APPLICATION INFORMATION

Genetic Analysis

USING THE AGENCOURT® AMPURE® AND AGENCOURT CLEANSEQ® METHODS TO MANAGE A VARIETY OF INPUT SAMPLE QUALITY

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Abstract

Sequencing core facilities today are faced with the challenge of accepting a variety of input sample quality and sample types. This challenge can be highlighted when BigDye[1], a major component of sequencing reactions, is diluted under commonly utilized reaction conditions and then applied for genotyping by sequencing. Here we present sample purification methods that assist in addressing the varying input sample quality encountered by facilities performing PCR[2] amplification and sequencing for SNP detection.

Introduction

The Agencourt AMPure and Agencourt CleanSEQ procedures utilize Agencourt SPRI® (Solid Phase Reversible Immobilization) magnetic bead technology for nucleic acid purification. When used in combination for PCR purification and sequencing reaction cleanup respectively, they produce superior sequencing data that makes accurate basecalling easier, which is important for applications like genotyping.
The unique combination of these two products successfully tolerates a wider variety of PCR and sequencing reaction product quality without the loss of final sequencing data quality. Data quality is maintained despite the variance in sample quality due to a retention of a higher average fluorescence signal intensity. With alternative methods such as the combination of QIAGEN's QIAquick[1] PCR and Edge Biosystem's Performa[1] DTR purification kits, we found that the loss of fluorescent signal intensity adversely affected the ability to accurately call genotypes by sequencing data as BigDye dilution increased. Samples that are hard to amplify by PCR or have difficult sequencing regions also suffer loss of signal intensity as approximated by this BigDye dilution series. On the other hand, the higher signal retention by Agencourt AMPure and Agencourt CleanSEQ benefits sequencing of such samples, retaining the ability to accurately call genotypes.

Methods

PCR reactions were carried out using two amplicons: MMPII and TP53-Exon 5 with Corriell gDNA samples. Amplicons were produced by PCR (30 cycles), pooled and consolidated into one 96-well plate. A 15 µL sample of pooled PCR product was then purified using QIAGEN's QIAquick 96-well PCR purification plates or Agencourt AMPure, both in accordance with the manufacturers' standard recommended protocols. Following PCR purification, sequencing reactions were set up and run at varying BigDye dilutions ranging from 1/4 to 1/32. A 3 µL sample of Agencourt AMPure purified product was carried into sequencing reactions. Sequencing reactions were then purified with Agencourt CleanSEQ or Edge Performa plates according to manufacturers' recommendations.

Results

Two PCR amplicons (Amplicon 1 and Amplicon 2) were generated and 15 µL of each was purified with either Agencourt AMPure reagent or QIAGEN QIAquick 96 columns. Both the QIAquick and Agencourt AMPure methods produced similar yields ranging from 52 to 339 ng of purified PCR Product.

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Amplicons 1 and 2 were then analyzed in sequencing reactions with varying BigDye dilutions down to 1/32 using Edge Biosystem's Performa DTR and Agencourt CleanSEQ. The Performa DTR system showed effective purification down to a 1/16 BigDye dilution without compromising sequencing pass rates. However, at 1/32 BigDye, the signal intensity for the Performa DTR system fell below detectable limits and no sequencing data was obtained (Figures 1A and 1B). In comparison, the Agencourt CleanSEQ process was able to effectively produce quality sequencing data down to 1/32 BigDye dilutions (Figures 1A and 1B). The effects of a 1/32 dilution BigDye reaction can be observed further when the accuracy of genotype calls between the two methods is compared. Agencourt CleanSEQ was able to accurately call known genotypes in a controlled experiment at the 1/32 level (Figure 2).

Accuracy of Heterozygote Calls

BigDye Dilution	Agencourt CleanSEQ (% Accuracy)	Edge Biosystems Performa DTR (% Accuracy)
1/4X	100	100
1/8X	100	100
1/16X	100	100
1/32X	100	0

Figure 2. At each concentration of BigDye, SNP calls were examined for 12 individuals in 2 amplicons and 5 SNP locations. The table above shows the number of correct heterozygote calls made using each chemistry and the number that are present. Edge Biosystems failed to call at 1/32X due to lack of good genotype quality sequence at that concentration of BigDye.

Conclusions

The choice of sample purification methods applied in core sequencing facilities has a large effect on final sequencing data quality. When examining the effects of accurately called genotypes by sequencing, the combination of Agencourt AMPure and Agencourt CleanSEQ technologies produced superior results in comparison to QIAquick 96 PCR purification and Edge Biosystem's Performa DTR. Retention of higher average fluorescence signals maximizes sequencing resolution, pass rates and quality Phred 20 scores. The ability of Agencourt AMPure and Agencourt CleanSEQ to retain higher average signal intensities as BigDye dilutions increase enables the processing of a wider variety of input sample quality without the loss of final data quality. This also approximates the loss in signal seen with difficult to sequence or poorly prepared templates. In these situations, the combination of Agencourt AMPure and Agencourt CleanSEQ methods would provide similar benefits. The Agencourt SPRI-based method's scalability and automation-friendly format also allows for a streamlined sample process while minimizing reagent costs.

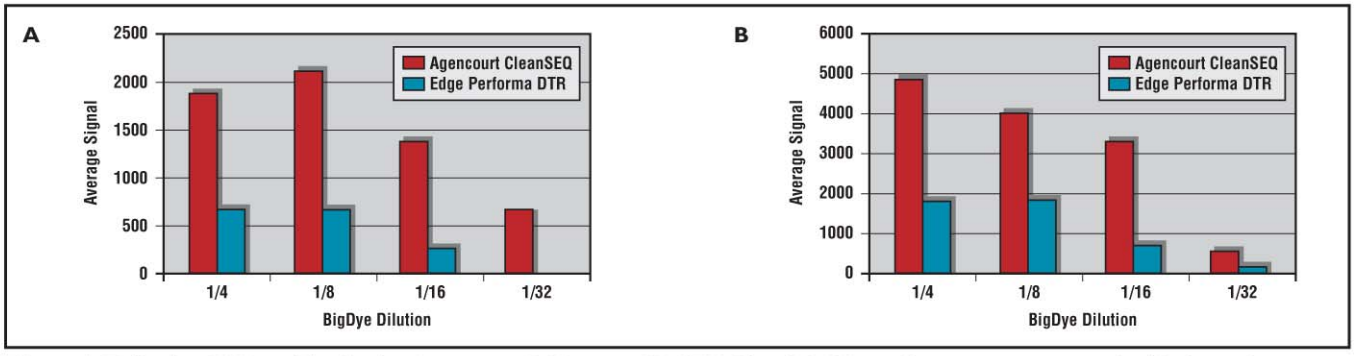

Figure 1. Following PCR purification by Agencourt AMPure or QIAGEN Qiaquick-96, amplicons were sequenced with decreasing amounts of BigDye reagent and purified via Agencourt CleanSEQ or Edge Biosystems Performa DTR plate as described. The average fluorescent signal obtained during sequencing for each clean up method is reported for A) amplicon 1 and B) amplicon 2.

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