Full Compatibility of the SEQueaky Kleen™ H_2O Dye Terminator Removal Kit With the Beckman Coulter CEQ 8000 Genetic Analysis System

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Introduction

Capillary sequencers are used in various fields today, including genomics, because of their simplicity, speed, and high sensitivity. In particular, capillary fluorescence sequencers that recognize dye terminators are now considered a standard tool. Despite improved sensitivity, capillary sequencers have a problem: The sequencing reaction occasionally must be repeated due to interference from nonspecific fluorescent signals (dye blobs) caused by unbound dye terminators. Unbound dye terminators should be removed after the cycle sequencing reaction; however, some fluorescent materials are very difficult to remove effectively.

The SEQueaky Kleen H_2O dye terminator removal kit (catalog #732-6500) is compatible with a wide variety of dye terminators and sequencers and provides highly reproducible sequencing results. The following is an example of a SEQueaky Kleen application where the Beckman Coulter CEQ 8000 genetic analysis system was used.

Method

The DTCS quick start kit for dye terminator cycle sequencing (Beckman Coulter) was used for the cycle sequencing reaction. The reaction system consisted of 8.0 µl DTCS quick start master mix, 1.0 µl (250 ng) pUC18 as template, 3.0 µl (4.8 pmol) primer [M13 –47 sequencing primer (5'-CGCCAGGCTTTTCCCAGTCACGAC-3')], and 8.0 µl sterile water. The sequencing reaction protocol was 20 sec at 96°C, 20 sec at 50°C, and 4 min at 60°C for 30 cycles. After 30 cycles, the samples were incubated at 4°C.

The storage buffer was removed from the SEQueaky Kleen H_2O filter plate by centrifuging it for 2 min at 750 x g in an S2096 horizontal rotor on an Allegra 21R centrifuge (Beckman Coulter). Each of the sequencing reactions was added to the SEQueaky Kleen H_2O filter plate after cycle sequencing was completed. The filter plate was then centrifuged for either 2 or 5 min at 750 x g (see Table). The purified samples were dried and dissolved in 30 µl of the supplied sample loading solution, which was recommended for use with the CEQ 8000.

Samples were sequenced using the CEQ 8000 genetic analysis system. Capillary electrophoresis conditions were: capillary temperature 50°C, voltage 4.5 kV, electrophoresis time 85 min. The sequencing analysis software installed on the CEQ 8000 was used for analysis.

Table. SEQueaky Kleen H_2O kit protocols

Standard protocol	Optimum protocol
1. Centrifugation at 750 x g for 2 min	1. Centrifugation at 750 x g for 2 min
2. Sample added to filter plate	2. Sample added to filter plate
3. Centrifugation at 750 x g for 2 min	3. Centrifugation at 750 x g for 5 min
4. Sample collection	4. Sample collection

Results and Discussion

The accuracy, speed, and number of bases identified are important in sequencing. Among them, the most important is the sequencing accuracy. We studied the conditions that would provide the best sequencing performance using the CEQ 8000 genetic analysis system, DTCS quick start kit, and SEQueaky Kleen H_2O kit. We focused particularly on the effect of sample elution time during dye terminator removal on the sequencing accuracy and signal level.

Centrifugation for 5 min using the SEQueaky Kleen H_2O kit yielded a better signal than centrifugation for 2 min (Figures 1 and 2), although the efficiency of dye terminator removal was high in both cases. Sequencing results for a 5 min centrifugation are shown in Figure 3. Equivalent results can also be achieved using a solution centrifuged for 2 min and then for an additional 3 min (data not shown). Therefore, a 5 min centrifugation is probably more suitable for most samples. The type of centrifuge used will also affect the optimal elution conditions. For centrifuges with a high/low speed feature, we recommend the maximum setting for faster and more accurate purification.

Conclusions

Optimal dye terminator removal for sequencing with the DTCS quick start kit and the CEQ 8000 genetic analysis system was tested using the SEQueaky Kleen H_2O kit. With the SEQueaky Kleen H_2O kit, unbound dye terminators are removed equally efficiently whether centrifuged for 5 min or 2 min (the normal sample elution time). However, stronger signals can be obtained with a 5 min centrifugation, and a wider variety of samples can be accommodated.

For more information, request Bio-Rad bulletin 2807, Comparison of Commercial Dye Terminator Removal Kits.

Acknowledgement

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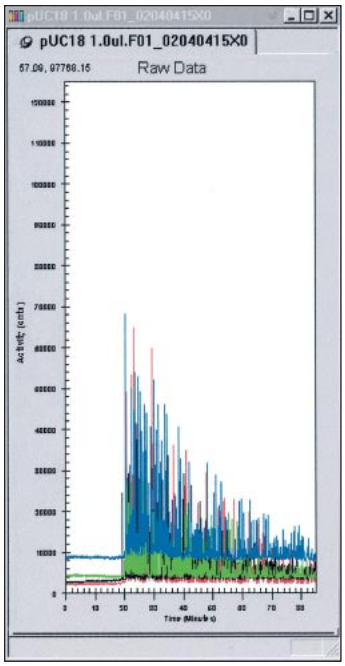

Fig. 1. Signal levels with a 2 min centrifugation.

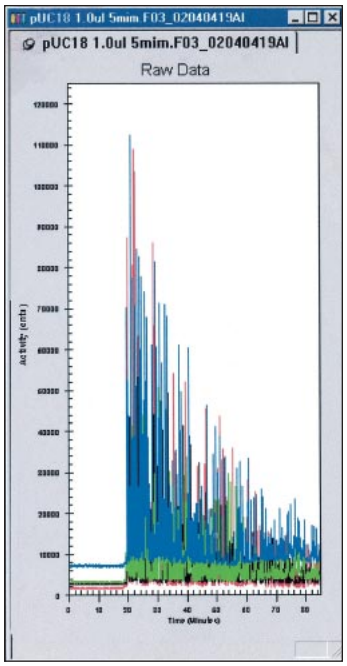

Fig. 2. Signal levels with a 5 min centrifugation.

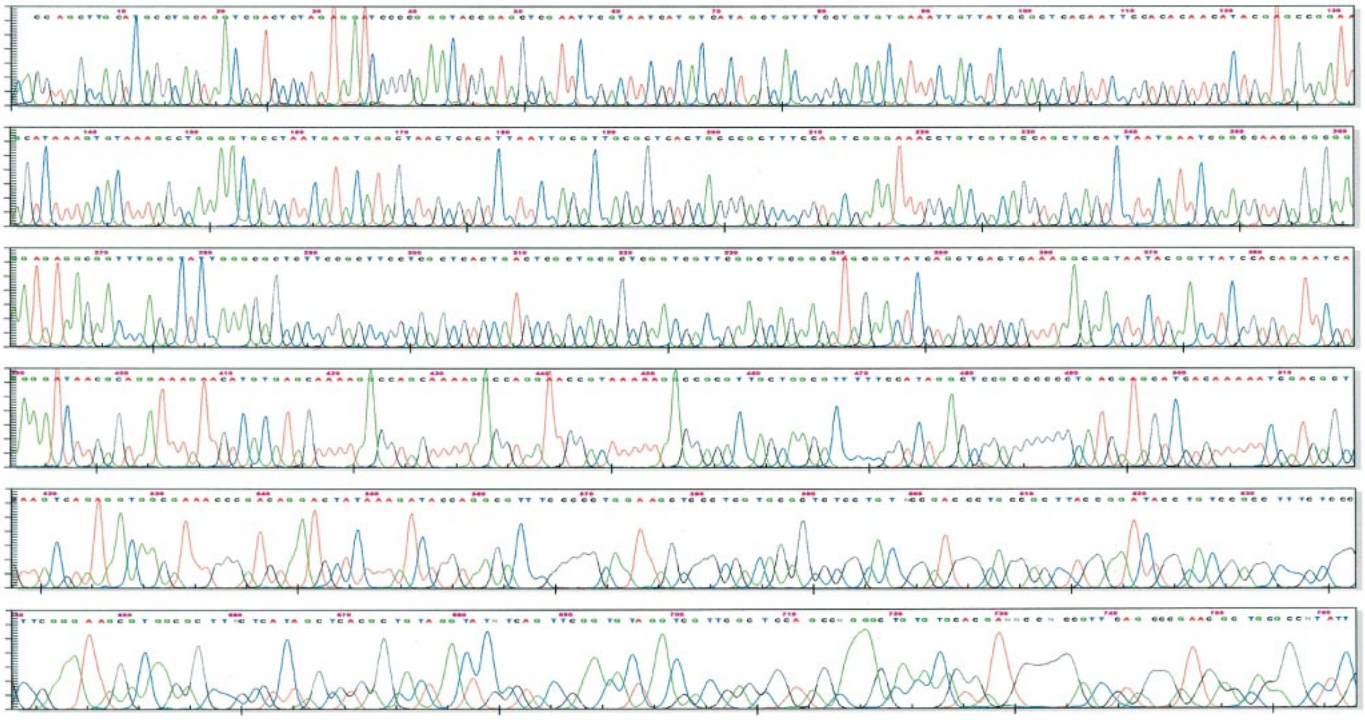

Fig. 3. Sequencing data with a 5 min centrifugation.

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