illustra AutoSeq G-50 and AutoSeq 96 G-50

Introduction

illustra™ AutoSeq™ G-50 and the new illustra AutoSeq 96 G-50 products provide a convenient and simple solution for dye terminator removal from sequencing samples prior to analysis. illustra AutoSeq G-50 is useful for handling small numbers of samples and illustra AutoSeq 96 G-50 is designed for parallel processing of up to 96 samples (Fig 1). We introduce illustra AutoSeq 96 G-50 for use with MegaBACE™ and illustra AutoSeq 96 G-50 for use with Applied Biosystems Inc., (ABI) sequence analyzers, respectively.

Purification of large numbers of sequencing reactions using single columns is time consuming, expensive, and tedious. In addition, ethanol precipitation—which is the standard laboratory method for dye terminator removal—is inconsistent with regards to sample recovery and purity. illustra AutoSeq G-50 and AutoSeq 96 G-50 provide faster, more consistent results than ethanol precipitation, with greater ease-of-use for multiple samples.

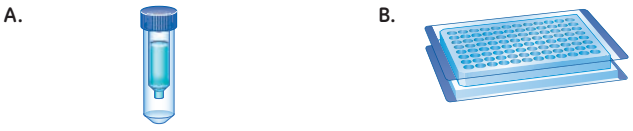

Fig 1. (A) illustra AutoSeq G-50 column (B) illustra AutoSeq 96 G-50 plate

illustra AutoSeq G-50 and AutoSeq 96 G-50 deliver:

- **Speed:** Pre-equilibrated columns and 96-well plates allow you to go from sample application to collection of purified DNA product in less than 4 min for the column and 15 min for the 96-well plate
- **Simplicity:** Quick reference protocol card provides instructions at a glance for experienced users
- **High-quality DNA:** Provides equivalent Phred 20 read length results as compared with DyeEx™ 2.0 Spin Kit from Qiagen™ and Edge Biosystems' Performa™ V3 DTR 96-Well Short Plate

Method overview

The use of relatively small volumes (5 or 10 µl) in sequencing reactions is becoming increasingly popular with researchers who perform high-throughput sequencing on ABI analyzers because of reduced costs while maintaining good signal strength and Phred 20 read length. illustra AutoSeq products contain a pre-equilibrated and pre-weighed amount of Sephadex™ G-50 DNA media for optimal sequencing reaction purification. The purification process involves gel filtration and centrifugation to separate sequencing reaction products from excess dye terminators. The exclusion properties that allow the sequencing product to pass through the gel whilst retaining the smaller dye terminator and salt impurities depend on factors such as the type of resin used, sample volume, product size, and the *g* forces employed in the purification process. The protocols provided with the illustra AutoSeq products have been optimized for the purification of dye terminator sequencing reactions (Fig 2).

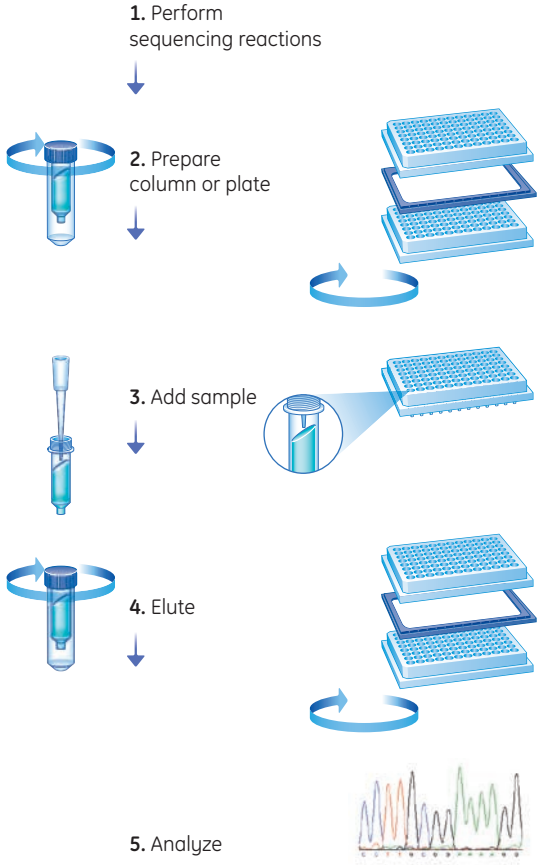

Fig 2. Schematic representation of the method employed by illustra AutoSeq G-50 dye terminator removal products.

High-quality sequencing analysis

Phred quality (q) values express the probability of correctly calling a base-peak in a sequencing electropherogram and they are based on key parameters such as resolution, spectral cross talk, and uniformity of peak spacing (1–3). The q value is logarithmically proportional to the probability of correctly calling the identified peak (i.e., a Phred value of 20 is an accuracy of 99%, Phred 30 is 99.9% accurate). The minimal acceptable value for sequencing is a Phred value of 20. For this study, we defined sequencing read length as the total number of bases with a Phred value of 20 or greater (q > 20) using a 10-base moving window average.

Tests using 20 µl reaction volumes showed no significant differences in Phred 20 read length obtained with the Qiagen DyeEx 2.0 Spin Kit and the illustra AutoSeq G-50 dye terminator removal kit (Fig 3) indicating that the illustra AutoSeq G-50 dye terminator removal kit can be used to generate high-quality sequencing results.

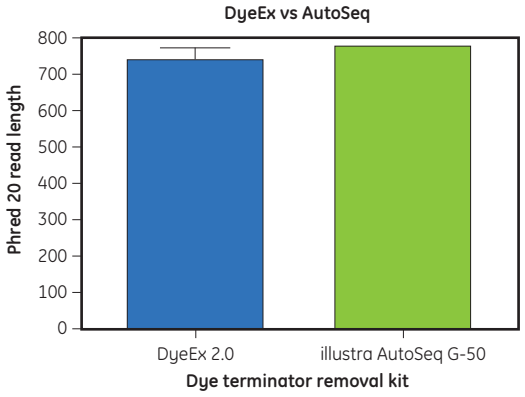

Fig 3. Phred 20 read length obtained using DyeEx 2.0 and AutoSeq G-50 kits (p = 0.2061). A bulk stock of sequencing reactions with BigDye™ Terminator v3.1 Cycle Sequencing Kit was prepared using a pUC-based plasmid containing the TP53 open reading frame (Tumor protein p53) as template and M13-forward or -reverse sequencing primers. Aliquots from this bulk stock were purified. Three sequencing reactions using the forward primer and three reactions using the reverse primer were purified by 3 different researchers. Purified sequencing samples were analyzed by the Qiagen Sequencing Service for analysis using an ABI PRISM™ 3730XL DNA analyzer.

We checked for data accuracy at the beginning of the sequence by examining electropherograms for the absence of dye blobs and noting the read length at which the Phred score reached ≥ 20. Dye blobs were absent from all the samples tested and there was no significant difference in the read length at which the Phred score reached 20 or more (p = 0.6579) between illustra AutoSeq-G50 and DyeEx 2.0 columns. A typical electropherogram image depicting the quality and accuracy of data generated with the illustra AutoSeq-G50 Kit is shown in (Fig 4).

High-throughput sequencing analysis

Test reactions using both 5 and 10 µl sequencing reaction volumes and 1 µl of dye terminator mix were performed. There was no significant difference in the Phred 20 read lengths for reactions purified with the illustra AutoSeq 96 G-50, Qiagen's DyeEx 96 Kit, and Edge Biosystems' Performa product (Fig 5).

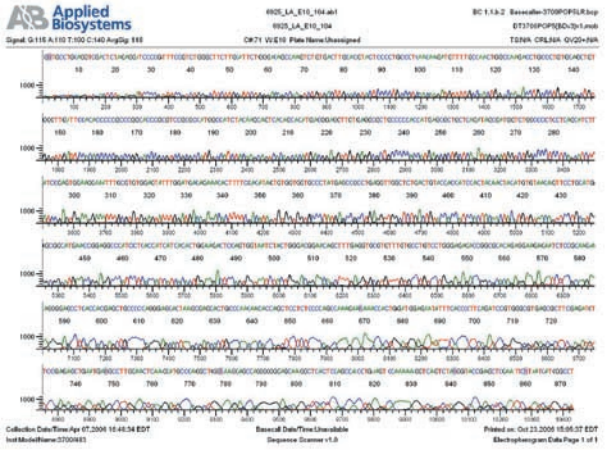

Fig 4. Typical electropherogram of sequencing reactions purified using illustra AutoSeq G-50.

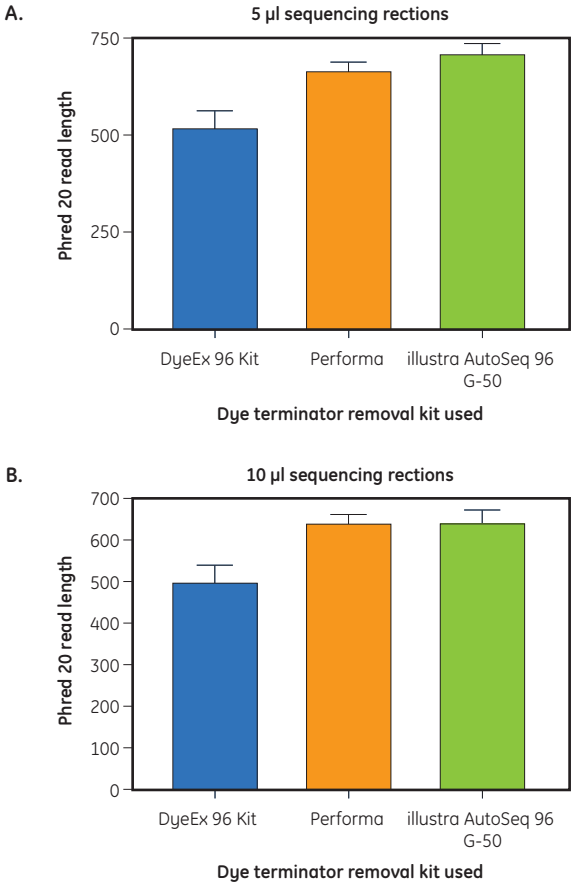

Fig 5. Phred 20 read length obtained from different reaction volumes (p > 0.05). A bulk stock of sequencing reactions was prepared with ABI's BigDye Terminator v3.1 Cycle Sequencing Kit using a pUC-based plasmid containing the Tumor protein p53 open reading frame (ORF) as template and M13-forward or -reverse sequencing primers. Aliquots from this bulk stock were purified. Purified sequencing samples were analyzed by the Qiagen Sequencing Service for analysis on an ABI 3730XL. To test for well-to-well variability, 6 samples of forward primers and another 6 samples of reverse primers were purified with the 96-well purification kits.

Summary

illustra AutoSeq G-50 and illustra AutoSeq 96 G-50 kits are designed for the rapid removal of unincorporated dye terminators from sequencing reactions. The purified DNA product routinely generates high-quality sequencing results. We now provide two new AutoSeq 96-well format kits for use on MegaBACE and ABI sequence analyzers, respectively. The versatility of the illustra AutoSeq product range enables you to take advantage of using small volumes of reagents for sequencing reactions without compromising sequence quality.

References:

1. Ewing, B. and Green, P. Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Res.* **8**, 186-194 (1998).
2. Ewing, B *et al*. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Res.* **8**, 175-185 (1998).
3. Paegel, B. M. *et al*. High throughput DNA sequencing with a microfabricated 96-lane capillary array electrophoresis bioprocessor. *Proc. Natl. Acad. Sci. U S A.* **99**, 574-579. (2002).

Ordering information

illustra AutoSeq G-50 (50 columns)	27-5340-01
illustra AutoSeq G-50 (250 columns)	27-5340-02
illustra AutoSeq G-50 (1000 columns)	27-5340-03
illustra AutoSeq 96 G-50 for MegaBACE Analyzers (10 × 96-well plates)	28-9094-90
illustra AutoSeq 96 G-50 for ABI Analyzers (10 × 96-well plates)	28-9034-27

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