Use of ADS™ Sequencing Reaction Cleaning Beads for Sanger Sequencing

ABSTRACT

We compared different sequencing dye removal methods for cleaning up sequencing reactions. Our results indicate that ADS™ Sequencing Reaction Cleaning Beads provide uniform signal intensity for both small and long extension products. Spin column and ethanol precipitation, on the other hand, tend to lose small extension products and therefore generate weak signals closer to the primer. Compared to ethanol precipitation, use of ADS™ Sequencing Reaction Beads saves cleaning time significantly. Overall, ADS Sequencing Reaction Cleaning Beads are cost-effective, easy to use, fast to operate, and easy to adapt for high-throughput sequencing reaction cleaning workflow.

INTRODUCTION

Sanger sequencing is the most commonly used and cost-effective method for the identification and confirmation of DNA mutations in small DNA regions and in low-throughput settings. A high-quality template and dye chemistry are certainly important for the success of the sequencing reaction and to generate extension products. Purification of the sequencing reaction is also important to remove interference contaminants, especially of unincorporated ddNTP dye terminators. The unremoved dye terminators can form dye blobs to block normal sequence reading and jeopardize sequencing quality.

Several commercial kits are available for removing the dye terminators but they are either expensive or time consuming, especially for preparation of multiple samples. While sequencing cost continues to drop to meet the market needs, it is critical to lower the sequencing cost from all steps and still maintain sequencing quality.

We have developed and optimized ADS™ Sequencing Reaction Cleaning Beads that are cost-effective and time-saving. Our bead costs a fraction of what the regular commercial kits cost. Our beads reduce wash steps and take only 20 minutes to complete the purification steps. Considering sequencing reaction purification of 96 well samples for 3730xl Genetic analyzer, the time-saving benefit compared to spin columns is significant.

MATERIAL AND METHODS

Sequencing Reaction: Cycle sequencing was performed in 10 µl volume (4.8 µl H2O, 0.4 µl BD3, 2 µl DNA, 1 µl primer, 1.8 µl 5x buffer) in ABI GeneAmp PCR System 9700. The cycling protocol was 95°C for 1 min, followed by 30 cycles of 95°C for 10 sec, 50°C for 5 sec, and 60°C for 4 min. The final step was 4°C hold.

Reagents for Sanger Sequencing Dye Removal: ADS™ Sequencing Reaction Cleaning Beads (ADS Beads) are from AdvancedSeq. All the other dye removal kits or PCR purification kits are purchased from different vendors and will be named as “vendor X kit” in this application note.

Purification of sequencing reactions was performed following protocols from the manufacturers. The ethanol purification protocols using ethanol with EDTA and ethanol with EDTA and NaAC were from DNA Sequencing by Capillary Electrophoresis, Applied Systems Chemistry Guide (Third Edition). The product from the 10 µl sequencing reaction using ADS beads was eluted in 50 µl ADS elution buffer, and the product from the 10 µl sequencing reaction using other purification methods was eluted in 25 µl ADS elution buffer. The purified products wereloaded into ABI PRISM 3130X/Genetic Analyzer to generate DNA sequence data. Sequence data were analyzed using ABI sequence analysis software v5.4.

RESULTS AND DISCUSSION

Sequence Data Comparison

First, we compared the sequencing performance after dye terminator purification using ADS Beads, two methods of ethanol precipitation, and Vendor Q spin column purification (Figure 1). The sequencing raw datashows that reaction cleaned with ADS beads generates higher signal intensity (at least for the first 150 base reading) compared with results from other purification methods. It is also obvious that the initial readable bases (the first 45) have much higher signals from ADS Beads purification compared to other methods. This result indicates that ethanol precipitation and spin column purification have lost some smaller extension products.

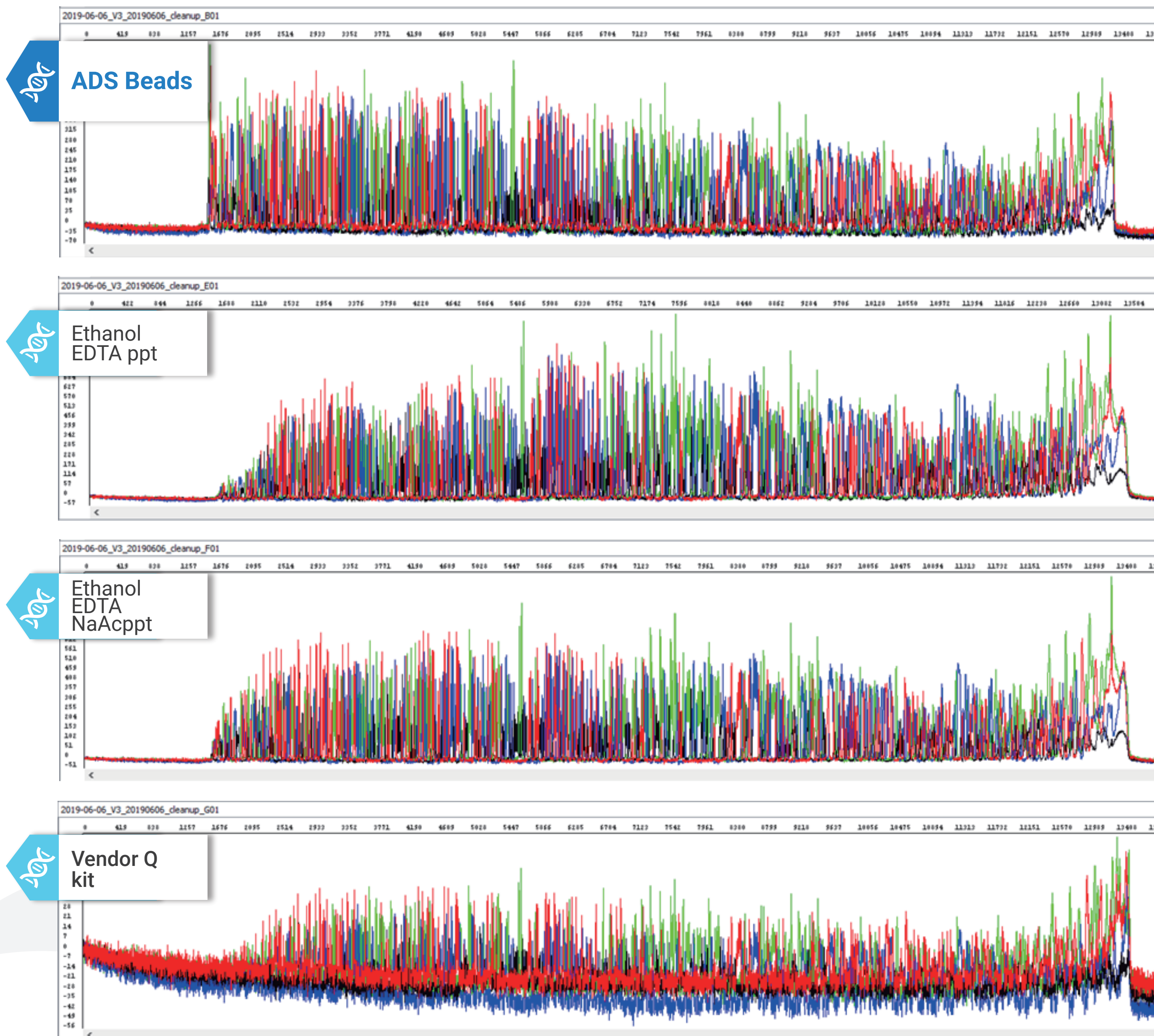

Figure 1. Sequencing raw data comparison for the same sequencing reaction purified by ADS Beads, two methods of ethanol purification, and Vendor Q kit spin column purification.

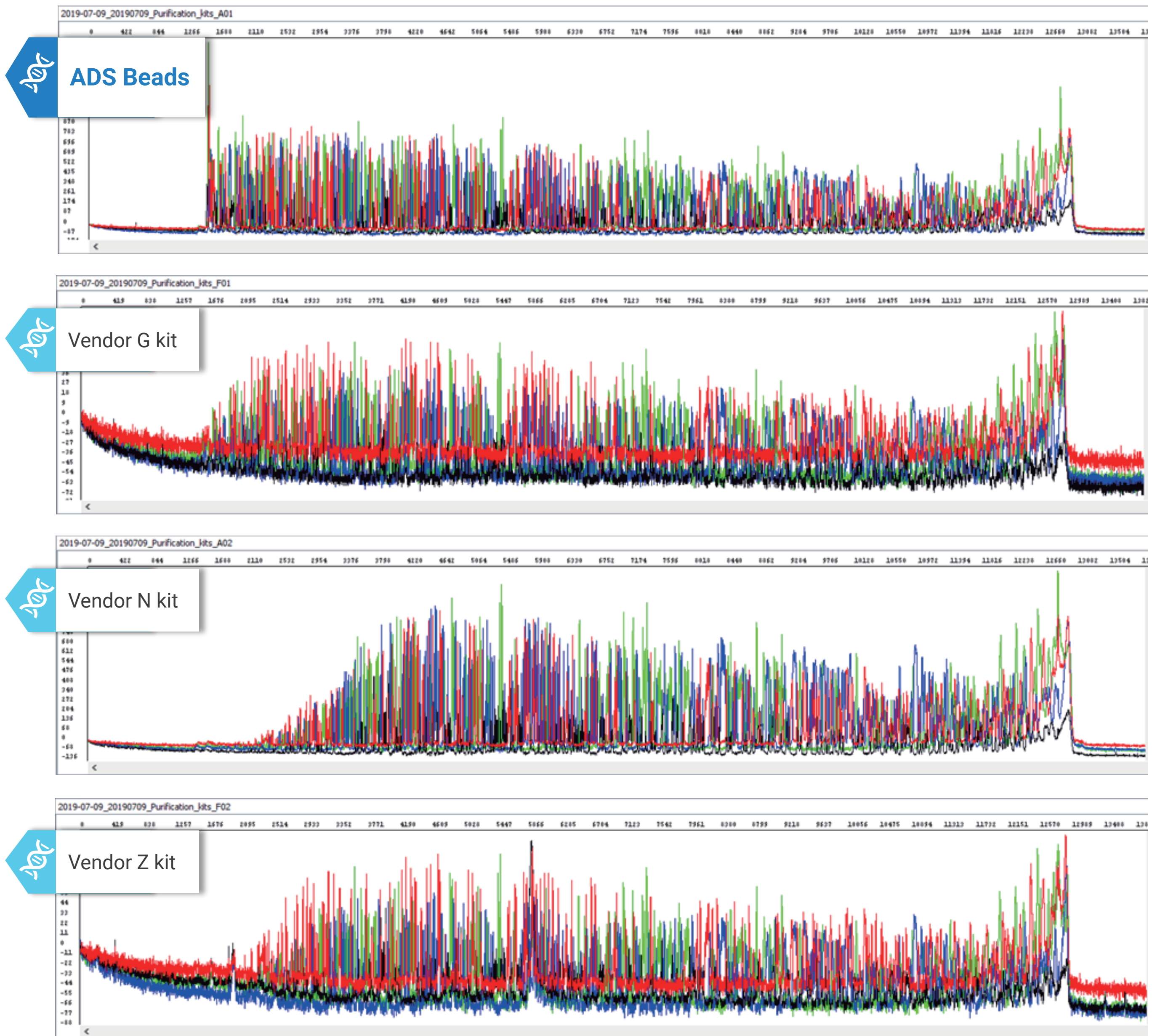

Figure 2. Sequencing raw data comparison for the same sequencing reaction purified by ADS Beads, a dye removal spin column kit from Vendor G, and other two DNA spin column purification kits from vendors N and Z.

When the sequencing performance for reactions purified by ADS Sequencing Reaction Cleaning Beads were compared with a dye removal spin column Vendor G kit, and two other DNA purification kits from Vendor N and Z, similar results as shown in Figure 1 were observed. These results suggested that all the spin column purification methods were losing smaller extension products and have overall lower signal intensity. This was trueeven for the Vendor G kit, which is specifically used for dye terminator removal.

We have found the above DNA purification spin columns, even those indicated as products to purify fluorescent labeled DNA fragments in their product description, are not good choices for sequencing reaction cleaning. On the other hand, some of the products we tested were good for sequencing reaction cleaning, but did not have good PCR product recovery (data not shown). Therefore, the two different cleaning sequencing steps, which focus on different needs, require different cleaning products, although the possibility of a single product being optimized for both purposes cannot be ruled out.

Sequence Analysis Report

A summary of thesequence analysis report generated using Sequencing Analysis v.5.4 softwareon the sequencing results shown in Figures 1 and 2 is depicted in Tables 1 and 2. The ADS beads gave the best value of all the methods tested in terms of the length of read (LOR), sample score and quality value (QV). Although the two ethanol precipitation methods gave good LOR, sample score and QV, it took100 minutes to purify the cycle sequencing products using these methods. In contrast, it took20 minutes to clean the sequencing product using ADS Sequencing Reaction Cleaning beads and 10-15 minutes to clean the sequencing products using spin columns. Although spin columns are easy and fast, they are more expensive and they lose smaller extension products. In addition, for high-throughput purification, spin columns are also cumbersome while magnetic beads are faster and easier to operate for multiple sample purification.

Table 1. Sequencing analysis report for sequencing results shown in Figure 1.

	ADS Beads	Ethanol_EDTA	Ethanol EDTA_NaAc	Vendor Q kit
Length of Read (LOR)	842	839	827	777
Sample Score	47	47	47	37
Quality Value (QV)	824	821	821	708
Cleanup Time	20min	100min	100min	15min

Table 2. Sequencing analysis report for sequencing results shown in Figure 2.

	ADS Beads	Vendor N kit	Vendor G kit	Vendor Z kit
Length of Read (LOR)	787	746	759	411
Sample Score	45	42	42	28
Quality Value (QV)	803	777	789	445
Cleanup Time	20min	15min	10min	10min

Taking into account all the criteria, including reagent cost, time consumed, sequence quality, length of read and sample score, cycle sequencing reactions purified using ADS Beads generate more favorable sequencing results compared with the other seven purification methods. Therefore, the cost-effective and time-saving ADS Sequencing Reaction Cleaning Beads are a better choice for Sanger sequencing dye terminator purification to obtain optimal sequencing quality.