DNA analyzer (Applied Biosystems) under standard conditions.

For BAC fingerprinting, a recently developed protocol was applied (Luo et al. 2003). This protocol uses the SNaPshot kit (Applied Biosystems) and five restriction endonucleases to generate a large number of small restriction fragments derived from BAC clones labeled with four different fluorescent dideoxynucleotides supplied in the kit.

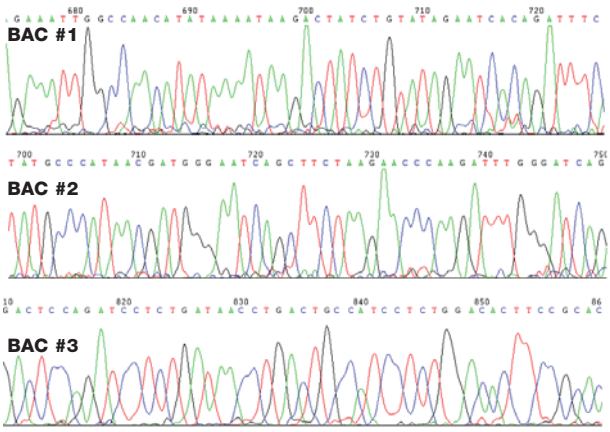

Fig. 2. Sequencing of three randomly chosen BACs using T7 primer. At least 550 and up to 800–900 bases with quality scores ≥20 were produced for each sample.

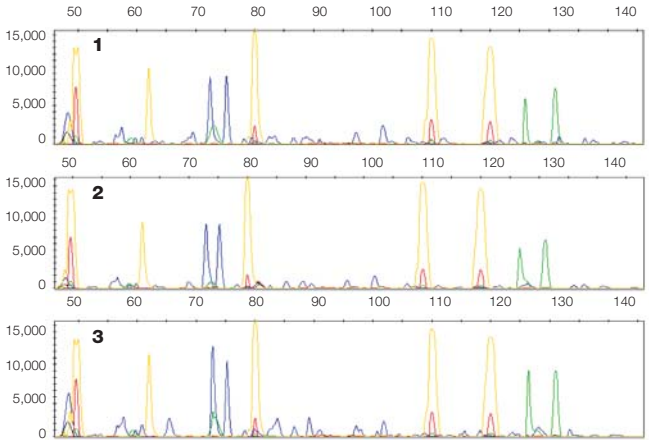

Fig. 3. Reproducibility of fingerprinting profiles generated for three independent preparations of the same BAC clone. Profiles were produced by labeling digested BACs using the four-colored SNaPshot kit followed by analysis with GeneMapper software (Applied Biosystems). The x-axis represents the sizes of fingerprint fragments in nucleotides; the y-axis represents intensities in relative fluorescent units. Yellow peaks are a GeneScan-120 LIZ size standard (Applied Biosystems). Note that in the window shown, only one fragment (~48 bases, black peak) in each profile was detected using dTAMRA dye due to the low guanosine content of this BAC (guanosine is a target for dTAMRA-labeled ddCTP in the SNaPshot kit).

Results

Protocol Optimization

For BAC preparation, a few minor modifications were made to the existing Aurum plasmid 96 protocol. First, the volume of *E. coli* cultures was increased to 3 ml, since BACs are low copy number molecules (1–2 per bacterial cell), and the yield of BACs is usually significantly lower than that of conventional cloning plasmids. After adding the neutralization solution (potassium acetate), the blocks were inverted 20 times and incubated for 10 min on ice to ensure proper separation of circular BAC DNA from the *E. coli* chromosomal DNA and insoluble cell compartments. The elution solution for the last step was warmed to 65°C to increase the efficiency of recovery of BAC DNA from the column. The yield of BAC DNA using this optimized protocol was ~2 µg per prep.

BAC Quality

Isolated BACs were assessed for quality by their performance in downstream applications. Yield of the BAC preps across the 96-well plate was analyzed by endonuclease digestion of several random clones (Figure 1). Typical sequencing results are shown in Figure 2; in total, 72 BAC ends were sequenced. The reproducibility of BAC digestion followed by four-color fluorescent labeling of digestion products is shown in Figure 3. All peaks, including overlapping fragments of similar sizes and different colors, were detected with 95% reproducibility.

Conclusions

The Aurum plasmid 96 protocol reliably allows high-throughput isolation of BAC clones of consistently high quality throughout a 96-well plate. When the culture volume was increased to 3 ml (from 1.0–1.3 ml recommended for plasmids) and the protocol slightly modified, ~2 µg per prep of BAC DNA was obtained. This is enough for sequencing from both ends of the clone.

Alternatively, a single prep is sufficient for fingerprinting of the BAC and developing reliable physical contigs of a genomic region of interest.

Acknowledgements

I thank Dr M Luo, Wheat Genomics Group, University of California, Davis, for providing the BAC fingerprinting protocol.

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