High-throughput purification of BACs with the new R.E.A.L.™ Prep 96 Protocol

Large plasmid constructs such as BACs, PACs, and P1s can now be purified on a high-throughput level using the optimized protocol provided in R.E.A.L.™ Prep 96 Plasmid and BioRobot™ Kits.

BAC purification with R.E.A.L. Prep 96 offers:

- A fast and efficient high-throughput procedure
- BAC DNA for sequencing and screening applications
- Multiwell format from culture to DNA
- Manual processing or automated purification on the BioRobot 96OO

Fast, high-throughput procedure

The Rapid Extraction Alkaline Lysis (R.E.A.L.) procedure allows up to 96 samples to be processed in parallel in just 60–75 minutes. Cultures grown in multiwell blocks are harvested and lysed using a modified alkaline lysis procedure (see flow chart). Denatured and precipitated proteins and carbohydrates are efficiently removed by vacuum filtration through the QIAfilter™ 96 plate. DNA in the lysates is then concentrated by isopropanol precipitation.

The procedure functions optimally when the same vector–host combination is used for all samples in a given block, creating standardized conditions. Since proper cultivation of BAC clones is a crucial factor for high-throughput BAC purification, the protocol for the R.E.A.L. procedure includes detailed recommendations for culturing large-plasmid–containing bacterial clones.

BAC DNA for sequencing and screening

Up to 800 ng large-construct DNA can be obtained from 2.5 ml bacterial culture grown in two 48-well blocks using the R.E.A.L. Prep 96 BAC purification procedure (Figure 1). The purified DNA is suitable for use in high-throughput applications such as restriction digestion (Figure 2) and BAC end sequencing (sequencing using the BAC insert proximal to the vector junction using priming sites within the vector, Figure 3). A detailed protocol for sequencing the purified BAC DNA is provided, along with useful hints for optimal cultivation of BAC clones. ▶

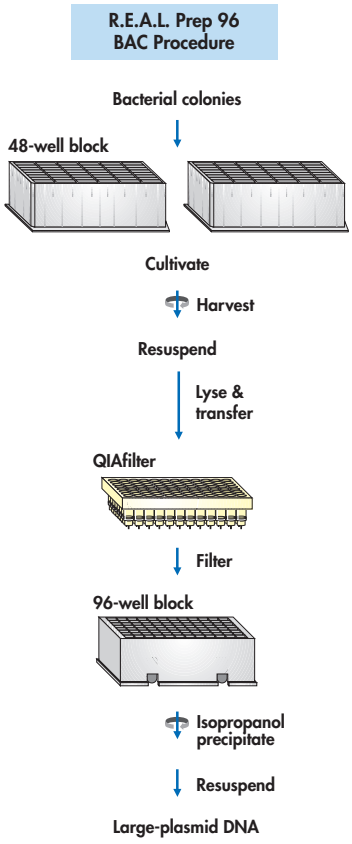

Figure 1 *High-throughput BAC plasmid isolation using R.E.A.L. Prep 96. The procedure can be performed manually or automated on the BioRobot 96OO.*

Complete Restriction Digestion of BAC Clones

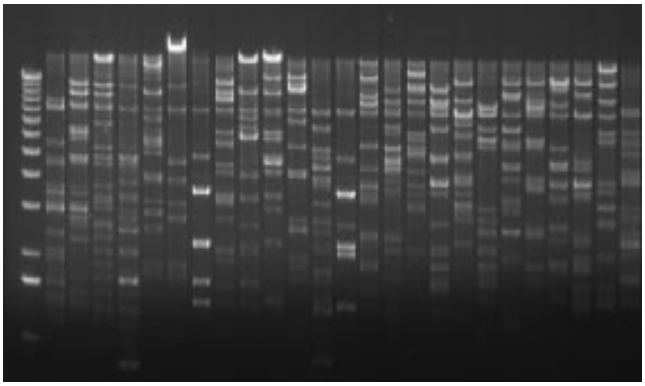

Figure 2 *Different BAC, P1, and PAC clones were purified from various libraries using the R.E.A.L. Prep 96 BAC BioRobot Protocol on the BioRobot 96OO. 7 µl of each sample were digested with EcoRI and the restriction digests were analyzed by agarose gel electrophoresis. Enzymatic reaction setup and gel loading were performed by the BioRobot 96OO. Marker (left lane): 1-kb ladder.*

Manual or automated processing

Large-plasmid purification with the R.E.A.L. Prep 96 BAC procedure is performed either manually using the QIAvac 96 manifold, or for increased productivity and efficiency, using the automated procedure on the BioRobot 96OO. The BioRobot 96OO multi-purpose workstation provides ready-to-run protocols including the R.E.A.L. BAC High-Speed Process, easy-to-use software, and fast, accurate liquid handling. It can also be used in subsequent applications with the purified BAC DNA, including set up of sequencing, restriction digestion, and amplification reactions, as well as analytical gel loading. Please call QIAGEN for more information on these applications. ■

Reliable BAC End Sequencing

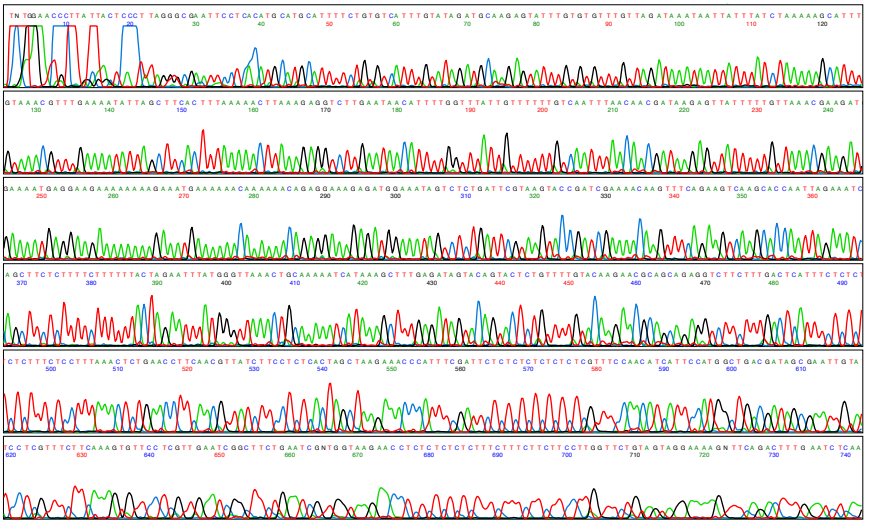

Figure 3 *BAC end sequence of an* Arabidopsis thaliana *BAC clone purified with the R.E.A.L. Prep 96 Plasmid Kit using the new BAC purification protocol and sequenced according to the sequencing protocol provided. The sequence was generated using the protocol in the handbook, universal primer, and BigDye™ terminator chemistry and analyzed on an ABI PRISM® 377XL Sequencer with a 36-cm well-to-read gel.*

Ordering Information

Product	Contents	Cat. No.
R.E.A.L. Prep 96 Plasmid Kit (4)*†	For 4 x 96 rapid extraction alkaline lysis minipreps: 4 QIAfilter 96 Plates, Square-Well Blocks, Tape Pads, Reagents, Buffers	26171
R.E.A.L. Prep 96 Plasmid Kit (24)*†	For 24 x 96 rapid extraction alkaline lysis minipreps: 24 QIAfilter 96 Plates, Square-Well Blocks, Tape Pads, Reagents, Buffers	26173
R.E.A.L. Prep 96 BioRobot Kit (4)†	For 4 x 96 automated rapid extraction alkaline lysis minipreps: 4 QIAfilter 96 Plates, Flat-Bottom Blocks, Square-Well Blocks, Reagents, Buffers, Tape Pads	961141
Accessories		
QIAvac 96	Vacuum manifold for processing QIAGEN 96-well plates,	19504
48-Well Blocks (24)	48-well blocks with 5-ml wells, 24 blocks per case	19577

**Requires use of QIAvac 96*

† BAC purification using the R.E.A.L. Prep 96 procedure requires the use of 48-well blocks to provide optimal cultivation conditions for the BAC clones. 48-well blocks are not included in R.E.A.L. Prep 96 Plasmid or BioRobot Kits and must be purchased separately.